CoronaVac

O que a ciência comprova

fundação butantan

INSTITUTO BUTANTAN
A serviço da vida

SÃO PAULO
GOVERNO DO ESTADO
TRABALHO E RESPEITO POR VOCÊ

Sumário

1. Gera alta resposta imune

1.1 Vacina do Butantan tem eficácia global superior à exigida pela OMS

O Instituto Butantan e o Governo de São Paulo informam que a vacina contra o coronavírus obteve 50,38% de eficácia global no estudo clínico desenvolvido no Brasil, além de proteção de 78% em casos leves e 100% contra casos moderados e graves da COVID-19. Todos os índices são superiores ao patamar de 50% exigido pela OMS (Organização Mundial de Saúde).

Os resultados foram submetidos a um comitê internacional independente e já estão com a Anvisa (Agência Nacional de Vigilância Sanitária), que analisa o pedido de uso emergencial do imunizante no Brasil. A pesquisa envolveu 16 centros de pesquisa científica em sete estados e o Distrito Federal. O teste duplo cego, com aplicação da vacina em 50% dos voluntários e de placebo nos demais, envolveu 12,5 mil profissionais de saúde.

"É uma excelente vacina esperando para ser usada em um país onde morrem, no momento, em torno de mil pessoas por dia. Esperamos que as autoridades entendam o momento e ajudem nossa população a receber as vacinas o mais rapidamente possível", afirmou o Diretor do Instituto Butantan, Dimas Covas.

"Os dados são extremamente importantes no impacto da saúde pública, impedindo que as pessoas adoeçam de forma grave e sobrecarreguem hospitais. É a possibilidade de impedirmos que as pessoas morram", disse o Secretário de Estado da Saúde, Jean Gorinchteyn. "Temos uma vacina que foi testada na vida real, no meio de uma pandemia e naqueles que eram mais expostos", acrescentou.

O estudo verificou que a menor taxa foi registrada em casos de infecções muito leves, considerados score 2 e verificados em pacientes que receberam placebo. De uma amostragem de 9,2 mil participantes, 85 dos casos muito leves foram de pessoas que receberam vacina, e 167 em voluntários que tomaram placebo.

Já o resultado de eficácia dos casos leves, classificado como score 3, em pacientes que precisaram receber alguma assistência, foi de 77,96%, sendo que sete pessoas haviam recebido a vacina, e outras 31, placebo.

Para os casos moderados e graves que necessitaram de hospitalização, a eficácia foi de 100%. Nenhum paciente infectado que recebeu a vacina do Butantan precisou de internação. Entre os que tomaram placebo, houve sete pacientes que precisaram de internação.

Todo os voluntários são profissionais de saúde, com risco muito alto e contínuo de exposição ao coronavírus. Eles receberam duas doses da vacina, com intervalos de duas semanas entre cada aplicação. A pesquisa também demonstrou que o imunizante é extremamente seguro - nenhuma reação adversa grave foi registrada entre os participantes.

A vacina é desenvolvida pelo Butantan há pouco mais de seis meses, em parceria internacional com a biofarmacêutica Sinovac Biotech, sediada em Pequim. O produto é baseado na inativação do vírus SARS-CoV-2 para induzir o sistema imunológico humano a reagir contra o agente causador da COVID-19. A tecnologia é similar à de outras vacinas amplamente produzidas pelo instituto de São Paulo.

Em novembro, a revista científica Lancet, uma das mais importantes no mundo, publicou os resultados de segurança da vacina do Butantan nas fases 1 e 2, realizados na China, com 744 voluntários. A publicação mostrou que o produto é seguro e capaz de produzir resposta imune em 97% dos casos em até 28 dias após a aplicação.

O Butantan já dispõe de 10,8 milhões de doses da vacina em solo brasileiro. No final de março, a carga total de imunizantes disponibilizados pelo instituto é estimada em 46 milhões de doses. O Plano Estadual de Imunização tem início previsto para o próximo dia 25.

Publicado em: 14/01/2021

Estudo 1


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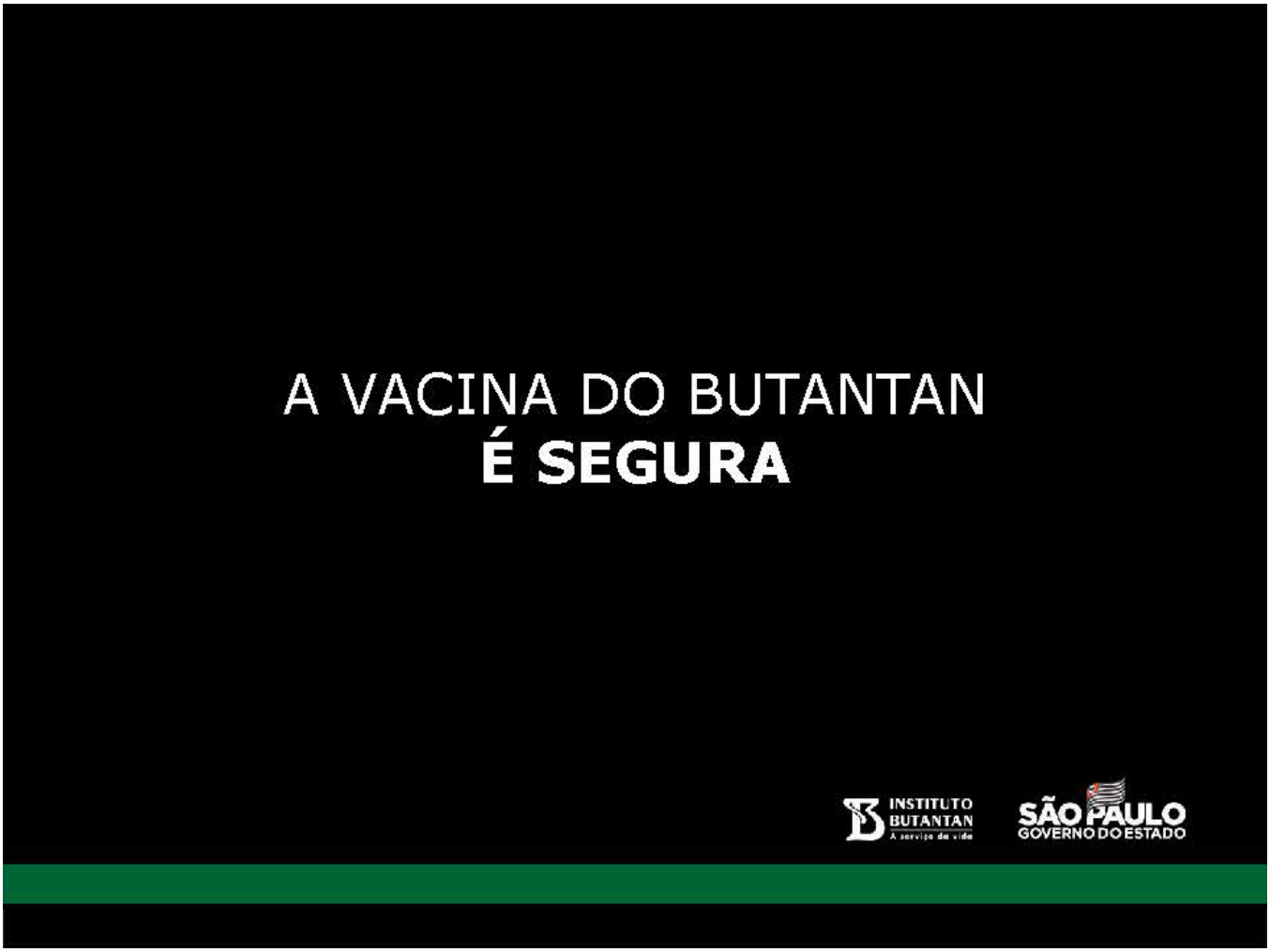
Vacina do Butantan
A vacina do Brasil
2021


A VACINA DO BUTANTAN
É SEGURA
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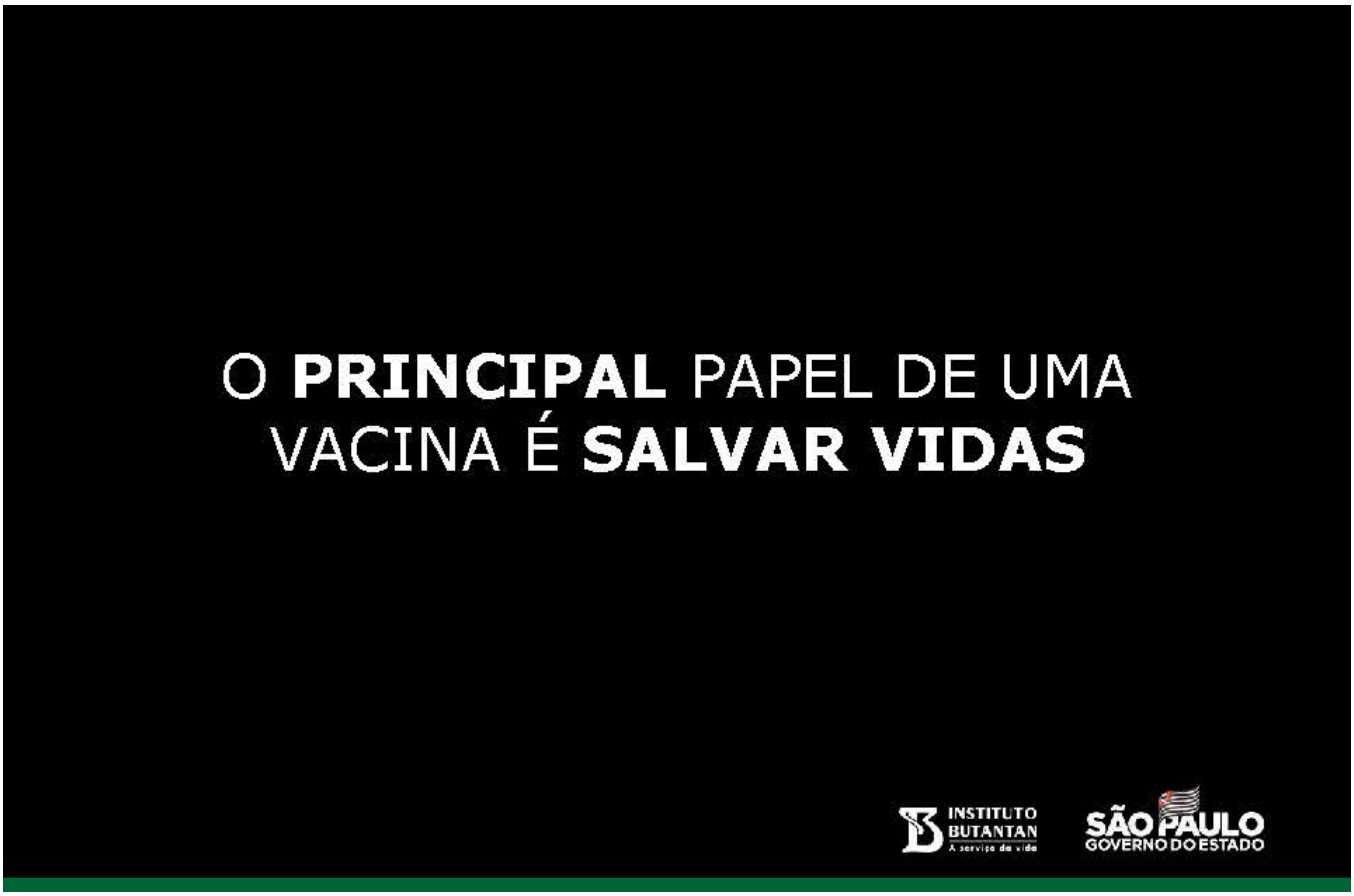
O **PRINCIPAL** PAPEL DE UMA VACINA É **SALVAR VIDAS**
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OS GRUPOS DE **MAIOR RISCO** SÃO SEMPRE **PRIORIDADES**
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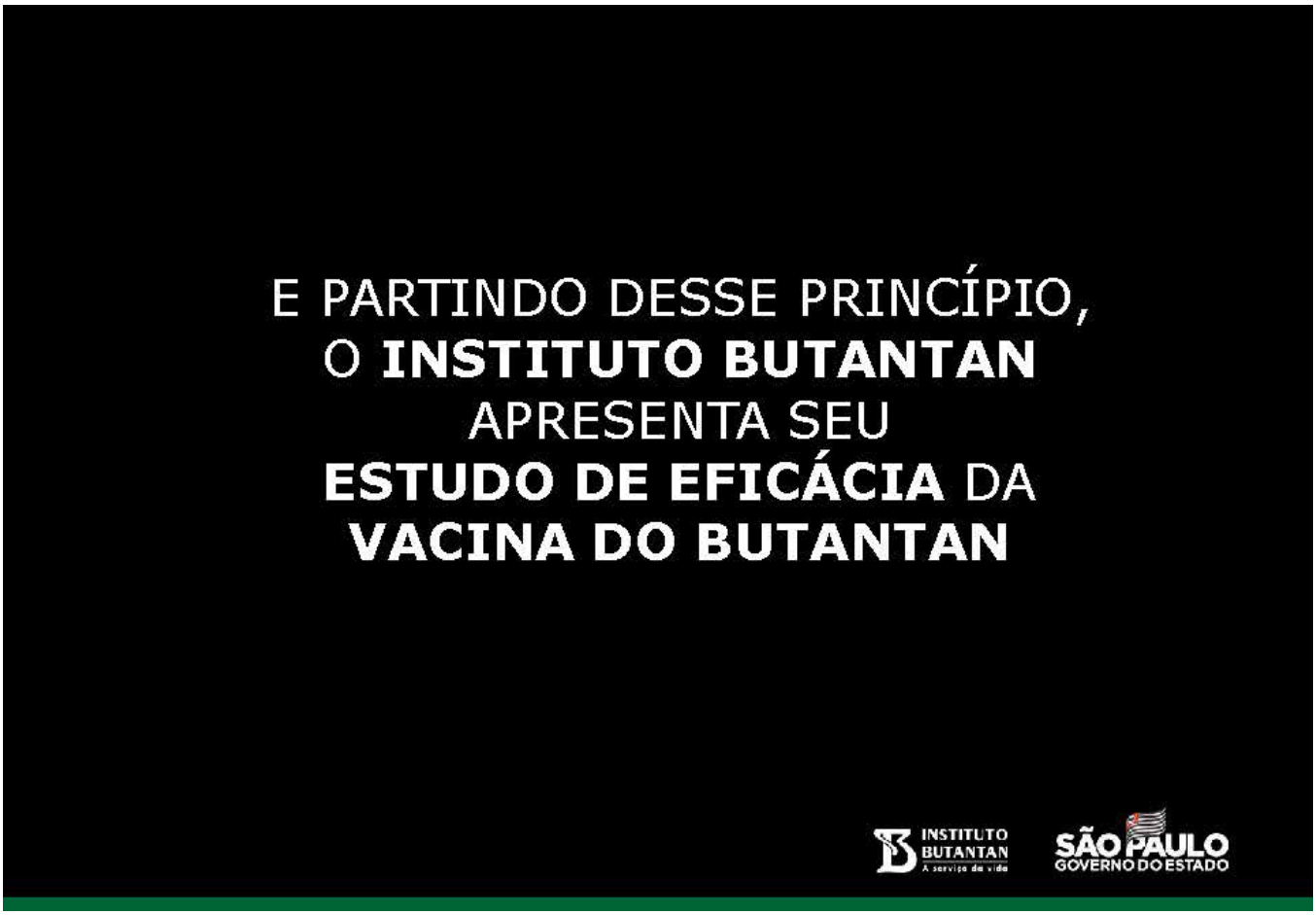
E PARTINDO DESSE PRINCÍPIO,
O INSTITUTO BUTANTAN
APRESENTA SEU
ESTUDO DE EFICÁCIA DA
VACINA DO BUTANTAN
INSTITUTO BUTANTAN
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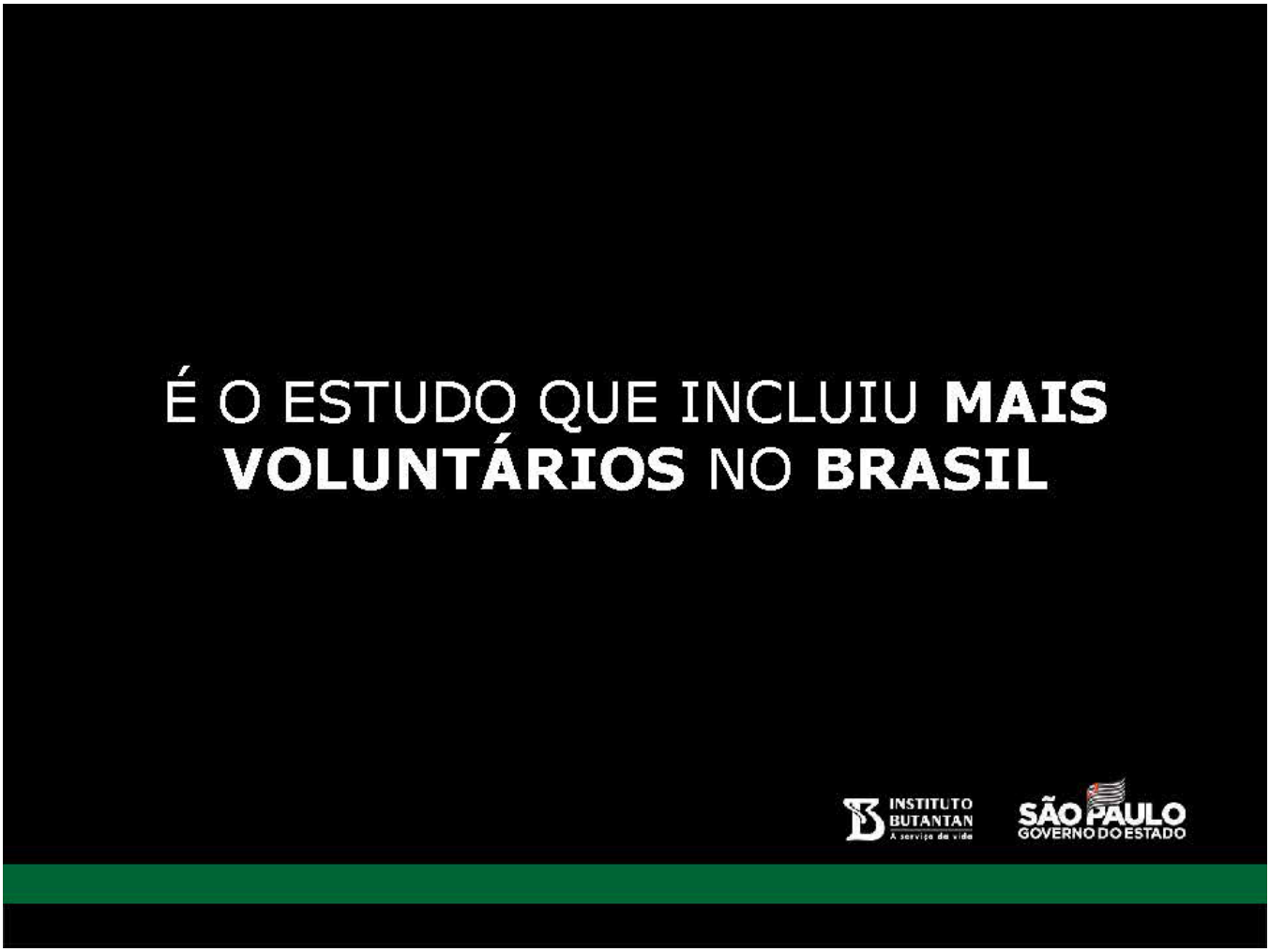
É O ESTUDO QUE INCLUIU MAIS
VOLUNTÁRIOS NO BRASIL
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Equipe PROFISCOV

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

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População de estudo

Único estudo feito exclusivamente em profissionais de saúde

13.060 voluntários cuidando de **pacientes com COVID-19**

O maior desafio para uma vacina

- Muito **alto risco de exposição**
- **Maior** dose **infectante**
- **Detecção precoce** de casos
- **Duas doses** com intervalo de duas semanas

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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

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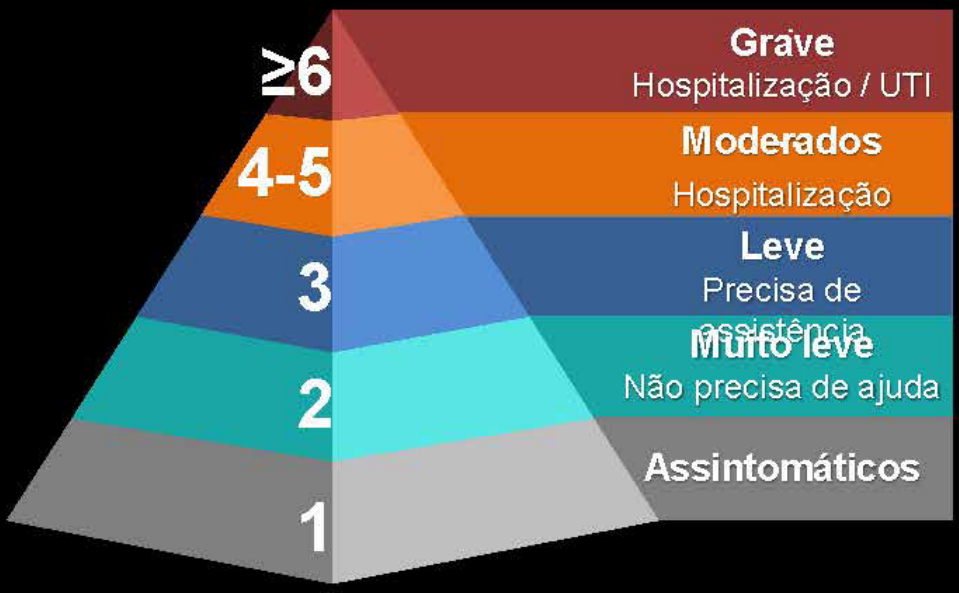
Escala de Progressão da OMS de COVID-19



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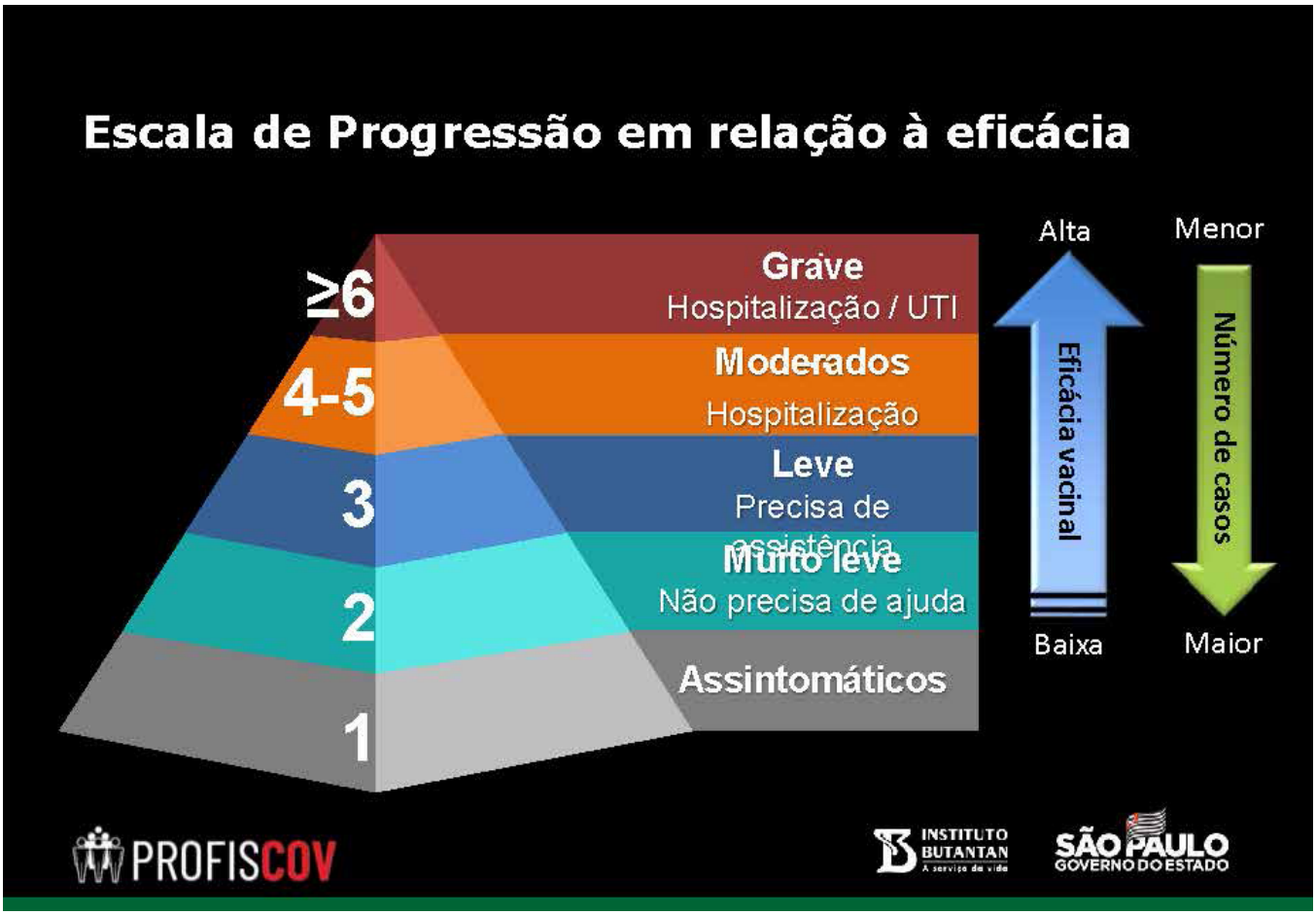
Escala de Progressão em relação à eficácia
≥6
4-5
3
2
1
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
Alta
Baixa
Eficácia vacinal
Menor
Maior
Número de casos
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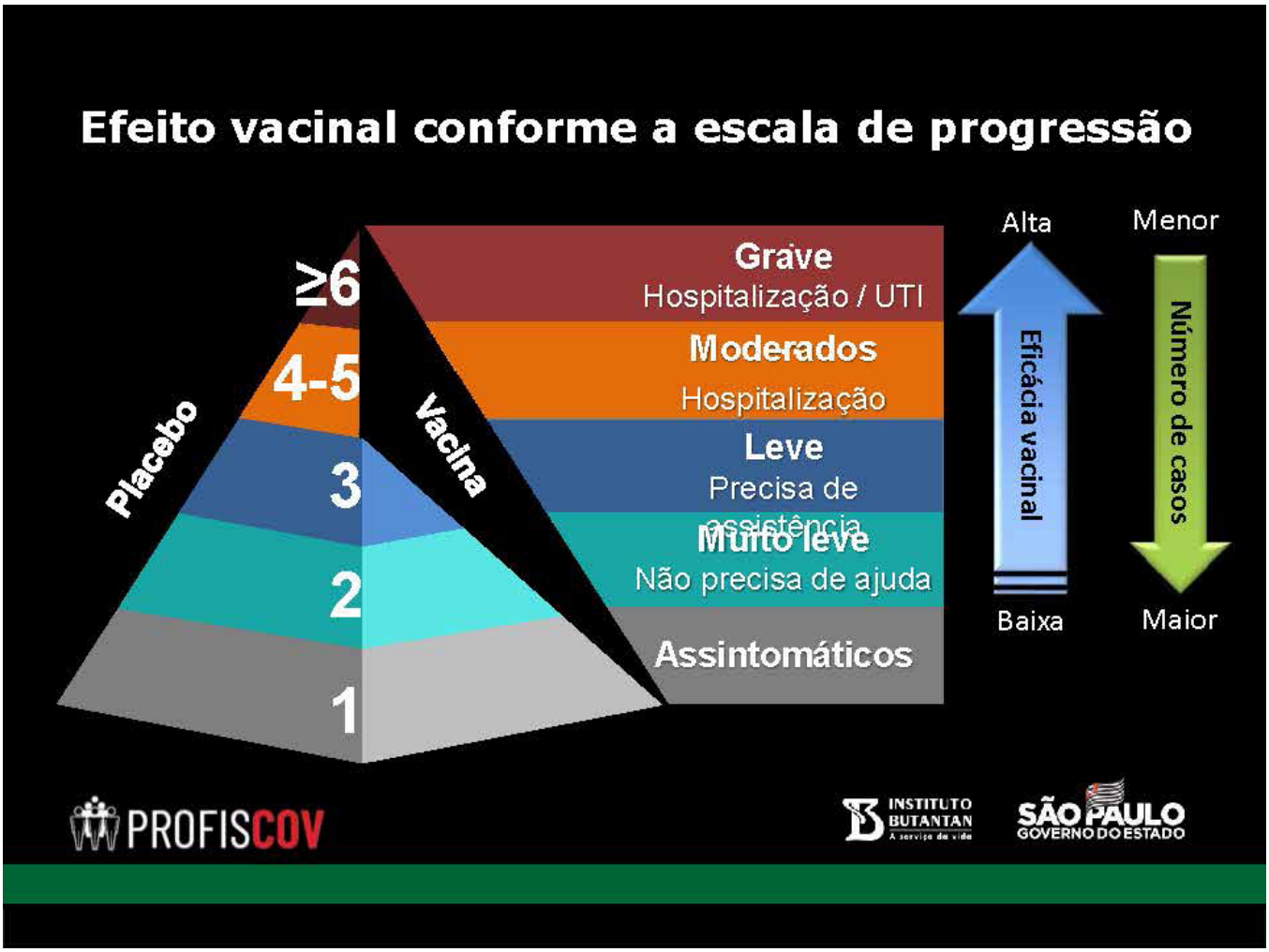
Efeito vacinal conforme a escala de progressão
Placebo
Vacina
≥6
4-5
3
2
1
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
Alta
Baixa
Eficácia vacinal
Menor
Maior
Número de casos
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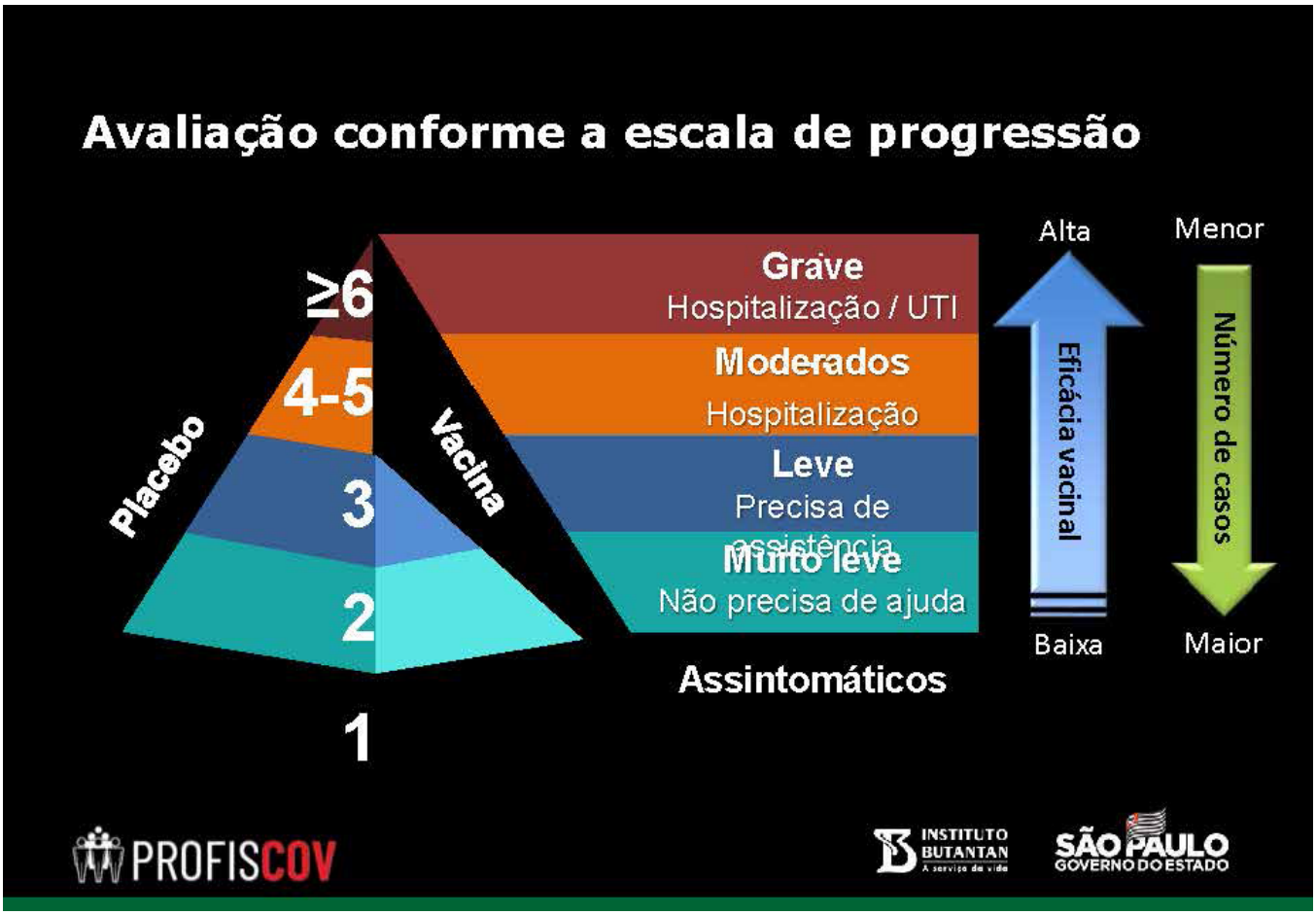
Avaliação conforme a escala de progressão
≥6
4-5
3
2
1
Placebo
Vacina
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
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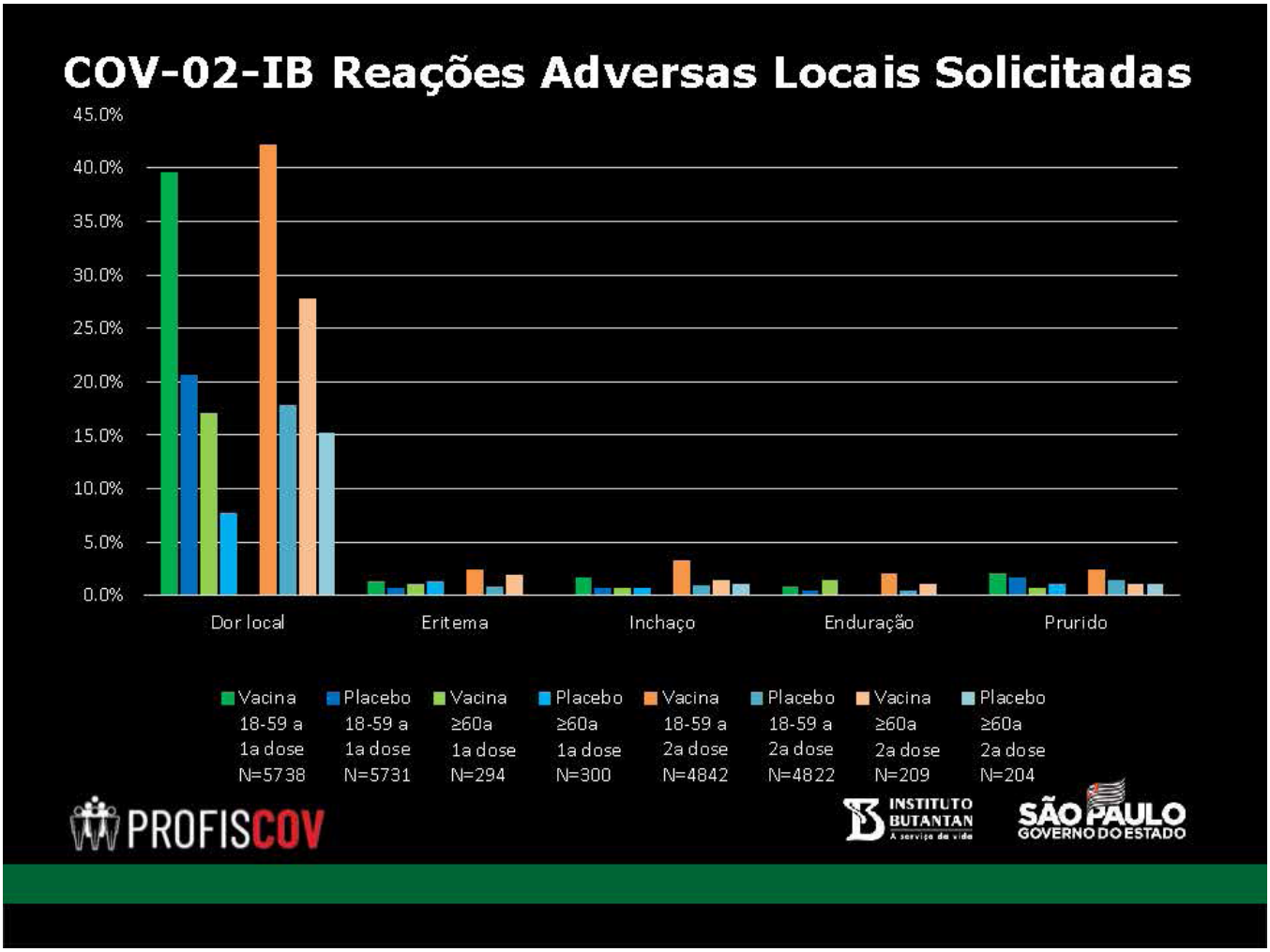
COV-02-IB Reações Adversas Locais Solicitadas
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Dor local
Eritema
Inchaço
Enduração
Prurido
Vacina 18-59 a 1a dose N=5738
Placebo 18-59 a 1a dose N=5731
Vacina ≥60a 1a dose N=294
Placebo ≥60a 1a dose N=300
Vacina 18-59 a 2a dose N=4842
Placebo 18-59 a 2a dose N=4822
Vacina ≥60a 2a dose N=209
Placebo ≥60a 2a dose N=204
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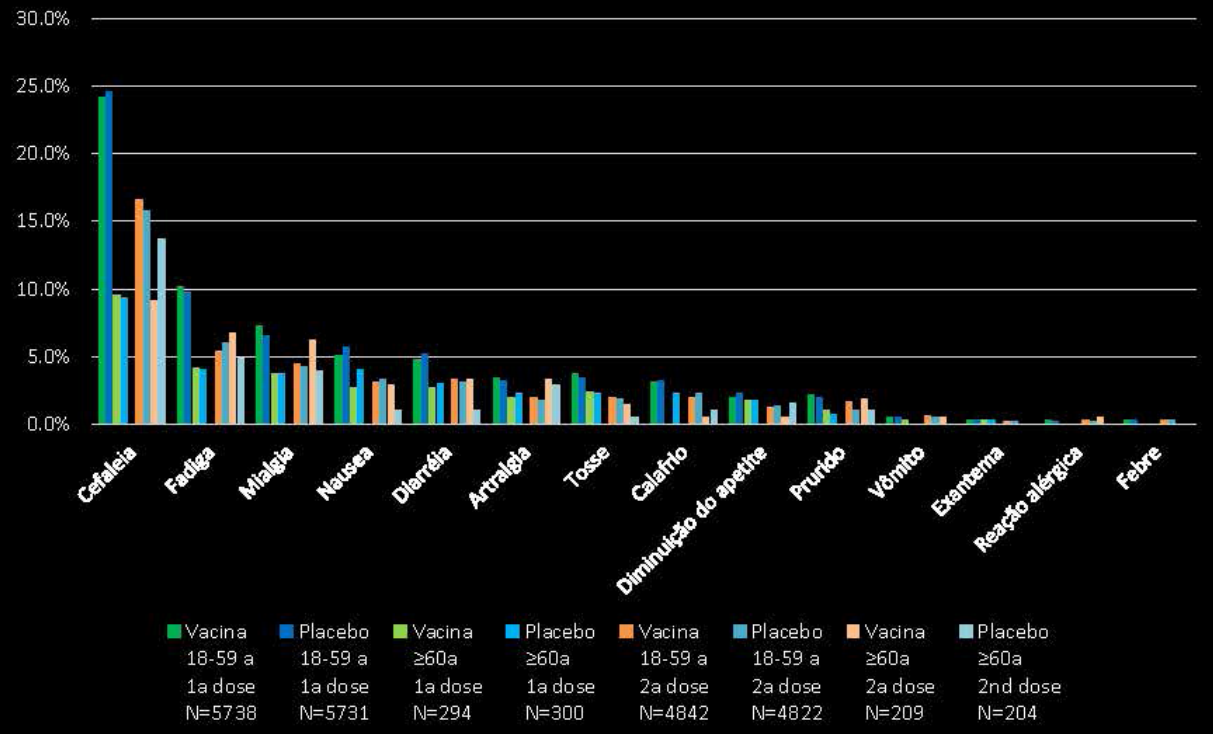
COV-02-IB Reações Adversas Sistêmicas Solicitadas



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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**

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Resultados de eficácia - Score 4 ou superior 9242 participantes
Vacina
n=4653
Placebo
N=4599
≥6
4-5
3
2
1
Placebo
Vacina
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
0
(0)
7
(0,99)
100%
p=0,4967
Casos (incidência 100s pessoas-ano)
PROFISCOV
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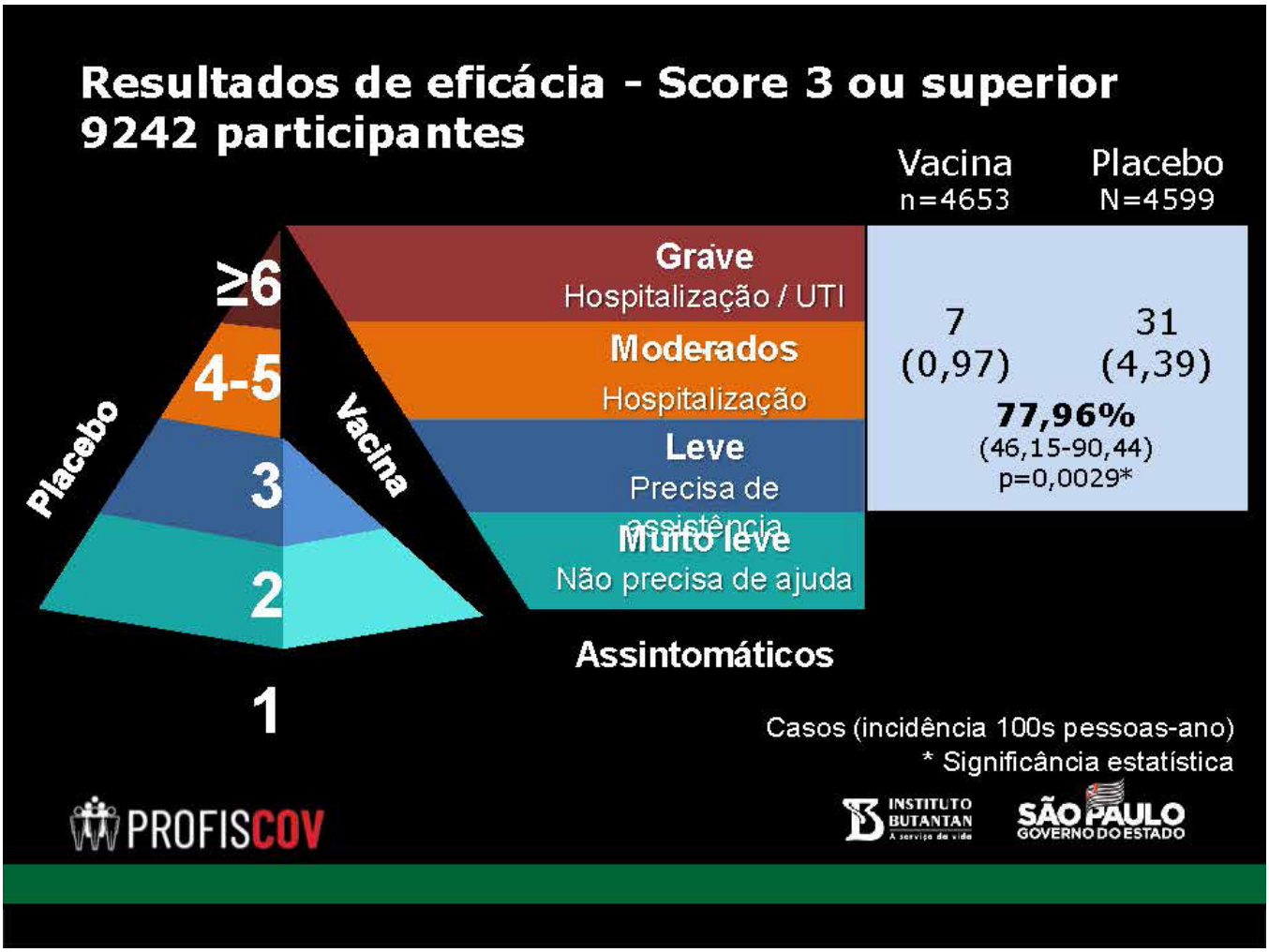
Resultados de eficácia - Score 3 ou superior 9242 participantes
Vacina
n=4653
Placebo
N=4599
≥6
4-5
3
2
1
Placebo
Vacina
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
7
(0,97)
31
(4,39)
77,96%
(46,15-90,44)
p=0,0029*
Casos (incidência 100s pessoas-ano)
* Significância estatística
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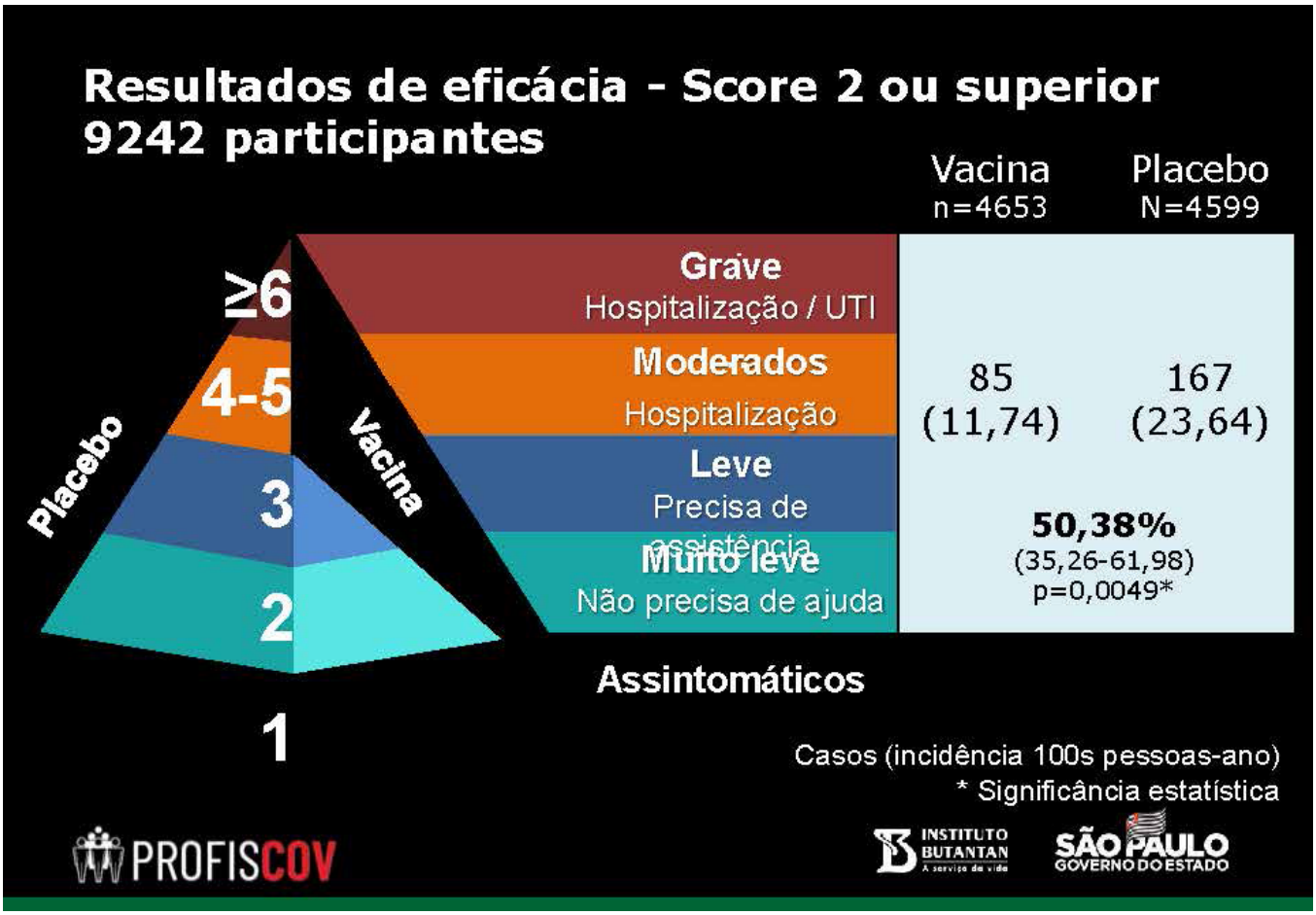
Resultados de eficácia - Score 2 ou superior
9242 participantes
Vacina
n=4653
Placebo
N=4599
≥6
4-5
3
2
1
Placebo
Vacina
Grave
Hospitalização / UTI
Moderados
Hospitalização
Leve
Precisa de assistência
Muito leve
Não precisa de ajuda
Assintomáticos
85
(11,74)
167
(23,64)
50,38%
(35,26-61,98)
p=0,0049*
Casos (incidência 100s pessoas-ano)
* Significância estatística
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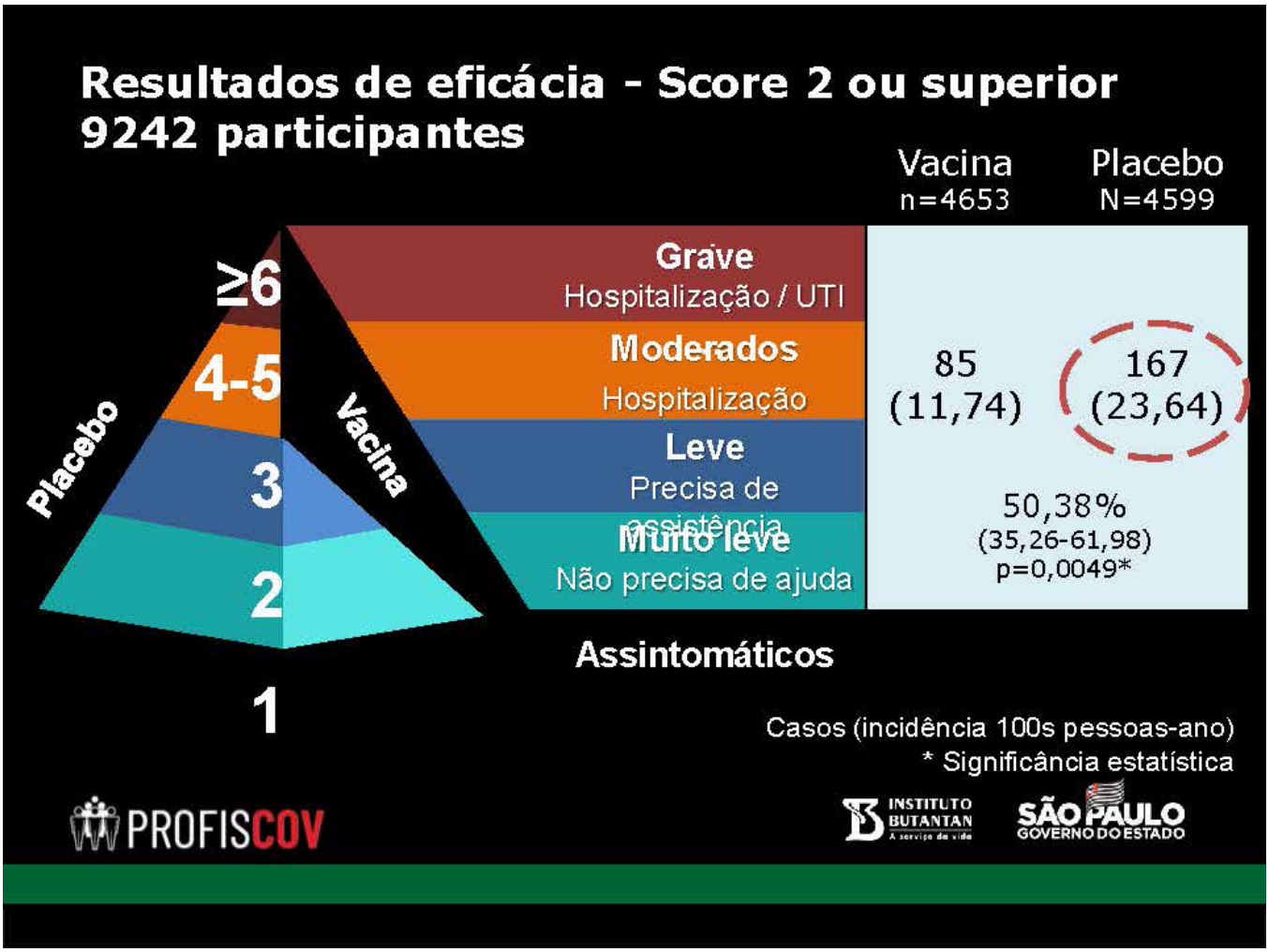
Resultados de eficácia - Score 2 ou superior
9242 participantes
Vacina
n=4653
Placebo
N=4599
≥6
4-5
3
2
1
Placebo
Vacina
Grave
Hospitalização / UTI
Moderados
Hospitalização
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50,38%
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p=0,0049*
Casos (incidência 100s pessoas-ano)
* Significância estatística
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Resumo de resultados de eficácia
9242 participantes

* Significância estatística

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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**

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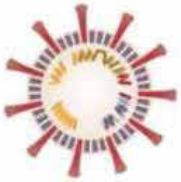
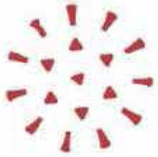
Estratégias

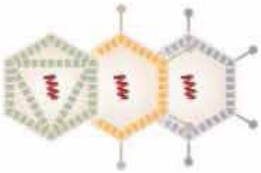
Vírus atenuado, morto ou VLP



Proteínas virais



Vetores virais



RNAm e DNA

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

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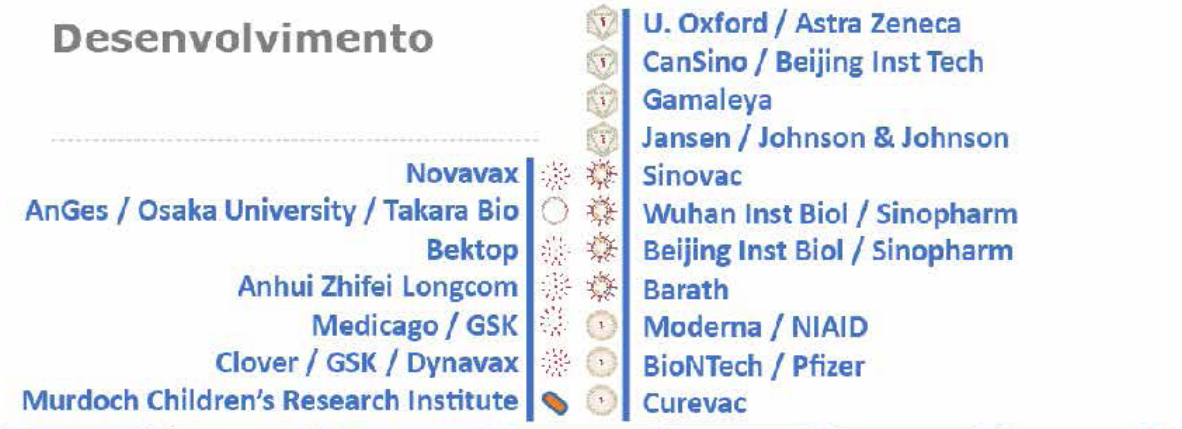
Desenvolvimento



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

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Eficácia dos estudos

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

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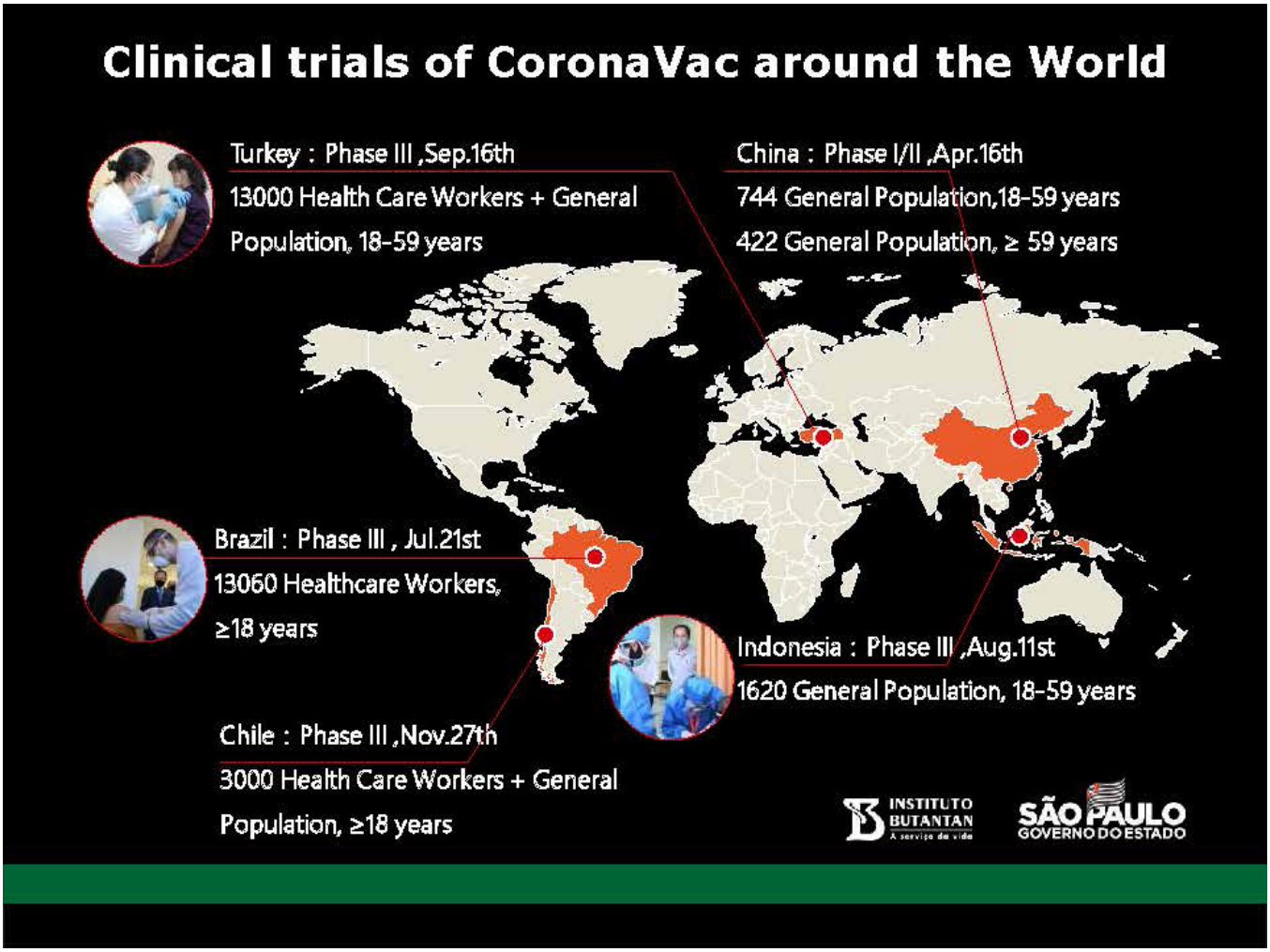
Eficácia dos estudos

- Pfizer 95%
- Moderna 94,1%
- AstraZeneca 62 a 90%
- Sinopharm 79%
- Gamaleya 90%
- Sinovac 50,3 a 100%

Pfizer			
Total	90.3	95	97.6
OMS 4		100	
Moderna			
Total	89.3	94.1	96.8
OMS 4		100	
AstraZeneca			
Total	54.8	70.4	80.6
BD/AD	67.4	90	97
AD/AD	28	60.3	78.2
OMS 4			
Sinopharm			
Total		79	
Gamaleya			
Total		91.4	
Sinovac			
OMS 2	35.26	50.39	61.98
OMS 3	49.15	77.96	90.44
OMS 4	95.42	100	100

Nenhuma das vacinas foi testada em um ambiente de incidência tão alta



Result of Phase I/II Study in China

1. Safety

No serious adverse reactions were observed in both vaccine group and placebo group

There was no significant difference between vaccine and placebo groups regarding to adverse reactions after vaccination

Majority of adverse reactions were grade 1

2.Immunogenicity

CoronaVac induced no less than 94.9% seroconversion of neutralizing antibody.

Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2020 Nov 17

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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

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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

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Obrigado

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

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

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

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

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

vacinal 81,3% da população adulta e 60,9% da população urbana de Serrana, o equivalente a cerca de 27 mil pessoas. Deste número, 16% eram idosos com mais de 60 anos.

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

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

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

Publicado em: 03/12/2021

Artigo 1

Original Research

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

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

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

Hugo Alberto Brango[3]

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

Elizabeth González Patiño[3]

Ana Paula Batista[3]

Barbara Marques Coutinho[2]

Gilberto Guedes de Padua[3]

Glenda Renata de Moraes[4]

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

Joane do Prado Santos[3]

Natasha Nicos Ferreira, M.D.[2]

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

Ricardo Haddad, Ph.D.[3]

Roberta de Oliveira Piorelli[3]

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

Simone Kashima Haddad[1]

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

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

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

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

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

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

Abstract

Background:

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

Methods:

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

Findings:

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

Interpretation:

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

(ClinicalTrials.gov Identifier, NCT04747821)

Funding

Fundação Butantan and São Paulo Research Foundation (FAPESP).

Introduction

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

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

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

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

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

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

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

Methods

Study design and participants

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

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

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

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

Randomization

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

Procedures

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

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

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

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

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

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

Outcomes

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

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

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

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

Statistical analysis

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

The model is written as follows:

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

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

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

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

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

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

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

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

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

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

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

Role of the funding source

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

Results

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Contributors

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

Data sharing

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

Declaration of interests

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

Acknowledgments

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

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

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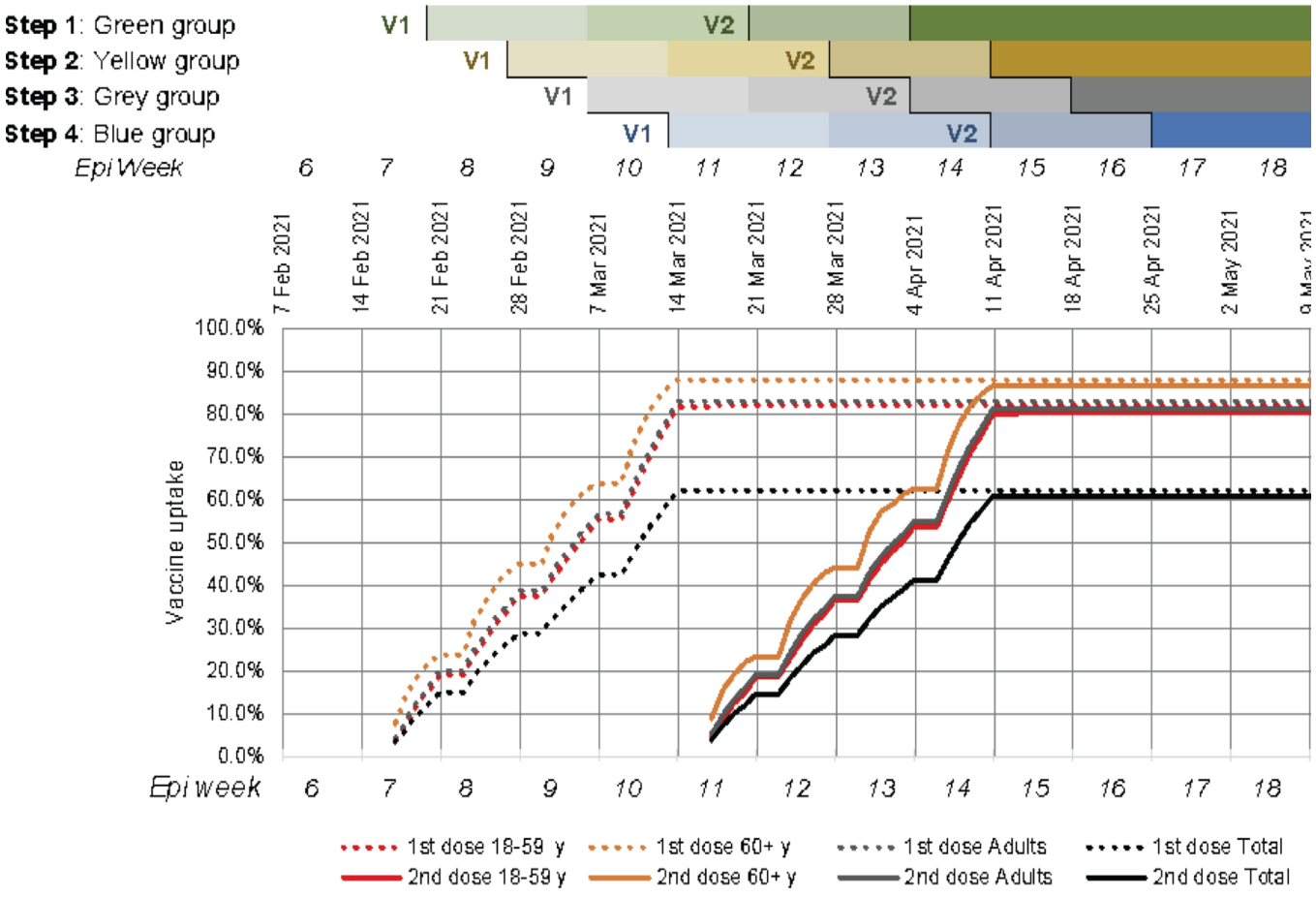
Figure legends

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.


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

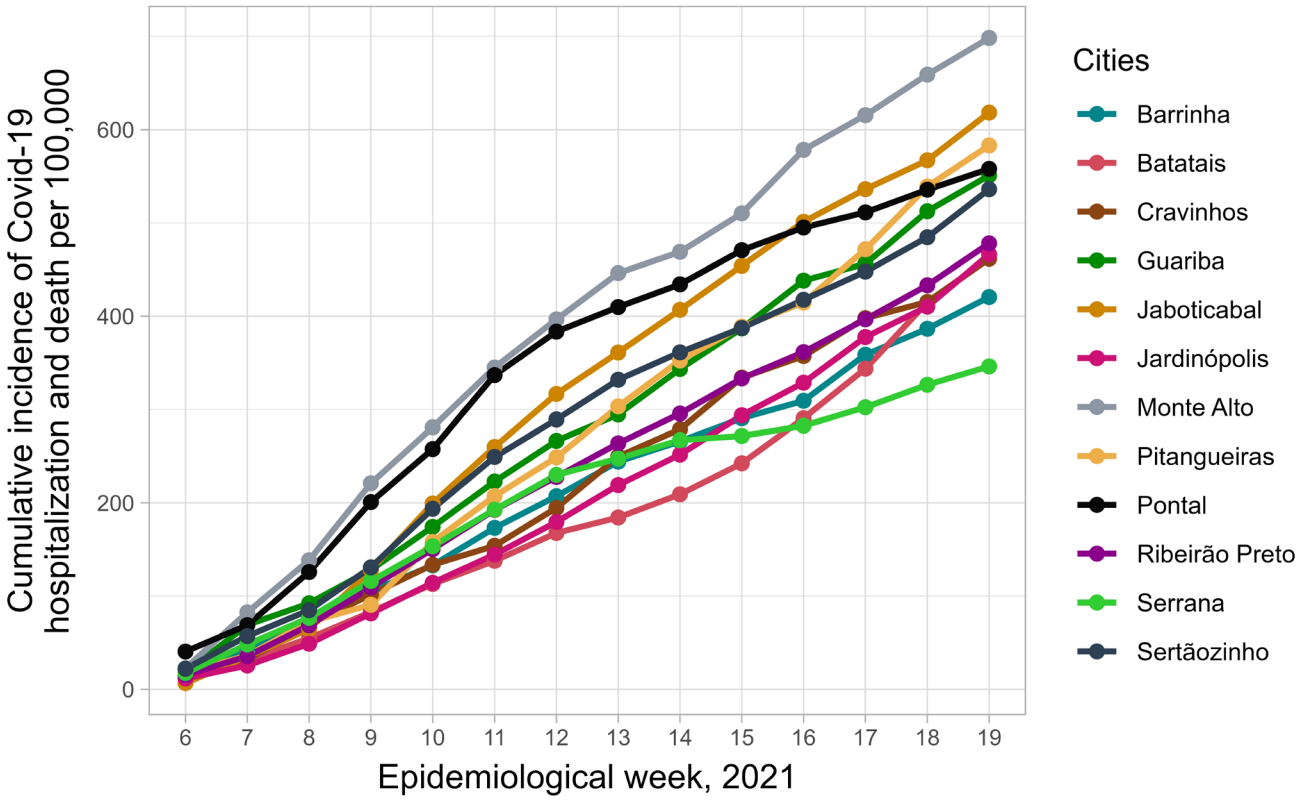

Cumulative incidence of Covid-19 hospitalization and death per 100,000
0
200
400
600
6
7
8
9
10
11
12
13
14
15
16
17
18
19
Epidemiological week, 2021
Cities
Barrinha
Batatais
Cravinhos
Guariba
Jaboticabal
Jardinópolis
Monte Alto
Pitangueiras
Pontal
Ribeirão Preto
Serrana
Sertãozinho

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

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

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

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

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

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

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

1.3 CoronaVac induz respostas de anticorpos rápidas e duradouras por até 12 meses, afirma estudo

Um estudo científico publicado na última semana por pesquisadores chineses do Centro de Controle e Prevenção de Doenças e da Universidade Médica Capital, ambos de Pequim, evidencia que a resposta imune humoral e celular induzida pela CoronaVac, vacina do Butantan e da Sinovac contra a Covid-19, permanece por um ano no organismo. O trabalho foi submetido à conceituada publicação médica britânica The Lancet, tendo sido publicado dia 19 sob a forma de preprint.

Foram analisados 150 voluntários, com idades entre 18 e 59 anos, que receberam as duas doses da vacina com 14 dias de intervalo. Para poder verificar a evolução do panorama imunológico dos participantes, amostras de sangue foram coletadas antes do recebimento da primeira dose da vacina, assim como decorridos um, três, seis e 12 meses após a segunda dose.

Os cientistas constataram que, um mês após a imunização completa, os anticorpos de ligação e os anticorpos neutralizantes surgiram rapidamente. A taxa soropositiva de anticorpos de ligação foi de 99% e a taxa de soroconversão de anticorpos neutralizantes foi de 50%. Do terceiro até o 12º mês após a imunização, houve uma ligeira diminuição ao longo do tempo nos anticorpos neutralizantes e anticorpos de ligação. Aos 12 meses, porém, os anticorpos de ligação e os neutralizantes ainda eram detectáveis.

Em termos mais técnicos, a secreção de interferon-gama (IFN-γ) e da interleucina 2 (IL-2) induzida especificamente por RBD (domínio de ligação ao receptor) persistiram em níveis elevados por até seis meses, e puderam ser observadas ao longo dos 12 meses de análise.

Além disso, células CD4 + TCM, CD4 + TEM, CD8 + TEM e CD8 + TE específicas para SARS-CoV-2 foram todas detectáveis e funcionais por até 12 meses após a administração da segunda dose.

Assim, os pesquisadores chineses constataram a persistência da resposta imune induzida pela CoronaVac, em um regime de duas doses. Foi comprovado que a vacina não apenas induziu ligações duráveis e respostas de anticorpos neutralizantes, como também células T de memória CD4 + e CD8 + específicas para SARS-CoV-2 por até 12 meses.

Publicado em: 22/11/2021

Artigo 2

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

Wei Zhao, Wei-xin Chen, Juan Li, Meng Cheng, Qin Li, Min Lv, Shan-Shan Zhou, Shuang Bai, Ya-li Wang, Li-chi Zhang, Peng Zhang, Jian Wang, Qun Zheng, Jiang Wu

Experimental Center for Basic Medical Teaching, School of Basic Medical Sciences, Capital Medical University (Q Zheng PhD); **Beijing Center for Disease Prevention and Control, Beijing Research Center for Preventive Medicine** (W Zhao PhD, W Chen MSc, J Li PhD, M Cheng MSc, Min Lv PhD, S Zhou MSc, S Bai MSc, Y Wang MSc, L Zhang MSc, P Zhang PhD, J Wang PhD, Prof J Wu); **Department of Laboratory, Yanjing Medical College, Capital Medical University** (Q Li PhD**).**

Correspondence to:

Prof Qun Zheng, Experimental Center for Basic Medical Teaching, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China

zhengqun@ccmu.edu.cn

or

Prof Jiang Wu, Beijing Center for Disease Prevention and Control, No.16, Hepingli middle street, Dongcheng District, Beijing, 100013, China.

wj81732@hotmail.com

Summary

Background Understanding immune memory to COVID-19 vaccines is critical for the design and optimal vaccination schedule for curbing the COVID-19 pandemic. Here, we assessed the persistence of humoral and cellular immune responses for 12 months after two-dose CoronaVac.

Methods Participants aged 18–59 years received two doses of 3 μg CoronaVac 14 days apart, and blood samples were collected before vaccination (baseline) and at 1, 3, 6, and 12 months after the second shot. Humoral responses of specific antibodies and neutralising antibodies were measured by using chemiluminescent immunoassay and wild-type SARS-CoV-2 microneutralisation assay, respectively. Cellular responses were measured by immunospot-based and intracellular cytokine staining assays. This trial is registered with ClinicalTrials.gov, NCT05072496.

Findings Total 150 participants were enrolled, and 136 of them completed the study through the 12-month endpoint. At 1 month after vaccination, binding and neutralising antibodies emerged rapidly, the seropositive rate of binding antibodies and seroconversion rate of neutralizing antibodies was 99% and 50%, respectively. From 3 to 12 months, the binding and neutralizing antibodies declined slightly overtime. At 12 months, the binding and neutralizing antibodies were still detectable and significantly higher than the baseline. IFN-γ and IL-2 secretion specifically induced by RBD persisted at high levels until 6 months, and could be observed at 12 months, while the levels of IL-5 and Granzyme B were hardly detected, demonstrating a Th1-biased response. Besides, specific $CD4^+$ T_{CM}, $CD4^+$ T_{EM}, $CD8^+$ T_{EM} and $CD8^+$ T_E cells were all detectable and functional up to 12 months after the second dose, as the cells produced IFN-γ, IL-2, and GzmB in response to stimulation of SARS-CoV-2 RBD.

Interpretation CoronaVac not only induced durable binding and neutralising antibody responses, but also SARS-CoV-2-specific $CD4^+$ and $CD8^+$ memory T cells for up to 12 months.

Funding Beijing Municipal Science & Technology Commission

Research in context

Evidence before this study

We searched PubMed for clinical trials published from the inception of the database to Oct 8, 2021, with the search terms “SARS-CoV-2”, “vaccine”, and “immune persistence”; no language restrictions were applied. We initially identified 206 references but this number decreased to 11 when we included the term “clinical trial”. Of these references, 3 of which report human clinical trials of SARS-CoV-2 vaccines. In the first study, six healthcare workers who contracted SARS-CoV-2 received the BNT162b2 mRNA COVID-19 vaccine, and had markedly higher neutralizing antibodies than those infected naturally. In the second study, 54 participants with HIV received two doses of ChAdOx1 nCoV-19, and there is no difference in magnitude or persistence of SARS-CoV-2 spike-specific humoral or cellular responses compared with participants without HIV. In the third study, the titrate of SARS-CoV-2 spike-specific IgG at day 320 after receiving a single dose of AstraZeneca ChAdOx1 declined to less than a third of the peak level, although the levels remained higher than the baseline. In the same study, a third injection boosted antibodies to a level that correlated with high efficacy after the second dose and boosted T-cell responses as well.

Added value of this study

To our knowledge, the present study is the first to report clinical data about immune persistence of an inactivated COVID-19 vaccine, which was monited for 12 months. Specific binding and neutralising antibodies peaked at 1 month after the second shot, and then dropped overtime, but remained significantly higher than baseline at 12 months. ELISpot responses showed that cytokine secretion was heavily biased toward to Th1 (IFN-γ and IL-2) rather than Th2 (IL-5) pathway, indicating that CoronaVac mainly induced a Th1-biased cellular immune response. Additionally, IFN-γ- or IL-2-producing $CD4^+$ and $CD8^+$ T cells were noted and detectable throughout the full observation period of 12 months following the boost.

Implications of all the available evidence

The CoronaVac, an inactivated COVID-19 vaccine, induced durable humoral and cellular immune responses for 12 months after the second shot, which would be valuable in restricting the COVID-19 pandemic. The mechanism of immune memory for the inactivated COVID-19 vaccine, of course, needs further investigation.

Introduction

COVID-19 is a worldwide emergency.[1] The urgent need for safe and effective interventions to mitigate the global spread of SARS-CoV-2 has prompted international efforts to develop vaccines. As of Oct 8, 2021, twenty-four COVID-19 vaccines have been approved for use[2] and more than 6.44 billion doses have been administered.[3] However, compared with other vaccines, the time interval between research and development and application of COVID-19 vaccines is very short, the underlying immunological mechanisms are not well-understood, such as antibody persistence, immune memory, etc. Therefore, it is important that more follow-up studies need to investigate the kinetics of neutralising antibody and immune memory of T and B cells, which will not guide the design of vaccination schedule, but also improve efficacy of vaccines.

CoronaVac (Sinovac Life Sciences, Beijing, China) is an inactivated vaccine against COVID-19, which has been currently approved for emergency use in China[4], and has also been included in the World Health Organization's (WHO) emergency use listing.[5] The data derived from phase 1-3 trials have shown that inactivated COVID-19 vaccines are effective, immunogenic and safe in children and adolescents aged 3–17 years,[6] and adults aged 18 years and older.[4] Here, we reported the status of persistence of antibodies and cellular responses within 12 months after two-dose of CoronaVac.

Methods

Study design, participants and collection of samples

The prospective cohort study was performed to evaluate the immunogenicity of an inactivated COVID-19 vaccine (CoronaVac, Sinovac Life Sciences, Beijing, China) in adults aged 18–59 years and followed up for 12 months after two vaccinations.
This trial was run at Beijing Center for Disease Prevention and Control (CDC), China. Participants who were healthy, non-pregnant adults 18-59 years of age were recruited from staff at Beijing CDC and Huairou District CDC (Beijng, China). All participants

provided written informed consent before enrolment. The trial protocol was approved by the Ethics Committee of Beijing CDC (2020-28) and was performed in accordance with the requirements of Good Clinical Practice of China and the International Conference on Harmonisation. The main exclusion criteria included history of SARS-CoV, SARS-CoV-2, or Middle East respiratory syndrome infection, high-risk epidemiology history within 14 days before enrolment (eg, travel or residence history in communities with case reports, or contact history with someone infected with SARS-CoV-2), axillary temperature of more than 37·0°C, history of allergy to any vaccine component. A complete list of exclusion criteria is in the protocol.
The participants were administered 3 μg CoronaVac intramuscularly following a 2-shot vaccine schedule, 14 days apart. Following that, the samples, including serum, plasma, and peripheral blood mononuclear cells were collected for investigation of exploratory end.

Procedures

CoronaVac, an inactivated vaccine containing whole-virion SARS-CoV-2, was developed by Sinovac Life Sciences (Beijing, China), and has been approved in 40 countries for emergency use as of Sep 15, 2021.[4,7] Using a 2-dose regimen, the participants received CoronaVac intramuscularly on day 0 and day 14, respectively. Blood samples were collected from participants on the day 0 before vaccination (baseline) and at 1, 3, 6, and 12 months after the second shot for analysing immunogenicity of vaccination.

The commercial chemiluminescence detection kits (2019-nCoV IgG antibody detection kit, Bioscience Diagnostics, Tianjin, China) were employed to measure SARS-CoV-2 receptor-binding domain (RBD) specific IgG following manufacturer's instructions as described before.[8] The titrates of neutralising antibodies against live SARS-CoV-2 (virus strain: SARS-CoV-2/human/CHN/CN1/2020, GenBank number MT407649.1) were quantified using the micro cytopathogenic effect assay[6]. All procedures related to virus neutralisation test were performed in a level 3 biosafety laboratory from Sinovac

Life Sciences, following WHO recommendations.

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood samples before vaccination and at month 1, 3, 6, and 12 post-vaccination. Enzyme-linked immunospot (ELISpot) assays (Cellular Technology Limited, OH, USA) were used to evaluate cellular immune responses through measuring expression of interferon (IFN) γ, interleukin-2 (IL-2), IL-5 by PBMS stimulated with RBD according to manufacturer's standard protocol. All measurements were subtracted by the unstimulated control values, while the subtracted values were corrected to zero. In addition, Flow cytometry (BD FACSLyric™, CA, USA) was employed to analyze proportions of the $CD4^+$ memory T-cell and $CD8^+$ memory T-cell subsets. Furthermore, intracellular production of IFN-γ, IL-2, and Granzyme B (GrzB) by T cells stimulated with RBD was also analyzed using flow cytometry as previously described. [9,10] The data were analysed with FlowJo software (Ashland, OR, USA).

Outcomes

Overall objectives were to assess the durability of the SARS-CoV-2-specific immune responses after CoronaVac vaccination as two intramuscular doses 14 days apart for up to 12 months. The humoral immunogenicity outcomes include the titres of RBD-specific IgG antibodies and neutralising antibodies against live SARS-CoV-2 at baseline and 1, 3, 6, and 12 months after the second shot of the vaccination. The positive cutoff value for RBD-specific IgG antibodies was defined as the sample cutoff (S/CO) value ≥1.0. Seroconversion of neutralising antibodies was defined as a titer of 8 or higher for neutralizing antibodies to live SARS-CoV-2. The cellular immune response outcomes include ELISpot assays for measuring secretion of IFN-γ, IL-2, IL-5, and GrzB by PBMS. The results are expressed as the number of spot-forming cells (SFCs) per 1,000,000 cells. In the meanwhile, the proportion of memory T-cell responses was also measured by ICS assays across as the above time points of the blood collection.

Statistical analysis

The sample size for this study was based on practical considerations rather than statistical power calculations. The data of immunogenecity were analysed descriptively using SAS (version 9·4). Titres of specific binding antibodies against SARS-CoV-2 RBD were presented as sample cutoff values (S/CO) with 95% CIs. Efficacy of neutralising antibodies was prensented as geometric mean titres (GMTs) with 95% CIs. Cellular immune responses were presented as the number of spot-forming cells (SFCs) per 1 million cells or as a proportion of positive responders with 95% CIs. The geometric means were calculated with $\log_{10}$ values of the original data, then the two-sided 95% CIs were calculated using Student's *t* distribution, with subsequent antilog transformation applied. χ^2 test was used to analyse categorical data, and ANOVA test was used to analyse numerical data. When the overall difference across the five time points was significant, paied t-test was used to compare the differences between groups. Two-sided p-values of less than 0.05 were considered significant. Figures were made using GraphPad Prism 8.0.1.

This study is registered with ClinicalTrials.gov, NCT05072496.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Total 150 participants were enrolled this study. Among them, 145 participants received two dose of the investigational product, and 136 participants completed the scheduled visits 12 months after the second shot. Baseline demographic characteristics of the participants at enrolment were shown in figure1.

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

to a maximum S/COvalue of 11·26 (95% confidence interval [CI], 9·29to 13·24), and the seropositiverate was 99% (141 of 143 participants). Although the mean concentration of the RBD-specific IgG antibodies at 3 months (S/CO value 3·87, [95% CI 2·85–4·90]) was only one third of the peak level observed at the 1month, the seropositive rate still persisted at a high level (92%, 130 of 142). Thereafter, the antibody titers reached a plateau phase with only a gradual decline from 3 to 12 months (6 months S/CO value 3·68, [95% CI 2·43–4·94]; 12 months S/CO value 2·11, [95% CI 1·50–2·72]). The seropositive rates of RBD-specific IgG antibody were 77% (105 of 136) and 49%(67 of 136) at 6 and 12 months after the second vaccination, respectively.

As expected, there were no detectable titres of neutralising antibodies in serua of all study participants at baseline (figure 2). At 1month after the second vaccination, neutralising antibody titres increased substantially from baseline to a geometric mean titre (GMT) with peak level of 7·0 (95% CI 4·9–9·1), while the seroconversion rate was 50% (71of 143 participants). Similar to RBD-specific IgG antibody, at 3 months after the second vaccination, a rapid decline in GMT of neutralising antibody (4·4, 95% CI 2·3–6·4) was observed, followed by a plateau phase. Interestingly, GMT of neutralizing antibody did not decrease continuously at 6 months, but increased significantly compared with that at 3 months, reaching 5·3 (95% CI 3·1–7·4). At 12 months, GMT of the neutralising antibody decreased to 4·1 (95% CI 2·0–6·2), yet remained significantly higher than the baseline, and which there was no significant difference between the GMT of 3 months and 12 months after the second vaccination. The seroconversion rates of neutralising antibody at 3, 6, and 12 months were 21% (29 of 140), 35% (48 of 136), and 20% (27 of 136), respectively, which were consistent with the changing trend of neutralising antibody titres.

SARS-CoV-2 RBD-specific IFN-γ, IL-2, IL-5, and GrzB ELISpot responses were assessed at 1, 3, 6, and 12 months after the second vaccination in PBMCs of all participants (figure 3). IFN-γ responses were elicited in participants with a peak

frequnce (SFCs 1107·7, [95% CI 941·1-1274·3]) at 1 month after the second vaccination, and stabilized towards 3 months (SFCs 1093.1, [95% CI 931·8-1254·5]). Although some decline in SFCs was seen, relative high levels of IFN-γ responses persisted to 6 months (SFCs 772·6, [95% CI 614·6-930·7]). At 12 months, IFN-γ responses further declined but were still detectable (SFCs 123·3, [95% CI 64·5-182·2]). In addition, IL-2 responses were also noted at each time point after the second vaccination, and showed a similar pattern to IFN-γ responses: high levels of IL-2 responses persisted until the end of 6 months after the second vaccination. Although some participants had detectable IL-5 responses after vaccination, IL-5 responses were obviously lower than that of IFN-γ and IL-2 at each time point after vaccination, indicating a type 1 helper T-cell (Th1) biased cellular immune response. GrzB responses was not detecteble at each time point after vaccination.

Memory T-cell subsets, expression of IFN-γ, IL-2, and GrzB were ananlyzed by uisng ICS assays to evaluate the SARS-CoV-2 RBD-specific memory T cells in a subset of participants (N=119, in whom sufficient PBMC were available) (figure 4). The percentage of RBD-specific $CD4^+$ T central memory (T_{CM}) cells was significantly higher at 1 month (11·78%%) after the second vaccination than that of the baseline, repsenting 76% (86/113) of participants with detectable RBD-specific $CD4^+$ T_{CM} cells. Then, the fraction of RBD-specific $CD4^+$ T_{CM} cells slightly but significantly increased (15·25%) as compared with those of 1 month, declined until 6 months (1.97%), and stabilized towards 12 months (1·24%) after the second vaccination (figure 4). Coversingly, the percentages of subjects with detectable circulating SARS-CoV-2 RBD-specific $CD4^+$ T_{CM} cells were 86% (95 of 110), 59% (64 of 108), and 56% (65 of 117) at 3, 6, and 12 months after the second vaccination, respectively. In the meanwhile, the specific $CD8^+$ effector memory (T_{EM}) responses were also noted. A considerable fraction of RBD-specific CD8+ T_{EM} cells was observed at 1 month (9·48%), then the fraction of specific $CD8^+$ T_{EM} peaked at 3 months (12·14%),and thereafter dropped over time (6 months 5·73% and 12 months 0·89%). The proportion of subjects with detectable circulating SARS-CoV-2 RBD-specific $CD8^+$ effector memory (T_{EM}) cells

were 69% (78 of 113),78% (86 of 110), 56% (60 of 108), and 31% (36 of 117) of participants at 1, 3, 6, and 12 months after the last vaccination, respectively. Besides, we also observed that the fractions of $CD4^+$ T_{EM} and $CD8^+$ T_E cells specific to SARS-CoV-2 RBD increased over time and constituted up to about 7.51% of total peripheral blood $CD4^+$ T cells and about 8.74% of total peripheral blood $CD8^+$ T cells, respectively (figure 4).

As known, memory T cells, once they meet same antigen(s), can rapidly express a wide variety of cytokines to engage, recruit, or activate innate cells or other adaptive lymphocytes. To assess functionality of the SARS-CoV-2-specific memory $CD4^+$ and $CD8^+$ T cell responses, we further measured intracellular cytokines expressed by these cells in response to SARS-CoV-2 RBD stimulation (figure 4). IFN-γ cytokine-producing memory $CD4^+$ T and $CD8^+$ T cells exhibited similar kinetics, in which IFN-γ production started at 1 month, reached the peak at 3 or 6 months, and thereafter dropped over time (figure 4). It has been well known that GzmB is a type of cytotoxic granules produced by NK cells and activated CTLs.[11] As expected, the GzmB production by specific memory $CD4^+$ T and $CD8^+$ T cells increased rapidly at 1 month after the second vaccination, and maintained a high percentage to 3 months, and then gradually decreased. Interestingly, the fraction of $CD4^+$ T_{CM}, $CD4^+$ T_{EM}, $CD8^+$ T_{EM} , and $CD8^+$ $T_E$cells producing IL-2 continued to rise from 1 to 6 months after the second dose and maintained at a high level throughout the entire follow-up period (until 12 months). As shown in Fig4, the SARS-CoV-2-specific $CD4^+$ T_{cM}, $CD4^+$ T_{EM}, and $CD8^+$ T_{EM}, and $CD8^+$ T_E cells were all functional up to 12 months after the second dose, as the cells produced IFN-γ, IL-2, and GzmB in response to SARS-CoV-2-specific RBD. Therefore, CoronaVac is not only albe to elicit durable SARS-CoV-2-specific memory $CD4^+$T cells, but also SARS-CoV-2-specific memory $CD8^+$ T cells.

Dicussion

In the present study, we monitored the status of 12-month durability of humoral and cellular immune responses in 145 individuals who received two doses of CoronaVac (3

µg/per dose, with an interval of 14 days). Our findings extended previously reported results[4] and showed that SARS-CoV-2 RBD-specific binding and neutralisation antibody responses to immunozazition with CoronaVac decreased gradually with timebing, but remained significantly higher than baseline after 12 months. More importantly, it is the first time that status of robustly expanded SARS-CoV-2 RBD-specific memory $CD4^+$ and $CD8^+$ T cells in the peripheral circulation were monited through 12 months post booster vaccination. Furthermore, ELISpot responses and ICS used to characterize T cell cytokine responses showed that profile of cytokine secretion was mainly toward to Th1 (IFN-γ and IL-2) rather than Th2 (IL-5) pathway, suggesting that CoronaVac predominantly induces Th1-biased cellular immune responses. In addition, it is also worth to note that CoronaVac induced rapid and durable antibody responses as well as cellular immune responses for up to 12 months.

It is no doubt that understanding the duration of antibody responses to COVID-19 vaccine is the key to continuously prevent infection. Although correlates of protection against SARS-CoV-2 infection in human are not yet established,[12] the data od CLIA and micro cytopathogenic effect assay showed that binding and neutralizing antibodies elicited by two doses of CoronaVac were able to persist through 12 months after the second shot, indicating that CoronaVac has the potential to provide durable humoral immunity. However, to our knowledge at the moment, there are the limited data available showing that humoral responses to COVID-19 vaccines can last for the 12 months. It has been shown that the Moderna mRNA-1273 vaccine (the 100-µg per dose) produces high levels of binding and neutralizing antibodies that declined slightly overtime until 90 days after the booster vaccination.[12,13] Besides, a significant trend of waning antibody levels with time has been oberved in both AstraZeneca ChAdOx1 and Pfizer BNT162b2, with antibody levels reducing by about five-fold for ChAdOx1, and by about two-fold for BNT162b2, between 21–41 days and 70 days or more after the second dose, respectively.[14] At 320 days, titres of SARS-CoV-2 spike protein-specific IgG in AstraZeneca ChAdOx1 declined to less than a third of the peak titres, although it remained higher than the baseline after receivinga single dose of 5×10^{10} viral particles

booster vaccine.[15] Numerically, the humoral responses of CoronaVac are not as strong as other COVID-19 vaccines, however, we shoule bear that in our mind, i.e., it is difficult to directly evaluate the capcacies for producing antibodies among different vaccines without a head-to-head comparison due to heterogeneity of neutralization assays. Even though the same live virus is used for neutralization analysis, the results vary from laboratory to laboratory due to the lack of standardized laboratory methods for SARS-CoV-2 neutralization and experimental procedures, including virus titration, serum dilution, virus-serum neutralization, readout, and reporting methods.[16] Additionally, the relatively low humoral responses of CoronaVac in the present study might be associated with the relatively short vaccination schedule used. It has been shown that a more robust antibody response can be generated by the day 0 and 28 vaccination schedule as compared to the day 0 and 14 schedule. We current use, therefore, the day 0 and 28 vaccination as routine for CoronaVac.[4,8]

Although recent work has much focused on antibody responses, memory $CD8^+$ T cells play cruitical role in defencing virus infection through killing virus-infected cells and expressing relevant cytokines and cytolytic molecules.[17] In addition, $CD8^+$ T-cell responses may also contribute to protection, particularly in the setting of waning or borderline antibody responses,[18] or potentially against viral variants that are partially resistant to antibodies.[19] Previous studies on SARS and Middle East respiratory syndrome (MERS) have shown that the increases in specific antibodies are temporaryly, and that antibody levels decline quickly in patients after recovery, whereas the specific $CD4^+$ and $CD8^+$ T-cell responses play an essential role in the control of SARS and MERS.[20,21] Besides, some studies have shown that the reduction in the number of T cells is related to poor clinical outcomes and immune pathogenesis, while adequate T cell counts and appropriate effector function are associated with patients having mild disease symptoms or successful rehabilitation.[22] Grifoni et al. have reporte that circulating SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells are 100% and 70% respectively in a small group of COVID-19 convalescent patients (n=20).[23] a In addition, another study has shown that the percentages of $CD4^+$ and $CD8^+$ T cells

concomitantly increase from day 7 after infection, which persist for 7 days as the symptoms disappeared.[24] In contrast, in the present study we also interrogated the presence of functional $CD4^+$ and $CD8^+$ memory T cells in participants who received the vaccine. ELISpot results showed that RBD-specific T cells secreting IFN-γ and IL-2 persisted through 12 months after the second shot of vaccination. In the meanwhile, these SARS-CoV-2 RBD-specific memory $CD4^+$ and $CD8^+$ T cells still expressed detectable cytokines IFN-γ, IL-2, and GzmB throughout entire study duration. Together, these data demonstrate that CoronaVac are able to elicit SARS-CoV-2 RBD-specific memory $CD4^+$ and $CD8^+$ T cells, while these cells could be maintained and still have capacity producing effector cytokines after restimulation 12 months post boost. Although the classical immunological theory believes that the inactivated vaccines are not thought to induce CD8 T-cell responses, our data suggest that the structural integrity of whole SARS-CoV-2 might be the key to elicit antiviral $CD8^+$ memory T-cell responses. The exact mechanism behind this hypothesis, of course, needs further investigation.

Previous reports on the development of SARS and the Middle East respiratory syndrome (MERS) vaccine candidates have shown that there are some raised concerns related to antibody-dependant enhancement (ADE) and induction of Th2 responses.[25-27] In contrast, our data showed that profile of cytokine secretion was prodeminately Th1 (IFN-γ and IL-2) produced by BPBC stimulated with SARS-CoV-2 RBD compared to baseline of participants received CoronaVac, while concentrations of Th2 cytokine IL-5 were hardly detectable. Similarly, phenotyping by flow cytometry demonstrated that substantial IFN-γ- and IL-2-producing cells mainly were $CD4^+$ and $CD8^+$ T cells. Herein, subjects vaccinated with CoronaVac seemed to have predominant Th1 responses, but little to no Th2 cytokines. These results are consistent with a previous animal study,[28] and further proves the safety of CoronaVac.

However, it is notable that there are some limitations. First, because the participants involved in the study aged 18 to 59 years, the generalizability to those at risk for SARS-

CoV-2 infection and other regions requires to be further studied. Second, we did not perform a more in-depth T cell analysis before and after vaccination due to the limited volumes of blood samples available. Finally, due to the ethical issues, we could not assess the induction of tissue-resident memory T cells. These are being addressed by the ongoing clinical programme.

In conclusion, two-dose of CoronaVac not only induces durable binding and neutralization antibody responses, but also elicit SARS-CoV-2 RBD-specific memory $CD4^+$ and $CD8^+$ memory T cells for up to 12 months.

Contributors

All authors had full access to all data in the studies and had final responsibility for the decision to submit for publication. WZ, QL, PZ, QZ, and JW designed the study. QZ and JW worked as coprincipal investigators of this study. ML and LZ did the statistical analysis. WZ drafted the manuscript. QZ and JW critically reviewed and revised the manuscript. JL and SB led and participated in the site work, including the recruitment, follow-up, and data collection. WC, MC, SZ, SB, YW, and JW were responsible for laboratory analyses.

Declaration of interests

The authors declare that no competing interests exist.

Data sharing

We support sharing of the individual participant data. The individual participant data that underlie the results reported in this Article will be made available when the study is complete. Researchers who provide a scientifically sound proposal will be allowed access to the individual participant data. Proposals should be directed to the corresponding authors. These proposals will be reviewed and approved by the funder, investigator, and collaborators on the basis of scientific merit. To gain access, data requesters will need to sign a data access agreement.

Acknowledgments

This study was supported by the Beijing Municipal Science & Technology Commission (Z211100002521014). The authors would like to thank all the participants who volunteered for this study. We also wish to thank Prof Ying Sun (Capital Medical University School) for critical reading of this manuscript.

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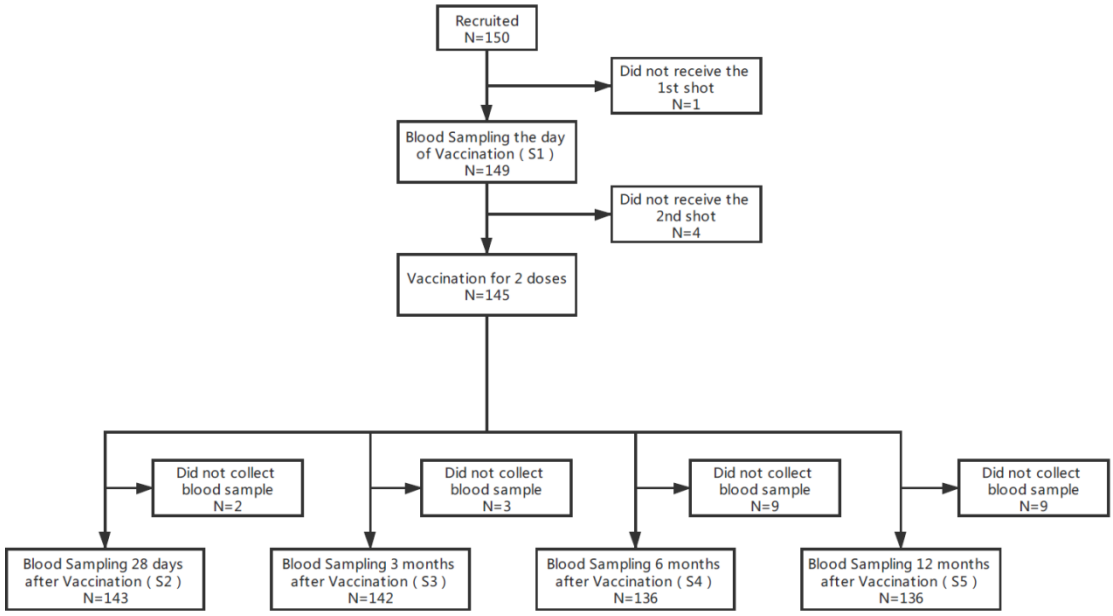

Figure 1: Design and Schedule of samples collection.

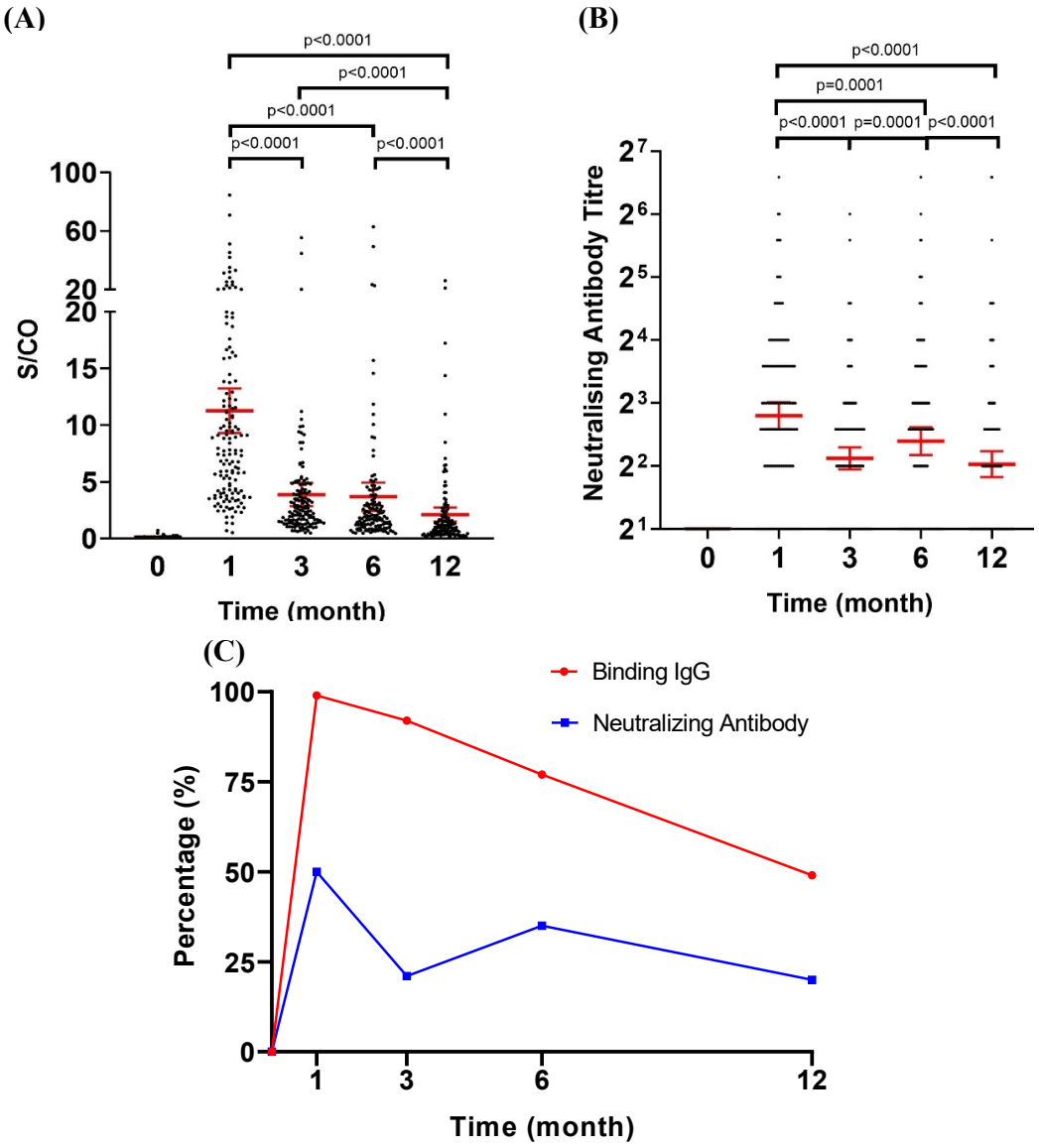

Figure 2: Status of sera IgG and neutralising antibody response following CoronaVac vaccination.

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

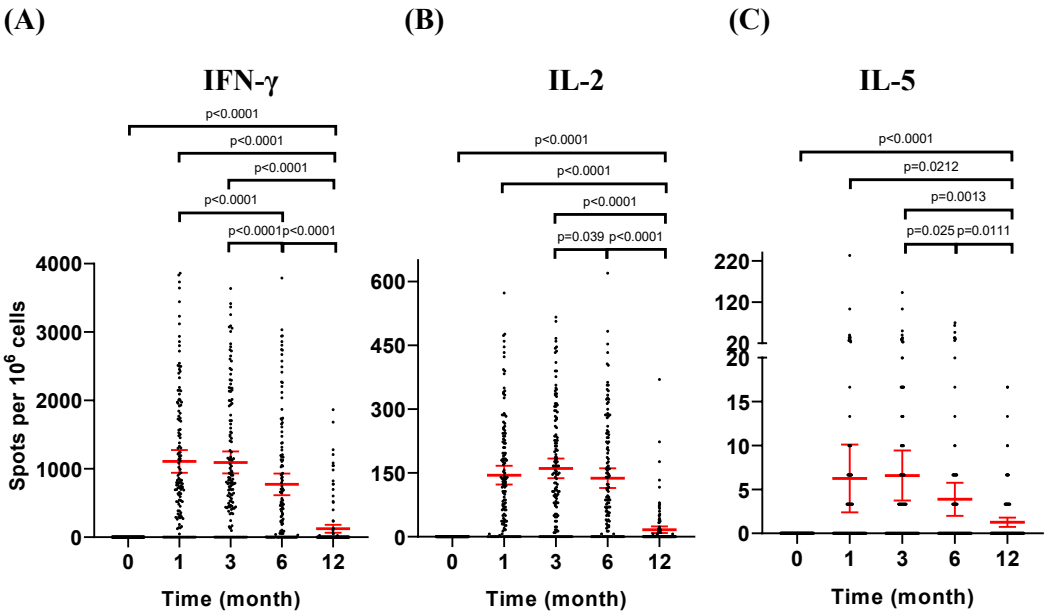

Figure 3: Status of specific T-cell responses following CoronaVac vaccination.

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.

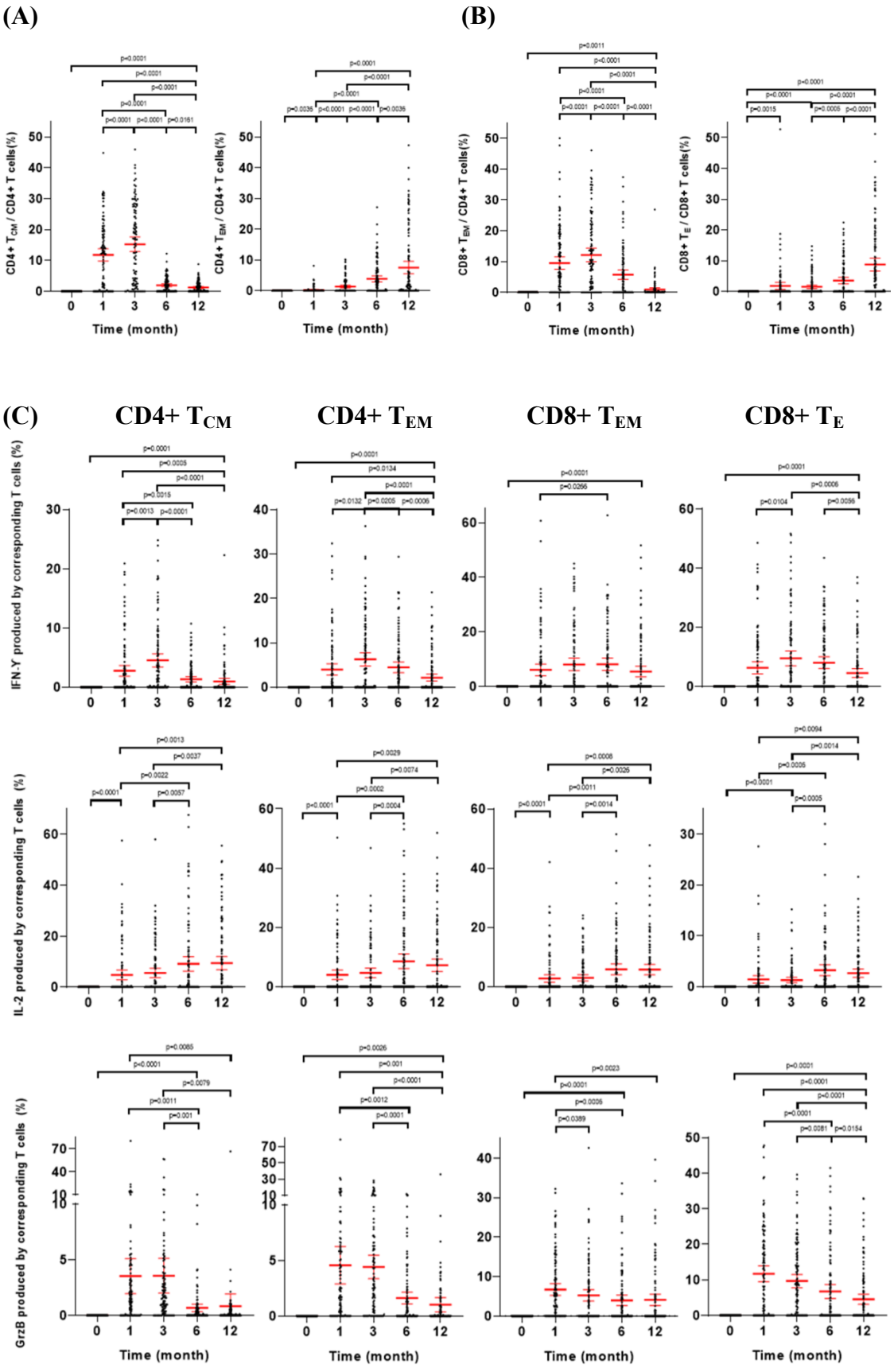

Figure 4：Status of distribution and expression of cytokines by T_{CM} and T_{EM}

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.

1.4 CoronaVac é segura, bem aceita pelo organismo e tem eficácia de 83,5%, de acordo com estudo clínico turco

Assim como já havia sido confirmado pelos ensaios clínicos de fase 3 conduzidos ao longo de 2020 no Brasil para avaliar a eficácia da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, um estudo da Universidade Hacettepe, com sede em Ancara, na Turquia, mostrou que o imunizante é 83,5% eficaz contra o SARS-CoV-2, além de ser seguro e bem tolerado pelo organismo. A pesquisa foi publicada em julho na revista científica The Lancet e também na Biblioteca Nacional de Medicina dos Estados Unidos, a maior biblioteca médica do mundo.

O estudo de fase 3, randomizado e duplo-cego, contou com a participação de 10.218 pessoas e foi feito entre 14 de setembro de 2020 e 5 de janeiro de 2021. Os voluntários foram avaliados sete, 14 e 28 dias depois de tomar cada uma das duas doses. Durante o acompanhamento médio de 43 dias, nove casos sintomáticos de Covid-19 foram confirmados no grupo que tomou a vacina e 32 casos foram relatados no grupo que tomou placebo. A CoronaVac preveniu hospitalizações em todos os voluntários, na comparação com os seis do grupo placebo. Não houve mortes nem no grupo que tomou a vacina e nem no grupo placebo.

Além disso, a CoronaVac induziu anticorpos em 89,7% dos participantes. Destes, 92% também produziram níveis protetores de anticorpos neutralizantes pelo menos 14 dias após a segunda dose da vacina.

O artigo destaca ainda que a vacina mostrou um perfil de segurança satisfatório, sem eventos adversos de grau 4 durante o período do estudo. A maioria dos efeitos adversos foi de grau 1 e ocorreu até sete dias após a injeção. A incidência total foi baixa (18,9%), e o principal sintoma foi fadiga.

"Nossos resultados mostram que a CoronaVac tem boa eficácia contra infecção sintomática por SARS-CoV-2 e Covid-19 grave com um perfil de segurança muito bom em uma população de 18 a 59 anos", afirmaram os autores do artigo. "A tolerabilidade da CoronaVac neste estudo foi excelente e a incidência de eventos adversos foi baixa."

Participaram do estudo voluntários de diferentes grupos de risco e ocupação, tornando os resultados bem próximos ao contexto do mundo real. Receberam a vacina 6.646 pessoas, sendo que 3.568 voluntários tomaram placebo (substância ou tratamento sem um princípio ativo, como uma injeção de soro fisiológico). Do total de participantes, 57,8% eram homens e 42,24% mulheres, todos entre 18 e 59 anos. Desse grupo, 3.675 pessoas eram profissionais de saúde e 1.463 eram obesos. E entre todos os participantes, 6.217 tinham algum tipo de comorbidade – a maioria relatou ter hipertensão.

O ensaio clínico de fase 3 realizado no Brasil pelo Butantan envolveu 16 centros de pesquisa científica em sete estados e no Distrito Federal. O teste duplo cego envolveu 12,5 mil profissionais de saúde, e obteve 62,3% de eficácia global em casos leves, moderados ou graves, num espaço de 21 dias ou mais entre as duas doses.

Publicado em: 21/09/2021

Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey

Mine Durusu Tanriover, Hamdi Levent Doğanay*, Murat Akova*, Hatice Rahmet Güner, Alpay Azap, Sıla Akhan, Şükran Köse, Fatma Şebnem Erdinç, Emin Halis Akalın, Ömer Fehmi Tabak, Hüsnü Pullukçu, Özgür Batum, Serap Şimşek Yavuz, Özge Turhan, Mustafa Taner Yıldırmak, İftihar Köksal, Yeşim Taşova, Volkan Korten, Gürdal Yılmaz, Mustafa Kemal Çelen, Sedat Altın, İlhami Çelik, Yaşar Bayındır, İlkay Karaoğlan, Aydın Yılmaz, Aykut Özkul, Hazal Gür, Serhat Unal*, and the CoronaVac Study Group†*

Summary

Background CoronaVac, an inactivated whole-virion SARS-CoV-2 vaccine, has been shown to be well tolerated with a good safety profile in individuals aged 18 years and older in phase 1/2 trials, and provided a good humoral response against SARS-CoV-2. We present the interim efficacy and safety results of a phase 3 clinical trial of CoronaVac in Turkey.

Methods This was a double-blind, randomised, placebo-controlled phase 3 trial. Volunteers aged 18–59 years with no history of COVID-19 and with negative PCR and antibody test results for SARS-CoV-2 were enrolled at 24 centres in Turkey. Exclusion criteria included (but were not limited to) immunosuppressive therapy (including steroids) within the past 6 months, bleeding disorders, asplenia, and receipt of any blood products or immunoglobulins within the past 3 months. The K1 cohort consisted of health-care workers (randomised in a 1:1 ratio), and individuals other than health-care workers were also recruited into the K2 cohort (randomised in a 2:1 ratio) using an interactive web response system. The study vaccine was 3 μg inactivated SARS-CoV-2 virion adsorbed to aluminium hydroxide in a 0·5 mL aqueous suspension. Participants received either vaccine or placebo (consisting of all vaccine components except inactivated virus) intramuscularly on days 0 and 14. The primary efficacy outcome was the prevention of PCR-confirmed symptomatic COVID-19 at least 14 days after the second dose in the per protocol population. Safety analyses were done in the intention-to-treat population. This study is registered with ClinicalTrials.gov (NCT04582344) and is active but no longer recruiting.

Findings Among 11 303 volunteers screened between Sept 14, 2020, and Jan 5, 2021, 10 218 were randomly allocated. After exclusion of four participants from the vaccine group because of protocol deviations, the intention-to-treat group consisted of 10 214 participants (6646 [65·1%] in the vaccine group and 3568 [34·9%] in the placebo group) and the per protocol group consisted of 10 029 participants (6559 [65·4%] and 3470 [34·6%]) who received two doses of vaccine or placebo. During a median follow-up period of 43 days (IQR 36–48), nine cases of PCR-confirmed symptomatic COVID-19 were reported in the vaccine group (31·7 cases [14·6–59·3] per 1000 person-years) and 32 cases were reported in the placebo group (192·3 cases [135·7–261·1] per 1000 person-years) 14 days or more after the second dose, yielding a vaccine efficacy of 83·5% (95% CI 65·4–92·1; p<0·0001). The frequencies of any adverse events were 1259 (18·9%) in the vaccine group and 603 (16·9%) in the placebo group (p=0·0108) with no fatalities or grade 4 adverse events. The most common systemic adverse event was fatigue (546 [8·2%] participants in the vaccine group and 248 [7·0%] the placebo group, p=0·0228). Injection-site pain was the most frequent local adverse event (157 [2·4%] in the vaccine group and 40 [1·1%] in the placebo group, p<0·0001).

Interpretation CoronaVac has high efficacy against PCR-confirmed symptomatic COVID-19 with a good safety and tolerability profile.

Funding Turkish Health Institutes Association.

Lancet 2021; 398: 213–22

Published Online July 8, 2021 https://doi.org/10.1016/S0140-6736(21)01429-X

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*Contributed equally

†Members of the CoronaVac Study Group and all participating sites are listed in the appendix (pp 15–16)

Department of Internal Medicine (Prof M D Tanriover MD), **Department of Infectious Diseases and Clinical Microbiology** (Prof M Akova MD, Prof S Unal MD), **and Department of Medical Microbiology** (H Gür MSc), **Hacettepe University School of Medicine, Ankara, Turkey; Hacettepe University Vaccine Institute, Ankara, Turkey** (Prof M D Tanriover, Prof M Akova, Prof S Unal); **Department of Gastroenterology, Turkish Republic Ministry of Health, İstanbul Provincial Health Directorate, University of Health Sciences İstanbul Ümraniye Training and Research Hospital, İstanbul, Turkey** (Prof H L Doğanay MD); **Department of Infectious Diseases and Clinical Microbiology, Ankara Yıldırım Beyazıt University, Ankara City Hospital, Ankara, Turkey** (Prof H R Güner MD); **Department of Infectious Diseases and Clinical Microbiology, Ankara University School of Medicine, Ankara, Turkey** (Prof A Azap MD); **Department of Infectious Diseases and Clinical Microbiology, Kocaeli University School of Medicine, Kocaeli, Turkey** (Prof S Akhan MD); **Department of Infectious Diseases, Turkish**

Introduction

The COVID-19 pandemic continues to affect individuals and populations, magnifying socioeconomic and health inequalities globally.[1–4] Vaccination is a crucial measure in breaking the transmission chain of SARS-CoV-2 infections. Among several vaccines against SARS-CoV-2, 13 in clinical development are inactivated vaccines, two of which are already in phase 4 trials. Although the basic cultivation techniques using Vero cells and inactivation strategies are similar, inactivated vaccines differ in the isolated virion strains and the adjuvants used.[5,6] The potential advantages of inactivated vaccines are non-replicability in the host, non-transmissibility, and the induction of a broad range of humoral and cellular

Republic Ministry of Health, İzmir Provincial Health Directorate, İzmir University of Health Sciences Tepecik Training and Research Hospital, İzmir, Turkey (Prof Ş Köse MD); Department of Infectious Diseases, Turkish Republic Ministry of Health, Ankara Provincial Health Directorate, Ankara Training and Research Hospital, Ankara, Turkey (Prof F Ş Erdinç MD); Department of Infectious Diseases and Clinical Microbiology, Bursa Uludağ University Health Application and Research Centre, Bursa Uludağ University Hospital, Bursa, Turkey (Prof E H Akalın MD); Department of Infectious Diseases and Clinical Microbiology, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, İstanbul, Turkey (Prof Ö F Tabak MD); Department of Infectious Diseases and Clinical Microbiology, Ege University School of Medicine, İzmir, Turkey (Prof H Pullukçu MD); Department of Chest Diseases, Turkish Republic Ministry of Health, İzmir Provincial Health Directorate, University of Health Sciences Dr Suat Seren Chest Diseases and Surgery Training and Research Hospital, İzmir, Turkey (Ö Batum MD); Department of Infectious Diseases and Clinical Microbiology, İstanbul University, İstanbul School of Medicine, İstanbul, Turkey (Prof S Şimşek Yavuz MD); Department of Infectious Diseases and Clinical Microbiology, Akdeniz University School of Medicine, Antalya, Turkey (Prof Ö Turhan MD); Department of Infectious Diseases and Clinical Microbiology, Turkish Republic Ministry of Health, İstanbul Provincial Health Directorate, Prof Dr Cemil Taşcıoğlu City Hospital, İstanbul, Turkey (M T Yıldırmak MD); Department of Infectious Diseases and Clinical Microbiology, Acıbadem University Atakent Hospital, İstanbul, Turkey (Prof İ Köksal MD); Department of Infectious Diseases and Clinical Microbiology, Çukurova University Balcalı Hospital Health Application and

Research in context

Evidence before this study

We searched PubMed for research articles published up to April 28, 2021, with no language restrictions, using the terms "SARS-CoV-2" OR "COVID-19" AND "vaccine" AND "clinical trial" AND "efficacy". We found four articles reporting the interim efficacy and safety results of phase 3 trials: ChAdOx1 nCoV-19 vaccine (University of Oxford–AstraZeneca) showing an efficacy against symptomatic COVID-19 of 62·1% (95% CI 41·0–75·7) with two standard doses and 90·0% (67·4–97·0) with a low dose followed by a standard dose; Gam-COVID-Vac (Gamaleya National Research Centre for Epidemiology and Microbiology) showing an efficacy of 91·6% (85·6–95·2); mRNA-1273 SARS-CoV-2 vaccine (Moderna) showing an efficacy of 94·1% (89·3–96·8), and BNT162b2 mRNA COVID-19 vaccine (Pfizer–BioNTech) showing an efficacy of 95% (90·3–97·6). The results of the ENSEMBLE trial showed that the efficacy of a single dose of the Ad26.COV2.S vaccine (Janssen Research and Development) against moderate to severe or critical COVID-19 with onset at least 14 days after administration was 66·9% (adjusted 95% CI 59·0–73·4) and at least 28 days after administration was 66·1% (55·0–74·8), and higher efficacies were obtained for severe or critical COVID-19. In the world's first publicly reported animal trial of a SARS-CoV-2 candidate vaccine PiCoVacc, thereafter named CoronaVac in clinical trials, Gao and colleagues showed that the vaccine induced the production of SARS-CoV-2-specific neutralising antibodies in animals and provided complete protection against SARS-CoV-2 challenge in non-human primates. Phase 1/2 studies of CoronaVac showed a good safety and tolerability profile, and a dosage of 3 μg produced seroconversion rates of 92·0% with a 14-day immunisation schedule and 97·0% with a 28-day schedule in participants aged 18–59 years, and 98·0% with a 28-day schedule in participants aged 60 years and older in phase 2 trials.

Added value of this study

This study reports the interim analysis of a double-blind, randomised, placebo-controlled phase 3 clinical trial to assess the efficacy and safety of the inactivated and aluminium hydroxide-adsorbed SARS-CoV-2 vaccine in Turkey, in which both high-risk health-care workers and volunteers with an average COVID-19 exposure risk in the community were recruited. CoronaVac showed an efficacy of 83·5% for preventing PCR-confirmed symptomatic COVID-19, with no cases of COVID-19 requiring hospitalisation. The incidence of adverse events was low (18·9%). Preliminary immunogenicity results revealed that CoronaVac induced anti-receptor-binding domain antibodies in 89·7% of participants. The vaccine is stored and transported at 2–8°C and was granted emergency use authorisation for mass vaccination in Turkey on Jan 13, 2021.

Implications of all the available evidence

The world needs every possible dose of any safe and effective vaccine against SARS-CoV-2. Although novel genetic vaccine production platforms hold great potential for the rapid and adaptable mass production of vaccines, traditional platforms have a long experience of producing safe and tolerable vaccines with good immunogenicity. The results of this interim analysis have shown that CoronaVac fulfils the critical or minimal requirement of vaccines for the indication of pandemic use, hitting above the minimum efficacy of 50% as specified by the WHO target product profile as an option for mass vaccination. WHO has given emergency use approval to another inactivated vaccine from a different Chinese producer (Sinopharm-Beijing) and our results add to the existing evidence on safety and efficacy of inactivated vaccines for prevention of COVID-19.

responses against different epitopes. Their production and scale-up are relatively easy in the context of good yield production systems and the availability of biosafety level 3 facilities.[7] Disadvantages include limited immunogenicity requiring adjuvants to enhance the immune response, large quantities of live virus to be handled, and the integrity of antigens or epitopes that should be verified.[8]

CoronaVac, an inactivated whole-virion SARS-CoV-2 vaccine candidate developed by Sinovac Life Sciences (Beijing, China), has been in phase 3 trials since mid-2020 in Brazil, Indonesia, Chile, and Turkey. As of April 28, 2021, it has been approved in 22 countries for emergency use.[9] In this Article, we present the interim safety and efficacy results of a phase 3 trial in Turkey investigating the use of CoronaVac in adults.

Methods

Study design and participants

We did a double-blind, randomised, placebo-controlled, case-driven phase 3 clinical trial to assess the safety and efficacy of the inactivated SARS-CoV-2 vaccine CoronaVac among volunteers in Turkey.

Volunteers aged 18–59 years with no history of COVID-19 were screened for eligibility. Exclusion criteria included (but were not limited to) positive PCR and total antibody tests for SARS-CoV-2; pregnancy, breastfeeding; known allergy to components of the study vaccine or placebo; recent (within the past 6 months) or planned use of immunosuppressive therapy, or use of immunoglobulins or any blood products within the past 3 months; asplenia; history of bleeding disorder; alcohol or drug abuse; and any confirmed or suspected autoimmune or immunodeficiency disease. The study protocol containing the full list of eligibility criteria is available online.[10]

Participants were recruited in two consecutive cohorts (K1 and K2) at 24 centres (appendix p 8) in Turkey between Sept 15, 2020, and Jan 6, 2021. K1 included actively working health-care workers such as doctors, nurses, and technicians working in health-care facilities,

including but not confined to COVID-19 areas, and was launched to closely observe the safety of the vaccine before proceeding with the community. K2 included subjects representing the community in addition to health-care workers included in K1.

During the study, the Ministry of Health gave an emergency use authorisation for CoronaVac on Jan 13, 2021, and started an immediate vaccination programme initially for health-care workers and later for the public, prioritising older adults (aged ≥65 years). Although recruitment of volunteers was ongoing at this time, to comply with the principles of the Declaration of Helsinki regarding using a placebo for human subjects in medical research, the ethics committee suggested discontinuing the masking and injection of participants in the placebo group. Consequently, the placebo recipients were offered vaccines, first in K1 and later in K2.

The study protocol was approved by the clinical research ethics board of Hacettepe University (approval number 2020/10-26, July 16, 2020). The entire study protocol was published previously and is available on the Hacettepe University Vaccine Institute website.[10] Signed informed consent was obtained from participants before screening.

Randomisation and masking

Randomisation into vaccine and placebo groups was done on day 0, at a 1:1 ratio in K1 and a 2:1 ratio in K2, using an interactive web response system (Omega-CRO, Ankara, Turkey). Participants and practitioners were masked to the group allocation. The masking was removed in the event of a medical emergency requiring acute intervention, upon the responsible investigator's approval and the data and safety monitoring board's knowledge.

Procedures

Oropharyngeal and nasopharyngeal swabs were obtained from all participants for baseline PCR testing with a Bio-Speedy Direct RT-qPCR SARS-CoV-2 detection kit (Bioeksen, Istanbul, Turkey) on a Bio-Rad CFX96 Touch platform (Hercules, CA, USA), and serum total SARS-CoV-2 antibody testing was done. The ADVIA Centaur COV2T assay (Siemens Healthcare Diagnostics, Erlangen, Germany), a fully automated one-step antigen sandwich immunoassay using acridinium ester chemiluminescence technology, was used to detect total antibodies (IgG and IgM) against the SARS-CoV-2 spike protein receptor-binding domain (RBD) in serum samples. This assay is semiquantitative and has a lower detection threshold value (1 sample-to-cutoff ratio). All PCR and serum antibody tests were done at two central laboratories.

The study vaccine is an inactivated whole-virion vaccine with aluminium hydroxide as the adjuvant, prepared with a novel coronavirus (CZ02 strain) inoculated in African green monkey kidney cells (Vero cells). The inactivation process is done by adding β-propiolactone in the virus harvest fluid at a ratio of 1:4000 and inactivating at 2–8°C for 12–24 h. One dose of COVID-19 vaccine contains 3 μg of SARS-CoV-2 virion in a 0·5 mL aqueous suspension for injection with 0·45 mg/mL of aluminium. The placebo contained all ingredients except the inactivated virus, in prefilled syringes. The injections were given in two doses, 14 days apart, intramuscularly in the deltoid muscle. As the placebo and study vaccine looked exactly the same, they were administered by staff masked to group allocation. Details of the procedures on visit dates and the pharmacological properties of the investigational product are provided in the appendix (pp 1–2).

Symptom-based active surveillance was done to detect participants with symptoms suggestive of COVID-19 during follow-up (appendix pp 3–4). Anyone with at least one of the following symptoms for 2 days or more underwent PCR testing: fever or chills; cough; dyspnoea; fatigue; muscle or body pain; headache; new loss of sense of smell or change in taste; sore throat; nasal congestion or rhinorrhoea; nausea or vomiting; and diarrhoea. Cases of SARS-CoV-2 infection were classified according to the scale of clinical progression proposed by WHO.[11] Clinical outcomes were assessed in a blinded manner.

Sampling for immunogenicity analyses was planned in a subgroup of volunteers selected sequentially. As the immunogenicity and T-cell response analyses are ongoing, we only report the initial results of the anti-RBD antibody tests and neutralising antibody assays gathered at least 14 days after the second dose of vaccine or placebo. Virus neutralisation assays were done in an in-house microtitre plate, as described by Hanifehnezhad and colleagues.[12] Five-fold diluted serum samples, starting from 1:5, were mixed with an equal volume of 100 median tissue culture infectious dose of SARS-CoV-2 Ank1 isolate (1:10 000) in quadruplicate and incubated for 1 h at 37°C for neutralisation. The serum–virus mixtures were subsequently inoculated onto 90% confluent Vero E6 cells grown in 96-well plates. The assay was evaluated via inverted microscope when a 100% cytopathic effect was observed in the virus control wells. Reciprocals of serum dilutions inhibiting at least 50% of virus infectivity were expressed as mean antibody titre (SN_{50}).

Outcomes

The primary outcome was the incidence of symptomatic COVID-19 cases confirmed by RT-PCR at least 14 days after the second dose of vaccination, assessed in the per protocol population. Secondary outcomes were the incidence of symptomatic COVID-19 cases confirmed by RT-PCR at least 14 days after the first dose (assessed in all participants who received at least one dose); incidence of hospitalisation or mortality at least 14 days after the second dose; the incidence of COVID-19 cases confirmed by RT-PCR at least 14 days after the second dose; the seroconversion rate, seropositivity rate, geometric mean

Research Centre, Adana, Turkey (Prof Y Taşova MD); **Department of Infectious Diseases and Clinical Microbiology, Marmara University School of Medicine, İstanbul, Turkey** (Prof V Korten MD); **Department of Infectious Diseases and Clinical Microbiology, Karadeniz Technical University School of Medicine, Trabzon, Turkey** (Prof G Yılmaz MD); **Department of Infectious Diseases and Clinical Microbiology, Dicle University School of Medicine, Diyarbakır, Turkey** (Prof M K Çelen MD); **Department of Chest Diseases, Turkish Republic Ministry of Health, İstanbul Provincial Health Directorate, University of Health Sciences İstanbul Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, İstanbul, Turkey** (Prof S Altın MD); **Department of Infectious Diseases and Clinical Microbiology, Turkish Republic Ministry of Health, Kayseri City Training and Research Hospital, Kayseri, Turkey** (Prof İ Çelik MD); **Department of Infectious Diseases and Clinical Microbiology, İnönü University Turgut Özal Health Centre, Malatya, Turkey** (Prof Y Bayındır MD); **Department of Infectious Diseases and Clinical Microbiology, Gaziantep University Şahinbey Research and Application Centre, Gaziantep, Turkey** (Prof İ Karaoğlan MD); **Department of Chest Diseases, Turkish Republic Ministry of Health, Ankara Provincial Health Directorate, Ankara Keçiören Sanatorium, Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey** (Prof A Yılmaz MD); **Department of Virology, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey** (Prof A Özkul DVM)

Correspondence to:
Prof Murat Akova, Department of Infectious Diseases and Clinical Microbiology, Hacettepe University School of Medicine, Hacettepe Mahallesi, Ankara, 06230, Turkey
makova@hacettepe.edu.tr

See Online for appendix

titre or geometric mean increase in neutralising antibody and IgG 14 days and 28 days after each dose; the incidence of adverse reactions from the day of first vaccination to 28 days after the second dose; the incidence of adverse reactions and adverse events within 7 days after each dose; and the incidence of serious adverse events from the first vaccination to 1 year after the second dose (appendix pp 5–7).

For evaluating the efficacy of CoronaVac, COVID-19-free person-years were calculated for both study groups. Accordingly, the time from the anticipated date of prevention (14 days after the administration of the second dose) to either the date of unmasking or date of an RT-PCR-confirmed diagnosis of COVID-19 was ascertained for each participant and summed to calculate the total person-years without the disease. Total person-years were divided by the number of participants diagnosed with COVID-19 to ascertain the vaccine efficacy in intervention and placebo groups.

Participants were questioned about all adverse events during all visits and through automated phone calls via an interactive voice response system (appendix pp 3–4). Predefined symptoms (solicited events) and other unspecified symptoms (unsolicited events) reported by the participants were recorded. All adverse events were assessed by study investigators for severity and causality. Any adverse event assessed by study investigators as possibly, probably, or definitely related to a study product was defined as an adverse reaction. All safety data, until the date of unmasking and data cutoff, were recorded and analysed in the current report. Further safety data are still being obtained in an open-label follow-up study.

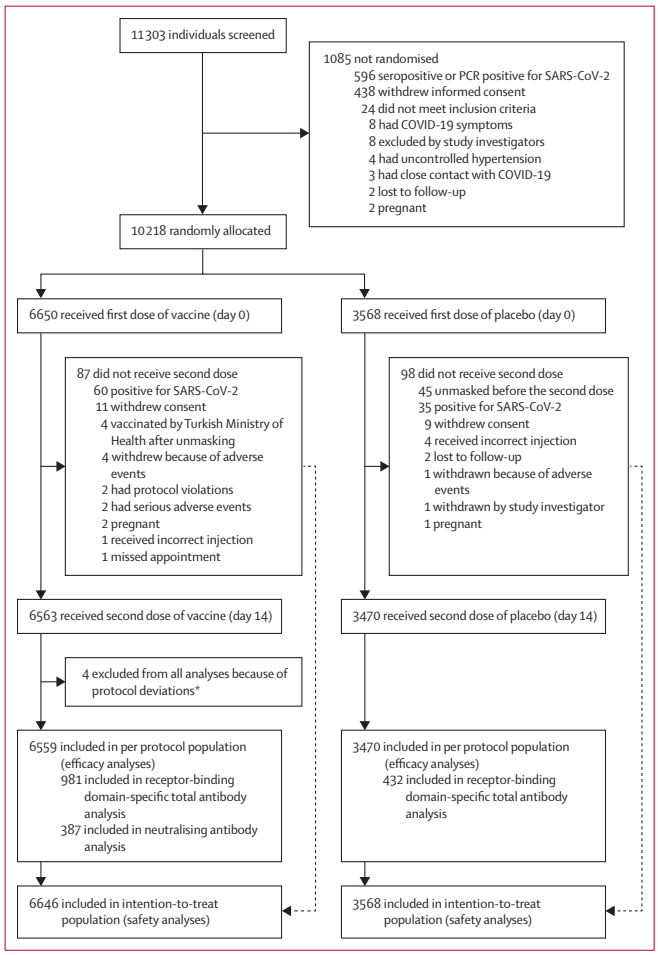


Figure 1: **Trial profile**
*Four participants in the vaccine group received two doses of the study product; however, because they were older than 59 years on the day of randomisation, they were excluded from all safety and efficacy analyses due to protocol violation.

Statistical analysis

For K1, the estimated sample size in both study groups was 588, based on assumptions that the risk of infection with SARS-CoV-2 would be 5% for the placebo group and 2% for the vaccine group. Considering a 10% dropout rate and 5% baseline seropositivity or RT-PCR positivity, it was calculated that 680 subjects would be screened in both groups of K1. Total sample sizes were calculated as 7545 for the vaccine group and 3773 for the placebo group in order to be able to detect a minimum clinically significant difference of 1% (with estimated incidence rates of 1% for the vaccine group and 2% for the placebo group) in a two-sided hypothesis testing design with 95% CIs. With the addition of a 10% dropout rate and 5% seropositivity or RT-PCR positivity at baseline, the total sample size was determined to be 13 000 participants, of whom 1360 would be in K1 and 11 640 in K2.

The initial study protocol indicated that if the efficacy of the vaccine could be demonstrated with an interim analysis done with 40 confirmed cases of COVID-19, masking would be removed and participants in the placebo group would be offered CoronaVac. Because the study was initiated with health-care workers at high risk, it was estimated that 5% of the placebo group (29 participants) and 2% of the vaccine group (11 participants) would have to be infected to demonstrate a clinical efficacy of 60%. If those rates could not be obtained in K1, enrolment would begin for K2. The enrolment rate remained very low for K1 and, after an interim safety analysis on Nov 18, 2020, the data and safety monitoring board decided to start enrolment into K2. Although the prespecified number of COVID-19 cases for the interim efficacy analysis was 40, as the incidence throughout Turkey increased rapidly, the Ministry of Health asked for a preliminary analysis to be able to grant an emergency use authorisation for

CoronaVac. Therefore, a non-predefined interim analysis was done on Dec 24, 2020, with 29 cases, which showed an efficacy above 60%. Afterwards, as community vaccination commenced, study participants were unmasked starting with K1 in blocks. The masked follow-up of those participants continued until their code was unmasked, and 41 COVID-19 cases were attained by the time all of the codes were unmasked and the prespecified interim analyses for efficacy and safety were done. Therefore, the cutoff date for inclusion in the analyses of the primary efficacy outcome and the secondary efficacy outcomes was the unmasking date of each participant in both groups. The follow-up period was defined as the period (days) from the randomisation date to the unmasking date. The data lock date was March 16, 2021. Safety data in the CoronaVac intention-to-treat group were gathered in an unmasked manner after the unmasking date, and an extended safety analysis until the data lock date is also presented.

All analyses were done using SPSS for Windows (version 25.0). Descriptive analyses were presented using mean and SD for continuous variables and frequency and percentage for categorical variables. 95% CI was presented for efficacy, calculated as events per COVID-19-free person-years (ie, the sum of RT-PCR-confirmed COVID-19 cases divided by the sum of time from vaccine protection to diagnosis or unmasking).

Time to diagnosis of COVID-19 from the time of anticipated vaccine protection in both groups was presented with Kaplan-Meier survival curves. Safety analyses were done in the intention-to-treat population. Because the study product is an inactivated vaccine, a single dose was not expected to be as efficacious as two doses, and the primary efficacy analysis was therefore done in the per protocol population (defined as participants who received two doses of vaccine or placebo in accordance with group allocation. To compare adverse events between the study groups, the χ^2 test was used when the χ^2 condition was met; otherwise, Fisher's exact test was used. A Mantel-Haenszel test of trend was used in the analysis of the positive anti-RBD antibody results among age groups within both sexes. A log-rank test was used for the comparison of follow-up duration between the treatment groups. The independent data and safety monitoring board monitored the quality of evidence, adverse events, revisions in line with the current literature, individual privacy, and data reliability from the planning stage to the end of the study.

This study is registered with ClinicalTrials.gov (NCT04582344).

Role of the funding source

The Turkish Health Institutes Association (TUSEB) provided the funding for this study; approved the final protocol, final manuscript, and the decision to submit for publication, but had no role in data collection, data analysis, data interpretation, or writing of the report. Omega-CRO (Ankara, Turkey) acted as the contract research organisation representing TUSEB and contributed to correspondence between investigators, the ethics committee, and the Ministry of Health; monitoring, site management, storage, and distribution of the consumables; developing electronic case report forms, the interactive web response system, and the interactive voice response system; and data management, statistical analyses, and overall project management. Sinovac Life Sciences provided the investigational products and reviewed the data and final manuscript before submission; however, the authors retained editorial control.

Results

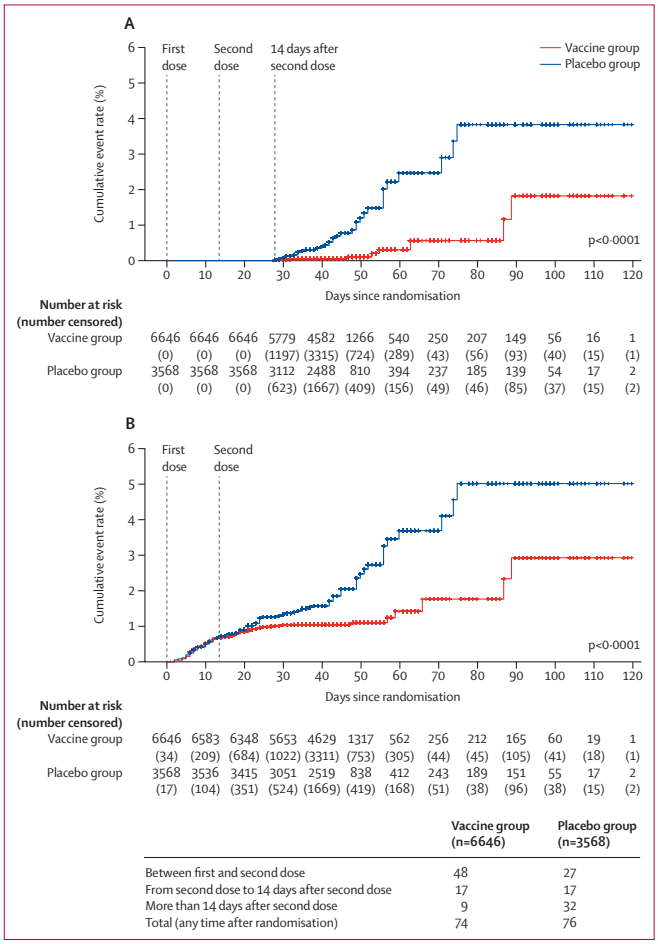
11 303 volunteers were screened for eligibility, and 10 218 were randomly allocated (6650 [65·1%] to the vaccine group and 3568 [34·9%] to the placebo group) between Sept 15, 2020, and Jan 6, 2021 (figure 1). After administration of the first dose and before receiving the second dose, 87 participants in the study group and 98 in the placebo group were excluded. After receiving two doses,

	Vaccine group (n=6646)	Placebo group (n=3568)
Age, years		
Median (IQR)	45 (37–51)	45 (37–51)
18–44	3259 (49·0%)	1764 (49·4%)
45–59	3387 (51·0%)	1804 (50·6%)
Sex		
Female	2831 (42·6%)	1476 (41·4%)
Male	3815 (57·4%)	2092 (58·6%)
Body-mass index*, kg/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



	Vaccine group (n=6646)	Placebo group (n=3568)
Between first and second dose	48	27
From second dose to 14 days after second dose	17	17
More than 14 days after second dose	9	32
Total (any time after randomisation)	74	76

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

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 SN_{50}s of at least 1/15 in 356 (92·0%) of the tested samples (figure 4).

Analyses of adverse events were done in the intention-to-treat population, which excluded four participants who had protocol deviations (n=10 214; figure 1). The vaccine showed a satisfactory safety profile, with no grade 4 adverse events or deaths during the study period. Six (0·1%) of 6646 participants in the vaccine group and one (<0·1%) of 3568 in the placebo group were withdrawn from the study because of adverse events. 3845 adverse events were

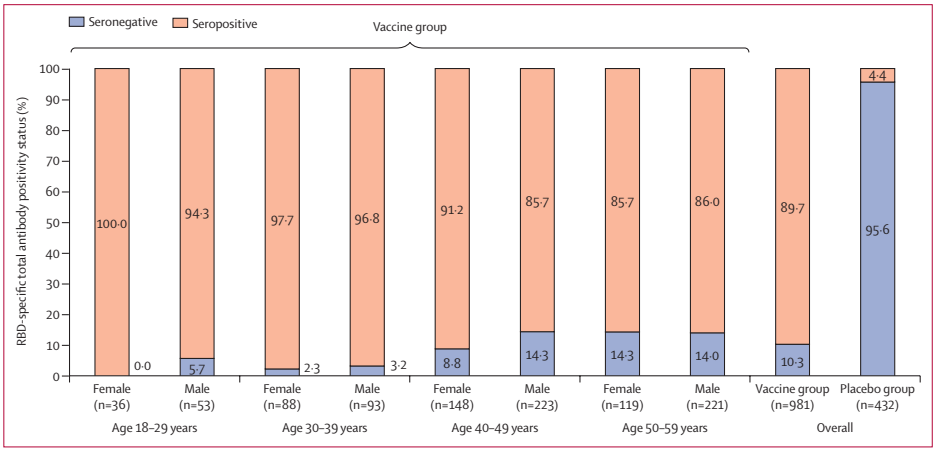


Figure 3: **Seropositivity of RBD-specific total antibodies in the vaccine and placebo groups 14 days after the second dose, by age and sex**
The participants with positive RBD-specific antibodies in the placebo group neither reported any symptoms during the follow-up nor had a laboratory confirmed diagnosis of COVID-19, probably representing cases with asymptomatic SARS-CoV-2 infection. RBD=receptor-binding domain.

reported among 1862 participants (1259 [18·9%] in the vaccine group and 603 [16·9%] in the placebo group, p=0·0108; figure 5A). Adverse events resolved in a median of 1 day (IQR 0–2). 3242 (84·3%) of 3845 adverse events were solicited (predefined) events, and were higher in the vaccine group (1148 [17·3%] participants) than in the placebo group (537 [15·1%], p=0·0039). Unsolicited (non-predefined) adverse events had a low incidence in both groups (figure 5A). Among all adverse events, 3469 (90·2%) were grade 1 and 3365 (87·5%) occurred within 7 days after injection. A comprehensive breakdown of adverse events is provided in the appendix (pp 10–14).

Local reactions were more commonly reported in vaccine recipients (180 [2·7%] participants) than in placebo recipients (52 [1·5%], p<0·0001). The most common solicited local reaction was inoculation site pain, which occurred significantly more frequently in the vaccine group (157 [2·4%] participants) than in the placebo group (40 [1·1%], p<0·0001). Other local adverse events, including erythema, paraesthesia, and swelling, were rare and did not differ significantly in incidence between groups (figure 5B).

The frequency of systemic adverse events was significantly higher in the vaccine group (1179 [17·7%] participants) than in the placebo group (571 [16·0%], p=0·0263). Events reported more frequently in the vaccine group than in the placebo group included fatigue (546 [8·2%] in the vaccine group *vs* 248 [7·0%] in the placebo group, p=0·0228), myalgia (267 [4·0%] *vs* 106 [3·0%], p=0·0071), chill (164 [2·5%] *vs* 63 [1·8%], p=0·0217), and nausea (46 [0·7%] *vs* 7 [0·2%], p=0·0008; figure 5C).

11 (0·1%) participants had serious adverse events during the study period (six [0·1%] in the vaccine group 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.

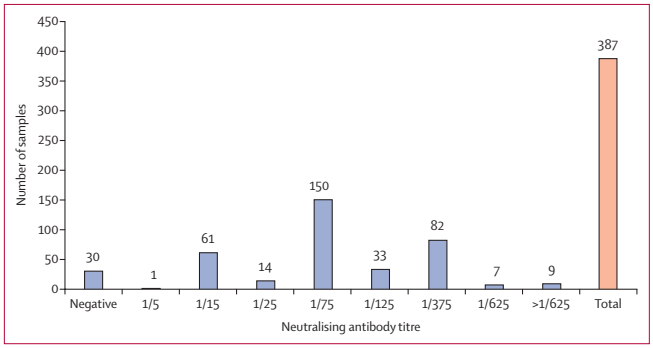


Figure 4: **Neutralising antibody titres among the subset of participants included in the immunogenicity analysis**

Discussion

This interim analysis indicated that, in a population aged 18–59 years, CoronaVac had high efficacy for preventing

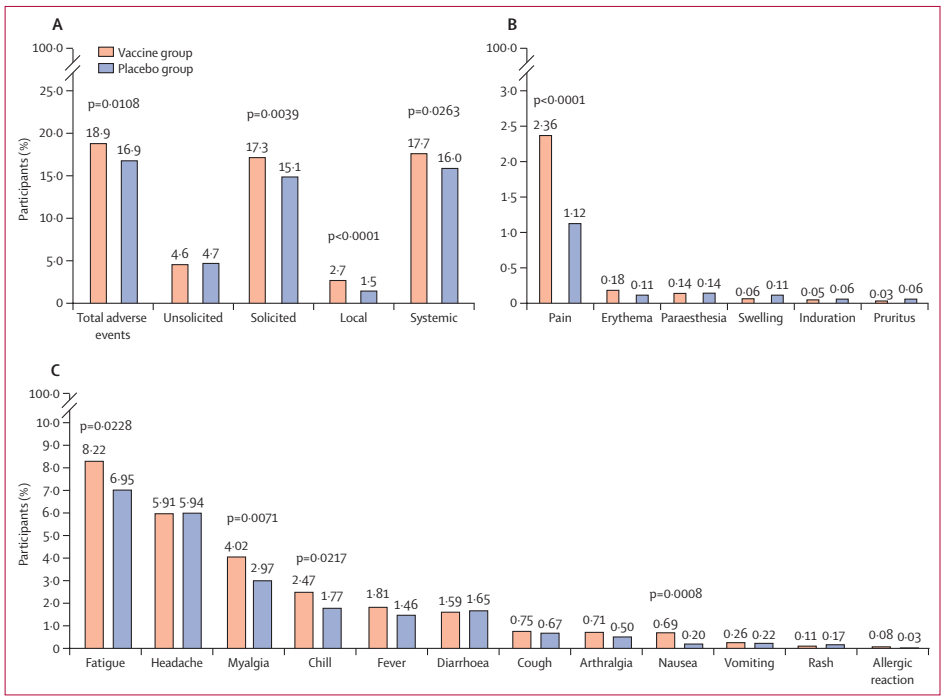


Figure 5: **Adverse events**
(A) Overall adverse events. (B) Local adverse events. (C) Systemic adverse events. p values are shown only for significant differences. See appendix (pp 10–12) for full data.

symptomatic COVID-19 (83·5% relative to placebo) and COVID-19-related hospitalisation (100%) at least 14 days after the second dose. Efficacy in subgroups was not a secondary outcome and the trial was not designed or powered to analyse the efficacy of the vaccine with regard to demographic variables and risk factors. Such analyses will require further trials designed accordingly. Anti-RBD antibodies developed in 89·7% of volunteers in a subset of our study sample, and 92·0% of those who were seropositive also produced protective levels of neutralising antibodies at least 14 days after the second dose of vaccine.

Inactivated SARS-CoV-2 vaccine candidates have shown promising results in preclinical trials.[13–15] Gao and colleagues[13] showed that, in mice, rats, and rhesus monkeys, 6 μg CoronaVac induced SARS-CoV-2-specific neutralising antibodies that effectively neutralised ten representative SARS-CoV-2 strains and provided complete protection against SARS-CoV-2 challenge in non-human primates. BBV152 (manufactured by Bharat Biotech), another inactivated vaccine, generated a quick and robust immune response with no histopathological changes in the lungs upon SARS-CoV-2 challenge in animal studies, provided adequate protection against SARS-CoV-2 infection in rhesus monkeys, induced T-helper-1 cell-skewed immune responses with elevated IgG2a/IgG1 ratios, and increased levels of SARS-CoV-2-specific IFNγ$^+$CD4$^+$ T-lymphocyte responses.[15,16] A phase 1 trial also revealed moderate seroconversion rates that persisted for up to 3 months after the second dose.[17,18] The immune response elucidated with inactivated vaccines is not confined just to the spike protein but rather to other SARS-CoV-2 proteins—the matrix proteins, envelope proteins, and nucleoprotein—which theoretically could be reflected as a vast array of immunogenic responses.[6,7] Voss and colleagues[19] showed that, in people previously infected with SARS-CoV-2, the plasma IgG response against SARS-CoV-2 was oligoclonal and more than 80% of spike protein IgG antibodies were directed towards non-RBD epitopes in the spike protein. This finding indicates that non-RBD-directed antibodies might have a role in protection against SARS-CoV-2 infection.

Phase 1/2 trials of CoronaVac in volunteers aged 18–59 years and older than 60 years showed that the vaccine doses and schedules investigated (3 μg or 6 μg, applied 14 days or 28 days apart) all had similar safety and immunogenicity profiles.[20,21] Considering the production

capacity and emergent need for vaccines, the 3 µg dose of CoronaVac has been suggested for efficacy assessment.[20] Palacios and colleagues[22] reported an overall efficacy of CoronaVac against symptomatic COVID-19 of 50·7% (95% CI 36·0–62·0) 14 days or more after the second dose; however, the efficacy in preventing the need for assistance (defined as a score ≥3 on the WHO Clinical Progression Scale) was 83·7% (58·0–93·7) and efficacy against moderate and severe cases was 100% (56·4–100·0). In a subset of participants, neutralising antibody assays showed that there were no significant differences in the frequency of seroconversion or geometric mean titres of neutralising antibodies against the B.1.128 variant compared with those against the P.1 and P.2 variants. The study cohort only included health-care workers actively working with COVID-19 patients, and a PCR-positive case with local symptoms (such as sore throat, nasal congestion, or rhinorrhoea) was considered as a failure of the vaccine, thus indicating that the vaccine might confer lower protection against asymptomatic or mildly symptomatic cases. The interim report of the phase 3 trial in Chile with a subset of 434 health-care workers, including those aged 60 years or older, revealed high seroconversion rates for specific anti-S1-RBD IgG and neutralising antibodies, along with a robust T-cell response.[23] The interim phase 3 results of other COVID-19 vaccines have shown efficacies ranging from 62·1% to 95%.[24–28] Higher and more rapidly established efficacies were observed with mRNA-based vaccines.[25,26] Considering the immunogenic mechanisms of inactivated vaccines, because one dose is not expected to be as efficacious as two doses, we did not expect to and could not show an early protective effect after the first dose, in contrast to findings with mRNA vaccines.

The tolerability of CoronaVac in this study was excellent and the incidence of adverse events, most of which were solicited systemic events, was low. The majority of the adverse events were grade 1 and occurred within 7 days after the injection. No grade 4 adverse events were observed and there was only one adverse event (an allergic reaction) that required hospitalisation.

The targeted sample size could not be reached because CoronaVac was granted emergency use authorisation by the Turkish Ministry of Health while the study recruitment was ongoing, and an immediate vaccination programme was initiated for health-care workers and later for the general public in Turkey. To comply with ethical standards, recruitment was closed earlier than planned and the placebo recipients were offered vaccines, depending on their vaccination priority.

The strengths of this study include the low dropout rate, reflecting the good tolerability of the vaccine. Additionally, the participants were from different risk groups and occupations, rendering the results of the study more generalisable to the real-world context. Additionally, active symptom surveillance was pursued to detect COVID-19 cases.

This study also has several limitations. First, the median follow-up period after randomisation to the date of unmasking was 43 days (IQR 36–48), which is a very short duration of follow-up. It is not possible to comment on the long-term protective effects of the two-dose immunisation schedule with this interim analysis.

Second, one should bear in mind that the study population consisted of relatively young (median age 45 years [37–51]) and healthy individuals with a low prevalence of chronic diseases, and the overall event rate was very low. Therefore, the generalisability of the findings of this interim analysis needs to be evaluated cautiously. In particular, the number of patients hospitalised with COVID-19 was quite low and the study population consisted of individuals at relatively low risk of severe or critical COVID-19, restricting our ability to make generalised conclusions about severe disease.

Third, the study used a 14-day interval immunisation scheme, whereas the community immunisation was with a 28-day interval. It has been claimed that, although 28-day immunisation schemes elucidated better immunogenicity after the second dose, longer intervals between the two doses are correlated with a higher probability of contracting COVID-19 before getting fully immunised and a great chance of emergence of mutant variants that can replicate in the setting of suboptimal levels of neutralising antibodies.[29] As our results pertain to the data before the emergence of variants of concern, we cannot comment on the efficacy of CoronaVac on the prevention of infection with mutant viruses. Although one of the prespecified outcomes was seroconversion, we have avoided using this term in our reporting of the results because the immunoassay we used was a semiquantitative assay. In fact, all of the participants were seronegative at the time of screening; therefore, the seropositivity 14 days after the second dose of vaccine would indicate seroconversion. However, we could not exclude the possibility that some samples with antibody levels below a sample-to-cutoff ratio of 1 might have very low concentrations of established antibodies. The current report neither involves data on the sequential serum neutralising antibody titres nor the magnitude of T-cell responses or the duration of protectivity. However, a study setting has been established to analyse the proliferation and functional capacity of $CD4^+$ and $CD8^+$ T cells, and the results of an initial study in a group of COVID-19 survivors have been reported by Tavukcuoglu and colleagues.[30] This setting is now being used to analyse the samples from selected participants of this trial to show the functional capacity of T cells induced by CoronaVac to reinvigorate antiviral immunity against SARS-CoV-2.

In summary, our results show that CoronaVac has good efficacy against symptomatic SARS-CoV-2 infection and severe COVID-19 (ie, that requiring hospitalisation), along with a very good safety profile in a population aged 18–59 years. Because this analysis included a very short

follow-up period before the emergence of viral variants and included a young and low-risk population, further data are needed on the performance of CoronaVac to demonstrate the efficacy of the vaccine against the variants of concern and the duration of protection, and to assess the safety and efficacy in older adult populations, adolescents, and children, and individuals with specific chronic diseases.

Contributors

The principal investigators, SU and MA, conceptualised and coordinated the study. SU, MA, MDT, and HLD drafted the manuscript. SU, MA, MDT, and HLD accessed and verified the data and contributed to the analysis and interpretation of the data. SU, MA, MDT, and HLD edited the manuscript. All authors were involved in organisation, coordination, conduct, and technical support of the study; collected data; critically reviewed the manuscript and approved the final version; had full access to all data in the studies, and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

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

Acknowledgments

We are grateful to all participants who volunteered to be part of this study and to all members of the clinical research teams of the participating sites. We thank TUSEB for funding the study and Omega-CRO for the statistical analyses and production of figures, and providing the study protocol. We also thank the members of the data and safety monitoring board for their contributions in the safe execution of this study.

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1.5 CoronaVac dobra número de anticorpos neutralizantes e aumenta IgG em 4,4 vezes em quem já teve Covid-19

Um estudo feito por pesquisadores da Universidade Médica de Chongqing, na China, com 85 pacientes recuperados de Covid-19 indica que a CoronaVac, vacina do Butantan e da farmacêutica Sinovac, é capaz de dobrar a quantidade de anticorpos neutralizantes e multiplicar em 4,4 vezes o nível de imunoglobulina IgG em quem já teve a doença. Os resultados preliminares foram divulgados na Cell Discovery, publicação que faz parte do grupo britânico Nature, no artigo Humoral responses in naive or SARS-CoV-2 experienced individuals vaccinated with an inactivated vaccine.

Os participantes da pesquisa tinham entre três e 84 anos e haviam se contaminado de Covid-19, em sua maioria, no início de 2020. Os pesquisadores aferiram os níveis de imunoglobulinas e de anticorpos neutralizantes nos pacientes convalescentes e selecionaram os cinco que apresentaram individualmente os menores indicadores ao final de 12 meses. Eles receberam duas doses de CoronaVac com intervalo de 21 dias.

O nível de anticorpos neutralizantes (que protegem contra uma eventual reinfecção pelo SARS-CoV-2) entre as pessoas que tiveram Covid-19, que era de 36 um dia antes da primeira dose, foi subindo até atingir 108 duas semanas após a segunda dose. No grupo controle, esse indicador alcançou 56 – ou seja, a quantidade de anticorpos neutralizantes gerados pela vacina em quem já teve Covid-19 foi o dobro na comparação com quem não havia tido a doença.

Entre os convalescentes, o nível da imunoglobulina (anticorpo) IgG, que era de 3,68 um dia antes da vacina, subiu para 47,74 duas semanas após a segunda dose de CoronaVac. É uma quantidade 4,4 vezes superior ao nível de 10,81 detectado no grupo controle. O IgG se relaciona à imunidade humoral, processo de defesa do organismo no qual atuam as imunoglobulinas encontradas na corrente sanguínea. A resposta imune humoral é crítica para o combate ao SARS-CoV-2 e também desempenha papel fundamental na prevenção de reinfecção viral.

Ao longo dos 12 meses de acompanhamento no universo dos 85 pacientes analisados, os níveis dos anticorpos neutralizantes diminuíram de 631 no final do primeiro mês para 84 no último mês. No caso da imunoglobulina IgG, o indicador caiu de 28,6 para 7,2 no mesmo período.

Os resultados da pesquisa sugerem que a CoronaVac estimula a memória humoral dos pacientes convalescentes, acelerando a produção de anticorpos neutralizantes e seu nível de circulação na corrente sanguínea.

Publicado em: 26/08/2021

Peng et al. *Cell Discovery* (2021)7:68
https://doi.org/10.1038/s41421-021-00311-z

Cell Discovery
www.nature.com/celldisc

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

Pai Peng[1], Hai-jun Deng[1], Jie Hu[1], Xiao-yu Wei[2], Jian-jiang Xue[3], Ting-ting Li[4], Liang Fang[2], Bei-zhong Liu[2], Ai-shun Jin[4], Feng-li Xu[1], Kang Wu[1], Quan-xin Long[1], Juan Chen[1], Kai Wang[1]✉, Ni Tang[1]✉ and Ai-long Huang[1]✉

Dear Editor,

The humoral immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is critical for the clearance of the virus and also plays a key role for the prevention of viral reinfection. It has been extensively reported that antibody response to SARS-CoV-2 tends to be diminished in course of time[1–3]. Thus, the durability of the protective immune response in coronavirus disease-2019 (COVID-19) recovered patients is of great interest. There is increasing appreciation of the key role that immunological memory plays in durable protective immunity after infections or vaccinations, even with lower antibody titers[4,5]. Inactivated vaccines as a conventional vaccine development have been shown to be effective among other viruses[6]. It has raised concern about the impact of prior infection by SARS-CoV-2 on the immune response induced by inactivated vaccines. For these reasons, we examined the humoral immunity in convalescent patients for 12 months postsymptom onset (PSO) and evaluated the immune response elicited by an inactivated vaccine in naive or COVID-19 recovered individuals.

170 blood samples from a follow-up cohort of 85 COVID-19 patients were collected over a 12-month period PSO (Supplementary Fig. S1a). Participants with 57.6% male and 42.4% female aged from 3 to 84 (median: 48 years) were enrolled (Supplementary Table S1). After the measurement of neutralizing antibodies (NAbs), five participants with low NAb titers were given two injections of CoronaVac vaccine (developed by Sinovac Life Sciences, China) 21 days apart for the study of immunological memory response. Meanwhile, 19 healthy individuals were recruited as the control group (Supplementary Fig. S1b, Table S2).

Anti-SARS-CoV-2 spike (anti-S) IgG/IgM/IgA and NAb titers were measured with previously described MCLIA kits and pseudovirus-based neutralization assay. Anti-S IgG and NAbs were still detectable in 95.5% (42 of 44) and 93.2% (41 of 44) serum samples, respectively, at 12 months PSO (Fig. 1a). Correlation between anti-S IgG levels and Nab titers ($r = 0.64$, $p = 5.8e{-}21$) was shown over the study period (Supplementary Fig. S2a). Nevertheless, during the 12-month follow-up visit in the COVID-19 recovery cohort, anti-S IgG/IgM/IgA and NAb titers represented a sustained decline (Fig. 1a, Supplementary Fig. S2b, c). For the neutralizing antibodies, median of NAb titers decreased from 631 at Month 1 to 604 at Month 3, to 134 at Month 8 and to 84 at Month 12. For the IgG antibodies, the median of signal-to-cutoff ratio (S/CO) dropped from 28.6 at Month 1 to 27.7 at Month 3, 11.5 at Month 8 and 7.2 at Month 12. At Month 12, the levels of specific antibodies were much lower than the levels at Month 1 (82.8%, 96.4%, and 89.4% decrease for IgG, IgM, and IgA antibodies, respectively). In addition, a longitudinal study was observed among nine participants provided samples at all follow-up time points. In spite of a general decline in humoral immune response, the dynamic changes showed significant variation between anti-S IgG/IgM/IgA antibodies and NAbs

Correspondence: Kai Wang (wangkai@cqmu.edu.cn) or Ni Tang (nitang@cqmu.edu.cn) or Ai-long Huang (ahuang@cqmu.edu.cn)
[1]Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, the Second Affiliated Hospital, Chongqing Medical University, Chongqing, China
[2]Yong-Chuan Hospital, Chongqing Medical University, Chongqing, China
Full list of author information is available at the end of the article
These authors contributed equally: Pai Peng, Hai-jun Deng, Jie Hu

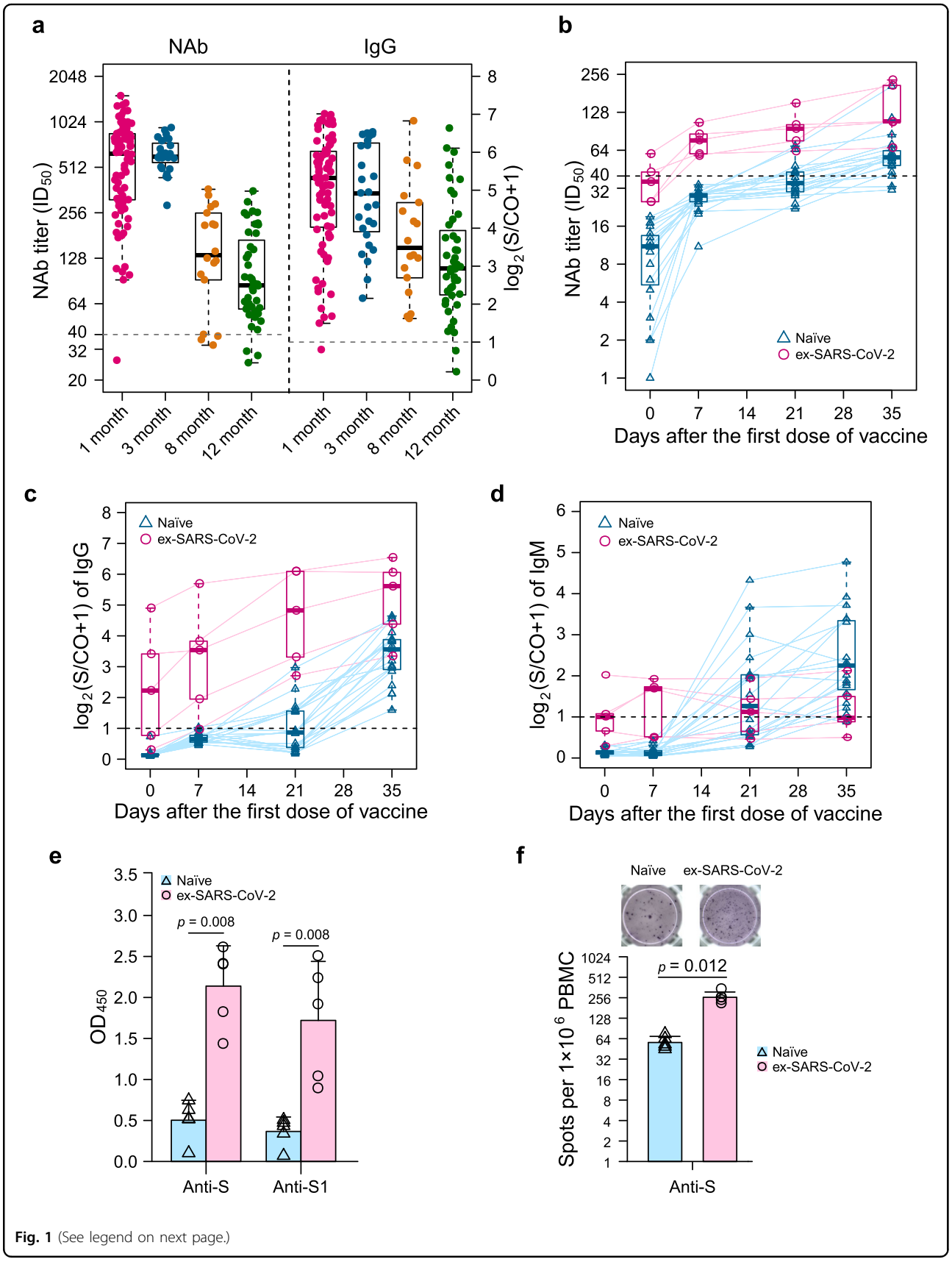

Fig. 1 (See legend on next page.)

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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 (ID_{50}) and the limit of detection (LOD) was 40; the signal to cut-off ratio (S/CO) of IgG/IgM/IgA above 1 was considered as positive. NAb titers (**b**), IgG (**c**), and IgM (**d**) levels of two cohort in which COVID-19 convalescent individuals or healthy participants were injected by two-dose inactivated vaccine CoronaVac; **e**, **f** the status of SARS-CoV-2 specific memory B cells in COVID-19 recovered individuals and naive individuals. Enzyme-linked immunosorbent assay (ELISA) (**e**) was performed to detected anti-S, anti-S1 IgG secreted by memory B cells and enzyme-linked immunosorbent spot assay (ELISpot) (**f**) was performed to analyze the number of antibody-secreting cells. OD denotes optical density, S spike protein and S1 fragment of spike glycoprotein. Empty triangles with red and empty circles with blue indicate healthy individuals and SARS-CoV-2 experienced individuals, respectively; the horizontal dashed lines denote the lower LOD. In **a**–**d**, boxes denote the median, first and third quartiles, while the whiskers show ×1·5 interquartile range (IQR) of antibody levels. In **e**, **f**, boxes and error bars denote mean ± standard deviation. Statistical analysis was performed with the use of the two-tailed, nonparametric Mann–Whitney *U* test.

(Supplementary Fig. S2d–g). Both IgM and IgA levels in 7 of 9 individuals reached peak at 1 month PSO and fell below the positive threshold thereafter. By contrast, IgG and NAbs decreased slowly and remains 100% (9/9) and 78% (7/9) positive at 12 months PSO.

Blood samples from two vaccination cohorts were collected pre-vaccination (day 0, the day before the first dose of vaccine) and 7, 21, 35 days after the first dose of vaccine (Supplementary Fig. S1b). The evaluation of immunological memory induced by the inactivated vaccine was performed by detection of specific antibodies and antibody-secreting memory B cells among participants. NAbs were detective only in COVID-19 recovered group within 7 days after the first dose of vaccine (median of NAb titers 36 on Day 0; 77 on Day 7; 95 on Day 21; and 108 on Day 35) (Fig. 1b). The median NAbs titer was 56 in the naive group 35 days after the first dose of vaccine. Due to the previous presence of SARS-CoV-2 specific antibodies, the majority of COVID-19 recovered individuals had detectable IgG from pre-vaccination to post-vaccination (median S/CO value before vaccination, 3.68; and 10.59, 27.33, and 47.74 on Day 7, 21, and 35 after vaccination, respectively) (Fig. 1c). In the naive group, anti-S IgG was detected with lower values than COVID-19 recovered individuals over 35 days after the first dose of vaccine (median S/CO value before vaccination, 0.10; and 0.57, 0.83, and 10.81 on Day 7, 21, and 35 after the first dose of vaccine, respectively). IgG levels of COVID-19 recovered individuals were 4.4 times that of naive individuals at Day 35 (median S/CO value, 47.74 vs 10.81). Interestingly, IgM titers increased over time in naive group, while no substantial changes displayed in COVID-19 recovered group (Fig. 1d). Furthermore, IgA of both groups remained at a low level, even staying below the positive threshold (Supplementary Fig. S3).

To further understand higher humoral response in COVID-19 recovered individuals after vaccination, SARS-CoV-2 specific memory B cells differentiated from peripheral blood mononuclear cells of 5 SARS-CoV-2 experienced and naive individuals before vaccination were determined by enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunosorbent spot assay (ELISpot). As expected, specific anti-S, anti-S1 fragment of spike glycoprotein (anti-S1) IgG and the number of anti-S IgG antibody-secreting cells presented higher levels in SARS-CoV-2 experienced group than the naive group (Fig. 1e, f).

Our findings demonstrated that anti-S IgG, IgM, IgA and NAb titers declined gradually over 1 year in patients infected with SARS-CoV-2. Even though antibody response of most participants remained detectable, the drop of more than 80% were shown in anti-S IgG, IgM, IgA, and NAb titers. To evaluate the duration of protective immunity against SARS-CoV-2, further surveillance is needed. Moreover, our results suggest that immunological memory mediated by an inactivated vaccine could recall higher response of IgG and NAb in COVID-19 recovered individuals with low NAb titers than in naive persons at 12 months PSO. After infection, SARS-CoV-2 specific memory B cells secreting antibody increased significantly in COVID-19 recovered individuals compared to healthy controls. It should be pointed out that maybe due to the cross-activity between SARS-CoV-2 and seasonal coronaviruses, SARS-CoV-2 S and S1-specific antibodies secreted by memory B cells were detected at baseline in naive persons[7].

Compared to our data, rapid immune response elicited by a single mRNA vaccine dose was showed in several SARS-CoV-2 recovery cohorts vaccinated by mRNA-based vaccines[8–11]. Further investigation is needed to answer the necessity of vaccination for SARS-CoV-2 experienced individuals, and to answer whether the immune response provides effective protection from reinfection in this special group, especially for SARS-CoV-2 variants.

The main limitation of this study is the small sample size and relatively short period for the observation of vaccination cohorts. Even though our data provided a hint about the role of memory B cell response in humoral response after vaccination or reinfection, a deeper investigation carried out by flow cytometry will be needed. An inactivated

virus vaccine including all components of SARS-CoV-2 might provide the distinct benefit to boost T-cell response against other SARS-CoV-2 proteins, but T-cell immunity was not investigated in our study.

Our results reveal the durability of immunological response 1 year after natural SARS-CoV-2 infection and the benefit from inactivated vaccines for COVID-19 recovered individuals. It provides more information about immunological characteristics of SARS-CoV-2 inactivated vaccines, thus will contribute to the development of vaccines and the new strategies of vaccination.

Acknowledgements
We thank Dr. Cheng-yong Yang for critical review of the manuscript. We acknowledge the arrangement of blood sample collection from Yongchuan Hospital Affiliated to Chongqing Medical University (Chongqing, China) and all participants involved in the study. This work had received funding support from the Natural Science Foundation Project of Chongqing (cstc2019jscx-dxwtBX0019), the Emergency Project from the Science & Technology Commission of Chongqing (cstc2020jscx-dxwtB0050, cstc2020jscx-fyzx0053), the Emergency Project for Novel Coronavirus Pneumonia from the Chongqing Medical University (CQMUNCP0302), a Major National Science & Technology Program grant (2017ZX10202203) from the Science & Technology Commission of China, Kuanren Talents Program of the second affiliated hospital of Chongqing Medical University, China Postdoctoral Science Foundation (2021M693924), and Chongqing Postdoctoral Science Special Foundation (2010010005216630).

Author details
[1]Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Institute for Viral Hepatitis, Department of Infectious Diseases, the Second Affiliated Hospital, Chongqing Medical University, Chongqing, China. [2]Yong-Chuan Hospital, Chongqing Medical University, Chongqing, China. [3]University-Town Hospital of Chongqing Medical University, Chongqing, China. [4]Department of Immunology, College of Basic Medicine, Chongqing Medical University, Chongqing, China

Author contributions
P.P. participated in data curation, formal analysis, funding acquisition, investigation, validation, and writing-original draft; J.H., F.L.X., K. Wu. participated in data curation, formal analysis, investigation, and methodology; H.J.D. participated in software, visualization, and writing-review and editing; J.C., Q.X.L., X.Y.W. and B.Z.L. participated in resources; J.J.X., T.T.L., A.S.J. participated in methodology and resources; K. Wang., N.T. and A.L.H. participated in conceptualization, formal analysis, funding acquisition, project administration, supervision, validation and writing-review and editing.

Conflict of interest
The authors declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41421-021-00311-z.

Received: 25 April 2021 Accepted: 11 July 2021
Published online: 17 August 2021

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1.6 Eficácia global da CoronaVac pode chegar a 62,3% com intervalo entre doses igual ou superior a 21 dias

Um artigo científico encaminhado por cientistas do Butantan em abril para a revista científica The Lancet mostrou que a eficácia da CoronaVac para casos sintomáticos atingiu 50,7% com 14 dias de intervalo entre as duas doses, mais do que os 50,38% divulgados em janeiro com base nos dados iniciais do estudo clínico de fase 3. Além disso, a eficácia global, que aponta a capacidade que o imunizante tem de proteger em casos leves, moderados ou graves, pode chegar a 62,3% se o espaço entre as duas doses for de 21 dias ou mais.

Os dados fazem parte de um aprofundamento dos estudos clínicos realizados em 2020 com mais de 12 mil participantes, todos profissionais da saúde. A pesquisa foi liderada pelo diretor de ensaios clínicos do Instituto Butantan, Ricardo Palacios. O artigo ainda diz que a eficácia mínima da vacina já aparece na segunda semana depois da primeira dose. Porém, para que a imunização fique completa, é necessário receber as duas doses.

Inicialmente, o estudo clínico de fase 3 indicava que, para os casos moderados e graves, que necessitam de assistência médica, a eficácia da vacina variava entre 78% e 100%. Nos resultados da nova pesquisa, no entanto, o imunizante se mostrou eficaz entre 83,7% e 100% dos casos. Isso significa que a CoronaVac tem a capacidade de reduzir a maioria dos casos que exigem algum cuidado médico.

O artigo ainda sugere que a CoronaVac, imunizante desenvolvido em parceria com a biofarmacêutica chinesa Sinovac, é capaz de proteger contra as variantes P.1 e P.2 do novo coronavírus.

O estudo foi conduzido entre 21 de julho e 16 de dezembro de 2020. Foram 12.396 voluntários em 16 centros de pesquisa brasileiros, e todos receberam ao menos uma dose da vacina ou placebo. No total, 9.823 participantes receberam as duas doses. Não houve óbitos por Covid-19 durante os testes.

Os dados foram divulgados na plataforma de preprints da revista The Lancet e estão em processo de revisão por pares.

Publicado em: 21/06/2021

Artigo 5

Article

Title Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: The PROFISCOV study

Ricardo Palacios, MD, PhD [1*]

Ana Paula Batista, RN [1]

Camila Santos Nascimento Albuquerque, RN [1]

Elizabeth González Patiño, PhD [1]

Joane do Prado Santos, BTech [1]

Mônica Tilli Reis Pessoa Conde, MD, PhD [1]

Roberta de Oliveira Piorelli, MD [1]

Luiz Carlos Pereira Júnior, MD, PhD [2]

Sonia Mara Raboni, MD, PhD [3]

Fabiano Ramos, MD, PhD [4]

Gustavo Adolfo Sierra Romero, MD, PhD [5]

Fábio Eudes Leal, MD, PhD [6]

Luis Fernando Aranha Camargo, MD, PhD [7]

Francisco Hideo Aoki, MD, PhD [8]

Eduardo Barbosa Coelho, MD, PhD [9]

Danise Senna Oliveira, MD, PhD [10]

Cor Jesus Fernandes Fontes, MD, PhD [11]

Gecilmara Cristina Salviato Pileggi, MD, PhD [12]

Ana Lúcia Lyrio de Oliveira, MD, PhD [13]

André Machado de Siqueira, MD, PhD [14]

Danielle Bruna Leal de Oliveira, PhD [15]

Viviane Fongaro Botosso, PhD [16]

Gang Zeng, PhD [17]

Qianqian Xin, PhD [17]

Mauro Martins Teixeira, MD, PhD [18]

Maurício Lacerda Nogueira, MD, PhD [19]

Esper G Kallas, MD, PhD [20]

On behalf of the PROFISCOV study group.

1. Clinical Trials and Pharmacovigilance Center, Instituto Butantan, São Paulo, Brazil.

2. Emilio Ribas Institute of Infectious Diseases. Secretary of Health of Sao Paulo State, São Paulo, SP, Brazil

3. Department of Infectious Diseases, Hospital de Clínicas, Universidade Federal do Paraná, Curitiba, PR, Brazil

4. Infectious Diseases Service, Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.

5. Center for Tropical Medicine, School of Medicine, University of Brasilia, Brasília, DF, Brazil

6. Universidade Municipal de São Caetano do Sul, São Caetano do Sul, SP, Brazil

7. Instituto Israelita de Ensino e Pesquisa Albert Einstein, São Paulo, SP, Brazil

8. School of Medical Science and Hospital of Clinics, State University of Campinas – UNICAMP, Campinas, SP, Brazil

9. Department of Internal Medicine, Ribeirão Preto Medical School, University of Sao Paulo, Ribeirão Preto, SP, Brazil.

10. Internal Medicine Department. School of Medicine, Universidade Federal de Pelotas. Pelotas, RS, Brazil
11. Department of Internal Medicine and Infectious Diseases, Julio Müller School Hospital, Federal University of Mato Grosso, Cuiaba, MT, Brazil.
12. Research Institute of Cancer Hospital, Barretos, SP, Brazil
13. Hospital Universitário Maria Aparecida Pedrossian, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil
14. Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil
15. Laboratory of Clinical and Molecular Virology, Department of Microbiology, Institute of Biomedical Science, University of Sao Paulo, São Paulo, SP, Brazil
16. Virology Laboratory, Development and Innovation Center (CDI) Instituto Butantan, Sao Paulo, SP, Brazil
17. Sinovac Biotech Co., Ltd, Haidian District, Beijing, People's Republic of China
18. Centro de Pesquisa e Desenvolvimento de Fármacos, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
19. Faculdade de Medicina de São José do Rio Preto (FAMERP), São José de Rio Preto, SP, Brazil
20. Department of Infectious and Parasitic Diseases, Clinicas Hospital, School of Medicine, University of São Paulo, São Paulo, SP, Brazil.

* Corresponding author: Ricardo Palacios, Clinical Research Medical Director, Instituto Butantan, São Paulo, Brazil, ricardo.palacios@butantan.gov.br, Phone +55-11-3723-2121

Abstract

Background

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

Methods

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

Findings

Between July 21 and Dec 16, 2020, 12 396 participants were enrolled and received at least one vaccine or placebo dose. There were 9,823 participants who received the two doses and were followed for at least 14 days and had, therefore, reached the final efficacy analysis. There were 253 confirmed COVID-19 cases in the cohort: 85 cases (11.0/100 person-year) among 4,953 participants in the vaccine group, and 168 cases (22·3/100 person-year) among 4,870 participants in the placebo group. The primary efficacy against symptomatic COVID-19 was 50·7% (95%CI 36·0-62·0). The

secondary efficacy against cases requiring assistance (score ≥3) and moderate and severe cases (score ≥4) were 83·7% (95%CI 58·0-93.7) and 100% (95%CI 56·4-100.0) respectively. All 6 cases of severe COVID-19 occurred in the placebo group. The incidence of adverse reactions, which was mainly pain at the administration site, was higher in the vaccine group (77·1%) than in the placebo group (66·4%). There were 67 serious adverse events reported by 64 participants and all were determined to be unrelated to vaccination, including two fatal cases. In a subset of participants, neutralizing antibody assays showed similar seroconversion and geometric mean titres against B.1.128, P.1, and P.2 variants.

Interpretation

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

Funding: Fundação Butantan, Instituto Butantan, and São Paulo Research Foundation - FAPESP (Grants 2020/10127-1 and 2020/06409-1)

Introduction

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

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

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

Methods

Study design and participants

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

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

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

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

Randomization and masking

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

Procedures

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

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

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

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

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

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

Outcomes

The primary endpoint was the efficacy of CoronaVac against confirmed symptomatic COVID-19 with onset at least 14 days after the second injection in the *per protocol* population. All the cases were judged by a blind independent clinical endpoint adjudication committee. Confirmed COVID-19 cases were defined as: 1) at least two consecutive days with one or more specific symptoms (cough, newly developed taste or smell disorders, shortness of breath or dyspnea); or 2) with two or more non-specific symptoms (fever [axillary temperature ≥37·5°C], chills, sore throat, fatigue, nasal congestion or runny nose, body pain, muscle pain, headache, nausea or vomiting, diarrhoea; or 3) imaging features of COVID-19; and 4) detection of SARS-CoV-2 nucleic acid in respiratory swab by RT-PCR. A case definition based on the U.S. Food and Drug Administration (FDA) criteria was also used as a sensitivity analysis.[9]. Following the latter criteria, a positive case was considered as anyone who presented at least one of the following symptoms for two days or more, with a positive SARS-CoV-2 RT-PCR result: fever or chills, cough, shortness of breath or difficulty in breathing, fatigue, muscle or body pain, headache, sore throat, nasal congestion or runny nose, nausea or vomiting, and diarrhoea. The primary efficacy was also evaluated in distinct subgroups, including age groups, race, and ethnic group, with or without underlying medical conditions, different vaccination intervals

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

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

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

Statistical analysis

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

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

Role of the funding sources

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

Results

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

Among those 12,396 participants, 5·1% were elderly participants aged 60 years or older, 64·2% were female, and most participants self-identified themselves as white (75·3%). More than half of the participants (55·9%) had underlying diseases, 22·5% of them were obese (BMI ≥30 kg/m²). The average age and BMI of participants were 39·5 years and 26·8 kg/m^2, respectively (Table 1).

All 12,396 participants were involved in the safety set (SS) and monitored for adverse events from the beginning of vaccination up until 12 months after the first dose vaccination. By the cut-off date, the incidence of adverse events and adverse reactions were 78·8% and 71·7%, respectively, by the cut-off date (Appendix p6). Generally, the vaccine group reported more adverse reactions than the placebo group (77·1% *vs*. 66·4%; p<0001), and most adverse reactions were solicited (73·1% *vs*. 60·0%, p<0·0001) (Figure 2A).

Among solicited adverse reactions, the incidence of local adverse reactions was 61·5% in the vaccine group, and this was higher than the 34·6% in the placebo group (p<0·0001). Local adverse reactions were mainly driven by pain at the injection site (60·3% *vs*. 32·5%, p<0·0001). All solicited local reactions were more frequently in the vaccine group, and the incidences were less than 6% in the vaccine group, except pain at the injection site (Figure 2B). Systemic adverse reactions were similar in the vaccine and placebo groups (48·4% *vs*. 47·6%, p=0·3882), including headache and

fatigue, the most common systemic symptom collected in this trial. Myalgia was more frequent in the vaccine group (11·7% *vs*. 10·5%, p=0·0257). Fever (≥37.8°C) was rare and only reported by 0·2% and 0·1% (p=0·2666) participants in the vaccine and placebo groups, respectively (Figure 2C). Unsolicited ARs were reported by 36·8% in the vaccine and 35·8% in the placebo groups (p=0·2177, Figure 2A). Only tremor, flushing and local reactions in the administration site (reported in an unsolicited period) showed higher incidence in the vaccine group. No difference was found for other unsolicited symptoms (Appendix p7-10).

In this study, 67 serious adverse events were reported by 64 participants, 33 in the vaccine group and 31 in the placebo group (Appendix p20-23). The overall incidence of SAE was 0·5%. All SAEs were determined as unrelated to the vaccine. Two deaths were reported in this trial: one case of cardiopulmonary arrest (placebo group), and one case of medication overdose (vaccine group); all of them unrelated to the vaccine. One additional death due to COVID-19 (placebo group) occurred as outcome on an ongoing case by the data cut time.

Among 9,823 participants in the *per protocol* analysis, 253 cases of symptomatic COVID-19 were reported during the primary efficacy analysis period (Table 2). There were 85 cases (11.0/100 person-year) among 4,953 participants in the vaccine group, and 168 cases (22·3/100 person-year) among 4,870 participants in the placebo group. The efficacy to prevent symptomatic COVID-19 was 50·7% (95%CI 35·9-62·0). Considering the α spending in the interim analysis, the corrected efficacy was 50.7% (95.4%CI 35·7-62·2). Sensitivity analysis of primary efficacy was conducted based on other case definitions, and the efficacy results ranged from 51·2% to 54·1% (Appendix p24).

A key secondary endpoint was to evaluate the efficacy to prevent COVID-19 disease at different clinical severities. There were 35 cases scored 3 and above, 10 cases scored 4 and above, 6 severe cases (including one fatal case) reported among the 9823 participants. For cases scored 3 and above, 5 cases were in the vaccine group, 30 were in the placebo group, resulting in a vaccine efficacy of 83·7% (95%CI 58·0-93·7). All cases scored 4 and above were in the placebo group, resulting in 100% vaccine efficacy against moderate and sever cases (95%CI 56·4-100·0).

Subgroup analyses were also conducted by the interval between two doses, the exposure status to SARS-CoV-2 pre-vaccination, age group, and underlying disease. Participants with two doses interval of fewer than 21 days showed similar efficacy (49·1%; 95%CI 33·0-61·4) as the primary efficacy analysis. For the small portion of participants who received two doses of vaccine or placebo with an interval of 21 days or more, the efficacy was calculated at 62·3% (95%CI 13·9-83·5). The efficacy was similar between different exposure status to SARS-CoV-2 pre-vaccination (Unexposed: 50·5%; Exposed: 49·5%), and between other age groups (18 to 59 years: 50·7%; ≥60 years: 51·1%). For participants with underlying diseases, a total of 130 cases were reported in this population, resulting in 48·9% efficacy (95%CI 26·6-64·5). For participants with cardiovascular disease, diabetes, and obesity, the efficacy was 39·5% (95%CI -66·4-78·0), 48·6% (95%CI -115·3-87·7) and 74·9% (95%CI 53·7-86·4), respectively. Two-hundred and fifty participants of Asian ethnicity reported 4 cases, of which 1 in the vaccine group and 3 in the placebo group, resulted in 66·0% efficacy (95%CI -226·8-96·5).

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

378 cases were reported after the first dose, of which 126 were in the vaccine group and 252 were in the placebo group, resulting in an efficacy of 50·8% (95%CI 39·0-60·3) after the first dose, similar to the calculated efficacy with the complete vaccination schedule. For 14 days after the first dose, 313 cases were collected among 11,431 participants, 94 were in the vaccine group and 219 were in the placebo group, resulting in an efficacy of 57·9% (95%CI 46·4-66·9) (Figure 3).

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

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

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

Discussion

The PROFISCOV study was designed to test CoronaVac in a group exposed to SARS-CoV-2 more often and at potentially higher infectious doses than in a community exposure. Using a smaller sample size compared to other large Phase III clinical trials with vaccine candidates, we were able to demonstrate that this vaccine was safe, well-tolerated, and efficacious. Efficacy to prevent any symptomatic COVID-19 started at 50.7% and became more extensive as disease severity increased. Of note, the case definition and professional profile of the study population allowed highly sensitive surveillance and the study was able to detect even the mildest cases of COVID-19. The conditions of this trial should be considered when the results are extrapolated to other populations or comparisons with other trials are suggested.

The vaccine performance met the requirements for Emergency Use Authorization in 32 countries and regions allowing a fast response to an ongoing public health emergency at a speed similar to other vaccine candidates receiving heavy subsidies from governments and international organizations.

One of the factors that might have affected the study's overall efficacy was the interval between two doses of 14 days. Although there were a limited number of participants in this study having doses with an interval of 21 days or higher, there was a trend to higher efficacy. Furthermore, previous neutralization data in adults were lower with a 14-days interval[3] , and, in this study, participants aged 60 years or more had a lower response than adults with the same 14-days schedule. These results contrast with previous studies where the immune responses in adults and elderly

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

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

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

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

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

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

Acknowledgments

First and foremost, we would like to thank the colleagues who volunteer to participate in this trial. Not only for the scientific contribution but for also staying at the frontlines caring for COVID-19 patients and their families and friends. The technical assistance of Jorge Flores, Christopher Gast and Chistian F. Ocklenhouse from PATH was key to make this study possible. We are very grateful to the members of the Data Safety Monitoring Board and the Clinical Endpoint Adjudication Committee that generously gave their knowledge, experience, and wise advice to the study at no cost. The PROFISCOV study group is in debt for all extraordinary efforts made by our colleagues Dimas Tadeu Covas, Rui Curi, Reinaldo Noboru Sato, Raul Machado Neto, Paulo Luis Capelotto, Tiago Tadeu Rocca, Cristiano Gonçalves Pereira, Gilberto Guedes de Padua, Ricardo Haddad, Natália Lamesa Ambrósio, Luiz Rogério Perilli, and Fernanda Aparecida Orsi Castilho from Instituto Butantan and Fundação Butantan to support this study. Sandra Yi O Cho and Bruno Antônio de Oliveira, from the Regulatory Affairs Area at Butantan, deserve our special gratitude for being the most constant fellows of the clinical research team. The vendors in a clinical trial have a critical role and we were lucky to have the most dedicated staff from IQVIA (Eduardo Tedeschi, Fernanda Nobrega, Renata Toledo, Tatiana Kasteckas, Guilherme Knorst, Nicolas Rodovalho, and colleagues), Allports (José Luiz Vaz Monteiro) and WorldCourier. We were able to move ahead with the extenuating journeys required for this study just because we have the care and the patience of our loved ones.

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Figure legends

Figure 1: Study Profile.

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

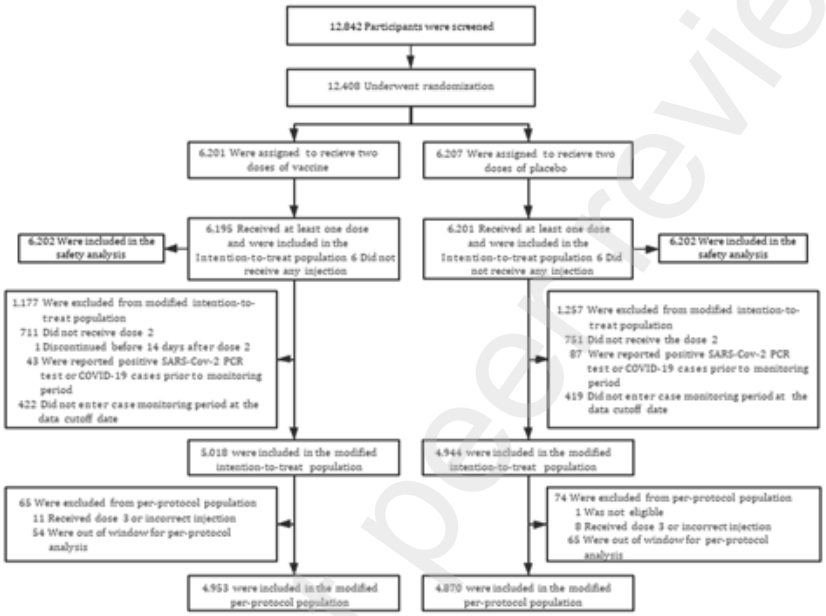


Figure 2: Overview of Adverse Reactions and Solicited Local/Systemic Adverse Reactions.

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

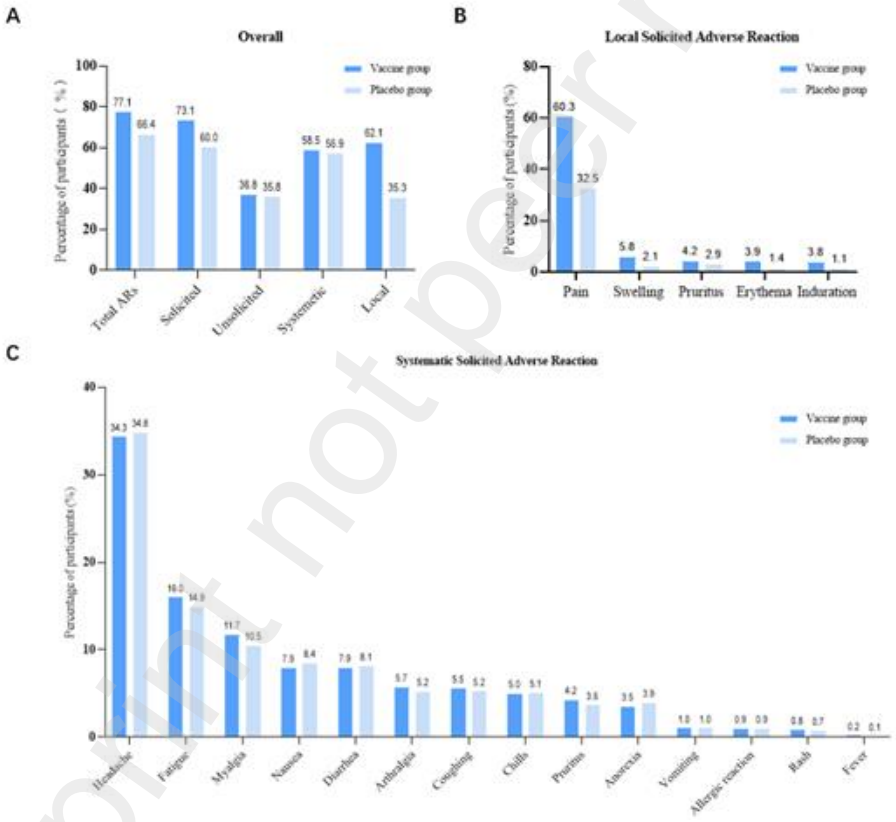
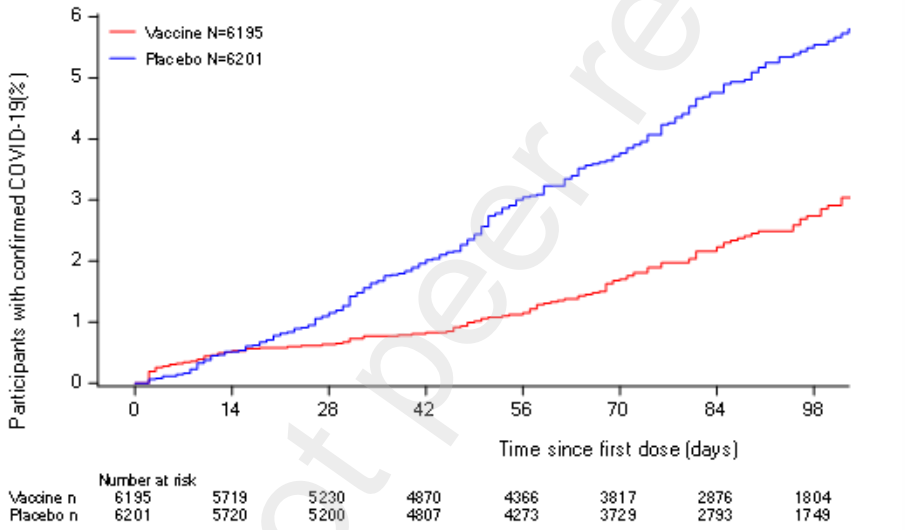


Figure 3. Efficacy of vaccine against COVID-19 cases after the 1st dose and the Kaplan-Meier cumulative incidence curves

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

A



B

Time	No. of cases	Vaccine n/N(incidence density)	Placebo n/N(incidence density per 100 person-year)	Efficacy (95%CI)
14 days after 1st dose	313	94/5717(8·0)	219/5714(19·0)	57·9 (46·4, 66·9)
14 days after 2nd dose	253	85/4953(11·0)	168/4870(22·3)	50·7 (35·9, 62·0)

Tables

Table 1: Baseline characteristics of participants who received at least one dose of vaccine or placebo

	Vaccine (N=6195)	Placebo (N=6201)	Total (N=12396)
Age Group			
18~59 years	5879 (94·9%)	5885 (94·9%)	11764 (94·9%)
≥60 years	316 (5·1%)	316 (5·1%)	632 (5·1%)
Gender			
Male	2270 (36·6%)	2171 (35·0%)	4441 (35·8%)
Female	3925 (63·4%)	4030 (65·0%)	7955 (64·2%)
Ethnic			
White	4685 (75·8%)	4633 (74·8%)	9318 (75·3%)
Multiracial	1012 (16·4%)	1065 (17·2%)	2077 (16·8%)
Black or African American	329 (5·3%)	319 (5·2%)	648 (5·2%)
Asian	148 (2·4%)	163 (2·6%)	311 (2·5%)
American Indian or Alaska Native	11 (0·2%)	13 (0·2%)	24 (0·2%)

	Vaccine (N=6195)	Placebo (N=6201)	Total (N=12396)
Underlying Disease	3441 (55·5%)	3484 (56·2%)	6925 (55·9%)
Cardiovascular disease	792 (12·8%)	773 (12·5%)	1565 (12·6%)
Diabetes	218 (3·5%)	197 (3·2%)	415 (3·4%)
Obesity	1386 (22·4%)	1403 (22·6%)	2789 (22·5%)
Age, years	39·42 (10·7)	39·59 (10·8)	39·50 (10·8)
BMI, kg/m^2	26·841 (5·1)	26·792 (5·3)	26·817 (5·2)

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

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 No. of cases	Vaccine n/N(incidence density)	Placebo n/N(incidence density per 100 person-year)	Vaccine Efficacy (95%CI)
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	Placebo	Vaccine Efficacy (95%CI)
		n/N(incidence density)	n/N(incidence density per 100 person-year)	
Cardiovascular disease	16	6/621(7·1)	10/608(11·6)	39·5(-66·4, 78·0)
Diabetes	8	3/175(11·2)	5/159(21·1)	48·6(-115·3, 87·7)
Obesity	63	13/1099(5·8)	50/1112(23·0)	74·9(53·7, 86·4)
Asian	4	1/125(5.38)	3/125(15.54)	66.02(-226.82, 96.47)

[1] The efficacy corrected based on the α spending in the interim analysis was 50.7% (95.4%CI: 35.7, 62.2).

[2] Calculated based on Poisson regression model

Appendix 1 Protocol violation

Table 1-1. Data set division of each protocol violation

No.	Protocol Violations	Efficacy Evaluation			Safety Evaluation		
		PPS	ITT	mITT	SS	SS1	SS2
1	Not vaccinated after randomisation	N	N	N	N	N	N
2	Received 1 dose vaccination	N	Y	N	Y	Y	N
3	Withdraw before 14 days after the second dose vaccination	N	Y	N	NA	NA	NA
4	Received 3 doses vaccination	N	Y	Y	Y	Y	Y
5	Participated in any COVID-19 vaccine clinical trial or vaccinated COVID-19 vaccine in the past	N	Y	Y	NA	NA	NA
6	Received the second dose vaccination beyond the window period	N	Y	Y	Y	Y	Y
7	Received wrong vaccine*	N	Y	Y	NA	NA	NA
8	The time of data analysis was before 14 days after the second dose vaccination	N	Y	N	NA	NA	NA
9	PCR positive between the first dose vaccination to the 14 days after the second dose vaccination	N	Y	Y	NA	NA	NA
10	Diagnosed COVID-19 between the first dose vaccination to the 14 days after the second dose vaccination	N	Y	Y	NA	NA	NA

*Details see Table 1-2.

Table 1-2. List of wrong vaccinations*

No. of subject	Wrong dose vaccination	No. of vaccine	Date of wrong dose vaccination	Describe of protocol violation
111451	1	111454	2020/8/6	
111577	2	111571	2020/8/25	
112384	1	112386	2020/8/20	
112538	2	114579	2020/9/4	
112828	2	111828	2020/9/8	
113046	2	113007	2020/9/9	
115170	2	115191	2020/9/23	
115191	2	115170	2020/9/23	
116623	1	116593	2020/9/17	
116737	2	wrong arm**	2020/10/1	Due to the error of the unblinded pharmacist, subject 116737 was assigned the wrong vaccine in V2.
116811	1	wrong arm**	2020/9/18	Due to the error of the unblinded pharmacist, subject 116811 was assigned the wrong vaccine in V1.
116881	1	wrong arm**	2020/9/18	Due to lack of supervision, the unblinded pharmacist assigned the wrong vaccine to subject 116881 in V1.
117927	2	118063	2020/10/9	
118339	1	wrong arm**	2020/9/26	Due to the error of the unblinded staff, an error occurred in the allocation of vaccine to subject 118339. Date of occurrence of PD: 2020-09-26
119167	2	119538	2020/10/20	
119278	1	wrong arm**	2020/10/3	Due to the absence of double review, subject 119278 was assigned the wrong vaccine in V1.

120446	1	120426	2020/11/6	
120579	1	Unknown**	2020/10/19	The unblinded monitor confirmed that subject 120579 was vaccinated on October 19, 2020, but the IWRS indicated that this assignment did not occur on that day. Therefore, it is unknown which vaccine the subject has been assigned.

*From the protocol deviation list provided by the monitor

**In the overall and corresponding dose safety analysis, from a conservative perspective, subjects with “wrong arm” and “unknown” are analyzed by vaccine group.

Appendix 2 Study sites

Table 2. Information of study sites

Code.	Study Site	Address	Principal Investigator
SAO06	Instituto de Infectologia Emílio Ribas	Sao Paulo, SP, Brazil, 01246-900	Luiz Carlos Pereira Júnior, MD, PhD
CWB01	Hospital das Clínicas da Universidade Federal do Paraná	Curitiba, PR, Brazil, 80060-900	Sonia Mara Raboni, MD, PhD
POA01	Hospital São Lucas da Pontifícia Universidade Catolica do Rio Grande do Sul	Porto Alegre, RS, Brazil, 90619-900	Fabiano Ramos, MD, PhD
BHZ01	Universidade Federal de Minas Gerais	Belo Horizonte, MG, Brazil, 30750-140	Mauro Martins Teixeira, MD, PhD
BSB01	Universidade de Brasília	Brasilia, DF, Brazil, 71691-082	Gustavo Adolfo Sierra Romero, MD, PhD
SCS01	Universidade Municipal de São Caetano do Sul	São Caetano do Sul, SP, Brazil, 09521-160	Fábio Eudes Leal, MD, PhD
SAO06	Instituto Israelita de Ensino e Pesquisa Albert Einstein	Sao Paulo, SP, Brazil, 05652-900	Luis Fernando Aranha Camargo, MD, PhD
VCP01	Hospital das Clínicas da UNICAMP	Campinas, SP, Brazil, 13083-888	Francisco Hideo Aoki, MD, PhD
RAO01	Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo	Ribeirao Preto, SP, Brazil, 14015-069	Eduardo Barbosa Coelho, MD, PhD
SAO01	Centro de Pesquisas Clínicas do Instituto Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo	Sao Paulo, SP, Brazil, 05403-000	Esper Georges Kallás, MD,PhD
PET01	Universidade Federal de Pelotas, Faculdade de Medicina. Departamento de Clínica Médica	Pelotas, RS, Brasil, 96030-002	Danise Senna Oliveira, MD, PhD
SJP01	Faculdade de Medicina de São José do Rio Preto - FAMERP	São José Do Rio Preto, SP, Brazil, 15090-000	Maurício Lacerda Nogueira, MD, PhD
CWB01	Universidade Federal de Mato Grosso, Faculdade de Ciências Médicas, Hospital Univeristário Júlio Müller.	Cuiabá, MT – Brasil, 78048-902	Cor Jesus Fernandes Fontes, MD, PhD
BAT01	Hospital de Amor	Barretos, SP, Brazil 14780-000	Gecilmara Cristina Salviato Pileggi, MD, PhD
CGR01	Hospital Universitário Maria Aparecida Pedrossian, Universidade Federal de Mato Grosso do Sul	Campo Grande, MS, Brazil, 79080-190	Ana Lúcia Lyrio de Oliveira, MD, PhD

RIO01	Instituto de Infectologia Evandro Chagas - Fiocruz	Rio De Janeiro, Brazil, 21710-232	André Machado de Siqueira, MD, PhD

Appendix 3 Adverse Events

Table 3-1. Overview of adverse events in subjects after vaccination

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total AEs	29041	5096(82·2%)	25619	4670(75·4%)	54660	9766(78·8%)	<0·0001
AEs related to vaccine	21162	4782(77·1%)	17270	4111(66·4%)	38432	8893(71·7%)	<0·0001
Solicited AEs	14949	4536(73·1%)	11119	3714(60·0%)	26068	8250(66·6%)	<0·0001
Unsolicited AEs	6213	2284(36·8%)	6151	2215(35·8%)	12364	4499(36·3%)	0·2177
Systemic AEs	14164	3625(58·5%)	14056	3525(56·9%)	28220	7150(57·7%)	0·0842
Local AEs	6998	3854(62·1%)	3213	2188(35·3%)	10211	6042(48·7%)	<0·0001
AEs within 60 min	611	460(7·4%)	525	413(6·7%)	1136	873(7·0%)	0·1064
AEs within 0-7 days	16583	4613(74·4%)	12625	3823(61·7%)	29208	8436(68·1%)	<0·0001
AEs in 8-28 days	4046	1619(26·1%)	4132	1615(26·1%)	8178	3234(26·1%)	0·9837
Grade 1 Adverse Event	17693	4652(75·0%)	13889	3901(63·0%)	31582	8553(69·0%)	<0·0001
Grade 2 Adverse Event	3306	1648(26·6%)	3158	1546(25·0%)	6464	3194(25·8%)	0·042
Grade 3 Adverse Event	144	98(1·6%)	205	128(2·1%)	349	226(1·8%)	0·0441
AEs unrelated to vaccine	7813	2398(38·7%)	8295	2442(39·4%)	16108	4840(39·0%)	0·3869

Table 3-2. Adverse reactions reported within 28 days after whole-schedule vaccination

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total adverse reactions	21162	4782(77·1%)	17270	4111(66·4%)	38432	8893(71·7%)	<0·0001
Solicited adverse reactions	14949	4536(73·1%)	11119	3714(60·0%)	26068	8250(66·6%)	<0·0001
Local adverse reactions	6767	3815(61·5%)	3074	2143(34·6%)	9841	5958(48·1%)	<0·0001
Vaccination site pain	5508	3742(60·3%)	2555	2014(32·5%)	8063	5756(46·4%)	<0·0001
Swelling	434	359(5·8%)	147	130(2·1%)	581	489(3·9%)	<0·0001
Pruritus	306	263(4·2%)	207	181(2·9%)	513	444(3·6%)	<0·0001
Redness	264	241(3·9%)	93	89(1·4%)	357	330(2·7%)	<0·0001
Induration	255	235(3·8%)	72	67(1·1%)	327	302(2·4%)	<0·0001
Systemic adverse reactions	8182	2999(48·4%)	8045	2947(47·6%)	16227	5946(48·0%)	0·3882
Headache	3034	2128(34·3%)	3098	2157(34·8%)	6132	4285(34·6%)	0·5583
Fatigue	1209	989(16·0%)	1164	922(14·9%)	2373	1911(15·4%)	0·1059
Myalgia	879	727(11·7%)	771	648(10·5%)	1650	1375(11·1%)	0·0257
Nausea	573	490(7·9%)	629	522(8·4%)	1202	1012(8·2%)	0·2939
Diarrhea	576	492(7·9%)	576	501(8·1%)	1152	993(8·0%)	0·7659
Arthralgia	411	353(5·7%)	369	321(5·2%)	780	674(5·4%)	0·2195
Cough	392	343(5·5%)	369	322(5·2%)	761	665(5·4%)	0·4254

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Chills	359	309(5·0%)	350	313(5·1%)	709	622(5·0%)	0·8693
Pruritus	315	263(4·2%)	266	225(3·6%)	581	488(3·9%)	0·0874
Appetite impaired	241	217(3·5%)	268	243(3·9%)	509	460(3·7%)	0·2169
Vomiting	64	61(1·0%)	66	61(1·0%)	130	122(1·0%)	1·0000
Hypersensitivity	66	58(0·9%)	68	58(0·9%)	134	116(0·9%)	1·0000
Rash	53	49(0·8%)	47	42(0·7%)	100	91(0·7%)	0·5281
Fever	10	9(0·2%)	4	4(0·1%)	14	13(0·1%)	0·2666
Unsolicited adverse reactions	6213	2284(36·8%)	6151	2215(35·8%)	12364	4499(36·3%)	0·2177
Tremor	10	10(0·2%)	1	1(0·0%)	11	11(0·1%)	0·0117
Complex local pain syndrome	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Wheezing	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Vaccination site pain	133	124(2·0%)	70	65(1·1%)	203	189(1·5%)	<0·0001
Vaccination site redness	19	17(0·3%)	10	10(0·2%)	29	27(0·2%)	0·2473
Vaccination site swelling	16	15(0·2%)	6	6(0·1%)	22	21(0·2%)	0·0781
Oedema	14	14(0·2%)	6	6(0·1%)	20	20(0·2%)	0·1150
Vaccination site induration	18	17(0·3%)	3	3(0·1%)	21	20(0·2%)	0·0026
Vaccination site warmth	10	10(0·2%)	5	5(0·1%)	15	15(0·1%)	0·3015

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Oedema peripheral	4	4(0·1%)	1	1(0·0%)	5	5(0·0%)	0·3749
Intestinal angina	5	5(0·1%)	3	3(0·1%)	8	8(0·1%)	0·7265
Paraesthesia oral	6	6(0·1%)	1	1(0·0%)	7	7(0·1%)	0·1249
Gastritis	4	4(0·1%)	2	2(0·0%)	6	6(0·1%)	0·6874
Abdominal pain lower	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Gastroesophageal reflux disease	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Muscular weakness	5	5(0·1%)	3	3(0·1%)	8	8(0·1%)	0·7265
Joint swelling	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Ecchymosis	5	5(0·1%)	2	2(0·0%)	7	7(0·1%)	0·4530
Petechiae	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Alopecia	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Sinusitis	7	7(0·1%)	4	4(0·1%)	11	11(0·1%)	0·5486
Flushing	39	37(0·6%)	20	18(0·3%)	59	55(0·4%)	0·0142
Hyperaemia	13	13(0·2%)	10	8(0·1%)	23	21(0·2%)	0·3829
Hypoacusis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Photophobia	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Anxiety disorder	5	4(0·1%)	2	2(0·0%)	7	6(0·1%)	0·6874

Category	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Tachycardia	7	7(0·1%)	4	4(0·1%)	11	11(0·1%)	0·5486
Palpitations	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000

Table 3-3. Adverse reactions reported within 14 days after first dose vaccination

Category	Vaccine group (N=6196)		Placebo group (N=6200)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total adverse reactions	11658	4058(65·5%)	9964	3438(55·5%)	21622	7496(60·5%)	<0·0001
Local adverse reactions							
Vaccination site pain	2890	2750(44·4%)	1442	1387(22·4%)	4332	4137(33·4%)	<0·0001
Induration	90	88(1·4%)	35	34(0·6%)	125	122(1·0%)	<0·0001
Swelling	185	162(2·6%)	77	72(1·2%)	262	234(2·0%)	<0·0001
Redness	97	95(1·5%)	52	48(0·8%)	149	143(1·2%)	<0·0001
Pruritus	154	147(2·4%)	133	126(2·0%)	287	273(2·2%)	0·1993
Warmth	6	6(0·1%)	2	2(0·0%)	8	8(0·1%)	0·1794
Rash	5	4(0·1%)	2	2(0·0%)	7	6(0·1%)	0·4529
Systemic adverse reactions							
Fever	8	7(0·1%)	8	8(0·1%)	16	15(0·1%)	1·0000
Hypersensitivity	53	47(0·8%)	50	44(0·7%)	103	91(0·7%)	0·7537
Rash	42	36(0·6%)	32	30(0·5%)	74	66(0·5%)	0·4625
Diarrhea	502	451(7·3%)	512	454(7·3%)	1014	905(7·3%)	0·9450
Appetite impaired	208	188(3·0%)	231	213(3·4%)	439	401(3·2%)	0·2230

Category	Vaccine group (N=6196)		Placebo group (N=6200)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Vomiting	48	47(0·8%)	51	49(0·8%)	99	96(0·8%)	0·9185
Nausea	464	423(6·8%)	521	445(7·2%)	985	868(7·0%)	0·4599
Myalgia	686	604(9·8%)	631	545(8·8%)	1317	1149(9·3%)	0·0677
Headache	2615	1944(31·4%)	2726	1996(32·2%)	5341	3940(31·8%)	0·3348
Cough	380	337(5·4%)	364	318(5·1%)	744	655(5·3%)	0·4458
Fatigue	1016	860(13·9%)	943	798(12·9%)	1959	1658(13·4%)	0·1018
Arthralgia	331	293(4·7%)	308	276(4·5%)	639	569(4·6%)	0·4659
Chills	274	252(4·1%)	285	266(4·3%)	559	518(4·2%)	0·5596
Pruritus	243	213(3·4%)	226	194(3·1%)	469	407(3·3%)	0·3387
Oedema	8	8(0·1%)	3	3(0·1%)	11	11(0·1%)	0·1457
Chest pain	7	7(0·1%)	4	4(0·1%)	11	11(0·1%)	0·3873
Warm at the vaccination site	6	6(0·1%)	2	2(0·0%)	8	8(0·1%)	0·1794
Rash at the vaccination site	5	4(0·1%)	2	2(0·0%)	7	6(0·1%)	0·4529
Tremor	8	8(0·1%)	1	1(0·0%)	9	9(0·1%)	0·0214
Paraesthesia oral	5	5(0·1%)	1	1(0·0%)	6	6(0·1%)	0·1248
Lower abdominal pain	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248

Category	Vaccine group (N=6196)		Placebo group (N=6200)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Gastritis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248
Back pain	26	26(0·4%)	19	17(0·3%)	45	43(0·4%)	0·1733
Muscle spasms	4	4(0·1%)	2	2(0·0%)	6	6(0·1%)	0·4529
Muscular weakness	3	3(0·1%)	1	1(0·0%)	4	4(0·0%)	0·3748
Hyperhidrosis	12	12(0·2%)	7	7(0·1%)	19	19(0·2%)	0·2627
Ecchymosis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248
Alopecia	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248
Oral herpes	16	16(0·3%)	10	9(0·2%)	26	25(0·2%)	0·1681
Rhinitis	5	5(0·1%)	3	3(0·1%)	8	8(0·1%)	0·5075
Conjunctivitis	4	4(0·1%)	2	2(0·0%)	6	6(0·1%)	0·4529
Sinusitis	4	4(0·1%)	1	1(0·0%)	5	5(0·0%)	0·2185
Amygdalitis	2	2(0·0%)	2	1(0·0%)	4	3(0·0%)	0·6248
Flushing	18	18(0·3%)	13	12(0·2%)	31	30(0·2%)	0·2803
Palpitation	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	0·6248

Table 3-4. Adverse reactions reported within 28 days after second-dose vaccination

Category	Vaccine group (N=5453)		Placebo group (N=5481)		Total (N=10934)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total adverse reactions	9481	3294(60·1%)	7329	2418(44·3%)	16810	5712(52·2%)	<0·0001
Local adverse reactions							
Vaccination site pain	2746	2520(46·0%)	1188	1079(19·8%)	3934	3599(32·9%)	**<0·0001**
Induration	180	174(3·2%)	40	39(0·7%)	220	213(2·0%)	**<0·0001**
Swelling	265	235(4·3%)	76	70(1·3%)	341	305(2·8%)	**<0·0001**
Redness	186	174(3·2%)	51	51(0·9%)	237	225(2·1%)	**<0·0001**
Pruritus	174	154(2·9%)	109	89(1·6%)	283	243(2·2%)	**<0·0001**
Sclerosis at the vaccination site	2	2(0·0%)	0	0(0·0%)	2	2(0·0%)	0·5000
Epidermis exfoliation at the vaccination site	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Pustules at the vaccination site	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Systemic adverse reactions							
Fever	3	3(0·1%)	4	4(0·1%)	7	7(0·1%)	0·7258
Hypersensitivity	37	32(0·6%)	43	37(0·7%)	80	69(0·6%)	0·5482
Rash	25	25(0·5%)	25	23(0·4%)	50	48(0·4%)	0·8852
Diarrhea	335	300(5·5%)	340	296(5·4%)	675	596(5·5%)	0·9329

Category	Vaccine group (N=5453)		Placebo group (N=5481)		Total (N=10934)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Appetite impaired	126	110(2·0%)	143	131(2·4%)	269	241(2·2%)	0·1714
Vomiting	50	50(0·9%)	48	45(0·8%)	98	95(0·9%)	0·6805
Nausea	304	263(4·8%)	311	266(4·9%)	615	529(4·8%)	0·8586
Myalgia	526	439(8·0%)	478	403(7·4%)	1004	842(7·7%)	0·2365
Headache	1957	1354(24·7%)	1922	1317(24·2%)	3879	2671(24·4%)	0·5044
Cough	283	247(4·5%)	282	245(4·5%)	565	492(4·5%)	1·0000
Fatigue	593	496(9·1%)	636	538(9·9%)	1229	1034(9·5%)	0·1504
Arthralgia	229	187(3·4%)	202	178(3·3%)	431	365(3·3%)	0·6706
Chills	185	164(3·0%)	200	186(3·4%)	385	350(3·2%)	0·232
Pruritus	155	129(2·4%)	117	100(1·8%)	272	229(2·1%)	0·0615
Oedema	6	6(0·1%)	3	3(0·1%)	9	9(0·1%)	0·5076
Complex local pain syndrome	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Intestinal angina	3	3(0·1%)	1	1(0·0%)	4	4(0·0%)	0·6249
Gastritis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Pain in limb	29	25(0·5%)	18	15(0·3%)	47	40(0·4%)	0·1532
Neck pain	11	11(0·2%)	5	5(0·1%)	16	16(0·2%)	0·2098

Category	Vaccine group (N=5453)		Placebo group (N=5481)		Total (N=10934)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Dyspnea	19	18(0·3%)	10	10(0·2%)	29	28(0·3%)	0·1844
Rhinallergosis	8	8(0·2%)	5	5(0·1%)	13	13(0·1%)	0·5808
Erythema	36	35(0·6%)	25	23(0·4%)	61	58(0·5%)	0·1470
Ecchymosis	3	3(0·1%)	1	1(0·0%)	4	4(0·0%)	0·6249
Skin warm	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Pharyngitis	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Flushing	21	20(0·4%)	7	7(0·1%)	28	27(0·3%)	0·0190
Hyperaemia	6	6(0·1%)	5	4(0·1%)	11	10(0·1%)	0·7538
Eye irritation	4	4(0·1%)	3	2(0·0%)	7	6(0·1%)	0·6874
Anxiety disorder	5	4(0·1%)	1	1(0·0%)	6	5(0·1%)	0·3749
Tachycardia	5	5(0·1%)	2	2(0·0%)	7	7(0·1%)	0·4530

Table 3-5. Adverse events in subjects with concomitant diseases

Concomitant disease	Vaccine group (N=3447)		Placebo group (N=3478)		Total (N=6925)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Cardiovascular disease	2553	560/794(70·5%)	2083	480/771(62·3%)	4636	1040/1565(66·5%)	0·0006
Diabetes	802	150/219(68·5%)	554	123/196(62·8%)	1356	273/415(65·8%)	0·2543
Obesity	5147	1058/1388(76·2%)	4171	933/1401(66·6%)	9318	1991/2789(71·4%)	<0·0001
Chronic lung disease	7	4/5(80·0%)	2	1/4(25·0%)	9	5/9(55·6%)	0·2063
Malignant disease	85	19/27(70·4%)	87	18/25(72·0%)	172	37/52(71·2%)	1·0000

Table 3-6. Adverse reactions in subjects with concomitant diseases

Category	Vaccine group (N=3447)		Placebo group (N=3478)		Total (N=6925)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Total adverse reactions	12974	2701(78·4%)	10961	2413(69·4%)	23935	5114(73·9%)	<0·0001
Solicited adverse reactions	9046	2562(74·3%)	6962	2176(62·6%)	16008	4738(68·4%)	<0·0001
Local adverse reactions	3935	2134(61·9%)	1836	1235(35·5%)	5771	3369(48·7%)	<0·0001
Vaccination site pain	3143	2096(60·8%)	1512	1156(33·2%)	4655	3252(47·0%)	<0·0001
Swelling	277	225(6·5%)	96	84(2·4%)	373	309(4·5%)	<0·0001
Redness	156	141(4·1%)	55	52(1·5%)	211	193(2·8%)	<0·0001
Induration	162	147(4·3%)	43	38(1·1%)	205	185(2·7%)	<0·0001
Vaccination site pruritus	197	163(4·7%)	130	113(3·3%)	327	276(4·0%)	0·0017
Systemic adverse reactions	5111	1764(51·2%)	5126	1761(50·6%)	10237	3525(50·9%)	0·6653
Headache	1813	1241(36·0%)	1927	1297(37·3%)	3740	2538(36·7%)	0·2725
Fatigue	784	620(18·0%)	752	588(16·9%)	1536	1208(17·5%)	0·2414
Myalgia	552	448(13·0%)	502	417(12·0%)	1054	865(12·5%)	0·2165
Nausea	343	294(8·5%)	410	337(9·7%)	753	631(9·1%)	0·0950
Diarrhea	370	312(9·1%)	352	306(8·8%)	722	618(8·9%)	0·7360
Arthralgia	270	225(6·5%)	255	221(6·4%)	525	446(6·4%)	0·7693
Pruritus	210	167(4·8%)	174	145(4·2%)	384	312(4·5%)	0·1829

Category	Vaccine group (N=3447)		Placebo group (N=3478)		Total (N=6925)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Cough	263	226(6·6%)	236	202(5·8%)	499	428(6·2%)	0·2121
Chills	233	197(5·7%)	216	189(5·4%)	449	386(5·6%)	0·6374
Appetite impaired	150	132(3·8%)	171	154(4·4%)	321	286(4·1%)	0·2271
Rash	31	28(0·8%)	36	32(0·9%)	67	60(0·9%)	0·6978
Hypersensitivity	47	41(1·2%)	50	40(1·2%)	97	81(1·2%)	0·9113
Vomiting	40	38(1·1%)	44	39(1·1%)	84	77(1·1%)	1·0000
Fever	5	5(0·2%)	1	1(0·0%)	6	6(0·1%)	0·1232
Unsolicited adverse reactions	3928	1396(40·5%)	3999	1364(39·2%)	7927	2760(39·9%)	0·2802

Appendix 4 Serious Adverse Events

Table 4. Serious Adverse Events by System Organ Class/Preferred Term

SAE	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Overall SAE	34	33(0·5%)	33	31(0·5%)	67	64(0·5%)	0·9004
Infection and infestations	13	13(0·2%)	14	13(0·2%)	27	26(0·2%)	1·0000
COVID-19	2	2(0·0%)	9	9(0·2%)	11	11(0·1%)	0·0384
Appendicitis	5	5(0·1%)	1	1(0·0%)	6	6(0·1%)	0·2186
Pyelonephritis	2	2(0·0%)	2	2(0·0%)	4	4(0·0%)	1·0000
Severe acute respiratory syndrome (SARS)	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Vestibular neuronitis	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Urinary tract infection	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Diverticulitis	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Pelvic inflammatory disease	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Nasal abscess	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Injury, poisoning and procedural complications	4	4(0·1%)	5	5(0·1%)	9	9(0·1%)	0·7537
Road traffic accident	1	1(0·0%)	2	2(0·0%)	3	3(0·0%)	0·6247
Limb injury	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000

SAE	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Foot fracture	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Fall	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Ankle fracture	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Fracture	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Sacroiliac fracture	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Psychiatric disorders	3	3(0·1%)	2	2(0·0%)	5	5(0·0%)	1·0000
Suicidal ideation	2	2(0·0%)	0	0(0·0%)	2	2(0·0%)	0·5000
Bipolar disorder	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Suicide attempt	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Alcohol abuse	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Pregnancy, puerperium and perinatal conditions	1	1(0·0%)	3	3(0·1%)	4	4(0·0%)	0·3746
Abortion	1	1(0·0%)	2	2(0·0%)	3	3(0·0%)	0·6247
Foetal death	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
General disorders and administration site conditions	3	3(0·1%)	0	0(0·0%)	3	3(0·0%)	0·2499
Systemic inflammatory response syndrome	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Death	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000

SAE	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Chest pain	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Musculoskeletal and connective tissue disorders	2	2(0·0%)	1	1(0·0%)	3	3(0·0%)	1·0000
Arthralgia	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Intervertebral disc disorder	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Intervertebral disc protrusion	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Respiratory, thoracic and mediastinal disorders	3	3(0·1%)	0	0(0·0%)	3	3(0·0%)	0·2499
Dyspnea	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Asthma	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Acute pulmonary oedema	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Nervous system disorders	1	1(0·0%)	1	1(0·0%)	2	2(0·0%)	1·0000
Syncope	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Transient ischaemic attack	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Renal and urinary disorders	0	0(0·0%)	2	2(0·0%)	2	2(0·0%)	0·2497
Nephrolithiasis	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Obstructive nephropathy	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Gastrointestinal disorders	1	1(0·0%)	1	1(0·0%)	2	2(0·0%)	1·0000

SAE	Vaccine group (N=6202)		Placebo group (N=6194)		Total (N=12396)		*P* value
	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	No. of events	No. of subjects (%)	
Abdominal pain	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Haemorrhoids thrombosed	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Vascular disorders	2	2(0·0%)	0	0(0·0%)	2	2(0·0%)	0·5000
Deep vein thrombosis	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Hypertension	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Metabolism and nutrition disorders	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Hypokalaemia	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Cardiac disorders	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Cardio-respiratory arrest	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Reproductive system and breast disorders	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Endometriosis	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Skin and subcutaneous tissue disorders	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Rash	1	1(0·0%)	0	0(0·0%)	1	1(0·0%)	1·0000
Hepatobiliary disorders	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997
Cholelithiasis	0	0(0·0%)	1	1(0·0%)	1	1(0·0%)	0·4997

Appendix 5 Efficacy Analysis

Table 5-1. Efficacy analysis by case definitions

Case definition	Total No. of cases	Vaccine	Placebo	Vaccine Efficacy (95%CI)
		n/N(incidence density)	n/N(incidence density per 100 person-year)	
Case definition 1	253	85/4953(11·0)	168/4870(22·3)	50·7 (35·9, 62·0)
Case definition 2	261	87/4953(11·1)	174/4870(22·8)	51·2(36·9, 62·3)
Case definition 3	250	80/4953(10·4)	170/4870(22·7)	54·1 (40·1, 64·8)
Case definition 4	243	79/4953(10·5)	164/4870(22·2)	53·0(38·6, 64·1)

Table 5-2. Efficacy analysis by follow-up time after first-dose vaccination

Follow-up time (after first-dose vaccination)	Total No. of cases	Vaccine	Placebo	Vaccine Efficacy (95%CI)
		n/N(incidence density)	n/N(incidence density per 100 person-year)	
Within 14 days	63	32/6195(11·4)	31/6201(11·0)	-3·3(-4·8, -1·9)
Within 28 days	104	38/6195(5·7)	66/6201(9·8)	42·5(32·9,50·7)
Within 42 days	158	48/6195(8·1)	110/6201(18·5)	56·5(49·6,62·5)
Within 56 days	221	63/6195(7·6)	158/6201(19·1)	60·4(56·5,63·9)
Within 70 days	274	86/6195(8·0)	188/6201(17·7)	54·7(53·2,56·1)
Within 84 days	326	104/6195(8·2)	222/6201(17·7)	53·7(52·7,54·7)
Within 98 days	357	116/6195(8·4)	241/6201(17·6)	52·5(51·9,53·1)
14-28 days after 1 dose*	18	1/5709 (1·3)	17/5697 (21·6)	94·0 (55·1, 99·2)

*For participants who received only single dose vaccination.

Table 5-3. Efficacy analysis by exposure history to SARS-CoV-2

Exposure to SARS-Cov-2 pre-vaccination	Total No. of cases	Vaccine	Placebo	Vaccine Efficacy (95%CI)
		n/N(incidence density)	n/N(incidence density per 100 person-year)	
Unexposed				
Score 2 and above	200	67/3637(13·3)	133/3587(26·8)	50·5(33·6, 63·1)
Score 3 and above	27	2/3637(0·4)	25/3587(4·5)	92·1(66·7, 98·1)
Score 4 and above	10	0/3637(0·0)	10/3587(1·8)	100·0(56·0, 100·0)
Severe	6	0/3637(0·0)	6/3587(1·1)	100·0(16·3, 100·0)
Exposed				
Score 2 and above	9	3/401(5·9)	6/408(11·7)	49·5(-101·8, 87·4)
Score 3 and above	0	0/401(0·0)	0/408(0·0)	NE
Score 4 and above	0	0/401(0·0)	0/408(0·0)	NE
Severe	0	0/401(0·0)	0/408(0·0)	NE

Appendix 6 PROFISCOV Study Group

Instituto Butantan, São Paulo, SP, Brazil

Clinical Trials and Pharmacovigilance Centre

Ricardo Palacios, Mônica Tilli Reis Pessoa Conde, Roberta de Oliveira Piorelli, Elizabeth González Patiño, Hugo Alberto Brango García, Joane do Prado Santos, Rodrigo Piske Finotto, Ana Paula Batista, Camila Santos Nascimento Albuquerque, Flávia Marilia Cestari Magalhães, Carolina de Moura Albino, Rafaela Fernandes Silva, Paloma Bomfim, Luiz Henrique Moraes Caetano de Camargo, Mirian Nascimento

Quality Control Laboratory

Patrícia Dos Santos Carneiro Matheus Trovão de Queiroz, Rubia Galvão Claudio

Development and Innovation Center

Viviane Fongaro Botosso, Soraia Attie Calil Jorge, Fabyano Bruno Leal, Renato Mancini Astray

Scientific Development Center

Sandra Coccuzzo Sampaio Vessoni, Mauricio Cesar Ando, Guilherme Rabelo Coelho, Monique da Rocha Queiroz Lima

University of São Paulo

School of Medicine. São Paulo, SP, Brazil

Clinical Research Center II and Research Medical Laboratory – LIM 60, Department of Infectious and Parasitic Diseases, Clinicas Hospital

Esper Kallás , Amanda Caroline Ribeiro Sales, Amanda Nazareth Lara, Angela Carvalho Freitas, Angela Naomi Atomiya, Bárbara Labella Henriques, Camila Rodrigues, Camila Sunaitis Donini, Danielle Rodrigues Alves, Elizabeth de Faria, Fábio De Rose Ghilardi, Joana Ramos Deheinzelin, Jorge Salomão Moreira, Juliana Ishimine da Silva, Karine Armond Bittencourt de Castro, Leon Capovilla, Livia Zignago Moreira dos Santos, Luara Teófilo Pignati, Luiz Gonzaga Francisco de Assis Barros D'Elia Zanella, Mariana Maria Rocha Santos de Souza, Marília Bordignon Antonio, Marjorie Marini Rapozo, Michel Silvio Duailibi, Natacha regina de Moraes Cerchiari, Patricia Rocha de Figueiredo, Pedro Henrique Fonseca Moreira de Figueiredo, Raphaella Goulart de Souza Vieira, Renata Pissuto Pinheiro, Ricardo de Paula Vasconcelos, Rosário Quiroga Ferrufino, Simone de Barros Tenore, Tatiana Fiscina de Santana, Zelinda Bartolomei Nakagawa, Elaine Cristina Bau, Lilian Ferrari, Denivalda da Silva Gomes Araújo, Gabriela de Castro Keller, Ketlin Kauane Cordeiro Santos, Rosângela Vitória Soares Silva, Beatriz Sales Mourão, Taynan Ferreira Rocha, Carlota Miranda Paredes, Carolina Cardona Siqueira Lobo, Taís Vargas Freire Martins Lúcio, Athos Nascimento Souza, Elenn Soares Ferreira, Gabriel Lopes Borba, Neivaldo Fiorin, Thiago Evaristo Tavares Luzzi, Denise Sales Mourão, Ederson Santo Xavier, Nailson de Jesus Ramos das Virgens, Thiago Antonio do Nascimento, Bruna Samanta da Silva Moreira, Karine Milani da Silva Dias, Leandro Concolato Miranda, Mary Helen Oliveira Morais, Priscilla Almeida Souza, Rayana Silva Paes, Geovanna Guarnier Cardin Farias, Gustavo Coutinho Rezende, Rosimeire Aparecida da Silva Zabotto, Verônica dos Anjos Souza da Silva, Ana Paula da Silva Barros, Clemildes Vieira de Almeida, Gislayne Aparecida de Lima Marcelino, Jéssica Aparecida Soares, Josélia Bezerra dos Santos, Márcia Alves de São Pedro, Maria Esmelindra Monteiro de Moraes, Helena Tomoko Iwashita Tomiyama, Alberto Hiroyuki Tomiyama, Aline Tatiane Lumertz dos Anjos, Andrea Niquirilo, Claudia Satiko Tomiyama, Elisabeth Alves Pereira, Eric Silvestre, Maria Angelica Alcalá Neves, Raissa Reis Silva, Yasmine Perez Levy Ribeiro, Maria Cândida de Souza Dantas, Issler Moraes da Silva,Renan Fernandes Carvalho

Institute of Tropical Medicine (IMT-SP)

Ester Cerdeira Sabino, Maria Cássia Mendes Correa, Anderson de Paula, Tania Regina Tozetto Mendoza, Mariana Severo, Jaqueline Goes de Jesus, Flávia Sales, Erika Manuli, Darlan da Silva Cândido, Ingra Morales

Laboratory of Clinical and Molecular Virology, Department of Microbiology, Institute of Biomedical Science. São Paulo, SP, Brazil

Edison Luiz Durigon, Danielle Bruna Leal de Oliveira, Erika Donizette Candido, Guilherme Pereira Scagion

Department of Internal Medicine, Ribeirão Preto Medical School, Ribeirão Preto, SP, Brazil

Eduardo Barbosa Coelho, Silvia Caroline Santana Moura Carvalho, Bárbara Cristina Santana Mello, Fábio André Dias, Marina Silva Campos, Juliana Rezende, Marilda Aparecida Ribas, Soraya Regina Abu Jamra, Daniela Aparecida Lorencini, Simone Aparecida Mattos Garcia, Onildo Passafaro, Ariane Teixeira Vicente, Diogo Henrique Martins, Ana Carolina Conchon Costa, Fabiana Alves Rodrigues Druzili, Carolina Leite Freitas, Luciana Mara Cangemi, Aila Mabla Azarias de Castro, Diego Lorencini, Leila Cristina Franco dos Santos, Janaina de Andrade Pereira, Taiz Francine Brasil da Silva, Ramaiane Aparecida Pugnolli, Ana Rayza Palaretti, Ana Claudia Aparecida Rodrigues, Camila Pereira, Jessica Merighe Godoi, José Fernando Aguiar, Nathalia Ziegler Oliveira, Ana Carolina Andrade Emerenciano, Barbara Marques Coutinho, Andrea Moreira de Freitas, Fernanda Munari Meneguim, Bianca Rizzotto Ferreira, Luan Lucas Reis da Costa, Gabriel Bazo, Fabio da Veiga Ued, Tatiane de Paula Rosa, Daiana Cristina Lorencini Aguiar, Gabriela Gimenes Faustino Ilana, Amanda Hereman Malavasi, Mayara Gomes Brito, Lucas Braz Barbosa Coelho

Emilio Ribas Institute of Infectious Diseases, São Paulo, SP, Brazil

Luiz Carlos Pereira Junior, Ana Paula Rocha Veiga, Guilherme Assis dos Anjos, Alexandre de Almeida, Najara Ataide de Lima Nascimento, Diogenes Coelho Junior, Gabriela Prandi Caetano, Ana Carla Carvalho de Mello e Silva, Rafael Affini Martins, Natália Mercedes Cabral Amdi, Cinthya Mayumi Ozawa, Magna Magalhaes Siva, Anna Karina Queiroz Mostachio, Katia Sayama Tsutui, Ana Paula Augusto dos Santos, Alessandra Moreti dos Santos, Marcia Aparecida dos Santos Gouveia, Lilian Mathias Moreira, Léia Dias Barbosa, Margarete Rodrigues de Carvalho, Mirian Ishi, Mariana Takahashi Ferreira Costa, Nelson Alberto Freitas Guanez, Odijoselia Ferreira de Sá, Virginia Barbosa Leite, Luciana Elizabete Cunha, Ivan Máximo da Silva, Vinicius Silva Araújo, Marian Romero Soares Rodrigues, Adriano Samoel Batista de Souza Nascimento, Marco Aurélio Conceição, Catia Dionisio dos Santos, Luciana Aparecida Pereira, Alessandra de Fatima Margarida, Debora Carla dos Santos, Andréia Aparecida Hermann, Ana Kesia de Souza Lima, Nailda Dantas Nunes Leal, Sergio Luiz de Lara Campos, Mariane Pereira, Caroline Franco Zanotti, Paloma Martins Vieira, Paloma Priscila Rocha Aronca, Denis Jose Fumagali, Alpetras Martins Maciel, Thais Monteiro Nunes Pereira, Milton Tadeu da Silva, Bruno Tenório de Oliveira, Maria Elena Ana Correa da Silva, Margarete Leme, Mercia Rocha Moreira,

Silvia Garcia, Vlaudeflide dos Santos, Maria Elizabete Mendes Alves dos Santos, Bianca Carolina Ferreira, Sonia Silva Ferreira, Leticia Kadiri da Silva, Neide Aparecida Leite dos Santos, Fernanda Lima Arruda, Vilma Aparecida Adami, Claudia Solange da Silva

Faculdade de Medicina de São José do Rio Preto (FAMERP), São José de Rio Preto, SP, Brazil

Mauricio Lacerda Nogueira, Cassia Fernanda Estofolete, Karen Sanmartin Rogovsky, Samuel Noah Scamardi, Camilo Alberto Correa de Vasconcellos Dias, Altaís Helena Camargos Robles, Ingrid Emily Alencar Bento, Bruna Basaglia, Elis Regina da Silva Ferreira, Danatielle Mega Ferreira, Eliane Aparecida Fávaro Pereira, Ana Maria Dias de Sousa Marconi, Talita Garcia Lopes Viçoso, Bethania Cirqueira de Oliveira, Hellen Carla Padovani, Theo Rodrigues da Silva, Elissandra Josepha Eugenia Machado Batista, Francielli Regini Carvalho de Faria, Carla Gabriela de Lima Mattos, Kamilla Amaral David Rocha, Rafael Alves da Silva, Bárbara Boreli Almeida, Marini Lino Brancini, Solange de Fátima Vargas Scaranaro, Marilia Mazzi Moraes, Lorena Fernanda da Silva, Debora Rodrigues de Brito, Victor Miranda Hernandes, Sonia Aparecida Miranda, Debora Rodrigues de Brito, Andresa Lopes dos Santos, Henrique Munhoz Moya Gimenes, Silvio Orlando Isso, Perpetua Pereira Dias Fernandes, Eduarda Veronezi Claro, Raphaely Rodrigues Obara, Erica Seixas Floriano, Gislaine Celestino Dutra da Silva

School of Medical Science and Hospital of Clinics, State University of Campinas – UNICAMP, Campinas, SP, Brazil

Francisco Hideo Aoki, Mariangela Resende, Adriane Maira Delicio Abati, Paulo Afonso Martins Abati, Diego Cassola Pronunciato, Alisson Aliel Vigano Pugliesi, Letícia Pisoni Zanaga, Eduardo Hiroshi Tikazawa, Marcelo Gustavo Lopes, Giovana Cury Queiroz, Anna Kim, Maria Helena Pavan, Alex Bento de Carvalho, Ehideé Isabel Gómez La-Rotta, Fernanda Sucasas Frison, Edite Kazue Taninaga, Inajara de Cassia Guerreiro, Leila Tassia Pagamicce, Cleusa Gimenes dos Santos, Sofia Domingues Barthman, Maria Silvia Kroll, Elisangela Aparecida Ramos Domingos, Rosilene Balbina de Souza, Liene Gomes Magossi, Hamilton Bertan, Lara Paro Dias, Mayra Carvalho Ribeiro, Mariane Galvão Roberto Tavares, Mariana Vieira Morau, Rafael Nishimoto, João Kleber Novais Pereira, Regina Helena Dahas de Carvalho, Kely Carolyne Alves de Sousa, Nanci Michele Saita, Maria Valéria de Omena AThyde, Cristina Medeiros da Silva Aguilar, Valéria Galdino, Robson Pereira da Silva, Luisa Lazarini Rubio, Elisandra Valéria Negrison Calixto, Manoel Vicente Carminitti Feiteiro, Vagner Oliveira Duarte, Ronald Jorge Menghini dos Santos, Rosangela Aparecida dos Santos, Eliana Ferreira Paes, Ingrid Rocha Franco de Lima, Edilene Menezes Sabino, Marcos Roberto Guimarães, Karina Dias Teixeira Vieira, Diogo Rodrigues Machado, Maxlei Silveira Marini, Graciela Amaral, Fabio Augusto

Cavaglieri Feiteiro, Elaine Cristina Paixão de Oliveira, Talita Lobato de Souza, Vanessa Alcântara de Carvalho, Edjane de Oliveira Marinho Feiteiro, Victor Rodrigues Olivato, Letícia Pinheiro de Mattos, Francisca Pontes Santinoni, Jessica do Nascimento, Mariana de Campos, Natalie de Oliveira Alves

Universidade Municipal de São Caetano do Sul, São Caetano do Sul, SP, Brazil

Fabio Eudes Leal, Aline Lopes de Almeida, Maria Laura Mariano de Matos, Letícia Cleto Duarte Sugiyama, Gabriela Mora Santos, Cristiane Alves da Silva Ferraz, Caroline Machado Nunes, Leticia Reis Lopes, Amarilys Luiza Druziani, Marine Antunes Pires, Gabriela Júlio Fernandes Viana, Fernanda de Almeida Euclides, Sônia Yoo Im, Suzane Pereira da Silva, Claudia Cristina Ferreira Ramos, Carolina de Oliveira Silva, Giovana Ferreira Pinheiro, Isabelle Moiano Roseira Galo, Carina Vitória Paradas Dias, Livia Mirella Mengar, Nicole Gallone Rizzo, Renata Nunes Achar Fuji, Andrea de Barros Coscelli Ferraz, Michelle Cirilo, Andreia Inacio Avelino, Vanessa Vieira Hornink, Cristina Antonia de Jesus Catalã, Selma Arnold da Silva, Erika Regina Manuli, Milena Borges, Eric Boragan Gugliano, Milena Pereira Canavesi Caetano, Yara Chinaglia, Fernando Monteiro de Sá Luiz, Raquel da Silva Terezam, Giselia Sabino Cruz, Luma Ramirez de Carvalho, Ranilson José da Silva, Edvaldo Alves, Giovana Lourenço Munhoz, Luiza Caroline Rinaldi, Carla Cristina Munhoz, Maria José Barzan, Stella Maris Pereira Sobrinho Rodrigues, Bárbara Suéllen Guimarães Marin Ferreira, Tainaira da Silva Gramalio, Carla Andrea Ciarineli, Bruna Fornazieri Piotto, Kimberley Yaunde da Silva Matos

Instituto Israelita de Ensino e Pesquisa Albert Einstein, São Paulo, SP, Brazil

Luis Fernando Aranha Camargo, Carolina Devite Bittante, Telma Priscila Lovizio Raduan, Mariana Silva Soares, Maria Clara Pimentel Lopes, João roberto Resende Fernandes, Kamilla Ferreira de Moraes, Ariane Teixeira Vicente, Ericka Constantinov Oliveira, Aleksandra Cristina Mussi, Cristiane Okada, Bruna Camargo Gonçalves, Roberta Carolina Haddad, Caroline dos Reis Pedroso, Mariza Kogake Nocamatsu, Elke Ferreira Salim, Tarsila Gomes Feijão de Oliveira, Elaine de Jesus Santos, Bruna Camargo Gonçalves, Daniela Regina Gusmão Ferreira, Luciane dos Santos Vieira Alves, Hannah Maureen Garcia Mota, Mariana Gabriela Rodrigues, Gisele Alves Pinheiro, Luiza Blumer Ribeiro, Isabel Cristina dos Santos, Regiane Mendes Brandão, Ariana Silva de Lima Teixeira, José Carlos da Silva, Rosana Soares Gomes, Fernanda Oliveira Marcelino da Silva, Giselia Martins Dantas Silva, Aparecida Sampaio Sousa, July do Nascimento Alves, Thais Helena Costa Petrin, Daniela

Boschetti, Amanda Tarratacada Guimarães, Danilo Souza Vanglerini, Juliana Alves de Lima, Letícia de Mello Soares, Julia Alves de Lima, Adriana Guilherme, Lorena Silva Brasil, Felipe Rodrigues Nogueira Silva, Diana Freire de Brito, Rebeca Cezário Freire de Araújo, Sandra Rodrigues Rocha, Lucas Soares Cardoso dos Santos, Raquel Fidelis Teixeira Bonfim da Silva, Jeane Silva Menezes, Talita Santos Martins Vaglerini, Danielle Santana de Oliveira

Research Institute of Cancer Hospital, Hospital de Amor, Barretos, SP, Brazil

Gecilmara Cristina Salviato Pileggi, Paulo Tarso Oliveira, Raquel Aparecida Reis Teixeira, Luis Guilherme de Freitas Rodrigues, Guilherme Corraleiro Martins, Maria Luisa Corcoll Spina, Laila Genoefa Bortot, Renan Cesar Zanon Teixeira, Isabela Silva Pasqua, Janaina Zambon de Oliveira, Juliana Doblas Massaro, Julia Anelli Roncador, Karla de Picoli Alexandre, Daniel Aquilino Oliveira, Tais Vaz, Izabella da Silva Oliveira, Nathalia Martines Tunissiolli, Silvania Rodrigues dos Santos, Cristiane Lima do Carmo, Maicon Fernando Zanon da Silva, Felipe Alexandre Machado, João José Mandu Confetti, Fernanda Amâncio da Silva Giroli, Isabela Haddad Peron, Carla Roberta M. B. de Sousa, Joviany Talita da Silva, Camila Rodrigues Marques Fornazier, Stefani Regina da Costa Lopes, Aline Larissa Virginio da Silva, Beatriz da Silva Ventura, Layane Cristina dos Santos Vaz, Thiago Jose Donizete Simão, Thais Kapp Gonçalves

Centro de Pesquisa e Desenvolvimento de Fármacos, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Mauro Martins Teixeira, Astaruth Guimarães Froede, Alexandre Moraes Periard, Tallyne Tayna Barros Lana, Marina Neves Zerbini de Faria, Último Libânio da Costa, Carolina Morais Milan de Oliveira, Gabriela Carvalho Abreu, Erickson Ferreira Gontijo, Lisia Maria Esper, Angela Vieira Serufo, Fátima Maria Caldeira Brant Costa, Josiane Pinto Moreira Vaz, Rosângela Santos Pereira, Vitor Magno Cardoso Rocha, Rafael Leite Ribeiro dos Santos, Tarciana Batista Teixeira, Marlene do Rozario Silva, Miriam Siqueira Araújo, Luana Patrícia Gonçalves Silva, Josiane Lino dos Santos Frattari, Tatiana Dias Calábria Santos, Thais de Menezes Noronha, Alessandro Ferreira de Macedo, Ana Rosa Rodrigues Silva, Daniela Cristina de Oliveira Pontes, Jucelina Gonçalves de Oliveira, Lorena Augusta Coelho de Mattos, Priscila Sena Lopes, Maria Cecília da Silva Ferreira, Tânia Mara Gomes de Pinho, Amanda Lino dos Santos, Diego Wagner Tarquino, Luciana Mara Costa Moreira, Bruno Vinicius Santos Valiate, Raquel Patrocinio Simoes, Letícia Soldati da Silva, Déborah Caroline Salgado Stoupa, Phillip Diego Carajá, Ilma Marçal de

Souza, Patricia Candida Messias Santana, Lorrayne Madaline Costa Tarquinio, Renata de Jesus Oliveira, Eva Eunice de Fátima Nascimento, Girlane Santos de Oliveira, Amanda Mara Gomes de Pinho, Gislene Moreira

Department of Infectious Diseases, Hospital de Clínicas, Universidade Federal do Paraná, Curitiba, PR, Brazil

Sonia Mara Raboni, Cristiane Secco Rosário, Débora Carla Chong e Silva, Giovanni Luis Breda, Andrea Maciel de Oliveira Rossoni, Maria Cristina Vilatore Assef, Susane Edinger Pereira, Tony Tannous Tahan, Betina Mendez Alcântara Gabardo, Carlos Antônio Riedi, Cleverson Alex Leitão, Débora Silva Carmo, Fabricio Salles Rosa Solak, Juliana Mayumi Kamimura Murata, Leniza Costa Lima Lichtvan, Tyane de Almeida Pinto Jardim, Tiago Hessel Tormen, Elessandra Souza Bitencourt, Maria Gabriela Mendes Pereira da Costa, Aline de Fátima Bonetti, Joelize Claudiane Stanoga Denk, Gisele Weissheimer, Luciana Michele Mello, Natália Fracaro Lombardi Asinelli, Ana Beatriz Guedes Ribeiro, Antônio Eduardo Matoso Mendes, Juliane Carlotto, Mayara Caroline Barbieri, Simone Gomes de Souza, Tânia Maria Araújo, Vivian Carnier Jorge, Inajara Rotta, Gisele de Paula e Silva Carneiro Mendes de Souza, Indianara Rotta, Irene de Morais, Klezia Morais da Silva Belletti, Juracy Anisio da Silva Neta, Valquiria Daniele Casanova Antunes, Sabrina Karim Mota Ribeiro, Meuryely Euleny Macedo da Silva, Alice dos Santos Almeida, Patricia Aline dos Santos Pançolin, Ester Silvino da Costa Paris, Larissa Izabelle Machado, Genilberta de Meireles Biscarde, Jean Pierre Guinapo Franco Marques, Jennifer de Souza Anhaia, Maria Aparecida Pereira da Silva, Rosangela Aparecida de Almeida de Fitz, Simone Albertoni Souza, Simone Pereira Leal, Tiago Rodrigues de Souza, Vilmara Aparecida Salles Machado, Patricia Proença Santana, Atila Brizola Ribas, Bárbara Kathleen Gonçalves Poletto, Alexandre Paim Mendes, Daniel Barros Teixeira Leite, Eduarda Sampaio Lazzarotto, Erika Leticia da Rocha, Ione da Silva Santos Mocelin, Jaqueline Fiamoncini, Guilherme Paim Mendes, Larissa de Souza Ferreira, Maria Regina de Mattos, Liriane Aparecida Pedroso Vaz, Mariana Likoski Pereira, Nildson Eduardo Pinheiro Souto Junior, Lucienne Dronneau, Rosangela Salete Ribeiro dos Santos, Rosangela de Fátima Prestes, Vinicius Lemos Brito, Vilmara Heimoski Teixeira, Thays Fischer, Laura Holtman Ferreira, Suzana Beatriz Borsato Crissi, Andressa Zabudowski Schroeder, Leonardo Filipetto Ferrari, Guilherme Stapasolla Vargas Garcia, Barbara Kawano Raposo, Gustavo Osmarin Tosti, José Marcio Camargo Junior, Meire de Souza Vieira, Letícia Mari Tashima, Carolina Ayumi Ichi, Micaela Hedeke Paim Mendes, Isadora Heimoski Escobar, Alberto Memari Pavanelli, Daniel Balaban, Fernanda Panacioni, Isabella Chapieski, Luana Francine Anad, Eduardo Mendonça Soares, Luiza Moschetta Zimmermann, Isabela Cristine Torres, Jessica Castro da Silva

Infectious Diseases Service, Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Fabiano Ramos, Clarissa Tabajara Moura, Ingridy Cortes da Silveira, Cindy Johan Jara Hernandez, Hilda Janneth Monroy Hernandez, Jae En Chung, Daniel Fernando Arias Betancur, Luciana Zani Viegas da Silva, Ana Carolina Müller Beheregaray, Gabriel Bottin Marques, Débora Wilke Franco, Amabile Ribeiro de Oliveira, João Pedro Volkmann Amaral, Michelle Stump Viegas, Gabriel Azambuja Athaydes, Deise do Nascimento de Freitas, Andressa de Oliveira, Leydy Yhoana Quevedo Diaz, Leonardo Ferraz de Bittencourt, Hernando Salles Rosa, Laura Trevisan, Isabelli Guasso, Daiane Monteiro Marques, Kelli Regina Brand dos Santos, Alice de Lima Alves, Caroline Cavalheiro Biazussi, Thays Maiato Pereira, Aline Nunes Leal, Roseli Andreia Borges da Costa, Allan Pereira, Rafaella Ruiz do Couto, Luiz Airton Rocha de Freitas, Bruna Isabel Silveira Costa, Larissa Rafaela de Lima Sanches, Fernanda Paula Polaczinski Pich, Mariana Horn Scherer, Thamiris Carvalho Vargas, Mariana Horn Scherer, Rodrigo Volf dos Santos, Rodrigo Flores Fraga, Tasiana Aylen Cervellera Simonetti, Otávio Freitas de Moraes Ogrizek,Michelle dos Santos Leite, Janaina Moraes de Araujo, Carine Padilha de Oliveira, Karla Durante, Renata de Araujo Montechiaro, Elisandra Simonetti Cervellera, Bianca Luisa Rodrigues Lopes, Krislayne da Silva Rosa

Internal Medicine Department. School of Medicine, Universidade Federal de Pelotas. Pelotas, RS, Brazil

Danise Senna Oliveira, Adrienne Sassi de Oliveira, Bruna Gazoni de Souza, Carolina Avila Vianna, Daniel Brito de Araujo, Paulo Orlando Alves Monteiro, Susane Müller Klug Passos, Luciane Maria Alves Monteiro, Clara Camacho dos Reis, Eduardo Coelho Machado, Carla Vandame da Silva, Cristofer Magro, Lorena Zaine Matos Martinho, Caren Laís Seehaber Friedrich dos Santos, Giulya da Silva Ribeiro, Rodrigo de Almeida Vaucher, Bianca Machado de Ávila, Luana Bonow Wachholz, Bruna Quintana Nizoli, Fernanda Severo Sabedra Sousa, Maura Geovana Farias Soares, Aline Machado Carvalho, Daniela Nunes Schaun de Siqueira, Leandro Rodrigues de Rodrigues, Fabiana Kommling Seixas, Thaís Larré Oliveira, Bruna Silveira Pacheco, Tiago Vieiras Collares, William Borges Domingues, Adriane Geppert, Samuel Silva dos Santos, Marcia Bandeira da Luz Baladão, Charles Ramalho Ioost, Gilda Nara Oliveira Barros da Silva Marcos Dinael Klug Stifft, Tatiane Jacobsen Rodrigues, Tatiana Vieira Macedo, Camila Bonemann Bender, Mariana Gállio Fronza, Marcelo de Lima, Vanessa Gali, Isadora André Rosa Lopes, Paola Oteiro de Faria, Mariana de Oliveira Martins, Marcela Bihalva da Silva, Rita Oliveira dos Santos

Center for Tropical Medicine, School of Medicine, University of Brasilia, Brasília, DF, Brazil

Gustavo Adolfo Sierra Romero, Edison Tostes Farias, Alexandre Anderson de Sousa Munhoz Soares, Veronica Cristina de Melo Rocha, Renata Maria de Castro Martins, Rafael Gobbato Brandão Cavalcanti, Camila Rodrigues Ribeiro, Amanda Moreira Parente, Larissa Fernanda Santos Silva, Eduarda Jacinto Bauer, Isabela Roberta Chaves Nunes, Marcia Andrea Seibert Campara, Elizabete Cristina Iseke Bispo, Gyselle Alanna Mota, Larissa Santos Soares, Viviane da Silva Machado, Barbara Manuella Cardoso Sodré Alves, Clarisse de Mendonça Brito, Matheus Eça de Oliveira Felipe, Jacqueline Pereira Oliveira, Patricia Matias Pinheiro, Torlane Renne Dias Rodrigues, Naiara Daris dos Santos, Luciana Franca de Meneses, Fernando de Oliveira Garcia, Cleuza Feliciana de Oliveira, Renata Santos Souza de Amorim, Claudio Lopes Valentim, Lidiane da Silva Queiroz, Sandra Ribeiro Barbosa, Joyce Martins Xavier, Talita Barreto Figueredo, Fabiana Canavieira Araújo, Kaline de Oliveira Medeiros, Leidiane Aparecida Torres da Silva, Suzana Cardoso Mesquita, Patricia Pereira Pires de Sousa, Fernanda dos Santos Lopes, Alceu Sluzala, Camila Ferreira de Moura, Gabrielle Kefrem Alves Gomes, Sandra Maria Ferreira, Adriana Pereira Campos, Marcia Veloso Machado de Mendonça, Roberta Gonçalves da Silva, Juliette Cardoso de Santana, Luciene Valeria Salgado Costa Vasconcelos, Carolina Liane de Macedo Eloi, Jaqueline Martins Xavier

Department of Internal Medicine and Infectious Diseases, Julio Müller School Hospital, Federal University of Mato Grosso, Cuiaba, MT, Brazil

Cor Jesus Fernandes Fontes, Tiago Rodrigues Viana, Rafael Estevanovich Bertoldi Torres, Larissa Botelho Pedrini, William Kleyton de Melo Aguiar, Willian Benedito de Proença Junior, Caroline Reyes, Iury Jocemar Alves, Grasiela Panchoni Menolli, Thiago Ribeiro Nunes Domingues, Pedro de Carvalho Ferreira, Laressa Pereira Sari,,Fábio José da Silva, Gisiene Silva da Luz Moreira, Renata Ito de Araújo, Fabio Alexandre Leal dos Santos, Ruberlei Godinho de Oliveira, Alessandra Emanuelle Cunha Rodrigues, Edna Maria dos Santos, Rosane Christine Hahn, Antônio Gonçalves de Araújo Junior, Maria Luiza Ortiz Nunes da Cunha, Kleythiane Regina Oliveira Costa, Edvania Oliveira Vasconcelos, Juliana Lelis de Almeida, Maria Aparecida Cordeiro de Jesus, Laura de Sousa Dias, Nágisla Maria Benta de Miranda Monteiro, Marly Lopes Soares Monteiro, Ana Lucia Maria Ribeiro,,Jakeline Oliveira da Silva, Rosana Jesuina da Silva, Armando José Guevara Patino, Thaís Maciel Carlos, Palloma Stefany Silva Café, Raimundo Jose da Silva,,Cristina Alves Pereira dos Reis, Igor Gonçalves Baicere,,Fábio Assis de Campos Junior, Emanuelle Soares Camolesi, Pedro Augusto Uecker Paixão,Camila Neves Silva, Hélio Carlos Rocha de Carvalho Junior,Welliton Batista de Morais, Laís Maria de Assis e Silva

Hospital Universitário Maria Aparecida Pedrossian, Universidade Federal de Mato Grosso do Sul

Ana Lúcia Lyrio de Oliveira, Rivaldo Venâncio da Cunha, Déborah Lemes Nogueira, Iêda Maria Rodrigues Vilela Demirdjian, Keila Ventura Soares, Leonardo Martinez Lourenço de Oliveira, Matheus Baptista Passos, Michaela de Oliveira Tognni, Susan Gomez Chambi, Izilyanne Lucas Hoscher Romanholi, Fernanda Paes Reis, Izadora Bonfim, Lethicia Farias Marcino, Lívia Alves da Silva, Thays Cristina Ferreira Ramos, Wagner de Souza Fernandes, Alessandra Moura da Silva, Cristiane de Sena Silva,Ione Maria Lobo dos Santos, Luiz José Gonçalves,Marcos Geronimo da Silva, Maria Aparecida dos Santos Eloy,Carlos Alberto Martins da Rocha, Naiara Valera Versage, Patricia Matos Coelho Vila Real, Rafael Ovando Fraiha, Debora Martins Cardoso, Jaciara de Souza Correa, Helena Pereira Vargas, Marcos Antonio Sebastião, Suelen Kelee Amarilha Gimenes

Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

André Machado de Siqueira, Ellen Fraga Won Randow Falcão, Carolina Pereira Carvalho Lucas, Christiane Fialho Gonsalves, Guilherme Téo Pinto Santoro, Tatiana Fukui da Silveira, Marcelle Alaluna Freitas e Silva, Laura Santos Oliveira, Bianca Silveira Moreira, Eliesier da Silva Souza Filho, Marina Barreto Alvarenga, Claudia Roberto da Silva, Camila Rosas Neves, Thainá do Couto Nabarro Fraga, Camila Arantes Ferreira Brecht D'Oliveira, Lua Kaihe Varjão de Oliveira Cerqueira, ,Caroline Loureiro Hildebrant,,Fabrício da Silva Rangoni, Darlizy Rodrigues Santos, Katia Lucia da Silva Scherz, Karine Carrilho Santos, Vanessa da Silva Porto, Tábatha de Souza Vasconcelos, Cíntia Ferreira Gonçalves, Daniele dos Santos Silva Fernandes, Christiane Berger, Francielle Mayer Guimarães, Marcos Vinícius Souza de Almeida, Katia Regina de Oliveira Azevedo Rocha, Thays Basilio Oliveira, Ana Carolina Almeida Fortes, Katharina Ferreira Araújo, Barbara de Azevedo Scangarelli, Ana Carolina Castro de Jesus

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

Gang Zeng, Qianqian Xin, Weining Meng

1.7 CoronaVac produz anticorpos contra Covid-19 em 87% dos vacinados com duas doses na Indonésia

A CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, produziu anticorpos contra o SARS-CoV-2 em 87,15% dos imunizados pelo menos 28 dias após a segunda dose, segundo estudo realizado com milhares de pessoas na Indonésia.

Esse é o resultado do estudo clínico de fase 3 feito por cientistas da Faculdade de Medicina da Universitas Padjadjaran, em Bandung, e pelo Ministério da Saúde da Indonésia publicado em maio deste ano e revisado em setembro.

O ensaio clínico, randomizado, duplo-cego e controlado por placebo, foi realizado em um total de 1.620 adultos saudáveis com idades entre 18 e 59 anos, divididos aleatoriamente entre os que receberam as duas doses ou placebo, entre os meses de agosto, setembro e outubro de 2020.

Entre os que receberam as duas doses, a eficácia da CoronaVac foi de 65,30%, uma alta eficácia que segue o padrão demonstrado em estudos realizados com a vacina em outros países, como Turquia, Chile e Brasil.

CoronaVac evitou casos graves e mortes

Durante o período de vigilância do estudo, houve 49 casos de Covid-19 entre os voluntários. Destes, sete imunizados e 18 casos no grupo placebo foram sintomáticos e ocorreram entre um período de 14 dias a três meses após a segunda dose. Não houve relato de casos graves, críticos ou óbitos por Covid-19 entre os voluntários do estudo.

Para a avaliação de segurança, os eventos adversos solicitados e não solicitados foram coletados após a primeira e segunda vacinação em 14 e 28 dias, respectivamente. Amostras de sangue foram coletadas para um ensaio de anticorpos antes e 14 dias após a segunda dose.

A maioria das reações adversas foram classificadas como leves e a mais relatada foi dor no local da injeção.

Dos 1.620 participantes, 1.046 eram do sexo masculino (64,57%) e 574 do sexo feminino (35,43%).

Publicado em: 24/11/2021

Vaccine 39 (2021) 6520–6528

Contents lists available at ScienceDirect

Vaccine

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

A phase III, observer-blind, randomized, placebo-controlled study of the efficacy, safety, and immunogenicity of SARS-CoV-2 inactivated vaccine in healthy adults aged 18–59 years: An interim analysis in Indonesia

Eddy Fadlyana [a,*], Kusnandi Rusmil [a], Rodman Tarigan [a], Andri Reza Rahmadi [a], Susantina Prodjosoewojo [a], Yulia Sofiatin [a], Citra V. Khrisna [a], Rini Mulia Sari [b], Lilis Setyaningsih [b], Fikrianti Surachman [b], Novilia Sjafri Bachtiar [b], Hadyana Sukandar [a], Imam Megantara [a], Chrysanti Murad [a], Krisna Nur A. Pangesti [c], Vivi Setiawaty [c], Sunarjati Sudigdoadi [a], Yaling Hu [d], Qiang Gao [d], Cissy B. Kartasasmita [a]

[a] Faculty of Medicine, Universitas Padjadjaran /Dr. Hasan Sadikin General Hospital, Bandung, Indonesia
[b] PT. Bio Farma, Bandung Indonesia
[c] National Institute of Health Research & Development, Jakarta, Indonesia
[d] Sinovac Life Sciences Co., Ltd., Beijing, China

ARTICLE INFO

Article history:
Received 21 May 2021
Received in revised form 8 September 2021
Accepted 21 September 2021
Available online 24 September 2021

Keywords:
Adults
Efficacy
Safety
Immunogenicity
SARS-CoV-2 Inactivated Vaccine

ABSTRACT

Background: The WHO declared COVID-19 a pandemic on March 11th, 2020. This serious outbreak and the precipitously increasing numbers of deaths worldwide necessitated the urgent need to develop an effective severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine. The development of COVID-19 vaccines has moved quickly. In this study, we assessed the efficacy, safety, and immunogenicity of an inactivated (SARS-CoV-2) vaccine.

Methods: We conducted a randomized, double-blind, placebo-controlled trial to evaluate the efficacy, immunogenicity, and safety of an inactivated SARS-CoV-2 vaccine and its lot-to-lot consistency. A total of 1620 healthy adults aged 18–59 years were randomly assigned to receive 2 injections of the trial vaccine or placebo on a day 0 and 14 schedule. This article was based on an interim report completed within 3 months following the last dose of study vaccine. The interim analysis includes safety and immunogenicity data for 540 participants in the immunogenicity subset and an efficacy analysis of the 1620 subjects. For the safety evaluation, solicited and unsolicited adverse events were collected after the first and second vaccination within 14 and 28 days, respectively. Blood samples were collected for an antibody assay before and 14 days following the second dose.

Results: Most of the adverse reactions were in the solicited category and were mild in severity. Pain at the injection site was the most frequently reported symptom. Antibody IgG titer determined by enzyme-linked immunosorbent assay was 97.48% for the seroconversion rate. Using a neutralization assay, the seroconversion rate was 87.15%. The efficacy in preventing symptomatic confirmed cases of COVID-19 occurring at least 14 days after the second dose of vaccine using an incidence rate was 65.30%.

Conclusions: From the 3-month interim analysis, the vaccine exhibited a 65.30% efficacy at preventing COVID-19 illness with favorable safety and immunogenicity profiles.

1. Introduction

The coronavirus disease 2019 (COVID-19) has inflicted catastrophic damage to public health, economic, and social stability worldwide [1]. In December 2019, a series of pneumonia cases of unknown origin emerged in Wuhan, Hubei, China, with clinical a presentation resembling viral pneumonia. The outbreak began in early November or December and the number of cases quickly

Abbreviations: COVID-19, Coronavirus Disease 2019; ELISA, Enzyme Link Immunoassay; GMT, Geometric Mean Titer; IgG, Immunoglobulin G; rRT-PCR, Real-time Reverse Transcriptase-PCR; SARS, Severe Acute Respiratory Syndrome; WHO, World Health Organization.

* Corresponding author.
E-mail addresses: eddy.fadlyana@unpad.ac.id (E. Fadlyana), y.sofiatin@unpad.ac.id (Y. Sofiatin), rini.mulia@biofarma.co.id (R.M. Sari), lilis.setyaningsih@biofarma.co.id (L. Setyaningsih), fikrianti.surachman@biofarma.co.id (F. Surachman), novilia@biofarma.co.id (N.S. Bachtiar), imam.megantara@unpad.ac.id (I. Megantara), huyl@sinovac.com (Y. Hu), gaoq@sinovac.com (Q. Gao).

https://doi.org/10.1016/j.vaccine.2021.09.052
0264-410X/

rose. As of May 2020, >80,000 cases were confirmed in China, including healthcare workers, which resulted in>4,000 deaths [2–5]. The virus is airborne, highly transmissible between humans, and has a long and insidious incubation period. The outbreak rapidly escalated out of China and throughout the world, pushing the World Health Organization (WHO) to declare a pandemic on March 11th, 2020 [6]. As of December 20th, 2020, the number of COVID-19 cases was>75 million with over 1.6 million deaths occurring globally [7]. Based on a WHO report, by January 20th, 2021, there were 939,948 confirmed cases of COVID-19 with 26,857 deaths in Indonesia [8].

Currently, there is no effective treatment available for coronavirus infection. Vaccination is crucial for blocking the rapid spread of deadly infectious diseases, such as the highly contagious COVID-19, especially when effective treatments or cures are not available [9]. Significant efforts have been focused on the development of vaccines and therapeutic drugs. Over the past decade, the scientific community and the vaccine industry have been asked to respond urgently to epidemics including H1N1 influenza, Ebola, Zika, and most recently, SARS-CoV-2 [10]. The WHO is currently preparing a comprehensive analysis of vaccine and therapeutic drug candidates that may be effective against SARS-CoV-2 and will use an evidence-based framework to transparently select the most promising therapeutic and vaccine candidates to evaluate in the clinic [11]. Multiple SARS-CoV-2 vaccines types, such as DNA-based and RNA-based formulations, recombinant subunit-containing viral epitopes, adenovirus-based vectors, and purified inactivated virus are under development. Purified inactivated viruses have been traditionally used for vaccine development and have been found to be safe and effective for preventing many viral diseases including influenza and polio [12–14].

As of January 25th, 2021, there are 64 vaccines in human clinical trials and 20 have reached the final stages of testing. At least 173 preclinical vaccines are under active investigation in animals [15].The preclinical study results of inactivated SARS-CoV-2 Vaccine (Vero Cell), developed by Sinovac Life Sciences Co. Ltd. indicate that the vaccine provided partial or complete protection in macaques from severe interstitial pneumonia after a SARS-CoV-2 challenge without observable antibody dependent enhancement [16]. A phase I/II clinical trial has been conducted in China since April 2020. The preliminary results indicate a favorable safety and immunogenicity profile with a two-dose vaccine schedule. No significant changes in inflammatory factors were observed indicating a small risk of immunopathology induced by the SARS-CoV-2 vaccine [17].

In this article, we report the efficacy of inactivated SARS-CoV-2 vaccine in preventing COVID-19 including safety and immunogenicity data based on the phase III trial collected during a 3-month period after the second injection in 18–59 year-old subjects in Indonesia. This data set and trial results form the basis of an application for emergency use authorization in Indonesia.

2. Materials and methods

2.1. Study design and population

This study was an observer-blinded, randomized, placebo-controlled two arm with parallel groups, prospective intervention, phase III study that began in August 2020 in Bandung, Indonesia to evaluate the efficacy, immunogenicity, and safety of an inactivated SARS-CoV-2 vaccine and its lot-to-lot consistency. The main exclusion criteria included evolving mild, moderate, or severe illness, especially infectious disease or fever (body temperature $\geq$ 37.5℃) , patients with serious chronic diseases, positive result from a nasopharyngeal swab RT-PCR test, reactive IgG and IgM for SARS-CoV-2, women who are lactating, pregnant or planning to become pregnant during the study period, serious chronic diseases (serious cardiovascular disease, uncontrolled hypertension and diabetes, liver and kidney disease, malignant tumors, or any condition which according to the investigator may interfere with the assessment of the trial objectives), uncontrolled coagulopathy or blood disorders, history of asthma, history of allergy to vaccines or vaccine ingredients, history of confirmed or suspected immunosuppressive or immunodeficient state, or received in the previous 4 weeks a treatment likely to alter the immune response [intravenous immunoglobulins, blood-derived products, or long-term corticosteroid therapy (>2 weeks)], history of uncontrolled epilepsy or other progressive neurological disorders, and having received any vaccination within 1 month before or after administration of the study vaccine.

After being informed about the study and signing an informed consent form, the medical history of the subjects was evaluated, and they were provided a physical exam. The blinded investigator team evaluated the inclusion and exclusion criteria. Eligible subjects were randomly assigned at a ratio of 1:1 into two study arms to receive either 3 μg/0.5 mL dose of inactivated SARS-CoV-2 vaccine or placebo on day 0 and 14. The randomization list was generated automatically using the website, www.sealedenvelope.com, and the vaccinated arms were grouped into three different batch numbers (batch 1/batch 2/batch 3) of SARS-CoV-2 vaccine. The subjects were randomized and vaccinated per treatment group by an unblinded team. The alphabetical code remained confidential and maintained by the unblinded team and was not to be opened until the end of the study.

The study protocol, subject information sheet and consent forms, and the subject's diary card was approved by the Research Ethics Committee of Universitas Padjadjaran (Ethical Approval No. 669/UN6.KEP/EC/2020) and Indonesian Regulatory Authorities. This trial was conducted in accordance with ICH Good Clinical Practice guidelines, the Declaration of Helsinki, and local regulatory requirements. The clinical trial was registered at clinicaltrials.gov with entry number NCT04508075 and in the Indonesian Clinical Research Registry (INA-WXFM0YX).

2.2. Study vaccine

The study vaccine, developed by Sinovac Life Sciences Co., Ltd., was an inactivated SARS-CoV-2 whole virion vaccine with aluminum hydroxide as an adjuvant. The study vaccine was manufactured by inoculating novel coronavirus (CZ02 Strain) into African green monkey kidney cells (Vero Cell). The virus was successfully incubated, harvested, inactivated using β-propiolactone, concentrated, purified, and adsorbed by aluminum hydroxide. The bulk vaccine was then formulated with phosphate-buffered saline and sodium chloride as the inactivated final product. A dosage of 3 μg/0.5 mL was selected for this study. Three batches of study vaccine were used (20200308, 20200412, and 20200419). The placebo contained water for injection packaged in ampoules (0.5 mL/dose) and manufactured by PT Bio Farma. The study vaccine was administered intramuscularly into the left deltoid region by an unblinded investigator. The vaccine was stored at + 2℃ to + 8℃.

2.3. Surveillance for COVID-19 and efficacy assessment

The primary outcome of the study was to assess the efficacy of two doses of the inactivated SARS-CoV-2 vaccine in preventing COVID-19 cases compared with placebo. The primary efficacy endpoint was incidence of laboratory confirmed-symptomatic COVID-19 cases starting at 14 days following the second dose. COVID-19 case defined according to the case definition of the national guidelines for the diagnosis and treatment of COVID-19

in Indonesia [18]. Subjects were surveilled for COVID-19 disease after the first dose of vaccine by a combination of active and passive surveillance. The surveillance team performed monthly contact (by phone or text message) to actively collect information from subjects whether they have any symptoms suggesting COVID-19 disease or admitted to hospital for any reason. Any subject who has at least one specific symptoms (cough, taste or smell disorders, or dyspnea) or has two or more non-specific symptoms (fever, chills, sore throat, fatigue, nasal congestion or runny nose, body pain, muscle pain, headache, nausea, vomiting, or diarrhea) for at least two consecutive days was scheduled to have nasopharyngeal swab sample taken for SARS-CoV-2 rRT-PCR test. Subjects were also regularly reminded to report if they have any of the above symptoms.

The rRT-PCR was performed by the Central Laboratory of Universitas Padjadjaran. Nasopharyngeal samples were processed in a dedicated BSL-2 laboratory with BSL-3 practices under a certified Class II Biological Safety Cabinet. Once a clinical sample was treated with lysis buffer for RNA extraction, the samples then moved to a less restrictive environment to complete the RNA extraction and real-time RT-PCR. A 140 µl aliquot of the specimen was added to 560 µl of lysis buffer (Qiagen Viral Mini kit). RNA extraction was done based on the manufacturer's protocol and immediately processed for RT-PCR. The remaining nucleic acid was stored at –80℃ for sequence analysis.

The real-time reverse transcriptase-PCR (rRT-PCR) reagent kit from ABT (Beijing Applied Bioscience Technology) and the Multiple Real-Time PCR Kit for Detection of 2019-nCoV were used. The results were analyzed by software provided by the manufacturer of the Light Cycler (Roche). Comparative viral load was calculated using the CT (Cycle Threshold) values of consecutive specimens. The incidence of suspected COVID-19 cases within 14 days to 6 months after the second dose of immunization was analyzed to determine efficacy.

2.4. Immunogenicity assessment

To assess the immune response, 4 mL blood samples were collected from 540 subjects before the first injection (Day 0) and 14 days after the second injection. The ability of the antibodies present in the blood sample to bind to the receptor binding domain (RBD) of SARS-CoV-2 was assessed blindly using an enzyme-linked immunosorbent assay (ELISA) at the Clinical Trial Laboratory of Bio Farma. The ELISA titers were determined by end point dilution and calculated using GraphPad Prism version 8.4.3 software [19–21]. The antibody increment and GMT 14 days post-last immunization were evaluated. ELISA seropositive antibody IgG titer was defined as titer > 200 and seroconversion was defined as a four-fold increase of anti-RBD antibody IgG titer (ELISA) at 14 days after two doses of vaccine compared with the baseline. The neutralization of antibody (NAb) assay was also conducted at the National Intitute of Health Reasearch & Development. A four-fold increase in antibody titer compared with the baseline value was considered as the measure of seroconversion. Seropositivity was defined as detected antibody $\geq$ 1:4. The immunogenicity data were analyzed in the per protocol population using SPSS software. Pre-vaccination titer levels for subjects with zero titer were assigned a value of 200 for ELISA and 2 to enable GMT and titer increment calculations.

2.5. Safety assessment

Subjects were given diary cards to record solicited adverse events (local pain, redness, swelling, induration, fever, myalgia, and malaise) and unsolicited adverse events occurring within 30 min, 7 days, and 8–28 days following each dose. Pain was graded as mild (pain at injection site when touched), moderate (pain with movements), and severe (significant pain at rest). Redness, induration, and swelling intensity were measured using a plastic bangle and categorized as mild (<5 cm), moderate (5–10 cm), and severe (>10 cm). Fever was graded as mild (38.0–38.4°C), moderate (38.5–38.9°C), and severe ($\geq$39.0°C). Fatigue, myalgia, and unsolicited events were graded as mild (no interference with activity), moderate (some interference with activity not requiring medical intervention), and severe (prevents daily activity, requires medical intervention).

Any serious adverse events were reported up to 6 months after the second dose. Diary card was reviewed by the blinded investigator at 14 days following the first injection, 14, and 28 days after the second injection. The safety data were reviewed by a Data Safety Monitoring Board (DSMB) and analyzed in the intention-to-treat population using SPSS software.

2.6. Sample size determination and statistical analysis

The study was powered for efficacy analysis. Sample size was determined based on 95% confidence interval and 80% power. Assuming that 2% of the population would develop COVID-19 infection in the placebo arm, a minimum of 810 subjects in each vaccinated and placebo group would provide 80% power to reject the null hypothesis of no difference if the true efficacy was 60% with a 5% dropout rate. In this study, the total cohort was 1620 subjects with 810 subjects in the vaccinated group and 810 subjects in the placebo group.

Vaccine efficacy (VE) will be estimated by (1 - RR) $\times$ 100, where RR (relative risk) is calculated as the incidence in the vaccinated group divided by the incidence in the placebo group per person-years.

To analyze the immunogenicity, GMTs comparation between vaccine and placebo group was calculated after logarithmic transformation using *t*-test or ANOVA (F-test). Serum immune response proportions (seropositive rate, seroconversion) and vaccine lot-to-lot comparison was calculated using Chi-square test. The incidence rates of solicited and unsolicited adverse events between both groups were analyzed using Chi-square test. A p-value of<0.05 was considered to be significant.

3. Results

3.1. Study population

Between August 11, 2020, and October 21, 2020, a total of 1819 participants were screened and 199 subjects were excluded due to not meeting the inclusion criteria or meeting one of the exclusion criteria. From 1620 subjects randomized in the study, there were 17 subjects that withdrawn from the study prior to the second dose [Fig. 1]. The first 540 participants were included in the immunogenicity subset group.

There were 1046 male participants (64.57%) and 574 female participants (35.43%). The participants were come from various age distribution from 18 to 59 years with average 35.5 ± 11.2 years old. Among the subset immunogenicity subjects, there were 314 male participants (58.15%) and 226 female participants (41.85%) with an average age of 35.82 years ± 11.4 years old. The details of the demographic data are provided in Table 1.

All study vaccines were administered according to the randomization list. Treatment compliance was defined as receiving both doses of vaccine/placebo within the specified time period. For the 540 participants in the immunogenicity subset, 10 subjects withdrew prior to the second dose vaccination and not included in the immunogenicity analysis. Meanwhile, 1 subject withdrew after

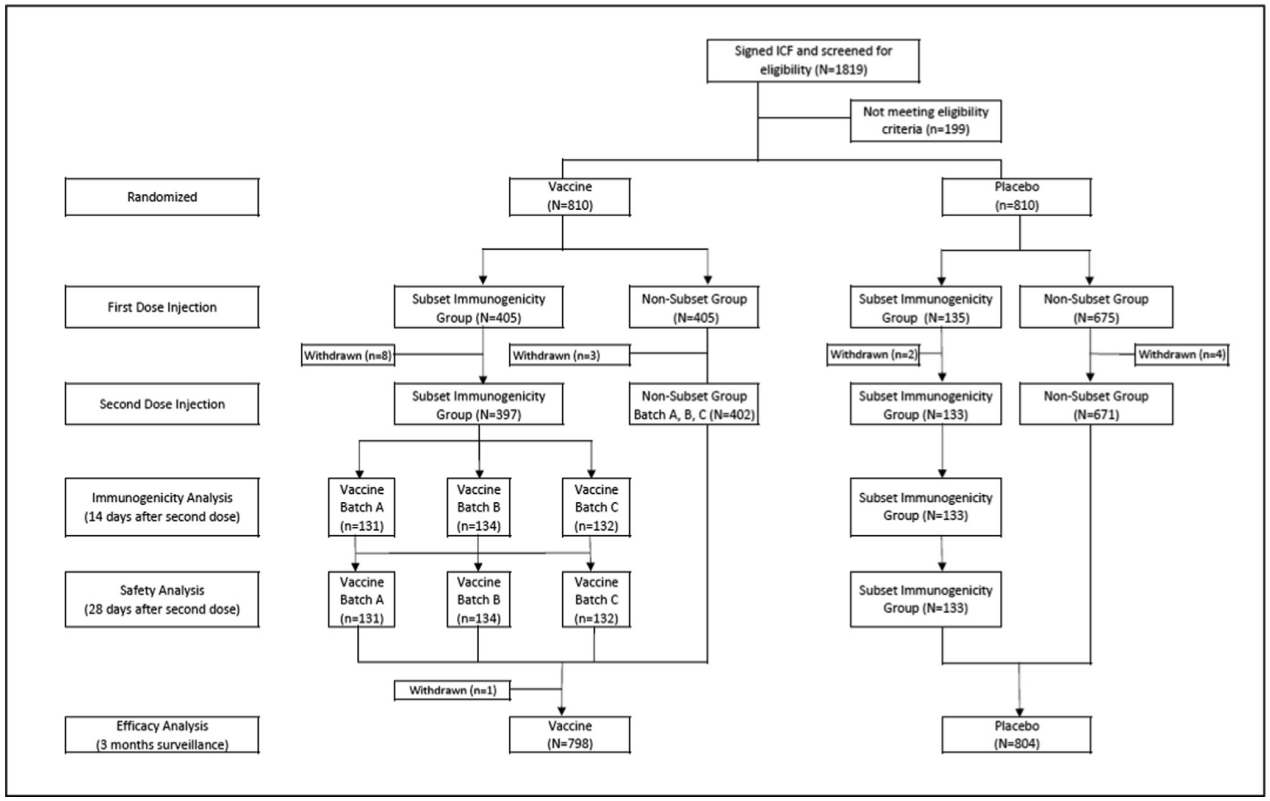


Fig. 1. Participant Disposition.

Table 1
Demographic Data.

Parameter	Vaccine (N = 811)	Placebo (N = 809)	Total (N = 1620)
Mean age [years] (SD)	35.6 (11.3)	35.4 (11.0)	35.5 (11.2)
Mean height [m] (SD)	1.63 (0.09)	1.63 (0.09)	1.63 (0.09)
Mean weight [kg] (SD)	65.6 (13.5)	64.8 (13.6)	65.2 (13.5)
BMI (kg/m^2)	24.8 (4.4)	24.5 (4.5)	24.6 (4.5)
Sex n(%)			
Male	505 (62.3)	541 (66.8)	1046 (64.57)
Female	305 (37.7)	269 (33.2)	574 (35.43)
Demographic Data in the Immunogenicity Subset Group			
Parameter	Vaccine (N = 405)	Placebo (N = 135)	Total (N = 540)
Mean age [years] (SD)	36.0 (11.5)	35.3 (10.9)	35.82 (11.4)
Mean height [m] (SD)	161.8 (8.9)	161.7 (9.8)	161.8 (9.2)
Mean weight [kg] (SD)	64.6 (13.2)	65.9 (13.6)	64.9 (13.3)
BMI (kg/m2)	24.6 (4.3)	25.2 (4.7)	24.75 (4.4)
Sex n(%)			
Male	229 (56.5)	85 (63.0)	314 (58.15)
Female	176 (43.5)	50 (37.0)	226 (41.85)

Abbreviations: N = number of participants, SD = Standard deviation.

Table 2
Treatment Compliance in Immunogenicity Subset Group.

	Vaccine n (%)	Placebo n (%)	Total N (%)
Subjects screened for RT-PCR test	405	135	540
Subjects screened for IgM/IgG test	405	135	540
Subjects enrolled	405	135	540
First vaccination completed	405	135	540
Second vaccination completed	397	133	530
Intention-to-treat population (for safety and efficacy analysis)	405	135	540
Per-protocol population (for immunogenicity analysis 14 days after last injection)	397	133	530

the second dose of the study vaccine. These dropout subjects included 9 from the vaccinated group and 2 from the placebo group. The details for treatment compliance in the subset immunogenicity group are presented in Table 2. Early withdrawal resulted from consent withdrawal by the subject or the subject met the contraindication criteria for the second vaccination (not in healthy condition during the second vaccination schedule). The study results presented in this article are based on a preliminary immunogenicity and safety data analysis of 540 subjects in the immunogenicity subset group, whereas the efficacy results are based on preliminary efficacy data from 1620 subjects with median ~ 2.5 months of surveillance period.

3.2. Efficacy

During the surveillance period, 320 COVID-19 suspect cases and 49 laboratory confirmed COVID-19 cases were collected. From these 49 confirmed COVID-19 cases, 25 cases (7 cases in the vaccine group and 18 cases in the placebo group) were symptomatic and occurred from 14 days following the second dose up to 3 months. There were no severe, critical, or deaths of laboratory confirmed COVID-19 cases observed [Table 3].

Vaccine efficacy was defined as percentage reduction in relative risk using the ratio of incidence rate in the vaccine group and placebo group. Incidence rate was calculated by the number of subjects with laboratory-confirmed COVID-19 divided by the total

Table 3
Summary of Primary Efficacy Endpoint.

	Vaccine			Placebo			
Endpoint	No. of cases	Mean follow-up days	Incidence rate (per 100 person years)	No. of cases	Mean follow-up days	Incidence rate (per 100person years)	Vaccine Efficacy (%)
Symptomatic confirmed laboratory cases COVID-19 starting 14 days after second injection	7	80.78		18	72.08		65.30%
			3.904			11.25	
Severe	0		0	0		0	
Critical	0		0	0		0	–
Death	0		0	0		0	

number of subjects at risk adjusted by time (person years). The vaccine showed 65.3% efficacy in preventing symptomatic COVID-19.

3.3. Immunogenicity

3.3.1. Antibody IgG titer by ELISA

The seropositive rate of SARS-CoV-2 IgG antibody in the vaccine group at 14 days after the second injection was 99.74%. The seropositive rate in the vaccine group increased significantly compared with the placebo group. The seroconversion rate at 14 days after the second injection in the vaccine group was 97.48% which was significantly different compared with a 0.75% seroconversion rate in the placebo group. There was a 23.5-fold increase of IgG antibody GMT at 14 days after the second injection in the vaccine group, whereas there was no significant increase of GMT in the placebo group. The results of the IgG analysis using ELISA are presented in Table 4.

3.3.2. Neutralization antibody

Neutralization antibody seropositive was defined as a titer $\geq$ 1:4 and seroconversion was defined as a change from a titer < 1:8 to a titer $\geq$ 1:8; or a 4-fold increase from baseline if the titer at baseline $\geq$ 1:8. After the full schedule of vaccine administration, the seropositive rate of SARS-CoV-2 antibody using the neutralization assay in the vaccine group at 14 days was significantly different compared with that of the placebo group. The seroconversion rate 14 days after the second injection in the vaccine group was 87.15% with no seroconversion in the placebo group. There was a 7.88-fold increase of antibody neutralization GMT at 14 days after the second injection. The neutralization antibody results are presented in Table 4.

3.3.3. Lot-to-lot consistency

Another objective of the study was to evaluate the consistency of 3 batches of inactivated SARS-CoV-2 vaccine. The IgG antibody seropositive rate for the three batches of vaccine (batch numbers 20200308, 20200412, and 20200419) were 100%, 99.25%, and 100%, respectively, whereas the seroconversion rates were 96.18%, 97.76%, and 98.48%, respectively for the 14 day time point after the second vaccination. The GMT of the three batches was 5093.78, 5421.63, and 5032.34, respectively, for the 14 day time point after the second injection.

Table 4
Antibody Titer between the Vaccine and Placebo Groups.

Antibody Titer	Time Point	Parameter	Group		p-value
			Vaccine (N = 397)	Placebo (N = 133)	
IgG (ELISA)	**V1**	Seropositive rate n(%)	44 (11.08)	14 (10.53)	0.859**
		(95% CI)	(8.36–14.55)	(6.37–16.89)	
		GMT*	220.27	220.37	0.990***
		(95% CI) Median	(212.87–227.93)	(206.45–235.24)	
			200.00	200.00	
	V3	Seropositive rate n(%)	396 (99.74)	7 (5.29)	<0.001**
		(95 %CI)	(99.26–100)	(1.47–9.06)	< 0.001**
		Seroconversion n(%)	387 (97.48)	1 (0.75)	< 0.001***
		(95% CI)	(95.43–98.63) 5181.19	(0.13–4.14)	
		GMT* (95% CI) Median	(4746.13–5656.14)	223.61	
			5333.35	(209.08–239.47)	
				200.00	
Neutralization Antibody	**V1**	Seropositive rate n(%) (95% CI)	0 (0–0.96)	0 (0–2.81)	–
		GMT* (95% CI) Median	2.00 (–)	2.00 (–)	
			–	–	–
	V3	Seropositive rate n(%)	380 (95.72)	1 (0.75)	<0.001**
		(95% CI)	(93.25–97.31)	(0.13–4.14)	< 0.001**
		Seroconversion n (%) (95% CI)	346 (87.15)	0 (0.00)	< 0.001***
		GMT* (95% CI)	(83.50–90.09)	(0–2.81)	
		Median	15.76	2.02	
			(14.57–17.04)	(1.98–2.05)	
			16	2	

*) The comparison results after logarithmic transformation. **) Chi-square test; ***) *t*-test.
V1 = before injection;
V3 = 14 days after second injection;
IgG seropositive = titer > 200; seroconversion = four-fold increasing anti-RBD antibody IgG titer compare to baseline 14 days after the second dose.
Antibody neutralization seropositive = titer $\geq$ 1:4; seroconversion = a change from seronegative (titer < 1:8) to seropositive (titer $\geq$ 1:8); or a 4-fold increase from baseline titers if titer at baseline $\geq$ 1:8.

We compared the proportion of participants with seropositive and seroconversion between the 3 batches of SARS-CoV-2 vaccine. The results indicated that there was no significantly different proportion between the 3 vaccine batches as shown in Table 5.

After the full schedule of vaccine, the seropositive rate of SARS-CoV-2 antibody as determined by the neutralization assay for batch numbers 20200308, 20200412, and 20,200,419 at 14 days after the second injection was above 94%. The seroconversion rate for each vaccine batch at 14 days after the second injection was 90.08%, 88.81%, and 82.58%, respectively. There was an increase of 7 to 8-fold for neutralization antibody GMT in all batches at 14 days following the second injection.

3.4. Safety

Within the immunogenicity subset group (n = 540), the majority of the reported local reactions was local pain, whereas the most common systemic event was myalgia. In the vaccine group, local pain was reported by 33.5% and 30.5% of the subjects after the first and second injection, respectively [Fig. 2]. In the placebo group, local pain was reported by 23.7% and 30.1% of the subjects after the first and second injection, respectively. In the vaccine group, myalgia was reported by 25.6% and 19.9% of the subjects after the first and second injection, respectively. In the placebo group, myalgia was reported by 12.6% and 9.0% of the subjects after the first and second injection, respectively. Based on the system organ class, majority of the unsolicited adverse event was categorized in the nervous system diseases category, specifically headache [Table S1].

The intensity of the adverse events was mostly mild in the vaccine and placebo groups. After the first injection, the percentage of mild adverse events in the vaccine and placebo groups was 54.3% and 46.7%, respectively. After the second injection, the percentage of mild adverse events in the vaccine and placebo groups were 47.9% and 42.9%, respectively. There was a significant difference in the distribution of severe adverse reactions after the second dose between the vaccine and placebo groups, with a higher proportion in the placebo group. Moderate adverse reactions after the first dose in the vaccine groups were significantly higher than the placebo group.

Of the 1620 subjects enrolled to the study, there were nine serious adverse events (SAE) that occurred in all subjects with a classification not related to vaccine products (five SAEs). One SAE was very unlikely and three SAEs were reported as less likely to be related to the vaccine product as assessed by the DSMB.

4. Discussion

The efficacy of 2 doses of SARS-CoV-2 vaccine at preventing COVID-19 was evaluated up to 6 months after the second dose of injection. However, this interim report consisted of an efficacy analysis of 1620 participants within 3 months following the final dose of study vaccine. The efficacy analysis was performed based on the primary endpoint for all enrolled subjects with a data cut-off date of January 9th, 2021. The efficacy in preventing symptomatic confirmed cases of COVID-19 occurring at least 14 days after the second dose of vaccine was 65.30% (person years) with 7 COVID-19 cases occurring in the vaccine group and 18 COVID-19 cases occurring in the placebo group. There were no severe, critical, or incidents of death from laboratory confirmed COVID-19 infection.

Table 5
Comparison of Antibody Titer in Different Vaccine Batches.

Antibody	Time Point	Parameter	Batch			p-value**
			Batch 20200308 (n = 131)	Batch 20200412 (n = 134)	Batch 20200419 (n = 132)	
IgG (ELISA)	**V1**	Seropositive rate n(%)	14 (10.70)	16 (11.94)	14 (10.61)	0.927**
		(95% CI)	(6.47–17.14)	(7.48–18.52)	(6.42–17.02)	
		GMT*)	215.16	223.40	222.26	0.384***
		(95% CI)	(205.70–225.05)	(208.36–239.52)	(209.08–236.27)	
		Median	200.00	200.00	200.00	
	V3	Seropositive rate n (%)	131 (100)	133 (99.25)	132 (100)	0.374**
		(95% CI)	(97.15–100)	(95.89–99.87)	(97.17–100)	
		Seroconversion n (%)	126 (96.18)	131 (97.76)	130 (98.48)	0.476**
		(95% CI)	(92.38–98.36)	(93.62–99.24)	(94.64–99.58)	
		GMT*)	5093.78	5421.63	5032.34	
		(95% CI)	(4369.78–5937.59)	(4656.29–6312.77)	(4314.30–5869.76)	0.898***
		Median	5105.05	5787.62	5302.40	
Neutralization Antibody	**V1**	Seropositive rate n(%)	0	0	0	–
		(95% CI)	(0–2.85)	(0–2.94)	(0–2.91)	
		GMT*)	2.00	2.00	2.00	–
		(95% CI)	–	–	–	
		Median	–	–	–	
	V3	Seropositive rate n (%)	126 (96.18)	127 (94.78)	127 (96.21)	0.803**
		(95% CI)	(91.38–98.36)	(89.61–97.45)	(91.44–98.37)	
		Seroconversion n (%)	118 (90.08)	119 (88.81)	109 (82.58)	0.150**
		(95% CI)	(83.76–94.11)	(82.35–93.10)	(75.21–88.10)	
		GMT*)	15.97	16.59	14.75	0.470***
		(95% CI)	(14.03–18.18)	(14.47–19.02)	(12.78–17.02)	
		Median	16.00	16.00	16.00	

*) The comparison results after logarithmic transformation. **) Chi-square test; ***) ANOVA (F-test).
V1 = before injection.
V3 = 14 days after second injection.
IgG seropositive = titer > 200; seroconversion = four-fold increasing anti-RBD antibody IgG titer compare to baseline 14 days after the second dose.
Antibody neutralization seropositive = titer ≥ 1:4; seroconversion = a change from titer < 1:8 to titer ≥ 1:8; or a 4-fold increase from baseline titers if titer ≥ 1:8 14 days after the second dose.

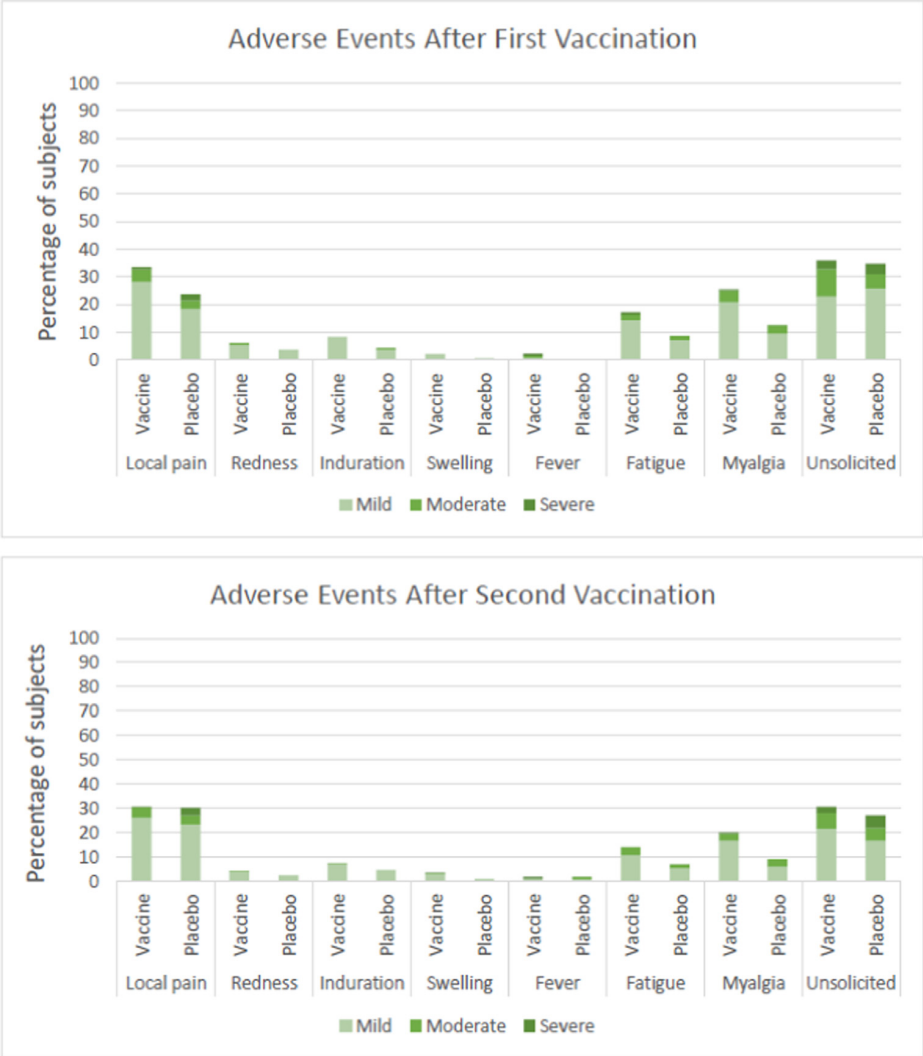


Fig. 2. Adverse Events occurring after the First and Second Vaccine Injection.

A phase III study for the study vaccine was also conducted in Brazil, Turkey, and Chile. Each country has a specific study design depending on its pandemic situation, but the main design is similar. Efficacy data from other countries may support the registration in each country. Based on the interim result, vaccine efficacy in Brazil and Turkey was 50.65% and 83.5%, respectively [22,23]. Vaccine effectiveness study was conducted in Chile with result of 65.9% [24]. The variability of efficacy result between the countries may reflect variance in study characteristics such as population, testing rate/capture of milder case, and force of infection [22].

The efficacy results in this study were higher compared with that of the same study in Brazil. The Brazilian study showed that after 14 days following vaccination with 2 doses of vaccine using a 0 and 14 day schedule, the efficacy rate against COVID-19 was 50.65% for all cases, 83.70% for cases requiring medical treatment, and 100.00% for hospitalized, severe, and fatal cases. This may be the result of Brazil having a high-risk population, particularly health care workers, thus leading to a higher COVID-19 infection rate. In contrast, the Indonesian study used the general population with a smaller occupational exposure to COVID-19 infection [22,25].

Efficacy is one of the key indices to evaluate a vaccine. It measures the effect of vaccination by calculating the proportionate reduction in cases among vaccinated subjects in a double-blind placebo-controlled randomized clinical trial. VE is measured by calculating the risk of disease among vaccinated and unvaccinated subjects and determining the percent reduction in risk of disease relative to the unvaccinated group. The greater the percent reduction of illness in the vaccinated group, the higher the VE [26–28].

In this study, the most common adverse events were pain at the site of injection and myalgia which were reported in vaccine and placebo recipients and with a significantly higher proportion of participants in the vaccinated group compared with the placebo group. Most adverse events were mild or moderate in severity. In the vaccine group, fever was reported in 2.5% of the participants after the first dose and 1.8% after the second dose of vaccine. No significant differences in proportion between the vaccine and placebo group were observed. Overall, reactogenicity events were mild and resolved within a couple of days after onset. These results indicate that the vaccine was well-tolerated. The occurrence of fever following vaccination with SARS-CoV-2 inactivated vaccine was lower compared with other COVID-19 vaccine candidates, such as the novel chimpanzee adenovirus vector vaccine, ChAdOx1 nCoV-19 viral-vector vaccines (18% in participants without paracetamol), or RNA vaccines (16% in younger vaccine recipients and by 11% of older recipients reported after the second dose) [29,30].

The immune response based on the seropositive and seroconversion rate of SARS-CoV-2 antibody IgG titer using ELISA at 14 days after the second injection were 99.74% and 97.48%, respectively. The IgG antibody GMT before injection and 14 days after the second injection were 220.27 and 5181.19, respectively. The seroconversion rate of RBD-specific IgG in this study were similar to that of the phase II study which was 97% [GMT 1094.3 (95% CI 936.7–1278.4)] at 14 days following the second dose [17].

The immune response based on the seropositive and seroconversion rate of SARS-CoV-2 neutralizing antibody using the neutralization assay in the vaccine group at 14 days after the second injection were 95.72% and 87.15%, respectively. The neutralization antibody GMT was 15.76 at 14 days after the second injection. The study vaccine phase I/II clinical trials conducted in China in April 2020 to evaluate the safety and immunogenicity of 2 doses of vaccine at intervals of 0 and 14 days (emergency schedule) and 0–28 days (routine schedule). In the phase I/II trials, it was found that immune responses induced by the day 0 and 28 vaccination schedule were larger than those induced from the day 0 and 14 vaccination schedule. In the phase 2 trial, the seroconversion rate of neutralizing antibodies to live SARS-CoV-2 for the same dosage used in this study were 92% with a GMT of 27.6 (95% CI 22.7–33.50) at 14 days after the second dose and 94% with a GMT of 23.8 (95% CI 20.5–27.7) at 28 days after the second dose in the day 0 and 14 vaccination cohort. Meanwhile, the seroconversion rate was 97% with a GMT of 44.1 (95% CI 37.2–52.2) at 28 days after the second dose in the day 0 and 28 vaccination cohort. However, based on the phase I/II clinical trial results, this study used the emergency vaccination schedule (day 0 and 14) which may be suitable for emergency use during the COVID-19 pandemic since antibody responses may be induced within a relatively short period of time [17].

Comparing the three different batches of vaccine (batch number 20200308, 20200412, and 20200419), we observed no significant differences in the proportion of participants with seropositive and seroconversion rates based on ELISA and neutralization assay, which demonstrated good consistency between each batch of the SARS-CoV-2 vaccine. The results of this interim report show the efficacy above the value required by the WHO [31].

Currently this study is still on-going to evaluate antibody persistence and efficacy up to 6 months after the second dose of vaccine. One limitation of our study is that it only assesses the efficacy of healthy adults aged 18–59 years with a limited number of subjects. Therefore, it still requires further research to obtain vaccine efficacy, safety, and immunogenicity data in the population aged 60 years of age and over, with or without comorbidities.

5. Conclusion

Based on the interim analysis, the vaccine showed a 65.30% efficacy at preventing COVID-19 illness with a good safety and immunogenicity profile.

Declaration of Competing Interest

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

Acknowledgements

The authors would like to thank all the subjects who participated in this study, the head of the Bandung District Health Office, the Dean of Faculty of Medicine Universitas Padjadjaran, the head and staff of Puskesmas Garuda, Puskesmas Dago, Puskesmas Ciumbuleuit, Puskesmas Sukapakir, Klinik Kesehatan UNPAD, for their support.

We would also like to thank our Sub-Investigators, Susi Susanah, Prayudi Santoso, Rudi Wicaksana, Yovita Hartantri, Hendarsyah S., Viramitha Kusnandi Rusmil, Reni Ghrahani, Mia Milanti Dewi, Akbar Tirtosudiro, Riyadi, Yudith Ermaya, Fina Meilyana Andriyani for their valuable contribution to the study.

Appendix A. Supplementary data

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

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1.8 Estudos confirmam segurança da vacina contra coronavírus desenvolvida em parceria com o Butantan

A vacina Coronavac, desenvolvida em parceria com o Instituto Butantan, se mostrou segura e com bom índice de imunogenicidade. A constatação é de estudo publicado ontem pela farmacêutica chinesa Sinovac Life Science. O estudo analisou o comportamento de 600 voluntários vacinados na China durante a fase 2 dos testes clínicos.

Cada voluntário recebeu 2 doses, sendo metade a vacina propriamente dita e a outra metade placebo. De acordo com o que foi identificado nos estudos, não existe nenhuma preocupação com relação a segurança da vacina utilizada nos voluntários. Dentre as principais reações está leve dor no local da aplicação.

A vacina desenvolvida pela Sinovac Life Science é uma das mais promissoras do mundo porque utiliza tecnologia já conhecida e amplamente aplicada em outras vacinas. O Instituto Butantan avalia que sua incorporação ao sistema de saúde deva ocorrer mais facilmente.

O laboratório asiático já realizou testes em cerca de mil voluntários na China, nas fases 1 e 2. Antes, o modelo experimental aplicado em macacos apresentou resultados expressivos em termos de resposta imune contra o coronavírus.

A farmacêutica forneceu ao Butantan as doses da vacina para a realização de testes clínicos de fase 3 em voluntários no Brasil, com o objetivo de demonstrar sua eficácia e segurança.

Caso a vacina seja aprovada será realizada a transferência de tecnologia para produção em escala e fornecimento gratuito pelo SUS. Os passos seguintes serão o registro do imunizante pela Anvisa (Agência Nacional de Vigilância Sanitária) e distribuição em todo o Brasil.

Publicado em: 11/08/2020

Full title

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

Running title

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

Author List:

Yanjun Zhang*, Ph.D., zhangyjtt@126.com, Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China.

Gang Zeng*, Ph.D., zengg@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Hongxing Pan*, M.Sc., panhongxing@126.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Changgui Li*, Ph.D., changguili@aliyun.com, National Institutes for Food and Drug Control, Beijing.

Biao Kan*, Ph.D., kanbiao@icdc.cn, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China.

Yaling Hu*, M.Sc., huyl@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Haiyan Mao, M.Sc., hymao@cdc.zj.cn, Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China.

Qianqian Xin, Ph.D., xinqq@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Kai Chu, M.Sc., chukai19812007@163.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Weixiao Han, M.Sc., hanwx@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Zhen Chen, M.Sc., robbieagassi@hotmail.com, National Institutes for Food and Drug Control, Beijing.

Rong Tang, B.A., tangrongtr@126.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Weidong Yin, MBA, yinwd@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Xin Chen, B.A., kjhszxchenxin@163.com, Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China.

Xuejie Gong, B.A., gongxj@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Chuan Qin, Ph.D., qinchuan@pumc.edu.cn, Key Laboratory of Human Disease Comparative Medicine, Chinese Ministry of Health, Beijing Key Laboratory for Animal Models of Emerging and Remerging Infectious Diseases, Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Comparative Medicine Center, Peking Union Medical College, Beijing, China.

Yuansheng Hu, M.P.H., huys@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Xiaoyong Liu, B.A., 317840532@qq.com, Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China.

Guoliang Cui, B.A., cuigl@sinovac.com, Sinovac Life Sciences Co., Ltd., Beijing,

China.

Congbing Jiang, B.A., snjkws@126.com, Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China.

Hengming Zhang, M.Sc., zhanghm@sinovac.com, Sinovac Biotech Ltd., Beijing, China.

Jingxin Li, Ph.D., jingxin42102209@126.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Minnan Yang, Ph.D., 28453073@qq.com, CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Xiaojuan Lian, MBA, lianxj@sinovac.com, Sinovac Life Sciences Co., Ltd., Beijing, China.

Yan Song, B.A., songyanww@126.com, Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China.

Jinxing Lu¶, Ph.D., lujinxing@icdc.cn, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China.

Xiangxi Wang¶, Ph.D., xiangxi@ibp.ac.cn, CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Miao Xu¶, Ph.D., xumiaobj@126.com, National Institutes for Food and Drug

Control, Beijing.

Qiang Gao¶, M.Sc., gaoq@sinovac.com, Sinovac Life Sciences Co., Ltd., Beijing, China.

Fengcai Zhu¶, M.D., jszfc@vip.sina.com, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China.

Footnote

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

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

Clinical Trial number: NCT04352608

Funding Project:

National Key Research and Development Program (2020YFC0849600)

Beijing Science and Technology Program (Z201100005420023)

78 **ABSTRACT**

79 BACKGROUND

80 The top priority for the control of COVID-19 pandemic currently is the development
81 of a vaccine. A phase 2 trial conducted to further evaluate the immunogenicity and
82 safety of a SARS-CoV-2 inactivated vaccine (CoronaVac).

83 METHODS

84 We conducted a randomized, double-blind, placebo-controlled trial to evaluate the
85 optimal dose, immunogenicity and safety of the CoronaVac. A total of 600 healthy
86 adults aged 18-59 years were randomly assigned to receive 2 injections of the trial
87 vaccine at a dose of 3 μg/0.5 mL or 6 μg /0.5mL, or placebo on Day 0,14 schedule or
88 Day 0,28 schedule. For safety evaluation, solicited and unsolicited adverse events
89 were collected after each vaccination within 7 days and 28 days, respectively. Blood
90 samples were taken for antibody assay.

91 RESULTS

92 CoronaVac was well tolerated, and no dose-related safety concerns were observed.
93 Most of the adverse reactions fell in the solicited category and were mild in severity.
94 Pain at injection site was the most frequently reported symptoms. No Grade 3 adverse
95 reaction or vaccine related SAEs were reported. CoronaVac showed good
96 immunogenicity with the lower 3 μg dose eliciting 92.4% seroconversion under Day
97 0,14 schedule and 97.4% under Day 0,28 schedule. 28 days after two-dose
98 vaccination, the Nab levels of individual schedules range from 23.8 to 65.4 among
99 different dosage and vaccination schedules.

100 CONCLUSIONS

101 Favorable safety and immunogenicity of CoronaVac was demonstrated on both
102 schedules and both dosages, which support the conduction of phase 3 trial with
103 optimum schedule/dosage per different scenarios.

104 **Keywords:** COVID-19; SARS-CoV-2; Inactivated vaccine; Clinical Trial.

BACKGROUND

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

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

METHODS

TRIAL DESIGN AND OVERSIGHT

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

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

Before enrollment, written informed consent was obtained from each participant. The main exclusion criteria included high-risk epidemiological history, positive IgG, IgM or nucleic acid test of pharyngeal or anal swab, axillary temperature >37.0□, allergy to a vaccine component, and other unsuitable conditions.

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

VACCINE

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

SAFETY ASSESSMENT

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

IMMUNOGENICITY

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

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

NEGATIVE STAIN

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

STATISITICAL ANALYSIS

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

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

RESULTS

STUDY POPULATION

From 29 April to 5 May 2020, 600 subjects were enrolled and randomly assigned to receive first of the CoronaVac or placebo dose. All subjects were included into the safety assessment. During this trial, 297 subjects put on Day 0,14 schedule and 294 subjects following Day 0,28 schedule were included in the per-protocol cohort for immunogenicity analysis. These subjects received the 2 injections, attended all visits and gave planned blood sample. Information about study enrollment, randomization, and vaccination is shown in Fig. S1.

Baseline demographic characteristics at enrollment were similar among these groups in terms of sex, mean age, height, and weight (Table 1).

Table 1. Baseline Characteristics of the Study Participants.*

Characteristics	3 μg Group	6 μg Group	Placebo	*P*
Day 0,14 schedule				
N	120	120	60	
Age (years)	42.0±10.2	42.4±9.0	43.6±7.6	0.5543
Gender (male/female)	54/66	48/72	25/35	0.7305
Height (m)	1.7±0.1	1.6±0.1	1.6±0.1	0.3864
Body weight (kg)	67.8±11.7	68.7±11.5	68.4±10.9	0.8258
BMI (kg/m2)	24.9±3.6	25.5±3.2	25.5±3.0	0.2930
Day 0,28 schedule				
N	120	120	60	

Age (years)	41.5±9.6	40.6±9.9	44.3±8.4	0.0472
Gender (male/female)	63/57	63/57	30/30	0.9417
Height (m)	1.7±0.1	1.7±0.1	1.7±0.1	0.9433
Body weight (kg)	70.0±11.8	70.0±12.2	72.1±12.2	0.4704
BMI (kg/m2) §	25.2±3.1	25.2±3.3	26.1±3.1	0.1741

209 * Plus-minus values are means ±SD.

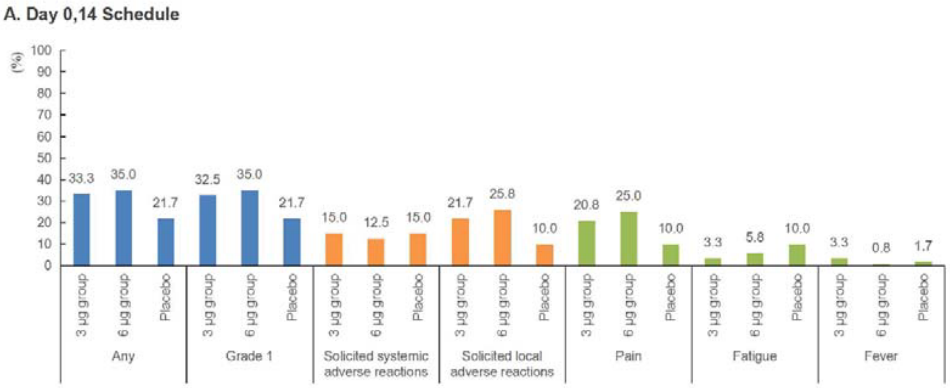
210 § BMI=body mass index.

211

212 **ADVERSE REACTIONS**

213 For subjects in Day 0,14 schedule, the incidence rates of adverse reactions in 6 μg, 3
214 μg and placebo group were 35.0%, 33.3% and 21.7%, respectively; while the
215 corresponding incidence rates were 19.2%, 19.2% and 18.3% in Day 0,28 schedule,
216 respectively. Within each schedule, there was no significant difference in the
217 occurrence of adverse reactions among all vaccine and placebo groups (Fig. 1). Most
218 of the adverse reactions were solicited adverse reactions and mild in severity. After
219 each injection, pain at the injection site was the most frequently reported local
220 symptoms, which reported in 61 subjects (20.3%) on Day 0,14 schedule and 31
221 subjects (10.3%) on Day 0, 28 schedule. (Additional detailed results related to adverse
222 reactions are available in Table S1).

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



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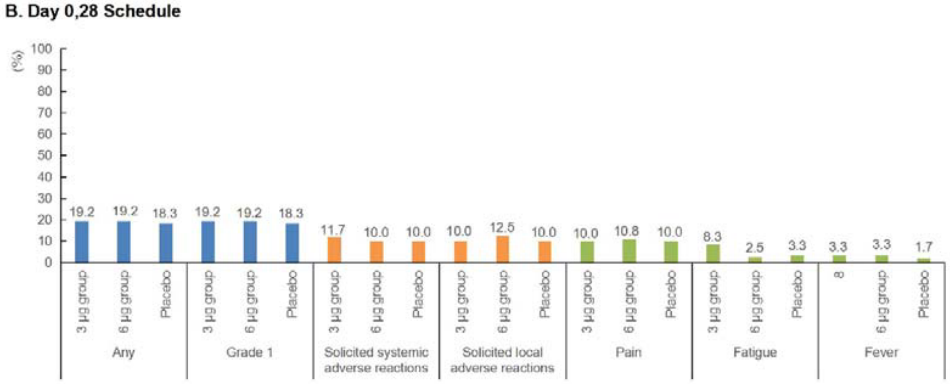

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228 **Figure legends**

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.

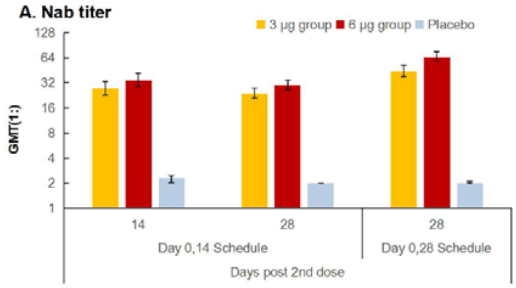
232

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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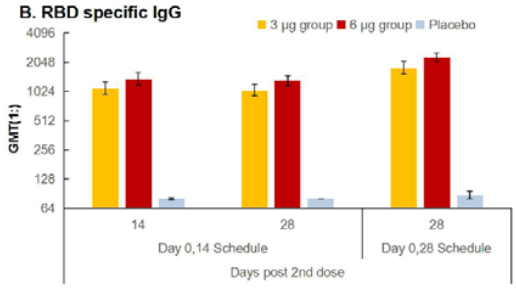

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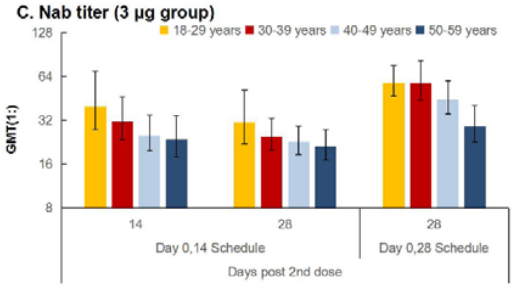

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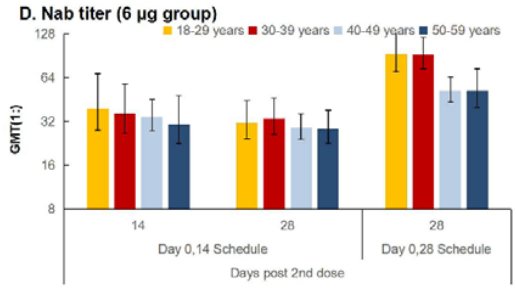

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254 **Figure legends**

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

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

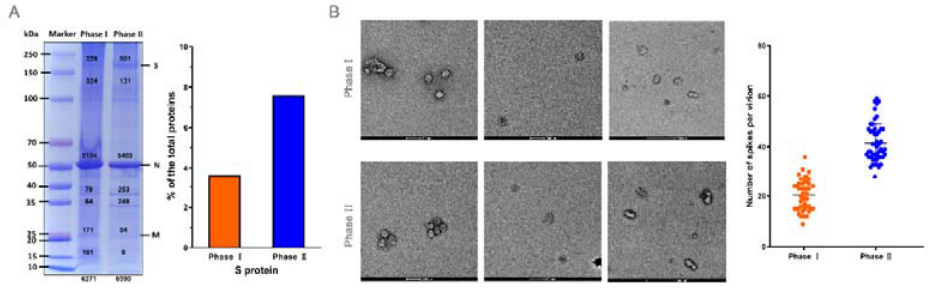

Figure legends

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

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

DISCUSSION

This trial demonstrated that the 2 doses of different dosage of CoronaVac were well tolerated and immunogenic in healthy adults aged 18-59 years. The incidence rates of adverse reactions in the 6 μg and 3 μg group were comparable, indicating that there was no dose-related aggravating concern on safety. Furthermore, no SAEs related to vaccine occurred, and most adverse reactions reported were generally assessed to be mild. The safety profile of CoronaVac is comparable to that observed in our phase 1 clinical trial [see the coordinated submission], and to other inactivated vaccine formulations manufactured by Sinovac.[4,5] Compared with other COVID-19 vaccine candidates, the incidence rate of fever was relatively low in our clinical trial, which further indicates that CoronaVac was well tolerated.[6-10]

It's worth noting that the immune responses elicited in phase 2 were much better than those recorded in phase 1, with seroconversion rates over 90%. Our preclinical investigations had revealed that cell culture technology closely correlated with viral propagation and affected viral morphology, protein composition and prefusion conformation of spikes.[3] In both preclinical study and phase 1 trials, a 50-liter culture of Vero cells grown in the Cell Factory system was used, while an optimized process for growing cells using a highly automated bioreactor, where cell culture parameters like dissolved oxygen, pH, and CO_2/O_2 gas levels, were controlled precisely, was developed for producing the CoronaVac for phase 2 trial. To deduce the reasons underlying the enhanced protective immune responses observed in phase 2 trial, we examined the molecular differences between the CoronaVac used in phase 1 and 2 trials. Protein composition analysis of the purified inactivated SARS-CoV-2 virions indicated that the bioreactor-produced CoronaVac possessed higher redundancy of

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

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

Nabs play an important role in virus clearance and have been considered as a key immune correlate for protection or treatment against viral diseases. Twenty-eight days after the two-dose vaccination, the Nab levels of individual schedules range from 23.8 to 65.4 in phase 2, which was lower than those of convalescent patients tested by the same method in the same laboratory, of which the Nab average level was 163.7.[11] We

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

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

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

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

not include data on more susceptible populations, such as elderly or with comorbidity; and also the immune persistence is not available yet, which need to be further studied. Thirdly, we didn't compare the neutralizing antibody titers induced by CoronaVac and convalescent COVID-19 patients in parallel, however, we conducted this detection of convalescent serum specimens with same procedure performed in this phase 2 trial.

In conclusion, favorable safety and immunogenicity of CoronaVac was demonstrated on both schedules and both dosages in this phase 2 clinical trial, which support the conduction of phase 3 trial with optimum schedule/dosage per different scenarios. Currently, our first priority is to evaluate the protective efficacy of the 3 μg dosage under Day 0,14 schedule. Moreover, Day 0,28 schedule with 3 μg vaccine will also be adopted in our future phase 3 clinical trials.

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

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

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

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

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

Já o segundo estudo, publicado no último mês, foi feito com 330 profissionais de saúde do Hospital da Faculdade de Medicina da Universidade de Istambul-Cerrahpaşa, com idades entre 19 e 65 anos, que foram imunizados com a CoronaVac. Destes, 255 nunca tiveram a doença e 75 tinham história prévia de Covid-19 (cinco casos assintomáticos, 36 leves, 31 moderados e três graves).

Amostras coletadas 28 dias após a segunda dose mostraram soroconversão de anticorpos IgG em 100% dos previamente infectados e 99,2% dos não infectados. Em todos os participantes do estudo, a taxa de eficácia da CoronaVac foi de 99,4%.

No grupo sem infecção, o título médio de anticorpos foi de 48,4 UA/mL após a primeira dose do imunizante, que aumentou para 707,1 UA/mL depois da segunda dose. Já entre os profissionais com história prévia de Covid-19, a média de anticorpos era de 301,9 UA/mL antes da vacinação, elevando para 1331,2 UA/mL depois da primeira dose e se mantendo em níveis semelhantes após a segunda.

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

Publicado em: 11/08/2020

Artigo 8

Vaccine 40 (2022) 52–58

Contents lists available at ScienceDirect

Vaccine

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

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

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

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

ARTICLE INFO

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

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

ABSTRACT

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

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

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

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

1. Introduction

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

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

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

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

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

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

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

2. Methods

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

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

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

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

2.1. Statistical analysis

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

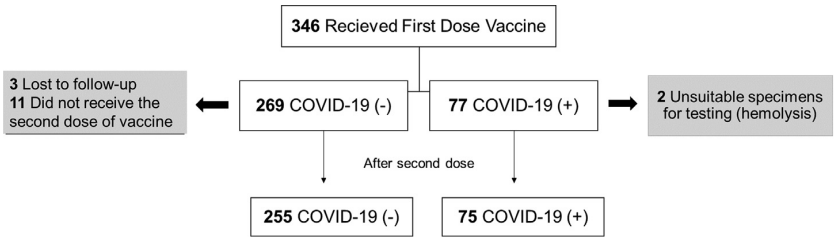

Fig. 1. Flowchart of volunteers participating in the Inactive SARS-CoV-2 Vaccine Efficacy Study.

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

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

3. Results

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

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

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

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

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

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

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

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

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

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

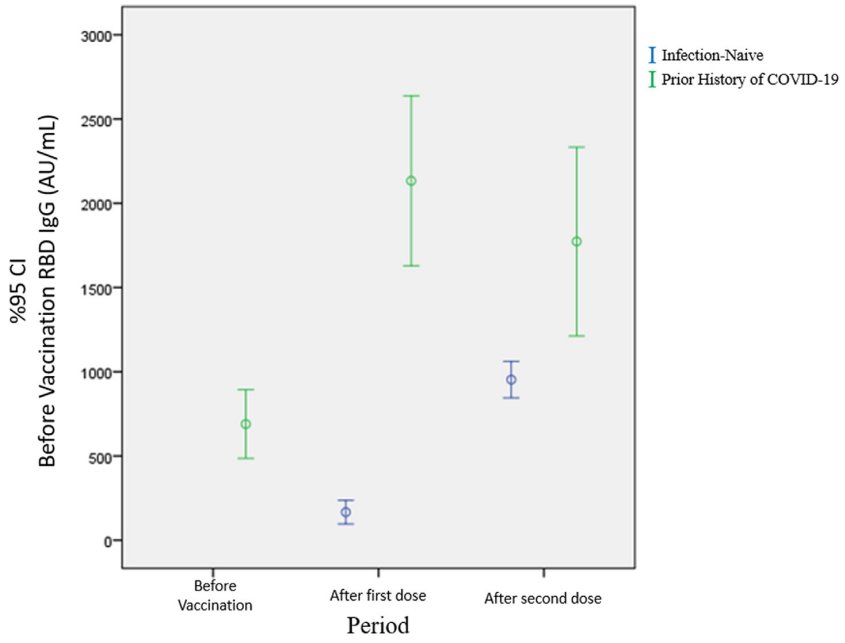

Fig. 2. SARS-CoV-2 IgG averages in blood samples taken at different times from healthcare workers who have prior history of COVID-19 and who are infection naïve.

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

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

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

IQR : Inter Quantile Range.

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

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

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

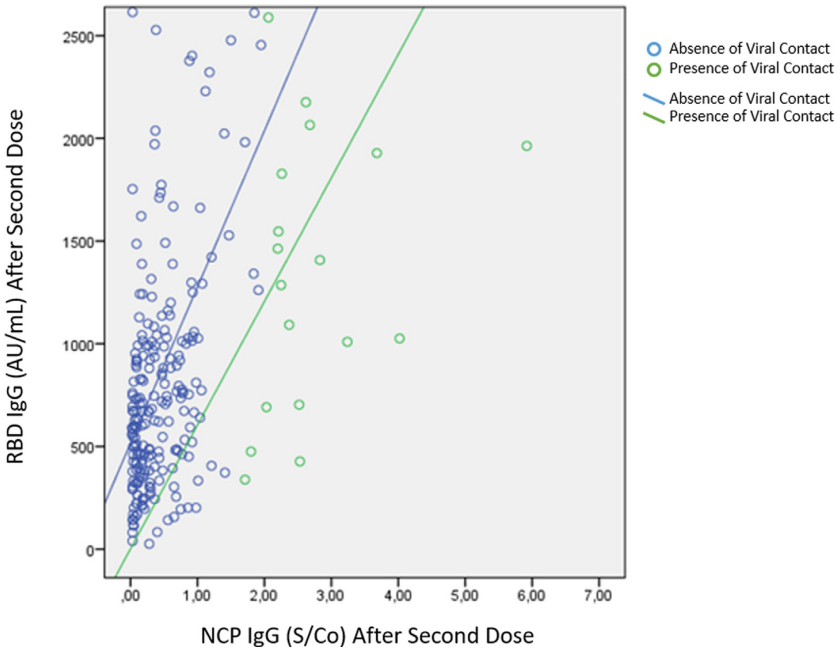

Fig. 3. SARS-CoV-II (RBD) IgG results by depending on viral contact in the Infection-Naive group.

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

Declaration of Competing Interest

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

Acknowledgment

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

Financial support

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

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

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

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

Published online: 29 Jul 2021.

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

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RESEARCH PAPER

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

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

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

ABSTRACT

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

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

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

Introduction

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

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

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

Table 1. Demographic and clinical features of study population.

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

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

Materials and methods

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

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

Results

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

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

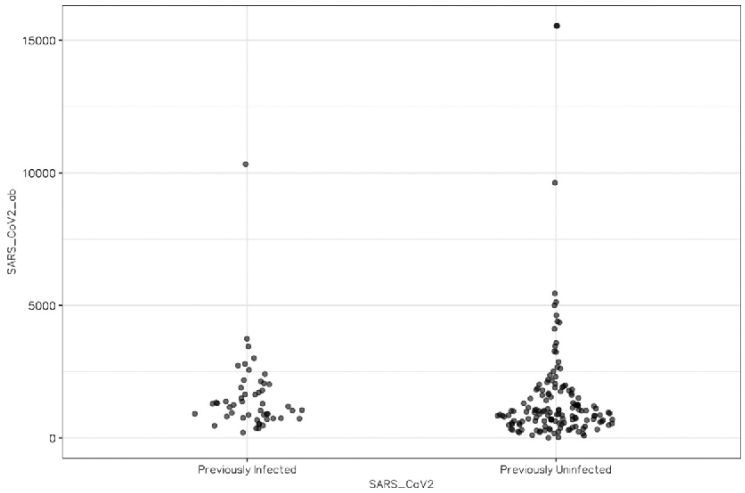

Figure 1. Anti-SARS-CoV-2 antibody responses after 2 doses of vaccine in health care workers concerning previous infection status. Anti-RBD antibody (Arbitrary unit per ml)

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

ORCID

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

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1.10 CoronaVac induz memória imunológica eficiente e semelhante à de pacientes convalescentes, mostra estudo chinês

Uma pesquisa publicada na revista Clinical Microbiology and Infection demonstrou que a CoronaVac, vacina do Butantan e da Sinovac, apresenta alta eficácia na resposta humoral (produção de anticorpos) e na resposta celular (células T CD4+ e CD8+) contra o SARS-CoV-2, e promove memória imunológica comparável à de pacientes convalescentes. O estudo foi conduzido por pesquisadores chineses da Universidade de Nanjing entre janeiro e fevereiro de 2021.

Os cientistas analisaram o perfil de resposta imune de 100 profissionais da saúde (37 homens e 63 mulheres) com idades entre 23 e 59 anos que foram vacinados com a CoronaVac. Amostras de sangue foram coletadas antes da primeira dose (T1), duas semanas após a primeira dose (T2), duas semanas após a segunda dose (T3) e oito a dez semanas após a segunda dose (T4).

Todos os participantes apresentaram soroconversão (produção de anticorpos) 14 dias após a segunda dose, sendo que 98% dos indivíduos produziram anticorpos IgG específicos contra a proteína Spike e 85% tinham anticorpos capazes de neutralizar o SARS-CoV-2.

Além disso, foram detectadas respostas potentes de células T CD4+ e CD8+ de memória, com níveis comparáveis aos encontrados em pacientes recuperados que já tiveram Covid-19. Segundo os autores, células T CD4+ e CD8+ específicas para o coronavírus já foram associadas à redução da gravidade da doença.

Os participantes do estudo também apresentaram células B de memória (produtoras de anticorpos) que foram mantidas até a análise final, oito a dez semanas após a segunda dose. Essas células são responsáveis por reconhecer os antígenos do vírus

e são capazes de reagir rapidamente à infecção.

Os pesquisadores afirmam que o estudo traz novas informações sobre a imunobiologia de vacinas de vírus inativado e pode ter implicações em estratégias vacinais no futuro. "Nós identificamos células T CD4+ de memória associadas às células B de memória específicas para proteína Spike e às células T CD8+ de memória, indicando um desenvolvimento convergente da imunidade adaptativa humoral e celular", destacam.

Fatores que interferem na resposta

Metade dos participantes recebeu a segunda dose da vacina com um intervalo de 14 a 21 dias, enquanto os outros 50 receberam a segunda dose 22 a 30 dias depois da primeira. O grupo imunizado com um intervalo maior entre as doses apresentou maiores taxas de anticorpos neutralizantes e uma maior porcentagem de células B específicas para a proteína Spike e de células de memória T CD4+ e CD8+.

A idade também influenciou a resposta imune: pessoas entre 20 e 40 anos apresentaram maiores títulos médios de anticorpos neutralizantes (GMT 42) do que indivíduos com mais de 40 anos (GMT 26). Apesar disso, ambos os grupos tinham níveis semelhantes de anticorpos IgG específicos para proteína Spike.

Publicado em: 26/10/2021

ARTICLE IN PRESS

Clinical Microbiology and Infection xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

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

Yuxin Chen [1, 5, †], Shengxia Yin [2, 5, †], Xin Tong [2, 5, †], Yue Tao [1], Jun Ni [1], Jie Pan [1], Ming Li [3], Yawen Wan [4], Minxin Mao [3], Yali Xiong [2], Xiaomin Yan [2], Yue Yang [2], Rui Huang [2, 5], Chao Wu [2, 5], Han Shen [1, *]

[1)] *Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China*
[2)] *Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China*
[3)] *Nanjing Drum Tower Hospital Clinical College of Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China*
[4)] *Department of Infectious Diseases, Nanjing Nanjing Drum Tower Hospital Clinical College of Xuzhou Medical University, China*
[5)] *Institute of Viruses and Infectious Diseases, Nanjing University, Jiangsu, China*

ARTICLE INFO

Article history:
Received 3 July 2021
Received in revised form
6 October 2021
Accepted 16 October 2021
Available online xxx

Editor: L. Kaiser

Keywords:
Adaptive responses
B cells
COVID-19
Inactivated virus vaccine
Neutralization
T cells

ABSTRACT

Objective: The dynamic adaptive immune responses elicited by the inactivated virus vaccine CoronaVac remain elusive.

Methods: In a prospective cohort of 100 healthcare professionals naïve for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) who received two doses of CoronaVac, we analysed SARS-CoV-2-specific humoral and cellular responses at four different timepoints, including before vaccination (T1), 2 weeks after the first dose (T2), 2 weeks after the booster dose (T3), and 8–10 weeks after the booster dose (T4). SARS-CoV-2-specific antibodies, serum neutralizing activities, peripheral B cells, $CD4^+$ and $CD8^+$ T cells and their memory subsets were simultaneously measured in this cohort.

Results: SARS-CoV-2 spike-specific IgG responses reached a peak (geometric mean titre (GMT) 54827, 30969–97065) after two doses and rapidly declined (GMT 502, 212–1190) at T4, whereas suboptimal IgA responses were detected (GMT 5, 2–9). Spike-specific circulating B cells (0.60%, 0.46–0.73% of total B cells) and memory B cells (1.18%, 0.92–1.44% of total memory B cells) were effectively induced at T3 and sustained over time (0.33%, 0.23–0.43%; 0.87%, 0.05–1.67%, respectively). SARS-CoV-2-specific circulating $CD4^+$ T cells (0.57%, 0.47–0.66%) and $CD8^+$ T cells (1.29%, 1.04–1.54%) were detected at T3. At T4, 0.78% (0.43–1.20%) of memory CD4+ T cells and 0.68% (0.29–1.30%) of memory CD8+ T cells were identified as SARS-CoV-2-specific, while 0.62% (0.51–0.75%) of $CD4^+$ T cells and 0.47% (0.38–0.58%) of $CD8^+$ T cells were SARS-CoV-2-specific terminally differentiated effector memory cells. Furthermore, age and interval between doses affected the magnitude of CoronaVac-induced immune responses. SARS-CoV-2 memory $CD4^+$ T cells were strongly associated with both receptor binding domain (RBD)-specific memory B cells (r 0.87, p <0.0001) and SARS-CoV-2-specific memory $CD8^+$ T cells (r 0.48, p <0.0001).

Conclusions: CoronaVac induced robust circulating and memory B cell and T cell responses. Our study offers new insight into the underlying immunobiology of inactivated virus vaccines in humans and may have implications for vaccine strategies in the future. **Yuxin Chen, Clin Microbiol Infect 2021;■:1**

Introduction

Vaccines are the cornerstone of the management of infectious disease outbreaks and the surest means to defuse pandemic risk. CoronaVac (Sinovac Biotech, China), a whole-virion chemically inactivated vaccine against severe acute respiratory syndrome

* Corresponding author: Han Shen, Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China.
E-mail address: shenhan10366@sina.com (H. Shen).
† Yuxin Chen, Shengxia Yin and Xin Tong contributed equally.

https://doi.org/10.1016/j.cmi.2021.10.006
1198-743X/

Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

coronavirus 2 (SARS-CoV-2), has so far been inoculated into at least 243 million individuals from more than 45 countries. A large, observational study in Chile indicated that two doses of CoronaVac had a vaccine effectiveness of 65.9% against coronavirus disease 2019 (COVID-19), 90.3% against intensive care unit admission and 86.3% against COVID-19-related death [1]. Nevertheless, few studies on CoronaVac recipients focused largely on binding and/or neutralizing antibodies (NAbs) as primary endpoints, while vaccine-induced cellular immune responses remain elusive.

It is well established that three fundamental components of the adaptive immune system (B cells, $CD4^+$ and $CD8^+$ T cells) are essential to control SARS-CoV-2 infection [2–7]. Despite the immune correlates of protection remaining unknown [8,9], antibodies and T-cell responses are important for the resolution of primary SARS-CoV-2 infection. Additionally, SARS-CoV-2 infection induced various immunological memory components displaying distinct kinetics [10].

Recently, we conducted a prospective, observational cohort study (NCT04729374) with 100 healthcare personnel in a tertiary hospital in Nanjing, China. Most sera elicited by two-dose CoronaVac were capable of effectively neutralize the ancestral strain, Alpha and Epsilon variants, but not Beta and Gamma variants bearing E484K mutation [11]. In this current study, we provided data from this cohort with new insights into the kinetics of vaccine-induced humoral and cellular immune responses, including circulating antibodies, antigen-specific B cells, $CD4^+$ and $CD8^+$ T cells, as well as their memory subsets at four timepoints extending up to 8–10 weeks post two-dose immunization. The impact of gender, age and interval between doses on the magnitude of vaccine responses were further analysed. The interrelationships between antibody and cellular responses were also evaluated.

Materials and methods

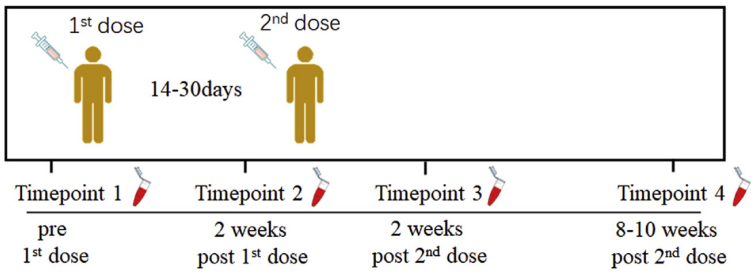
Study cohort and sample collection

A total of 100 healthcare professionals were enrolled in a prospective study (NCT04729374) from January to February 2021 in Nanjing Drum Tower Hospital. All participants tested negative for SARS-CoV-2 infection at screening and provided written informed consent. The clinical trial protocol was approved by the hospital ethics committee (2021-034-01). Two cohorts of COVID-19 convalescent patients were included, and their demographic characteristics are provided in Fig. 1. In the first cohort, serum samples were collected from 26 convalescent patients on a 4-week follow-up visit after hospital discharge, while peripheral blood mononuclear cells (PBMCs) from 12 convalescent patients were collected 16 months after COVID-19 infection in the second cohort.

SARS-CoV-2-specific humoral and cellular responses

The quantification of antigen-specific antibodies against SARS-CoV-2 and serum neutralization activities were performed as previously described [11,12]. Fluorescence-labelled ectodomain of the spike or receptor binding domain (RBD) proteins were used as probes to identify SARS-CoV-2-specific B cells and memory B cells. PBMCs were stimulated with SARS-CoV-2 peptide pools to measure antigen-specific $CD4^+$ and $CD8^+$ T cells. The details of peptide pools,

A



B

variables(n[%] or median[IQR]	COVID-19 vaccinee cohort (n=100)	convalescent COVID-19 patient cohort 1 (n=26)	convalescent COVID-19 patient cohort 2 (n=12)
Sex			
Male	37 (37%)	14 (53.8%)	6 (50%)
Female	63 (63%)	12 (46.2%)	6 (50%)
Age (yr)	35 (28, 41)	47 (34, 58)	50 (28, 59)
Age group, years			
18-30	35 (36%)	3 (11.5%)	2 (16.7%)
31-40	39 (38%)	4 (15.4%)	2 (16.7%)
41-50	16 (16%)	7 (26.9%)	2 (16.7%)
51-60	10 (10%)	4 (15.4%)	6 (50%)
Sample types	serum and PBMC	serum	PBMC
Sample collection data	Jan-May 2021	March-April 2020	May-Jun 2021
Disease onset time	NA	Jan-Feb 2020	Jan-Feb 2020
Disease severity			
Severe	NA	7 (26.9%)	2 (16.7%)
Non-severe	NA	19 (73.1%)	10 (83.3%)
Co-morbidities	2 (2%)	4 (15.4%)	0 (0%)

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

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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).

Immunoglobin (Ig) isotypes among the antigen-specific memory B-cell population shifted with time (Fig. 3A,B). After primary immunization, ~23% of RBD-specific memory B cells were IgG^+ and ~22% were IgM^+. The frequency of IgG^+ memory B cells surged to ~45% following the second dose, and slightly increased to ~55% 8–10 weeks after full vaccination. RBD-specific IgA^+ memory B-cell frequency was ~13% at both T2 and T3 and slightly increased to ~22% at T4.

B-cell analyses were extended to *in vitro* stimulation of memory B cells which differentiate into antibody-secreting cells (ASCs) by ELISPOT assay among a small portion of participants (Fig. 3D). The first dose induced positive spike-specific and RBD-specific B cells in 38.9% (21/54) and 22.2% (12/54) of subjects, respectively. The second dose further boosted spike-specific and RBD-specific antibody-secreting B cells in 57.7% (15/26) and 57.7% (15/26) of subjects, respectively. The frequency of spike-specific and RBD-specific memory B cells was stable at T4, and were detected in 70.2% (33/47) and 87.2% (41/47) of subjects.

The magnitude of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cell responses

SARS-CoV-2-specific T-cell responses were analysed by *ex vivo* stimulation with SARS-CoV-2 peptide pools covering the most commonly recognized T-cell epitopes [4], including S, M, E, N, ORF3a and ORF7/8 (Supplementary Material Fig. S4). Robust expanded SARS-CoV-2-specific $CD4^+$ T cells were detectable in 61.5% (48/78), 74.2% (69/93) and 75.0% (60/80) of the subjects at T2, T3 and T4, respectively (Fig. 4A). SARS-CoV-2-specific $CD4^+$ T-cell responses were also significantly elevated after the primary immunization (0.57%, 0.47–0.66%) compared to that at T1 (0.08%, 0.02–0.27%). The specific $CD4^+$ T cells (0.83%, 0.67–1.00%) elicited after two doses remained stable at T4 (1.22%, 0.96–1.48%).

Minimal circulating SARS-CoV-2 $CD8^+$ T-cell responses (0.06%, 0.05–0.07%) were detected at T1 baseline (Fig. 4B); 80% (52/65) of participants had detectable SARS-CoV-2 $CD8^+$ T-cell responses (0.69%, 0.54–0.84%) at T2. The boosting immunization induced 83.9% (78/93) of subjects with positive SARS-CoV-2 $CD8^+$ T-cell responses (1.29%, 1.04–1.54%), which steadily increased to 1.61% (1.21–2.02%) at T4. Spike-specific $CD4^+$ or $CD8^+$ T cells displayed a similar kinetic to the SARS-CoV-2-specific $CD4^+$ or $CD8^+$ T cells. Interestingly, CoronaVac also induced $CD4^+$ and $CD8^+$ T-cell responses specific to HCoV-OC43 and HCoV-299E spike glycoprotein (Supplementary Material Fig. S5).

At T4, 0.78% (0.43–1.20%) of memory $CD4^+$ T cells and 0.68% (0.29–1.30%) of memory $CD8^+$ T cells were identified as SARS-CoV-2-specific (Fig. 4C). Vaccinees had similar magnitudes of SARS-CoV-2-specific memory $CD4^+$ T cells, $CD8^+$ T cells and spike-specific memory $CD4^+$ T cells, but a lower level of spike-specific memory $CD8^+$ T cells, compared to convalescent donors. The majority of virus-specific $CD8^+$ T cells were identified as CD45RA CCR7 effector memory (T_{EM}) or $CD45RA^+CCR7$ terminally differentiated effector (T_{EMRA}) [13,14]. Among vaccinees at T4, 0.62% (0.51–0.75%) and 0.43% (0.30–0.57%) of $CD4^+$ T cells were SARS-CoV-2-specific T_{EMRA} and T_{EM}, respectively (Fig. 4D), whereas 0.48% (0.38–0.58%) and 0.79% (0.66–0.92%) of $CD8^+$ T cells were SARS-CoV-2-specific T_{EMRA} and T_{EM}, respectively (Fig. 4E). Convalescent patients displayed a similar proportion of virus-specific T_{EMRA} and T_{EM} as the vaccinees. Our data suggest that CoronaVac effectively induced virus-specific memory $CD4^+$ T cells and $CD8^+$ T cells as well as effector populations.

Factors associated with adaptive responses to SARS-CoV-2 inactivated virus vaccine

There were no relationships identified between gender and the magnitude of SARS-CoV-2-specific adaptive responses (Fig. 5A). Consistent with a previous report [15], the participants between 20 and 40 years old had significantly higher neutralizing titres (GMT 42, 33–52) against the ancestral strain, compared to the participants between 40 and 60 years old (GMT 26, 19–37) (Fig. 5B and Supplementary Material Fig. S6A). Despite the fact that young participants had a higher magnitude of serum neutralizing activities than elder individuals, both groups had a comparable level of anti-spike IgG, suggesting potential qualitative differences in spike-

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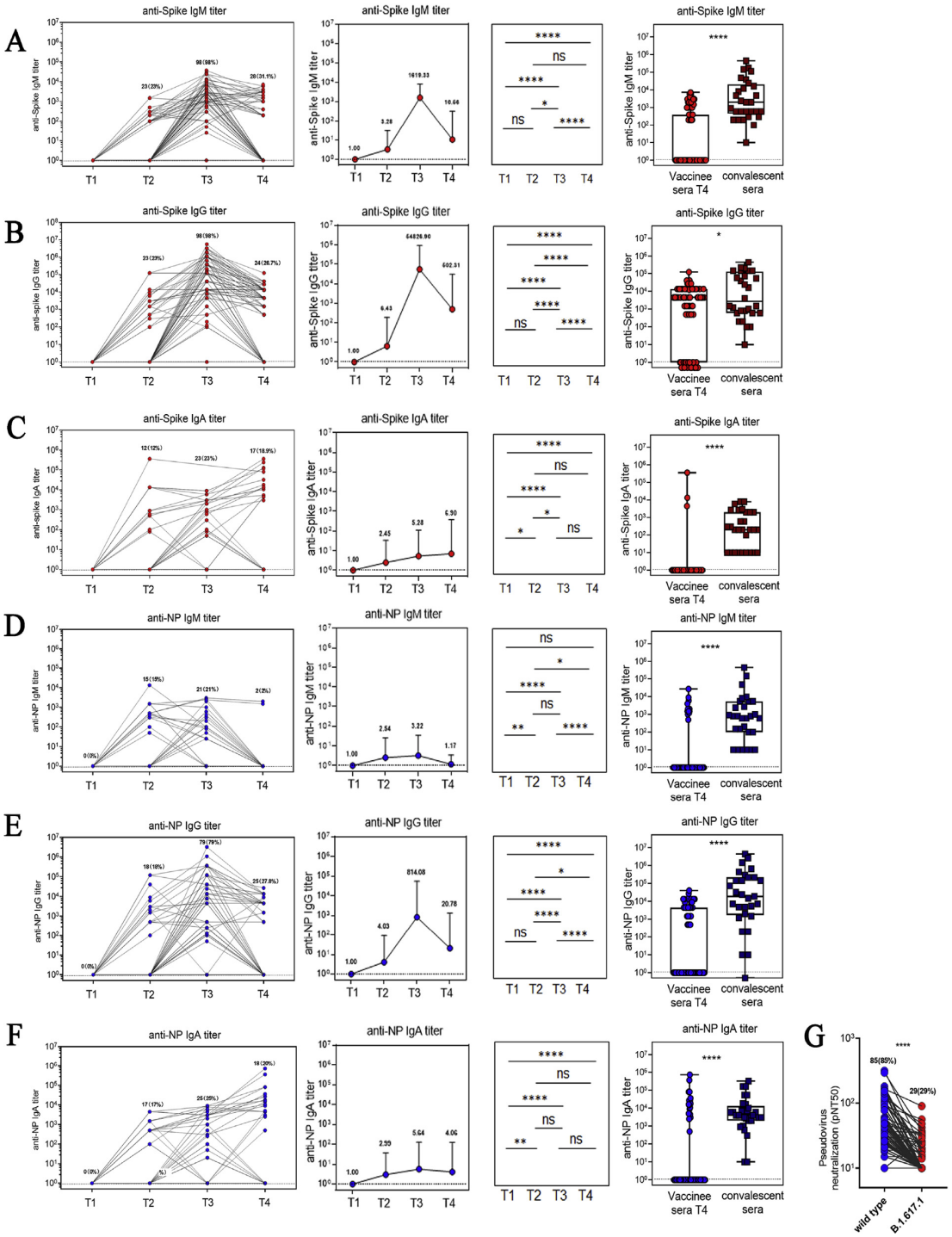

Fig. 2. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibody responses following CoronaVac immunization. (A–F) Dynamic antibody titres for (A) anti-spike IgM, (B) anti-spike IgG, (C) anti-spike IgA, (D) anti-NP IgM, (E) anti-NP IgG, and (F) anti-NP IgA at four time points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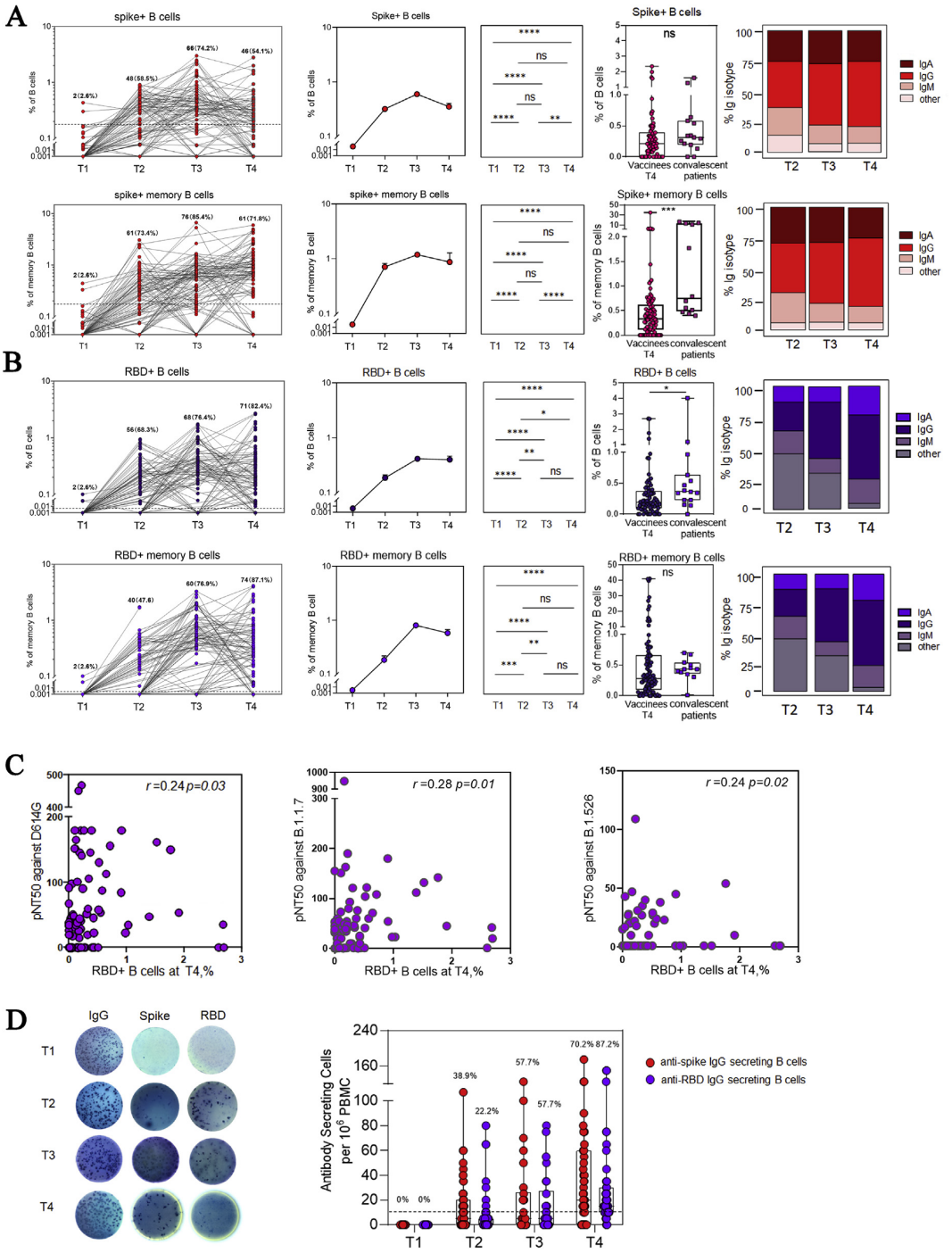

Fig. 3. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific circulating B cell and memory B cell responses following CoronaVac immunization. (A) Frequency of spike$^+$ B cells and spike$^+$ memory B cells over time in vaccinees. Frequency of spike$^+$ B cells or memory spike$^+$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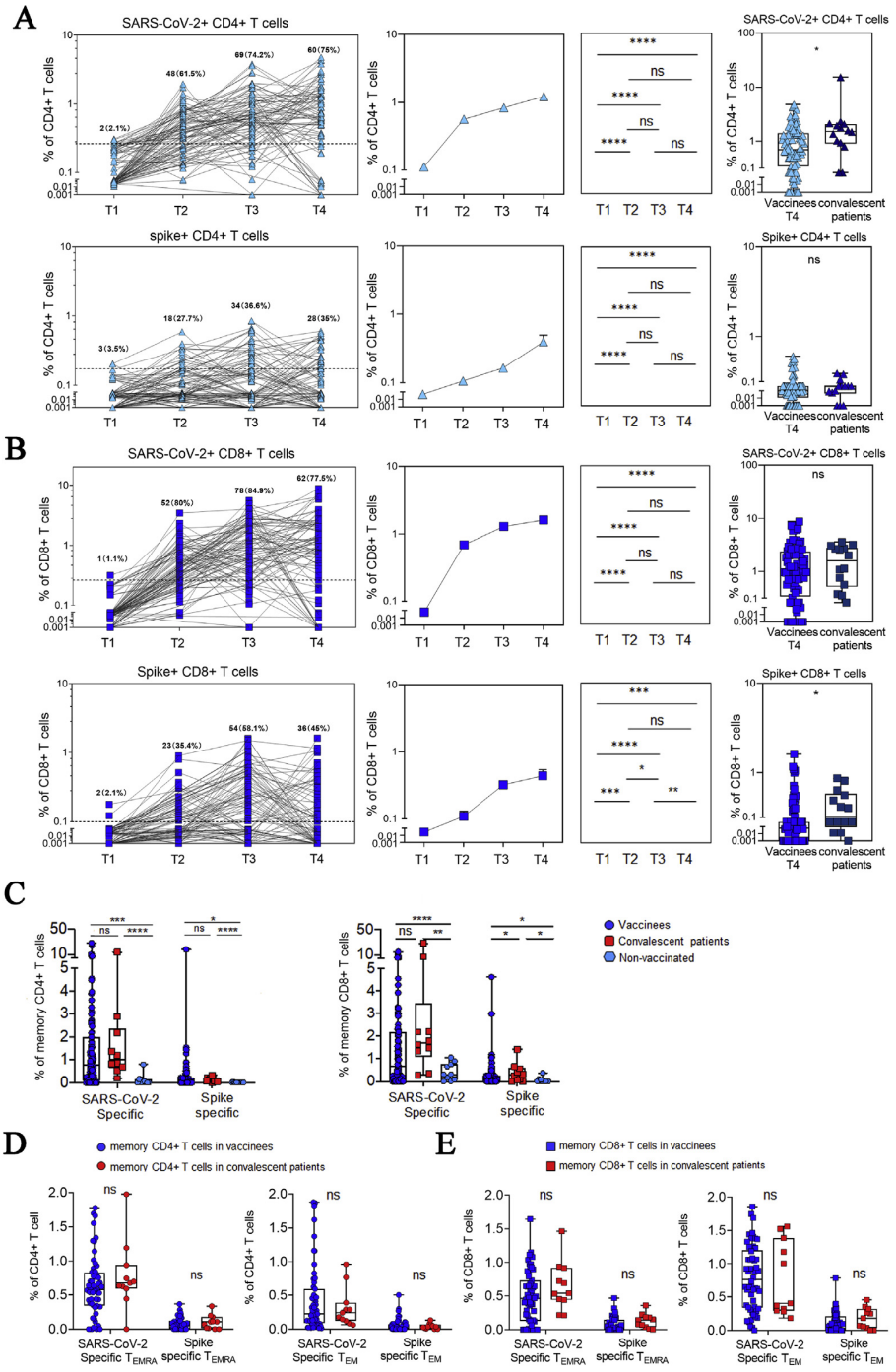

Fig. 4. Dynamic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific circulating $CD4^+$ and $CD8^+$ T cell responses following CoronaVac immunization. (A) Frequency of SARS-CoV-2-specific (top) and spike-specific (bottom) $CD4^+$ T cells over time among vaccinees, the magnitude of which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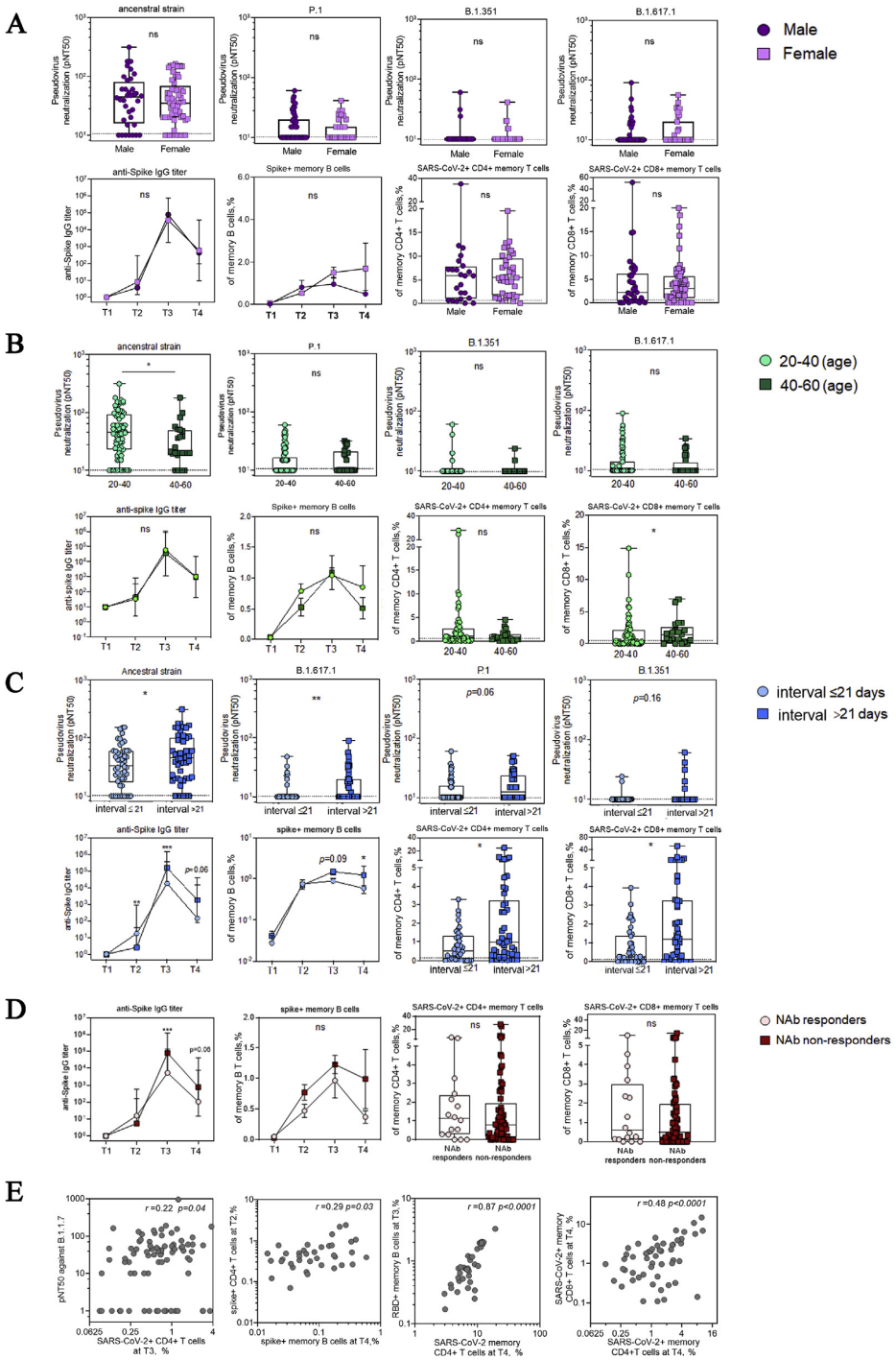

Fig. 5. Association of various factors with vaccine-elicited adaptive responses. (A-C) Serum titres that achieved 50% pseudovirus neutralization (pNT50) against the ancestral strain, the P.1, the B.1.351, the B.1.617.1, anti-spike IgG titre, the frequency of spike-specific memory B cells, the frequency of SARS-CoV-2-specific memory $CD4^+$ and $CD8^+$ T cells compared with (A) gender, (B) age, and (C) interval between doses. (D) Anti-spike IgG titre, the frequency of spike-specific memory B cells, and the frequency of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ memory T cells among neutralizing antibody (NAb) responders versus NAb non-responders. (E) Correlation analysis of pNT50 against B.1.1.7 and SARS-CoV-2-specific $CD4^+$ T cells at T3 timepoint, correlation analysis of spike-specific memory B cells at T4 and spike-specific $CD4^+$ T cells at T2, correlation analysis of RBD^+ memory B cells at T3 and SARS-CoV-2 memory $CD4^+$ T cells at T4, and correlation analysis of SARS-CoV-2-specific memory $CD8^+$ T cells at T4 and SARS-CoV-2-specifc memory $CD4^+$ T cells responses at T4. Statistics were analysed using unpaired Wilcoxon test between groups. * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001, ns, no significant difference.

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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 dpike-specific memory B cells, SARS-CoV-2 memory $CD4^+$ and $CD8^+$ T cells were detected at a similar magnitude as those in NAb responders. Whether these specific $CD4^+$ and $CD8^+$ T cells could also serve as surrogates for protective immunity remains to be determined. Meanwhile, we also identified SARS-CoV-2 memory $CD4^+$ T cells strongly associated with RBD-specific memory B cells as well as SARS-CoV-2 memory $CD8^+$ T cells, indicating a convergent development of humoral and cellular adapative immunity.

This study has some limitations. The follow-up observation time in our study was relative short, only extending up to 8–10 weeks post full vaccination. Besides, the alternative function of vaccine-elicited antibody such as antibody-dependent cell-mediated cytotoxicity (ADCC) [27] were not evaluated.

In summary, this study demonstrated multiple compartments of adaptive immunity elicited by an authorized inactivated vaccine in an integrated manner. Our study offers insight into the underlying immunobiology of inactivated virus vaccines in humans and may have implications for vaccine strategies in the future.

Author contributions

CW, HS and YC designed the study. YC, YT, YY and YX recruited the patients. JP and JN processed the blood samples, ML, YS and YW performed cellular analysis. TX and MM performed the antibody assay. RH, XY and HS analysed and interpreted the data. YC, SY and TX wrote the manuscript. All the authors revised the manuscript.

Transparency declaration

The authors declare that they have no conflicts of interest. This study was supported by Clinical Trials from the Affiliated Drum Tower Hospital, Medical School of Nanjing University (2021-LCYJ-PY-10), Nanjing Medical Science and Technique Development Foundation (QRX17141, YKK19056, YKK20058 and YKK20076), National Natural Science Foundation of China (82002133), and

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Fundamental Research Funds for the Central Universities (14380459).

Acknowledgements

We thank all vaccine recipients for providing serum samples.

Appendix A. Supplementary data

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

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Please cite this article as: Chen Y et al., Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2021.10.006

2. Combate as variantes do coronavírus

2.1 CoronaVac tem eficácia superior a 75% contra variantes alfa, gama e delta; apenas 2% dos chilenos vacinados na fase 3 desenvolveram Covid-19

Duas pesquisas publicadas nas últimas semanas por cientistas chilenos dão provas de que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, é eficiente no combate à Covid-19 e eficaz contra as novas variantes do SARS-CoV-2. No primeiro estudo, os indicadores de anticorpos neutralizantes gerados pelo imunizante foram acima de 97% contra a cepa original do vírus, acima de 80% contra as variantes alfa e gama e acima de 75% contra a variante delta. No segundo estudo, a eficácia da CoronaVac para evitar o desenvolvimento de casos de Covid-19 foi superior a 90% em um grupo de mais de duas mil pessoas.

Ambas pesquisas são de autoria de cientistas da Pontifícia Universidade Católica do Chile, do Instituto de Saúde Pública do Chile e da Universidade do Chile e foram publicadas na revista científica Frontiers of Immunology. A importância dos estudos se deve ao fato de que a vacinação no país andino foi feita preponderantemente com a CoronaVac, com 70% das pessoas recebendo o imunizante do Butantan. Até a última sexta (29), o governo chileno havia vacinado 97,1% da população, utilizando 36,8 milhões de doses em um esquema vacinal que incluiu dose de reforço.

Eficácia da CoronaVac contra as variantes do SARS-CoV-2

De acordo com o estudo Recognition of variants of concern by antibodies and T cells induced by a SARS-CoV-2 inactivated vaccine, publicado de forma preliminar em 21/10, a CoronaVac promoveu a secreção de anticorpos capazes de bloquear o domínio receptor-obrigatório (RBD, do inglês receptor-binding domain, partes específicas do coronavírus que lhe permitem invadir e infectar células humanas) de todas as variantes de preocupação do SARS-CoV-2. As taxas de soropositividade de anticorpos neutralizantes registradas foram acima de 97% para a cepa original, de mais de 80% para as variantes alfa e gama, de mais de 75% para a variante delta e de mais de 60% para a variante beta.

Para fazer essa análise, os pesquisadores avaliaram os voluntários inscritos no ensaio clínico de fase 3 que foram imunizados com duas

doses de CoronaVac no Chile. Após a administração da segunda dose, foram coletadas amostras de soro para medir a capacidade de neutralização de anticorpos contra as variantes de preocupação. "É importante ressaltar que, após a infecção por SARS-CoV-2, a capacidade de bloqueio de anticorpos de voluntários vacinados aumentou para todas as variantes testadas", ressaltaram os cientistas. Segundo eles, a imunização com CoronaVac em qualquer esquema estimula respostas celulares contra todas as variantes de preocupação e contribui para neutralizar a infecção causada pelo vírus.

Entre 2.263 chilenos vacinados com CoronaVac, apenas 45 desenvolveram Covid-19

Já o estudo Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination with an inactivated SARS--CoV-2 Vaccine, publicado no final de setembro, avaliou a segurança, a imunogenicidade e a eficácia da CoronaVac para evitar casos graves de Covid-19. Dos 2.263 indivíduos totalmente vacinados no final de junho de 2021, apenas 45 (ou seja, 1,99%) apresentaram sintomas de infecção sintomática decorridos 14 dias ou mais da segunda dose.

Destes 45, 43 desenvolveram quadros leves. As exceções foram dois casos de homens com mais de 60 anos. O primeiro deles, um homem de 62 anos com duas comorbidades (hipotireoidismo e obesidade), desenvolveu um quadro moderado e necessitou de oxigenação suplementar. O segundo, um homem de 69 anos com quatro comorbidades (obesidade, hipertensão, aorta bicúspide e fibrilação atrial), evoluiu para um quadro de maior gravidade e precisou de ventilação mecânica. Ambos se restabeleceram e passam bem.

Os pesquisadores salientaram que a vacinação com CoronaVac é eficaz. "Os casos da doença foram em sua maioria leves e não necessariamente se correlacionaram à falta de imunidade induzida pela vacina, sugerindo que outros fatores, a serem definidos em estudos futuros, poderiam levar à infecção sintomática após a vacinação com CoronaVac."

Publicado em: 10/11/2021

frontiers in Immunology

ORIGINAL RESEARCH
published: 09 November 2021
doi: 10.3389/fimmu.2021.747830

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

Felipe Melo-González[1,2†], Jorge A. Soto[1,2†], Liliana A. González[1,2†], Jorge Fernández[3†], Luisa F. Duarte[1,2†], Bárbara M. Schultz[1,2†], Nicolás M. S. Gálvez[1,2†], Gaspar A. Pacheco[1,2], Mariana Ríos[1,2], Yaneisi Vázquez[1,2], Daniela Rivera-Pérez[1,2], Daniela Moreno-Tapia[1,2], Carolina Iturriaga[4], Omar P. Vallejos[1,2], Roslye V. Berríos-Rojas[1,2], Guillermo Hoppe-Elsholz[1,2], Marcela Urzúa[4], Nicole Bruneau[3], Rodrigo A. Fasce[3], Judith Mora[3], Alba Grifoni[5], Alessandro Sette[5,6], Daniela Weiskopf[5], Gang Zeng[7], Weining Meng[7], José V. González-Aramundiz[8], Pablo A. González[1,2], Katia Abarca[1,4], Eugenio Ramírez[3], Alexis M. Kalergis[1,2,9*] and Susan M. Bueno[1,2*]*

[1] Millennium Institute on Immunology and Immunotherapy, Pontificia Universidad Católica de Chile, Santiago, Chile, [2] Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile, [3] Departamento de Laboratorio Biomédico, Instituto de Salud Pública de Chile, Santiago, Chile, [4] Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile, [5] Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), La Jolla, CA, United States, [6] Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego (UCSD), La Jolla, CA, United States, [7] Sinovac Biotech, Beijing, China, [8] Departamento de Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile, [9] Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus responsible of the current pandemic ongoing all around the world. Since its discovery in 2019, several circulating variants have emerged and some of them are associated with increased infections and death rate. Despite the genetic differences among these variants, vaccines approved for human use have shown a good immunogenic and protective response against them. In Chile, over 70% of the vaccinated population is immunized with CoronaVac, an inactivated SARS-CoV-2 vaccine. The immune response elicited by this vaccine has been described against the first SARS-CoV-2 strain isolated from Wuhan, China and the D614G strain (lineage B). To date, four SARS-CoV-2 variants of concern described have circulated worldwide. Here, we describe the neutralizing capacities of antibodies secreted by volunteers in the Chilean population immunized with CoronaVac against variants of concern Alpha (B.1.1.7), Beta (B.1.351) Gamma (P.1) and Delta (B.617.2).

Methods: Volunteers enrolled in a phase 3 clinical trial were vaccinated with two doses of CoronaVac in 0-14 or 0-28 immunization schedules. Sera samples were used to evaluate the capacity of antibodies induced by the vaccine to block the binding between Receptor Binding Domain (RBD) from variants of concern and the human ACE2 receptor by an in-house ELISA. Further, conventional microneutralization assays were used to test neutralization of SARS-CoV-2 infection. Moreover, interferon-γ-secreting T cells

OPEN ACCESS

Edited by:
Xuguang (Sean) Li,
Health Canada, Canada

Reviewed by:
Sathya Narayanan Thulasi Raman,
Health Canada, Canada
Robert J. Visalli,
Mercer University, United States

***Correspondence:**
Eugenio Ramírez
eramirez@ispch.cl
Alexis M. Kalergis
akalergis@bio.puc.cl
Susan M. Bueno
sbueno@bio.puc.cl

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Vaccines and Molecular Therapeutics, a section of the journal Frontiers in Immunology

Received: 26 July 2021
Accepted: 21 October 2021
Published: 09 November 2021

Citation:
Melo-González F, Soto JA, González LA, Fernández J, Duarte LF, Schultz BM, Gálvez NMS, Pacheco GA, Ríos M, Vázquez Y, Rivera-Pérez D, Moreno-Tapia D, Iturriaga C, Vallejos OP, Berríos-Rojas RV, Hoppe-Elsholz G, Urzúa M, Bruneau N, Fasce RA, Mora J, Grifoni A, Sette A, Weiskopf D, Zeng G, Meng W, González-Aramundiz JV, González PA, Abarca K, Ramírez E, Kalergis AM and Bueno SM (2021) Recognition of Variants of Concern by Antibodies and T Cells Induced by a SARS-CoV-2 Inactivated Vaccine. Front. Immunol. 12:747830. doi: 10.3389/fimmu.2021.747830

against Spike from variants of concern were evaluated in PBMCs from vaccinated subjects using ELISPOT.

Results: CoronaVac promotes the secretion of antibodies able to block the RBD of all the SARS-CoV-2 variants studied. Seropositivity rates of neutralizing antibodies in the population evaluated were over 97% for the lineage B strain, over 80% for Alpha and Gamma variants, over 75% for Delta variant and over 60% for the Beta variant. Geometric means titers of blocking antibodies were reduced when tested against SARS-CoV-2 variants as compared to ancestral strain. We also observed that antibodies from vaccinated subjects were able to neutralize the infection of variants D614G, Alpha, Gamma and Delta in a conventional microneutralization assay. Importantly, after SARS-CoV-2 infection, we observed that the blocking capacity of antibodies from vaccinated volunteers increased up to ten times for all the variants tested. We compared the number of interferon-γ-secreting T cells specific for SARS-CoV-2 Spike WT and variants of concern from vaccinated subjects and we did not detect significant differences.

Conclusion: Immunization with CoronaVac in either immunization schedule promotes the secretion of antibodies able to block SARS-CoV-2 variants of concern and partially neutralizes SARS-CoV-2 infection. In addition, it stimulates cellular responses against all variants of concern.

Keywords: CoronaVac, SARS-CoV-2, antibodies, vaccine, variants of concern, T cell immunity

INTRODUCTION

SARS-CoV-2 represents a global threat to public health and has been responsible for over 4 million deaths worldwide to date (1). After the spread of the original wild-type SARS-CoV-2 strain, multiple mutants have arisen around the world. Most of these circulating variants belong to the SARS-CoV-2 lineage B, in particular lineage B.1 (2). One of the most prevalent strains is the D614G, which displays a mutation in the C-terminal region of the Spike 1 (S1) domain outside the Receptor Binding Domain (RBD) (2). Although this mutant has been reported to be more infective, sera from convalescent patients and subjects vaccinated with mRNA vaccines are able to neutralize the D614G mutant to an extent similar to that of the ancestral strain, i.e. lineage B or wild type strain (2–5).

Current vaccination programs around the world are facing the threat of these circulating variants of concern of SARS-CoV-2, as they exhibit different mutations in the RBD and may evade antibody neutralization (2). To facilitate their identification, variants of concern are currently termed Alpha (B.1.1.7), Beta (B1.351), Gamma (P.1), and Delta (B.617.2) (6). Alpha (first identified in the UK), Beta (first identified in South Africa) and Gamma (first identified in Brazil) mutants share the N501Y mutation that has been linked with increased affinity of the Spike protein for the endogenous receptor human Angiotensin-converting enzyme 2 (hACE2) (7). Beta and Gamma mutants exhibit the E484K mutation, associated with an increased evasion of neutralizing antibodies (8–10). Furthermore, Beta and Gamma exhibit mutations in the residue K417 of the RBD but differ in the amino acid substitutions (K417N for Beta and K417T for Gamma), which may affect antibody binding (6). In addition, the Delta variant (first identified in India) is currently a cause of concern due to its high transmissibility and may even surpass other variants in this regard (11). Delta exhibits unique mutations (L452R, T478K and P681R), which may increase viral infectivity and viral fusion (12, 13). Considering the increased infectivity and death rates described for these variants, it is crucial to understand whether vaccination can induce protection against them (6).

Chile is among the countries with the highest percentage of vaccination worldwide (over 56% of the total population), and CoronaVac, an inactivated SARS-CoV-2 vaccine, represents 78.2% of the immunized population (14). A phase 3 clinical trial is being conducted in Chile, with two vaccination schedules: two doses separated by 14 days (0-14) or by 28 days (0-28), and the general population has received the latter schedule. CoronaVac is safe and induces humoral and cellular responses in vaccinated subjects from different age groups, and has been proven effective in remarkably reducing hospitalizations and death rates (15, 16). Here, we evaluate the blocking and neutralizing capacities of circulating antibody induced by CoronaVac in vaccinated volunteers for both schedules against the most prevalent variants in Chile. Blocking capacities against the RBD of variants Alpha, Beta, Gamma and Delta were tested with an in-house surrogate neutralization test (sVNT) and compared to the wild strain, included in the vaccine formulation. The neutralizing capacities of antibody were evaluated using a conventional plaque-reduction neutralization test (cVNT) for the D614G, Alpha, Gamma and Delta variants. Our data shows that vaccinated volunteers exhibit circulating

antibodies with neutralizing capacities against the different variants of concern, with a better response against the Alpha and Gamma variants, although inhibition of the binding between hACE2 and RBD from the Beta variant was also detected using sVNT. We also observed that CoronaVac promotes Interferon-γ (IFN-γ)-producing $CD4^+$ T cells against Spike peptides from variants of concern. These results suggest that the antibodies and cellular responses induced by the administration of two doses of CoronaVac would have a protective role against the several circulating variants of concern of SARS-CoV-2.

METHODS

Study Design and Volunteers

The clinical trial (clinicaltrials.gov NCT04651790) was conducted in Chile at eight different sites and evaluated two immunization schedules. This trial was approved by each Institutional Ethical Committee and the Chilean Public Health Institute (#24204/20) and conducted according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki (17), and local regulations. Volunteers were inoculated with either two doses of 3 μg (600SU) of CoronaVac at 0- and 14-days or 0- and 28-days post the first immunization (p.i.). Written informed consent was obtained from each participant. Exclusion criteria included history of confirmed symptomatic SARS-CoV-2 infection, pregnancy, allergy to vaccine components, and immunocompromised conditions. A complete list of inclusion and exclusion criteria has been published previously (15). A total of 2,302 volunteers were enrolled by March 19th, 2021, and a subgroup of 440 volunteers was chosen to evaluate their immune response. Demographic information, co-morbidities, nutritional status, immunization schedule, and dates of vaccination, were obtained at enrolment for all volunteers.

Procedures

Sera samples from the 0-14 and 0-28 immunization schedules were chosen among those that were previously confirmed as positive against wild-type SARS-CoV-2 through commercial kits (GenScript #L00847-A and BioHermes #COV-S41). A total of 42 samples (22 samples from the 0-14 schedule and 20 from the 0-28 schedule) were evaluated by sVNT. A total of 52 samples (34 samples from the 0-14 schedule and 18 samples from the 0-28 schedule) were evaluated by cVNT. Both groups included volunteers aged 18 to 59 years and over 60 years.

To assess the capacity of the antibodies against SARS-CoV-2 circulating variants of concern to inhibit RBD and hACE2 interaction in the samples from vaccinated volunteers, we performed in-house SARS-CoV-2 sVNT based on previous reports (18). RBD unconjugated proteins from wild-type (WT) SARS-CoV-2 (GenScript #Z03483) and the variants B.1.1.7 (GenScript #Z03533), B.1.351 (GenScript #Z03537) P.1 (SinoBiological #40592-V08H86) and B.1.617.2 (GenScript #Z03613) were conjugated to HRP using the HRP Conjugation Kit - Lightning Link (#ab102890) in a 2:1 mass ratio (HRP to RBD) following the instructions of the manufacturer. ELISA 96-well plates (SPL) were pre-coated with 100 ng per well of the recombinant hACE2 protein (GenScript #Z03484) in 50 μL of 100 mM carbonate–bicarbonate coating buffer (pH 9.6) ON at 4°C. Plates were then washed three times with PBS - 0.05% Tween 20 and blocked with PBS - 10% FBS for 2h at RT. The HRP-RBD conjugates obtained previously were then incubated with the serum sample in a final volume of 120 μL for 1 h at 37°C. Concentration of conjugates used were as follows: 3 ng of WT SARS-CoV-2, 0.75 ng of B.1.1.7, 3 ng of B.1.351, 3 ng of P.1 and 3 ng of B.1.617.2. Then, these mixtures were added into the 96-well plates coated with hACE2 and were incubated for 1 h at RT. Unbound HRP-RBD were removed washing five times with PBS - 0.05% Tween 20. Then, 50 μL of 3,3',5,5'-tetramethylbenzidine (TMB – BD #555214) was added. An equal volume of 2 N H_2SO_4 was added to stop the reaction, and optical densities (OD) values at 450 nm were read. The antibody titer was determined as the last fold-dilution with a cut-off value over 20% of inhibition. The percentage of inhibition was defined as: [OD_{450nm} value of negative control-OD_{450nm} value of sample]/[OD_{450nm} value of negative control*100]. Negative controls (corresponding to sera sample obtained before immunization) were included. For the cVNT, sera samples were two-fold serially diluted starting at a 4-fold dilution until a 512-fold. Then, samples were incubated for 1 h at 37°C with an equal volume of a SARS-CoV-2 33782CL-SARS-CoV-2 strain (lineage B, D614G), Alpha (B.1.1.7), Gamma (P.1) and Delta (B.1.617.2) variants. These variants were previously isolated by the Institute of Public Health of Chile from clinical samples. These mixtures were inoculated on confluent Vero E6 cell monolayers (ATCC CRL-1586) and cytopathic effect (CPE) was evaluated seven days later. Sera samples from uninfected patients (negative controls) and sera samples from confirmed COVID-19 patients (positive controls) were included. Plaque forming units were quantified by direct visualization and the titer of neutralizing antibodies was defined as the highest serum dilution that neutralized 100% of virus infection. Seropositivity rates were calculated as the percentage of the population evaluated that showed end titers ≥1/4 in both techniques.

To assess the cellular immune response, ELISPOT assays were performed using PBMCs from 18 participants, as described previously, using the human IFN-γ/interleukin-4 (IL-4)double-color ELISPOT (Immunospot) (15). Cells were stimulated for 48h in the presence of Mega Pools (MPs) of peptides derived from SARS-CoV-2 Spike WT, Alpha, Beta, Gamma and Delta at 37°C, 5% CO_2. As positive controls, an independent stimulation performed with 5 mg/mL of Concanavalin A (ConA) (Sigma Life Science #C5275-5MG) and with an MP of peptides derived from cytomegalovirus proteins (MP-CMV) for the stimulation of both $CD4^+$ and $CD8^+$ T cells. As a vehicle control, DMSO 1% (Merck #317275) was included. Spot Forming Cells (SFCs) were counted on an ImmunoSpot® S6 Micro Analyzer.

Statistical Analysis

Statistical differences were evaluated by Wilcoxon tests (for comparisons between two groups). Differences were considered

significant if the p value was under 0.05. All data were analyzed with GraphPad Prism 9.0.1.

RESULTS

To assess whether volunteers from the Phase 3 clinical trial being held in Chile exhibited antibodies able to inhibit the RBD of SARS-CoV-2 circulating variants of concern, we performed an in-house sVNT designed to evaluate the inhibition of the interaction between hACE2 and RBD, which has been previously shown to correlate with neutralizing antibodies (15, 18). Samples from volunteers immunized with two doses of CoronaVac in a 0-14 or 0-28 immunization schedule were tested. Levels of antibodies able to inhibit the interaction between hACE2 and RBD from circulating SARS-CoV-2 variants of concern combining both 0-14 and 0-28 immunization schedules are shown in **Figure 1A**. We report a 1.8-fold reduction of antibody titers that inhibit the variant Alpha, a 5.9-fold reduction of titers against the variant Beta, a 3-fold reduction of titers against the variant Gamma, and a 3.5-fold reduction of titers against the variant Delta, as compared to the WT strain. These reductions were associated with a decrease in GMT values, i.e., 29.5 (95% CI 20.1-43) for the WT strain, 16.0 (95% CI 10.9-23.5) for Alpha, 5.0 (95% CI 3.8-6.7) for Beta, 9.8 (95% CI 6.9-13.9) for Gamma, and 8.5 (95% CI 6.1-11.9) for Delta. Reductions seen for variants Beta, Gamma, and Delta were detected in both age groups. Interestingly, participants aged 18-59 years did not exhibit significant differences in the level of antibodies inhibiting the WT strain and the Alpha variant (**Supplementary Figure 1**). The seropositivity rate of the neutralizing antibodies in the population evaluated was 100% for the WT strain and 88.1%, 64.2%, 88.1% and 78.6% for Alpha, Beta, Gamma, and Delta, respectively.

For the 0-14 immunization schedule, antibodies that inhibit the variants Alpha, Beta, and Gamma were measured 28 days after administration of the second dose. GMTs of antibodies able to inhibit the RBDs (**Figure 1B**) are lower compared to the wild-type strain (17.6, 95% CI 10.2-30.1) and the lowest reported value were against the Beta variant (GMT 4.8, 95% CI 3.1-7.4, a 3.6-fold reduction) and Delta variant (GMT 7.8, 95% CI 4.7-12.9, a 2.3-fold reduction). In contrast, similar GMT values were found for the Alpha and Gamma variants (12.8, 95% CI 7.7-21.5 and 12.4, 95% CI 7.3-21.2, respectively). Similar values were found when samples were analyzed according to their age group, although volunteers aged 18 to 59 years old exhibited a significant decrease in antibodies against the Beta RBD and Delta RBD whereas volunteers over 60 years only exhibit a significant decrease against the Beta RBD (**Supplementary Figures 2A, B**). The seropositivity rate was 95.45% of the evaluated volunteers exhibiting neutralizing antibodies against the WT strain, while the percentages against the Alpha, Beta, Gamma and Delta variants were 86.36%, 63.64%, 86.36%, and 72.72%, respectively.

For volunteers of the 0-28 immunization schedule, increased GMT values in antibodies able to block the RBDs were found against the WT strain (52.0, 95% CI 33.2-81-3) compared to the GMTs for the WT strain observed in the 0-14 schedule, as observed in Fig 1C. These GMT values decreased when evaluating the circulating variants of concern (Alpha, 2.5-fold reduction, GMT 20.4, 95% CI 11.1-37.4; Beta, 9.8-fold reduction, GMT 5.3 95% CI 3.4-8; Gamma, 6.9-fold reduction, GMT 7.5, 95% CI 4.7-11.9; and Delta, 5.5-fold reduction, GMT 9.5 95% CI 5.9-15.4) (**Figure 1C**). Decreases in GMT values against the Beta, Gamma and Delta variants were seen for both age groups in this immunization schedule. However, volunteers aged 18-59 years exhibited a similar GMT between the WT strain and the Alpha variant (**Supplementary Figures 2C, D**). Seropositivity rates of antibodies measured for this schedule are showed in **Figure 1C** and are similar to those reported for the 0-14 schedule. The results indicate that 100% of the evaluated volunteers exhibited antibodies able to inhibit the WT strain, while percentages against the Alpha, Beta, Gamma, and Delta variants were 90%, 65%, 80% and 85%, respectively.

In order to further corroborate whether these antibodies were also able to neutralize viral infection in a cell culture, we performed cVNT for lineage B SARS-CoV2 (D614G) and the Alpha, Gamma, and Delta variants. The results obtained showed that, as compared to the D614G strain, there was a 2.33-fold decrease in neutralizing antibodies against the Alpha variant, a 4.73-fold reduction against the Gamma variant and a 9.46-fold reduction against the Delta variant (**Figure 2A**). This result suggests that CoronaVac induce the secretion of antibodies that can neutralize these variants, but at rates lower than those reported for the WT or the D614G strain. The GMT values obtained by cVNT for D614G strain and the Alpha, Gamma, and Delta variants were 74.8 (95% CI 59.8-93.6), 32.1(95% CI 20.1-51.1), 15.8 (95% CI 9.5-26.2) and 7.9 (95% CI 5.2-12), respectively. As also seen for sVNT, volunteers aged 18 to 59 years exhibit a significant decrease in neutralizing antibodies against Gamma, and Delta, whereas volunteers over 60 years old exhibited significantly decreased neutralizing antibodies against Alpha and Delta and a lower but insignificant decrease in neutralizing antibodies against Gamma (**Supplementary Figure 3**). The seropositivity rates of neutralizing antibodies for the Alpha, Gamma and Delta variants were 84.62%, 65.38% and 55.76% respectively, while for the D614G strain was 97.6% (**Figure 2B**). Further details regarding the values reported on **Figures 1** and **2** can be found in **Tables 1** and **2**.

We also evaluated whether nine volunteers infected with SARS-CoV-2 after their respective vaccination schedules were completed (breakthrough cases) produced antibodies inhibiting the RBDs of the different variants evaluated. **Figure 3** compares antibodies levels 28 days after the second dose of CoronaVac (pre-infection) and 28 days after the infection were detected (post-infection). Most of the volunteers exhibited a 10-fold increase in the GMT of antibodies able to inhibit the RBDs of the four variants evaluated (Alpha, Beta, Gamma and Delta), as compared to GMT observed for samples previous infection. Therefore, natural infection with SARS-CoV-2 increases the secretion of antibodies that can block the interaction of RBDs from the Beta, Gamma, and Delta variants with the hACE2

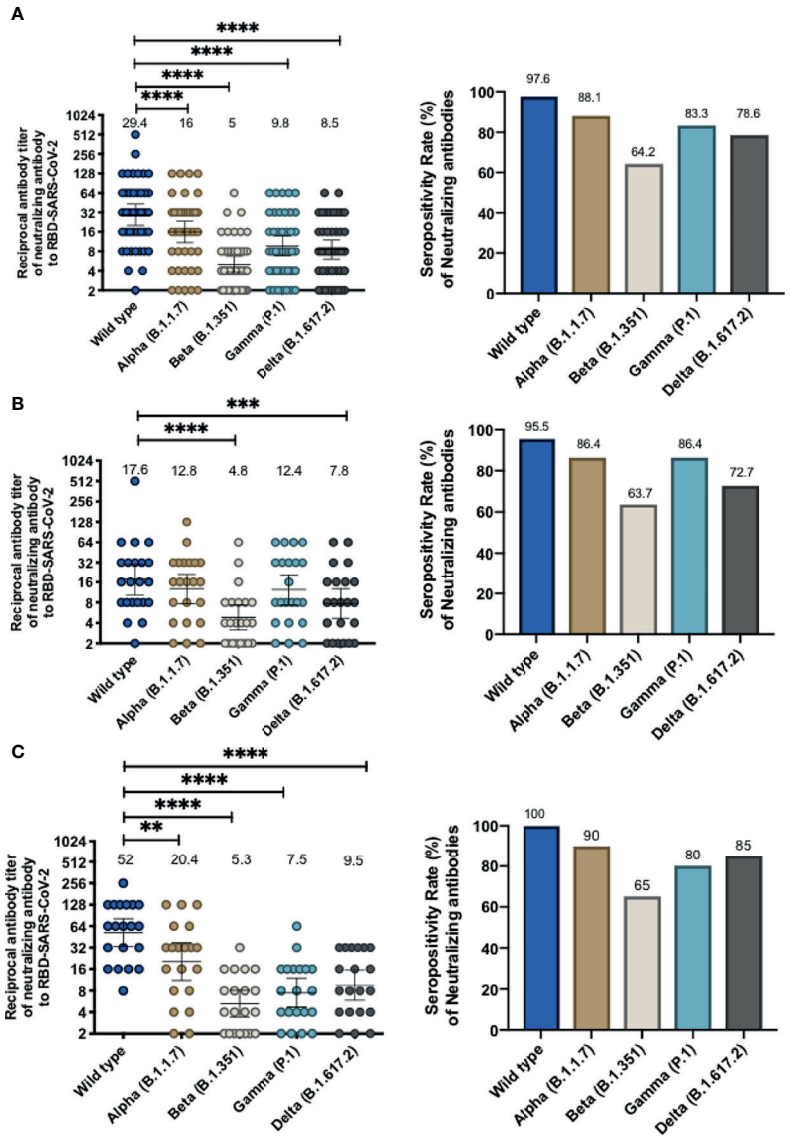

FIGURE 1 | Immunization with CoronaVac induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants after two immunizations in a 0-14 and 0-28 schedule. Antibody titers were evaluated with a surrogate virus neutralization assay (sVNT), which quantifies the interaction between S1-RBD from either WT SARS-CoV-2 or variants of concern (Alpha, Beta, Gamma, and Delta) and hACE2 on ELISA plates. Total neutralizing antibodies titer from volunteers vaccinated with CoronaVac, 28 days after the second dose and the seropositivity rate of neutralizing antibodies are shown for both vaccination schedules **(A)**, 0-14 schedule **(B)** and 0-28 schedule **(C)**. Numbers above the bars show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI in the graphs showing total antibody titers, and the number above bars show the percentage of seropositivity rate in the respective graphs. A Wilcoxon test analyzed data to compare against the wild-type RBD; $^{**}p < 0.005$, $^{***}p < 0.001$, $^{****}p < 0.0001$. The graph represents the results obtained for 22 volunteers for the 0-14 schedule and 20 volunteers for the 0-28 schedule.

receptor. However, further analyses are still required, as no characterization of the variants infecting these volunteers was performed.

Moreover, we have recently shown that CoronaVac is able to stimulate $CD4^+$ T cell responses against MPs of both Spike and Non-Spike peptides, displaying higher secretion of IFN-γ and expression of activation markers following vaccination in a 0-14 schedule, which peaks 14 days after the second dose (15). In order to evaluate anti-Spike $CD4^+$ T cell responses, we stimulated PBMCs of participants from both 0-14 and 0-28 schedules with Spike MPs from the WT strain and variants of concern and evaluated IFN-γ expression by ELISPOT (**Figure 4**). As previously reported, the subjects evaluated exhibited robust IFN-γ production following stimulation and we did not observe significant differences between PBMCs stimulated with any of the Spike MPs, suggesting that CoronaVac induces protective

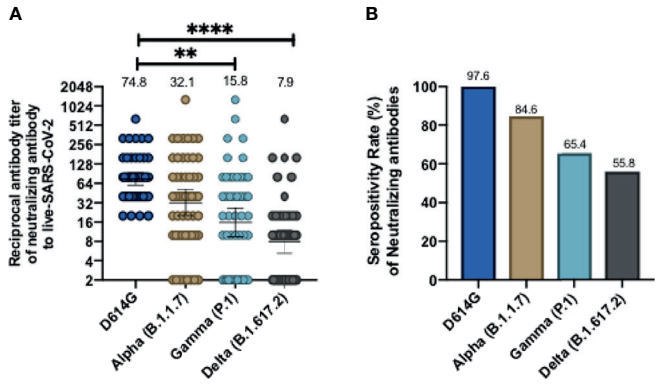

FIGURE 2 | CoronaVac immunization induces neutralizing antibodies against SARS-CoV-2 variants after two vaccine doses using a conventional virus neutralization test. Neutralizing antibody titers were evaluated by incubating the serum with a SARS-CoV-2 Chilean clinical strains and then added into Vero E6 cell for seven days. The neutralizing titer was determinate for the last dilution where no viral cytopathic effect was found in cells against wild type (D614G), and Alpha, Gamma and Delta variants. Consolidate neutralizing antibodies titer of both schedules is shown in **(A)**, and the seropositivity rate of neutralizing antibodies is shown in **(B)**. Numbers above the bars show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI in **(A)**, and the number above bars in **(B)** showed the seropositivity rate. A Wilcoxon test analyzed data to compare against the wild-type RBD; **p < 0.005, ****p < 0.0001. The graph represents the results obtained for 52 volunteers of both schedules.

cellular responses against all SARS-CoV-2 variants of concern. In addition, we observed low numbers of IL-4-secreting T cells in response to all of the MPs (**Supplementary Figure 4**), which is consistent with our previous data using the MP-S WT.

DISCUSSION

The current spread of multiple SARS-CoV-2 variants worldwide challenges the strategies of vaccination and represent a threat for potential new waves of infection. The inactivated SARS-CoV-2 vaccine CoronaVac has been proven to induce total IgG and neutralizing antibodies against the Spike protein in subjects vaccinated with either a 0-14 or 0-28 vaccination schedule, although those levels are lower as compared to other vaccines such as BNT16b2 and Moderna mRNA-1273 (15, 19, 20). Here we report that CoronaVac induces the secretion of neutralizing antibodies that recognize most of the variants of concern currently circulating in the population, as determined by sVNT and cVNT (**Figures 1**–**3**). Although the intrinsic characteristics for each of the techniques used in this report to evaluate circulating neutralizing antibodies in immunized volunteers were different, the results obtained were mostly equivalent for the WT strain, as described in our previous studies (15, 21). We found similar fold reductions in blocking and neutralizing antibodies against the variants Alpha and Gamma using both techniques, but a higher fold reduction against the Delta variant (3.5-fold reduction in the sVNT and 9.46-fold reduction in the cVNT) was observed. Moreover, when evaluating through cVNT, lower seropositivity rates were observed against the Gamma and Delta variants (65.4% and 55.8%, respectively) as compared to the results obtained by sVNT (83.3% and 78.57%, respectively), but we report a similar percentage of seropositivity for participants with circulating neutralizing antibodies against at least two of the variants with both techniques (88.1% by sVNT and 78.8 by cVNT) (**Tables 1** and **2**). These results are in line with previous reports that have shown a high correlation between these two techniques (15, 18). A recent study that

TABLE 1 | Seropositivity rates and geometric mean titer of antibodies that inhibit the RBDs of SARS-CoV2 variants, by sVNT.

Schedule	Indicators	Wild type	Alpha (B.1.1.7)	Beta (B.1.351)	Gamma (P.1)	Delta (B.1.617.2)	Seropositivity rate over 2 variants
0-14	Seropositivity n/N	21/22	19/22	14/22	19/22	16/22	19/22
	(%)	95.5	86.4	63.6	86.4	72.72	86.4
	GMT	17.6	12.8	12.4	4.8	7.8	N/D
	(95% CI)	10.3-30.2	7.7-21.5	7.3-21.2	3.2-7.4	4.7-12-9	(-)
0-28	Seropositivity n/N	20/20	18/20	13/20	16/20	17/20	18/20
	(%)	100	90.0	65.0	80.0	85.0	90.0
	GMT	52.0	20.4	7.5	5.3	9.5	N/D
	(95% CI)	33.1-81.4	11.1-37.4	4.7-11.2	3.4-8.1	5.9-15.4	(-)
Total	Seropositivity n/N	41/42	37/42	27/42	35/42	33/42	37/42
	(%)	97.6	88.1	64.3	83.3	78.57	88.1
	GMT	29.5	16.0	9.8	5.0	8.5	N/D
	(95% CI)	20.2-43.1	10.9-23.5	6.9-13.9	3.8-6.7	6.1-11.9	(-)

RBD, Receptor-binding domain; S, Spike; GMT, Geometric mean titer; N/D, Not determined.

TABLE 2 | Seropositivity rates and geometric mean titer of neutralizing antibodies against SARS-CoV2 variants by cVNT.

Schedule	Indicators	D614G	Alpha (B.1.1.7)	Gamma (P.1)	Delta (B.1.617.2)	Seropositivity rate over 2 variants
0-14	Seropositivity n/N	34/34	27/34	27/34	20/34	29/34
	(%)	100	79.4	79.4	58.8	85.2
	GMT	57.7	26.5	27.0	7.7	N/D
	(95% CI)	45.1-74.0	14.9-47.1	14.8-49.4	4.7-12-6	(-)
0-28	Seropositivity n/N	18/18	17/18	7/18	9/18	12/18
	(%)	100	94.4	38.9	50.0	66.6
	GMT	122.2	46.1	5.7	8.3	N/D
	(95% CI)	83.9-178.1	19.8-107.2	2.6-12.4	3.5-19.7	(-)
Total	Seropositivity n/N	52/52	44/52	34/52	29/52	41/52
	(%)	100	84.6	65.4	55.8	78.8
	GMT	74.8	32.1	15.8	7.9	N/D
	(95% CI)	59.8-93.6	20.1-51.1	9.5-26.2	5.2-12	(-)

GMT, Geometric mean titer; N/D, Not determined.

used the sVNT and cVNT to evaluate neutralizing antibodies against SARS-CoV-2 variants of concern in heterologous and homologous ChAdOx1 nCoV-19/BNT162b2 vaccination has shown high correlation between both assays (22).

Our results are in line with the effectiveness of CoronaVac observed in a study of elderly subjects vaccinated in Brazil, where the Gamma variant is the most prevalent SARS-CoV-2 strain and an effectiveness of 42% was reported (23). Furthermore, our data is consistent with a recent study in volunteers vaccinated with two doses of CoronaVac in China, which exhibit a 4.3-fold reduction of VNT in live neutralization assays against the Gamma variant compared to the WT strain and another study with individuals vaccinated with two doses of CoronaVac in Brazil, which reported reduced VNT against the isolates P.1/28 and P.1/30 as compared to the WT strain (a 3.1 and 2.6 fold reduction, respectively) (24, 25). Similarly, here we report a 4.73 fold reduction compared to the D614G strain using cVNT (**Figure 2**). In addition, other studies carried out in Chile using cVNT and pseudotyped viruses have reported a 7.51 and 2.33-fold reduction, respectively, in Gamma variant neutralization as compared to the WT strain in subjects vaccinated with CoronaVac (26, 27). The reduced neutralizing capacities reported against the Gamma variant have been related to the E484K mutation, which promotes the evasion of neutralizing antibodies (28). Importantly, the Gamma variant became one of the dominant SARS-CoV-2 strains in Chile during 2021 in parallel to the vaccination of Chilean population with CoronaVac (26). However, only 45 out of 2,263 participants of the phase 3 clinical trial carried out in Chile developed breakthrough cases following vaccination and among these individuals 96% developed mild disease, which suggests that CoronaVac is protective against SARS-CoV-2 and potentially against SARS-CoV-2 variants (21).

We also reported neutralizing responses against the Beta variant in subjects vaccinated with two doses of CoronaVac. A reduced inhibition of the interaction between hACE2 and RBD compared to the WT strain and a seropositivity of 64.2% was

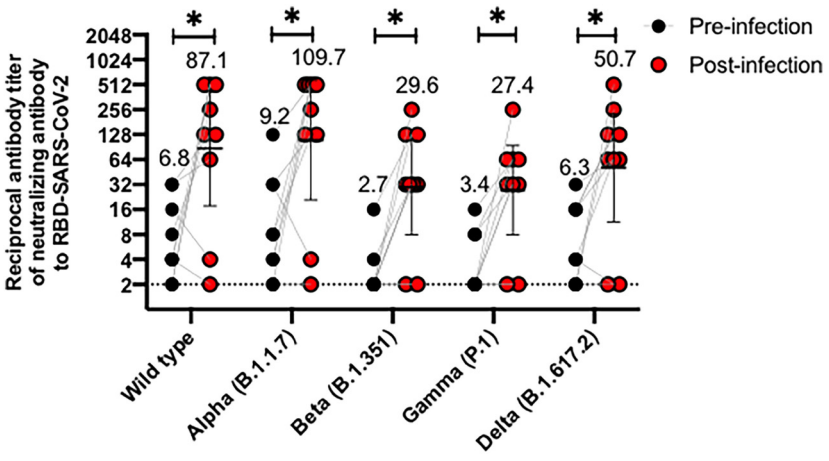

FIGURE 3 | CoronaVac immunization induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants in vaccine breakthrough cases after two vaccine doses. Antibody titers were evaluated with a surrogate virus neutralization assay (sVNT), which quantifies the interaction between S1-RBD from either Wild type SARS-CoV-2 or variants of concern (Alpha, Beta, Gamma, and Delta) and hACE2 on ELISA plates. Comparative data from vaccine breakthrough cases from both schedules are represented for each variant in two different point times, pre-infection (black circle) and post-infection (red circles). A Wilcoxon test analyzed data to compare against the wild-type RBD; *$p < 0.05$. The graph represents the results obtained for nine volunteers considering both schedules.

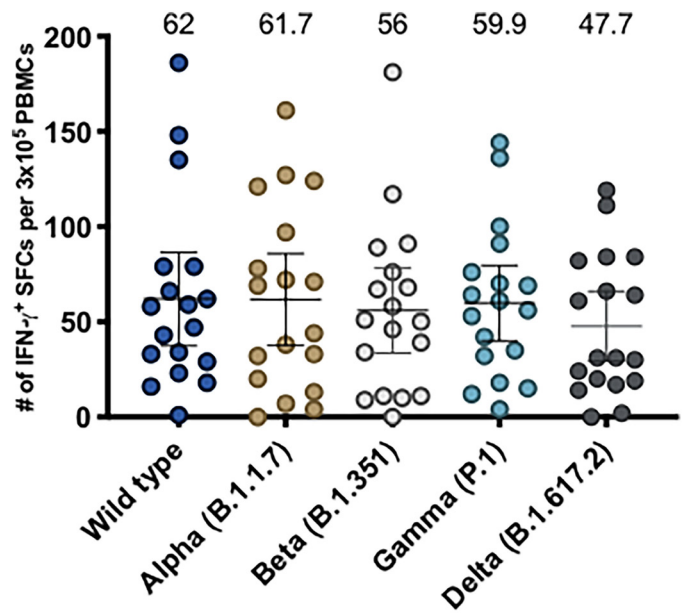

FIGURE 4 | Evaluation of cellular immune response through ELISPOT upon stimulation with Mega Pools of Spike peptides derived from SARS-CoV-2 WT and variants of concern in volunteers immunized with CoronaVac. Numbers of IFN-γ-secreting cells, determined through ELISPOT as spot forming cells (SFCs) were determined. PBMCs were stimulated with MP-S WT, MP-S Alpha, MP-S Beta, MP-S Gamma and MP-S Delta for 48 h for samples obtained 2 weeks after the second dose of volunteers of the 0-14 schedule (n = 11) and 0-28 schedule (n = 7). A total of 18 volunteers were evaluated. Data shown represents mean ± 95% CI and the mean is indicated above each bar. Statistical differences were evaluated by a one-way ANOVA followed by Dunnett's test for multiple comparisons against the MP-S WT.

reported using the sVNT, the lowest across all variants of concern analyzed (**Figure 1** and **Table 1**). These results are consistent with recent reports in cohorts from Thailand and China vaccinated with CoronaVac, in which reduced neutralization was reported using live virus neutralization (fold reductions of 22.1 and 5.7 compared to the WT strain, respectively) (24, 29) and also with the reduction in neutralizing responses observed in subjects vaccinated with the mRNA vaccine BNT162b2 for the Beta variant (4, 30). In line with the reports for the Gamma variant, the E484K mutation found in the Beta variant has been identified as the main mutation responsible for this effect as antibodies bind to RBD with less affinity.

Of note, we used the D614G variant in the cVNT, which exhibits a mutation outside of the RBD and we were able to observe effective neutralization against viral infection in all the subjects evaluated from both vaccination schedules and both age groups (**Figure 2**). These results support that CoronaVac is protective against the D614G variant, which is one of the most prevalent strains worldwide.

Our work also reported protection against the variant Delta. The Delta variant (first identified in India) exhibit the RBD mutations T478K, L452R and P681R and is currently a cause of concern due to its high transmissibility and may even surpass other variants in this regard (11). The Delta variant has been recently detected in Chile and it is becoming one of the dominant SARS-CoV-2 strains. Here we show using a RBD containing the mutations T478K and L452R present in the Delta variant that volunteers vaccinated with CoronaVac exhibit reduced blocking antibodies compared to the WT RBD but we report a seropositivity of 78.57% and 55.76% by sVNT and cVNT (**Tables 1** and **2**), respectively, which suggests that the vaccine confers protection against this variant. Our data is in line with the previously mentioned works from Thailand and China in volunteers vaccinated with 2 doses of CoronaVac, in which neutralization was evaluated by cVNT and reported fold reductions of 31.7 and 3.7 fold reduction, respectively, as compared to the WT strain, whereas we report a 9.46-fold reduction (24, 29). Similarly, mRNA vaccines induce neutralizing antibodies against the Delta variant but to a reduced extent compared to the WT strain (31, 32). Pseudoviruses carrying the L452R mutation display higher infectivity in cell culture and when incubated with sera from subjects vaccinated with Moderna mRNA-1273 or BNT16b2, as compared to the WT strain (13).

Our study also shows how subjects vaccinated with CoronaVac increase their blocking antibody GMTs following natural infection against the wild type strain and to a similar extent to the Alpha variant, but this increased GMT was lower for the variants Beta, Gamma and Delta (**Figure 3**). These findings are consistent with studies comparing different vaccine platforms against natural infection, which indicate that inactivated vaccines induce lower levels of neutralizing antibodies compared to natural infection with SARS-CoV-2, in contrast to mRNA vaccines, which exhibit comparable levels of neutralization, using live virus neutralization (20). In line with

this, cohorts from Thailand and Brazil vaccinated with CoronaVac exhibits lower neutralizing antibody titers against either the WT strain or variants of concern, compared to naturally infected individuals (25, 29). We have previously reported levels of neutralization in unvaccinated and naturally infected hospitalized individuals, which exhibit a robust neutralizing antibody response against wild-type SARS-CoV-2 (33). Although we did not perform cVNT for either breakthrough cases or naturally infected individuals against variants of concern, our results obtained by sVNT are in line with data from non-variant infected subjects, who also exhibit a similar reduction in neutralization against the variants Beta, Gamma and Delta (20).

Moreover, here we show that CoronaVac is able to stimulate T cell responses against Spike MPs from either WT strain or variants of concern and we did not see any significant differences (**Figure 4**). This is the first report to date to characterize T cell responses against SARS-CoV-2 Spike MPs in volunteers vaccinated with CoronaVac. Concordantly, MPs from variants of concern have been previously used to show that volunteers vaccinated with two doses of either Moderna mRNA-1273 or BNT16b2 exhibit IFN-γ-secreting T cells in response to these MPs and no significant differences were found (34). These results have been attributed to the high conservation of T cell epitopes in variants of concern, suggesting that vaccines can induce effective cellular responses against them. In addition, it is important to highlight that although the majority of the T cell responses are conserved and the variants do not mutate enough to disrupt the overall T cell repertoire, mutations are observed in other SARS-CoV-2 proteins and across variants (34). Therefore, it is likely that the induction of cellular responses against other SARS-CoV-2 proteins by CoronaVac may confer an advantage compared to other vaccines, considering that the inclusion of multiple antigens might increase the likelihood that more epitopes are conserved than having only one protein in the vaccine.

Importantly, a limitation of our study is that we were not able to characterize other non-neutralizing antibody functions that could be important in either vaccinated or convalescent subjects against variants of concern. Furthermore, *in vitro* evaluation of neutralizing antibodies does not necessarily correlate with protection against SARS-CoV-2 in vaccinated individuals. However, recent evidence supports that levels of neutralizing antibodies are predictive of protection against symptomatic SARS-CoV-2 infection (35). In addition, although cellular responses do not necessarily prevent infection, induction of cellular responses against variants of concern in individuals vaccinated with CoronaVac suggests that vaccinated individuals are protected from severe disease, which is supported from the results of the clinical trial performed in Chile with this vaccine (16, 21).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This trial was approved by each Institutional Ethical Committee and the Chilean Public Health Institute (#24204/20) and conducted according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization and visualization, AK, ER, SB, KA, PG, and JG-A. Methodology, RF, JM, JF, GZ, WM, AG, AS, and DW. Investigation, FM-G, JS, JF, NB, LG, BS, LD, NG, GAP, RB-R, GH-E, CI, DM-T, MR, DR-P, OV, MU, and YV. Funding acquisition, AK. Project administration, AK, KA, SB, PG, and JG-A. Supervision, AK, KA, SB, and PG. Writing – original draft, FM-G and JS. Writing – review and editing, AK, KA, SB, ER, PG, AG, AS, and DW. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by The Ministry of Health, Government of Chile supported the funding of the CoronaVac03CL Study, The Confederation of Production and Commerce (CPC), Chile, supported the funding of the CoronaVac03CL Study, the NIH NIAID under Contract No. 75N93021C00016 supports AS and Contract No. 75N9301900065 supports AS, DW. The Millennium Institute on Immunology and Immunotherapy, ANID - Millennium Science Initiative Program ICN09_016 (former P09/016-F) supports SB, KA, PG, and AK. The Innovation Fund for Competitiveness FIC-R 2017 (BIP Code: 30488811-0) supports SB, PG, and AK. SINOVAC contributed to this study with the investigational vaccine and experimental reagents.

ACKNOWLEDGMENTS

We would like to thank the support of the Ministry of Health, Government of Chile, Ministry of Science, Technology, Knowledge, and Innovation, Government of Chile, and The Ministry of Foreign Affairs, Government of Chile and the Chilean Public Health Institute (ISP). We also would like to thank Rami Scharf, Jessica White, Jorge Flores and Miren Iturriza-Gomara from PATH for their support on experimental design and discussion. We also thank the Vice Presidency of Research (VRI), the Direction of Technology Transfer and Development (DTD), the Legal Affairs Department (DAJ) of the Pontificia Universidad Católica de Chile. We are also grateful to the Administrative Directions of the School of Biological Sciences and the School of Medicine of the Pontificia Universidad Católica de Chile for their

administrative support. Special thanks to the members of the CoronaVac03CL Study Group [list in the Supplementary Appendix (SA)] for their hard work and dedication to the clinical study; the members of the independent data safety monitoring committee (members in the SA) for their oversight, and the subjects enrolled in the study for their participation and commitment to this trial.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.747830/full#supplementary-material

Supplementary Figure S1 | Immunization with CoronaVac induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants in participants aged 18-59 and ≥60 after two immunizations. Antibody titers were evaluated with a surrogate virus neutralization assay, which quantifies the interaction between S1-RBD from either Wild type SARS-CoV-2 or variants of concern (Alpha, Beta, Gamma and Delta) and hACE2 on ELISA plates. Results were obtained from participants vaccinated with CoronaVac, 28 days after the second dose in volunteers between 18-59 **(A)** and ≥ 60 **(B)** consolidating the data from both 0-14 and 0-28 schedules. Numbers above the bars show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI. A Wilcoxon test analyzed data to compare against the wild-type RBD; ****p < 0.0001. The graph represents the results obtained for 22 participants in the 18-59 years old group and 20 participants in the ≥60 years old group.

Supplementary Figure S2 | CoronaVac vaccination induces antibodies able to inhibit the interaction between hACE2 and S1-RBD from SARS-CoV-2 variants in participants aged 18-59 and ≥60 after two immunizations in both 0-14 and 0-28 schedules. Antibody titers were evaluated with a surrogate virus neutralization assay, which quantifies the interaction between S1-RBD from either Wild type SARS-CoV-2 or variants of concern (Alpha, Beta, Gamma and Delta) and hACE2 on ELISA plates. Results were obtained from participants vaccinated with CoronaVac 28 days after the second dose. For 0-14 schedule, volunteers between 18-59 and ≥ 60 are shown in **(A, B)**, respectively, and for 0-28, schedule volunteers between 18-59 and ≥ 60 are shown in **(C, D)**, respectively. The bars above show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI. A Wilcoxon test analyzed data to compare against the wild-type RBD; **p < 0.05, ***p < 0.005, ****p < 0.0001. The graph represents the results obtained for 12 participants in the 18-59 years old group and 10 participants in the ≥60 years old group in the 0-14 schedule and for 10 participants in the 18-59 years old group and 10 participants in the ≥60 years old group in the 0- 28 schedule.

Supplementary Figure S3 | CoronaVac immunization induces neutralizing antibodies against SARS-CoV-2 variants after two vaccine doses using a live virus test in volunteers aged 18-59 and over 60 years old. Antibody titers were evaluated by incubating the serum with a SARS-CoV-2 Chilean clinical strain and then added into Vero E6 cell for seven days. The neutralizing titer was determinate for the last dilution where no viralcytopathic effect was found in cells against wild type (D614G) and Alpha, Gamma and Delta variants. Consolidate neutralizing antibodies titer of volunteers from 0-14 and 0-28 schedules aged 18-59 years old are shown in **(A)**, while volunteer under 60 years old from 0-14 and 0-28 schedules are shown in **(B)**. The bars above show the Geometric Mean Titer (GMT), and the error bars indicate the 95% CI. A Wilcoxon test analysed data to compare against the wild-type RBD; *p < 0.05. The graph represents the results obtained for 42 volunteers of both schedules.

Supplementary Figure S4 | Evaluation of cellular immune response through ELISPOT upon stimulation with Mega Pools of Spike peptides derived from SARS-CoV-2 WT and variants of concern in volunteers immunized with CoronaVac. Numbers of IL-4-secreting cells, determined through ELISPOT as spot forming cells (SFCs) were determined. PBMCs were stimulated with MP-S WT, MP-S Alpha, MP-S Beta, MP-S Gamma and MP-Delta for 48 h for samples obtained 2 weeks after the second dose of volunteers of the 0-14 schedule (n = 11) and 0-28 schedule (n = 7). A total of 18 volunteers were evaluated. Data shown represents mean 95% CI and the mean is indicated above each bar. Statistical differences were evaluated by a one-way ANOVA followed by Dunnett's test for multiple comparisons against the MP-S WT.

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Conflict of Interest: Authors GZ and WM are employed by company SINOVAC Biotech. AS is a consultant for Gritstone Bio, Flow Pharma, Arcturus Therapeutics, ImmunoScape, CellCarta, Avalia, Moderna, Fortress and Repertoire.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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

The authors declare this study received the investigational product (placebo and vaccines) from the company SINOVAC Biotech. SINOVAC employees contributed to the conceptualization of the study (clinical protocol and eCRF design) but did not participate in either the analysis or interpretation of the data shown in this manuscript.

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frontiers in Immunology

ORIGINAL RESEARCH
published: 29 September 2021
doi: 10.3389/fimmu.2021.742914

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

Luisa F. Duarte[1,2†], Nicolás M. S. Gálvez[1,2†], Carolina Iturriaga[3†], Felipe Melo-González[1,2†], Jorge A. Soto[1,2†], Bárbara M. Schultz[1,2†], Marcela Urzúa[3†], Liliana A. González[1,2], Yaneisi Vázquez[1,2], Mariana Ríos[1,2], Roslye V. Berríos-Rojas[1,2], Daniela Rivera-Pérez[1,2], Daniela Moreno-Tapia[1,2], Gaspar A. Pacheco[1,2], Omar P. Vallejos[1,2], Guillermo Hoppe-Elsholz[1,2], María S. Navarrete[4], Álvaro Rojas[4], Rodrigo A. Fasce[5], Jorge Fernández[5], Judith Mora[5], Eugenio Ramírez[5], Gang Zeng[6], Weining Meng[6], José V. González-Aramundiz[7], Pablo A. González[1,2], Katia Abarca[1,3], Susan M. Bueno[1,2*] and Alexis M. Kalergis[1,2,8*]*

[1] Millennium Institute on Immunology and Immunotherapy, Santiago, Chile, [2] Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile, [3] Departamento de Enfermedades Infecciosas e Inmunología Pediátricas, División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile, [4] Departamento de Enfermedades Infecciosas del Adulto, División de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile, [5] Departamento de Laboratorio Biomédico, Instituto de Salud Pública de Chile, Santiago, Chile, [6] Sinovac Biotech, Beijing, China, [7] Departamento de Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile, [8] Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

OPEN ACCESS

Edited by:
Rong Hai,
University of California, Riverside, United States

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Daniel Ramos Ram, Beth Israel Deaconess Medical Center and Harvard Medical School, United States
Duo Xu, University of California, Riverside, United States

***Correspondence:**
Katia Abarca
kabarca@uc.cl
Susan M. Bueno
sbueno@bio.puc.cl
Alexis M. Kalergis
akalergis@bio.puc.cl

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Vaccines and Molecular Therapeutics, a section of the journal Frontiers in Immunology

Received: *16 July 2021*
Accepted: *06 September 2021*
Published: *29 September 2021*

Citation:
Duarte LF, Gálvez NMS, Iturriaga C, Melo-González F, Soto JA, Schultz BM, Urzúa M, González LA, Vázquez Y, Rios M, Berríos-Rojas RV, Rivera-Pérez D, Moreno-Tapia D, Pacheco GA, Vallejos OP, Hoppe-Elsholz G, Navarrete MS, Rojas Á, Fasce RA, Fernández J, Mora J, Ramírez E, Zeng G, Meng W, González-Aramundiz JV, González PA, Abarca K, Bueno SM and Kalergis AM (2021) Immune Profile and Clinical Outcome of Breakthrough Cases After Vaccination With an Inactivated SARS-CoV-2 Vaccine. Front. Immunol. 12:742914. doi: 10.3389/fimmu.2021.742914

Constant efforts to prevent infections by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are actively carried out around the world. Several vaccines are currently approved for emergency use in the population, while ongoing studies continue to provide information on their safety and effectiveness. CoronaVac is an inactivated SARS-CoV-2 vaccine with a good safety and immunogenicity profile as seen in phase 1, 2, and 3 clinical trials around the world, with an effectiveness of 65.9% for symptomatic cases. Although vaccination reduces the risk of disease, infections can still occur during or after completion of the vaccination schedule (breakthrough cases). This report describes the clinical and immunological profile of vaccine breakthrough cases reported in a clinical trial in progress in Chile that is evaluating the safety, immunogenicity, and efficacy of two vaccination schedules of CoronaVac (clinicaltrials.gov NCT04651790). Out of the 2,263 fully vaccinated subjects, at end of June 2021, 45 have reported symptomatic SARS-CoV-2 infection 14 or more days after the second dose (1.99% of fully vaccinated subjects). Of the 45 breakthrough cases, 96% developed mild disease; one case developed a moderate disease; and one developed a severe disease and required mechanical ventilation. Both cases that developed moderate and severe disease were adults over 60 years old and presented comorbidities. The immune response before and after SARS-CoV-2 infection was analyzed in nine vaccine breakthrough cases, revealing that six of them exhibited circulating anti-S1-RBD IgG antibodies with neutralizing capacities

after immunization, which showed a significant increase 2 and 4 weeks after symptoms onset. Two cases exhibited low circulating anti-S1-RBD IgG and almost non-existing neutralizing capacity after either vaccination or infection, although they developed a mild disease. An increase in the number of interferon-γ-secreting T cells specific for SARS-CoV-2 was detected 2 weeks after the second dose in seven cases and after symptoms onset. In conclusion, breakthrough cases were mostly mild and did not necessarily correlate with a lack of vaccine-induced immunity, suggesting that other factors, to be defined in future studies, could lead to symptomatic infection after vaccination with CoronaVac.

Keywords: CoronaVac, phase 3 clinical trial, SARS-CoV-2, COVID-19, vaccines, breakthrough cases

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus first identified in China, in December of 2019, and is responsible of the current worldwide pandemic with nearly 4 million deaths reported at the beginning of July 2021 (1, 2). Coronavirus disease 2019 (COVID-19) is the result of infection caused by this virus, a disease that ranges from mild respiratory symptoms in over 80% of the population to severe illnesses requiring oxygen assistance and invasive ventilation, which usually leads to fatal or life-threatening outcomes (3).

Vaccine development has become the main hope for reducing COVID-19 cases and the severity of this disease (4). Several vaccines have been developed through different molecular approaches (i.e., viral mRNA, viral recombinant proteins, recombinant viral vectors, or inactivated whole virus), and up to date, the World Health Organization (WHO) has granted emergency approval for the use of 10 of them (5). Despite their differences, all these vaccines have reported a protective immune response against SARS-CoV-2 infections in clinical trials (6). Several studies have reported the production of antibodies with neutralizing capacities, along with broad cellular immune responses that helps in the clearance of the virus (6–10). However, breakthrough cases, defined as the detection of SARS-CoV-2 RNA in people ≥14 days after they completed the immunization schedule, have been reported (11, 12). These cases push the scientific community towards a further characterization and comprehension of the immune response elicited upon vaccination, in order to achieve enhanced protective responses in all the population.

CoronaVac is an inactivated SARS-CoV-2 vaccine that has shown to be 65.9%, 87.5%, 90.3%, and 86.3% effective in preventing COVID-19 symptoms, hospitalization, ICU admission, and COVID-19-related death, respectively, as recently reported in a cohort of almost 10.2 million individuals in Chile (13). It has been reported that immunization with CoronaVac elicits an immune response directed against several viral components, beyond the spike (S) protein, after the administration of two doses, as evidenced by detecting IgG antibodies against N protein and a substantial $CD4^+$ T-cell response after *ex vivo* stimulation with a MegaPool (MP) of peptides covering the remainder "non-spike" SARS-CoV-2 proteome (7, 14, 15). Phase 3 clinical trials for this vaccine are being held in different countries around the globe (15, 16). Particularly in Chile, a clinical trial is undergoing to evaluate two different immunization schedules, with the second dose administered either 2 (0–14) or 4 (0–28) weeks after the first one (clinicaltrials.gov number: NCT04651790). Among 2,263 fully vaccinated volunteers, on June 25, 2021, a total of 45 COVID-19 cases (1.99%) have been reported occurring in the monitoring period (from 2 weeks after the second dose). Here, we report the clinical outcome and the immune response elicited by nine breakthrough cases detected among the 15 of the 450 volunteers enrolled in the immunogenicity branch of the phase 3 clinical trial, who already received both doses of CoronaVac. Evaluation of the humoral immune response considered the measurement of circulating anti-S1-RBD IgG antibodies and their neutralizing capacities as measured by two different techniques. Evaluation of the cellular immune response was performed through ELISPOT assays after *ex vivo* stimulation of peripheral blood mononuclear cells (PBMCs) with two sets of MP of peptides derived from the proteome of SARS-CoV-2 (17). A thorough understanding of the immune responses elicited after vaccination and as to how it correlates with the protection elicited after this and subsequent infections will provide valuable information that will improve the approaches currently being used to halt the COVID-19 pandemic and will also indicate whether an additional dose of currently approved vaccines is needed after a certain time span.

MATERIALS AND METHODS

Study Design, Volunteers, and Randomization

The clinical trial (clinicaltrials.gov NCT04651790) was conducted in Chile at eight different sites and evaluated two immunization schedules in a 1:1 ratio. This trial was approved by each Institutional Ethical Committee and by the Chilean Public Health Institute (#24204/20) and conducted according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki (18), and local regulations. Written informed consent was obtained from each participant. Volunteers included men and women aged ≥18, inoculated with two doses of 3 μg (600SU) of CoronaVac. One group received the second dose 2 weeks after the first dose (0–14 schedule), while a second group received the second dose 4 weeks after the first one (0–28 schedule). Exclusion criteria included, among others, history

of confirmed symptomatic SARS-CoV-2 infection, pregnancy, allergy to vaccine components, and immunocompromised conditions. A complete list of inclusion and exclusion criteria has been published previously (15).

A total of 2,302 volunteers were enrolled by March 19, 2021, of whom 2,263 received both doses. A subgroup of 450 volunteers was selected to evaluate their immune response, receiving randomly CoronaVac either in a 0–14 or a 0–28 immunization schedule (1:1 ratio). Demographic information, comorbidities, nutritional status, immunization schedule, and dates of vaccination were obtained at enrollment and registered in the electronic case-report form (eCRF) for all volunteers. Nutritional status was determined using a gender and body mass index (BMI) (19).

Breakthrough Case Follow-Up

Confirmed COVID-19 cases reported 14 days after the administration of the second dose of CoronaVac were identified following the protocol procedures for efficacy. Briefly, upon enrollment, participants were instructed to report through an electronic platform, e-mail, cell phone message, or telephone call, each time the definition for suspected positive case was met. A positive case was suspected if at least one of the following symptoms were present for over 2 days: fever or chills, coughing, shortness of breath or breathing difficulty, fatigue, muscle or body pain, headache, loss of smell or taste, sore throat, nasal congestion or runny nose, nausea or vomiting, and diarrhea. Upon the report, an evaluation visit was scheduled with a study physician, for 3 days after symptoms onset, to evaluate the presence of SARS-CoV-2 RNA by reverse-transcriptase quantitative PCR (RT-qPCR) in nasopharyngeal (NP) sample. If the sample was negative, and at least one symptom persisted, a second test was performed after 48 h. If a sample was positive, the clinical evolution of the case was closely monitored by the center personnel until its resolution. If hospitalization was required, information was obtained from relatives of the volunteer and from clinical reports.

Upon confirmation of positive cases, history of possible close contact with confirmed COVID-19 cases and the severity and duration of each signs and symptoms were registered. Severity was classified from grades 1 to 4, as published previously by the Food and Drug Administration (FDA) and the National Institutes of Health (NIH) (20, 21). Intensity of the disease was graded from score 1 to 9, as published previously by the WHO (22). The grading for severity criteria indicated in the protocol were either mild (symptomatic patients without viral pneumonia or hypoxia), moderate (clinical signs of pneumonia such as fever, coughing, shortness of breath, difficulty breathing but no signs of severe pneumonia, oxygen saturation ≥94% on room air), or severe {resting clinical signs indicative of severe clinical illness [respiratory rate (RR) ≥30/min; heart rate (HR) ≥125/min; oxygen saturation <94% at room air at sea level; PaO_2/FiO_2 <300 mm Hg], respiratory failure [requirement of high-flow oxygen, noninvasive ventilation, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO)], evidence of shock [systolic blood pressure (SBP) <90 mmHg, diastolic blood pressure (DBP) <60 mmHg, or requirement of vasopressors], significant acute renal, hepatic, or neurological dysfunction, admission to ICU, or death}. All this information was recorded in both the clinical file of the participant and the eCRF.

Procedures

To evaluate the immune response elicited upon immunization, peripheral blood samples were obtained for the isolation of serum and PBMCs. For volunteers from the immunogenicity branch, samples were collected before the first and the second dose and 2 and 4 weeks after the second dose. After COVID-19 confirmation by PCR, two additional peripheral blood samples were obtained about 2 and 4 weeks after symptoms onset (follow-up 1 and 2, respectively). Sera samples and PBMC were collected as previously reported (15) and stored at −80°C or in liquid nitrogen, respectively.

Circulating IgG antibodies specific against the RBD of the S1 protein of SARS-CoV-2 (S1-RBD) were measured using the COVID-19 Human Antibody Detection Kit (RayBio #IEQ-CoVS1RBD-IgG), following the instructions of the manufacturer. Sera samples were two-fold serially diluted, starting at a 200-fold dilution until a 6,400-fold dilution. The antibody titer was determined as the last fold dilution with an absorbance over the cut-off value. The cut-off value for each dilution was determined as 2.1 times the absorbance at 450 nm for a panel of 29 seronegative samples.

The neutralizing capacities of circulating antibodies were determined by two different techniques, i.e., through a surrogate virus neutralizing test (sVNT) and a conventional plaque-reduction neutralization test (cVNT). The sVNT were performed following the instructions of the manufacturer (BioHermes #COV-S41), and sera samples were 2-fold serially diluted starting at a 4-fold dilution until a 4,096-fold dilution. The percentage of inhibition was defined as follows: ($OD_{450\ nm}$ value of negative control − $OD_{450\ nm}$ value of sample)/($OD_{450\ nm}$ value of negative control × 100), and titers were reported as the reciprocal of the highest serum dilution required to achieve 30% of inhibition. Samples exhibiting <30% inhibitory activity at the lowest dilution tested (1:4) were assigned a titer of 2. For the cVNT, sera samples were 2-fold serially diluted starting at a 4-fold dilution until a 512-fold dilution. Then, samples were incubated with a SARS-CoV-2 clinical isolate (33782CL-SARS-CoV-2 strain) for 1 h at 37°C. The mixtures were then added to Vero E6 cell monolayers (ATCC CRL-1586), and cytopathic effect (CPE) was evaluated 7 days after infection. Positive and negative controls were held for each assay. CPE was evaluated by direct visualization, and the titer of neutralizing antibodies was defined as the latest fold dilution exhibiting 100% of infection inhibition and absence of CPE. A titer of 2 was assigned for samples showing CPE at the lowest dilution tested (1:4).

The cellular immune response was evaluated through ELISPOT assays, as described previously, using the human interferon (IFN)-γ/IL-4 double-color ELISPOT (Immunospot) (15). Cells were cultured for 48 h in the presence of four different SARS-CoV-2-specific MPs (17). Two of these MPs are composed of 15-mer peptides derived from the S protein (MP-S) and the remaining proteins of the viral particle (MP-R). The other two MPs are composed of 9- to 11-mer peptides from the whole proteome of SARS-CoV-2 (CD8-A and CD8-B). Positives and

negative controls were considered for each assay as reported previously (15, 17).

RESULTS

Clinical Features of Breakthrough Cases

From January 1 to June 25, 2021, 50 breakthrough cases were reported among the 2,263 vaccinated volunteers that had received two vaccine doses, of which 45 had over 14 days after the second dose (26 cases in the 0–14 schedule and 19 in the 0–28 schedule). Fifteen of these breakthrough cases were among the 450 volunteers in the immunogenicity branch. Eight of these had follow-up samples from days 14 and 30 after the start of symptoms of COVID-19, and one of them had a single follow-up sample taken 14 days after symptoms onset (Volunteer 1). All nine were Hispanic–Latin and were negative for the presence of circulating S- and N-SARS-CoV-2 IgG antibodies at recruitment. Six of them received the 0–14 immunization schedule and three the 0–28 immunization schedule (**Figure 1**). The demographic characteristics and relevant clinical history of cases are shown in **Table 1**.

Intensity and severity of the disease were mild, with a score of 2 in seven out of the nine cases (Volunteers 1, 2, 3, 5, 6, 8, and 9), and the symptoms exhibited by them in decreasing frequency were nasal congestion (seven cases), sore throat (six), loss of smell (six), headache (five), coughing (four), loss of taste (four), runny nose (four), fatigue or myalgia (three), dyspnea (one), nausea (one), and diarrhea (one). None of the seven cases exhibited fever or vomiting. Accordingly, the duration of each symptoms was nasal congestion (1–13 days), sore throat (1–12), loss of smell (3–10), headache (5–13), cough (1–8), loss of taste (3–10), runny nose (2–13), fatigue (4–12), myalgia (1–21), dyspnea (12), nausea (4), and diarrhea (4–5). Most of the symptoms recorded were grade 1 or 2. The clinical outcome of the COVID-19 disease for each volunteer is indicated in **Table 2**.

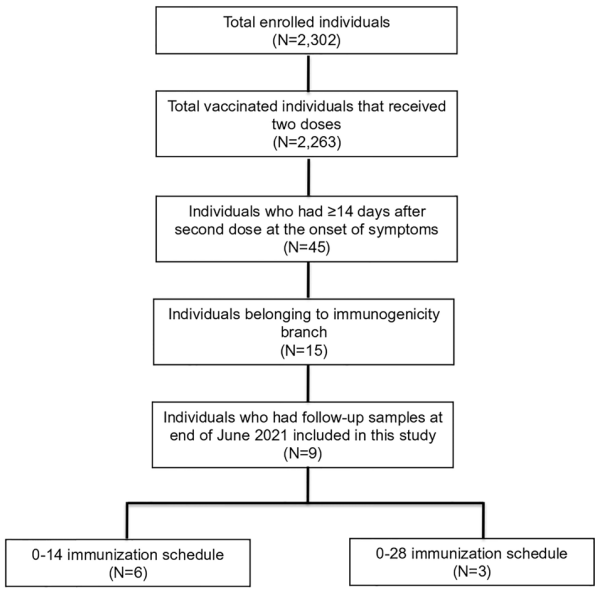

FIGURE 1 | Enrolled volunteers and breakthrough cohort included in this study. Nine of the 2,302 vaccinated individuals belonging to the clinical trial conducted in Chile were included in this study after confirming COVID-19 disease by reverse-transcriptase polymerase chain-reaction (RT-qPCR) assay. They were selected from 45 individuals who displayed symptoms after ≥14 days from the administration of the second dose of the vaccine because they were enrolled in the immunogenicity branch and further had at least one follow-up sample after symptoms onset at the end of June of 2021.

Two out of the nine breakthrough cases (Volunteers 4 and 7) reached a score over 2. The highest clinical score registered for Volunteers 4 was 5 (moderate), and for Volunteer 7 was 7 (severe). Volunteer 4 is a 62-year-old man, with a BMI of 29.3 (overweight) and is currently being treated for hypothyroidism (**Table 1**). The onset date was 122 days after the administration of the second dose (0–28 immunization schedule), and no close contact with a COVID-19-positive case was reported. The symptoms exhibited were fatigue, muscle pain, headache, nasal congestion, cough, and fever. After 6 days of disease development, Volunteer 4 was hospitalized due to persistent symptoms and the addition of shortness of breath to the list. A chest CT confirmed COVID-19 pneumonia. He was diagnosed with acute respiratory insufficiency and then received 4 L/min of oxygen by nasal cannula for 4 days. After this, he exhibited an overall improvement and recovery, with a total time of hospitalization of 8 days. Volunteer 7 is a 69-year-old man, with a BMI of 28.0 (overweight) and a history of arterial hypertension, bicuspid aorta, and atrial fibrillation. The onset date was 32 days after the administration of the second dose (0–28 immunization schedule), and close contact with a COVID-19-positive case was confirmed (his son). He presented respiratory symptoms and fever. Later, onset and persistence of malaise and fever, the onset of dyspnea, and the confirmation of COVID-19 pneumonia by a chest CT led to hospitalization. All the typical COVID-19 symptoms except nausea, vomiting, and diarrhea were reported after hospitalization. He received supplemental oxygen by nasal cannula and was transferred to ICU due to heart failure. He required mechanical ventilation for 6 days and eventually recovered, with a total time of hospitalization of 20 days.

Remarkably, as described below, two out of the nine breakthrough cases (Volunteers 2 and 6) exhibited a weak immune response upon immunization and infection. Volunteer 2 is a 48-year-old man, with a BMI of 28.9 (overweight) and a history of hypothyroidism, arterial hypertension, coronary heart disease (acute myocardial infarction on September 2020), fatty liver disease, and dyslipidemia under treatment. During his childhood, he was diagnosed with influenza-associated encephalitis (4 years old, hospitalized in ICU) and with uncomplicated diphtheria (6 years old). During his adulthood, he was diagnosed with a post-influenza pneumonia in 2000 and with a clinically suspected *Mycoplasma pneumonia* infection in 2018, both were treated with oral antibiotics. The symptoms onset was 26 days after the administration of the second dose (0–14 immunization schedule), and no contact with a COVID-19-

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

Volunteer	Biological Sex*	Age	Nutritional Status	BMI	Co-morbidities
1	F	46	Normal	23.2	Migraine syndrome, allergic rhinitis
2	M	48	Overweight	28.9	Arterial hypertension, coronary heart disease, hypothyroidism
3	F	24	Overweight	25.3	Allergic rhinitis, penicillin allergy
4	M	62	Overweight	29.3	Hypothyroidism
5	F	32	Normal	23.9	Allergic rhinitis
6	F	33	Normal	20.5	Hypothyroidism
7	M	69	Overweight	28.0	Arterial hypertension, bicuspid aorta, atrial fibrillation, nephrolitiasis
8	F	28	Overweight	27.3	None
9	F	59	G2 Obesity	36.4	Insulin resistance

**Gray shading, female; no shading, male.*

TABLE 2 | Clinical development of COVID-19 disease in the nine breakthrough cases described.

Volunteer*	Immunization schedule	Day of symptoms onset^	Possible close contact with COVID-19 case	Required Hospitalization	Highest clinical score
1	0–14	37	Yes	No	2
2	0–14	23	No	No	2
3	0–14	43	No	No	2
4	0–14	122	No	Yes	5
5	0–14	122	No	No	2
6	0–14	94	No	No	2
7	0–28	32	Yes	Yes	7
8	0–28	34	No	No	2
9	0–28	16	Yes	No	2

**Gray shading, female; no shading, male.*
^Days after the administration of the second dose.

positive case was reported. He presented fatigue, headache, nasal congestion, runny nose, coughing, and diarrhea. Volunteer 6 is a 33-year-old woman, with a BMI of 20.5 (eutrophic), and medical history of mononucleosis (2003), recurrent herpes simplex labialis (since 2003), hypothyroidism, and currently on oral contraceptive therapy. No contact with a COVID-19-positive case was reported, and the onset date was 94 days after the administration of the second dose (0–14 immunization schedule). She presented fatigue, muscular pain, loss of smell, loss of taste, sore throat, and nasal congestion.

Altogether, the immunization schedule, medical history, demographic characteristics, the symptoms onset day, reporting of close contact with COVID-19 confirmed cases, and the symptoms exhibited by all breakthrough cases are diverse, and an evident pattern of conditions leading to susceptibility towards SARS-CoV-2 infection is not observed.

Humoral Immunity in Breakthrough Cases

To evaluate the humoral immune response elicited by the nine breakthrough cases, circulating IgG antibodies specific against the S1-RBD of SARS-CoV-2 were evaluated as indicated in *Materials and Methods*. As shown in **Figure 2** (and individually for each volunteer in **Supplementary Figure S1**), three out of the six cases from the 0-14 immunization schedule (Volunteers 1, 3, and 5) exhibited detectable levels of IgG antibodies specific against the S1-RBD at 4 weeks after the administration of the second dose (**Figure 2A** and **Supplementary Figures S1A, C, E**). This was also found for all three subjects in the 0–28 immunization schedule, although Volunteer 7 showed a weak response (**Figure 2B** and **Supplementary Figures S1G–I**). Circulating antibodies specific against S1-RBD also increased drastically 2 and 4 weeks after disease onset for all volunteers, except for Volunteers 2 and 6, that exhibited no changes in their antibodies profile throughout the time points evaluated.

The neutralizing capacities of the circulating antibodies measured in these nine breakthrough cases were also evaluated by two different techniques, as indicated in *Materials and Methods*. As evaluated by sVNT, five out of six cases in the 0–14 immunization schedule exhibited detectable levels of neutralizing antibodies 4 weeks after the administration of the second dose (**Figure 3A** and **Supplementary Figures S2A–F**). As expected, Volunteers 2 and 6 exhibited a very weak neutralizing capacity at this time point evaluated. However, upon evaluation by cVNT, only three volunteers in the 0–14 immunization schedule (Volunteers 1, 3, and 5) showed detectable neutralizing response (**Figure 3C**), which is in line

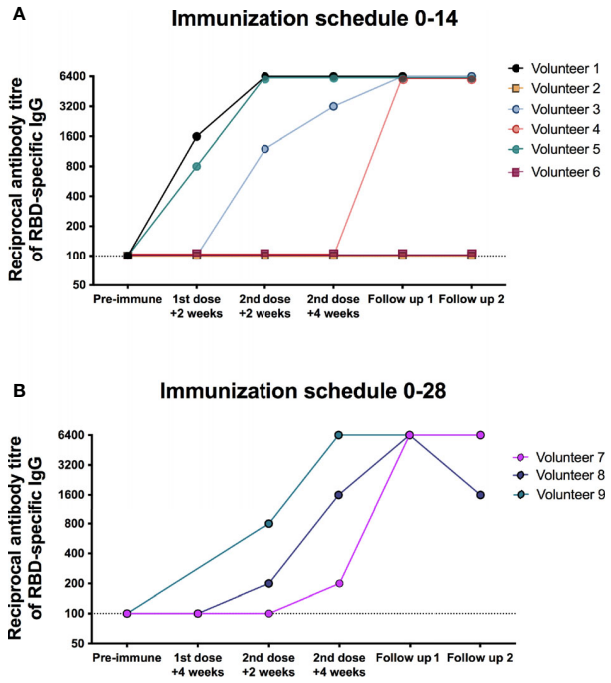

FIGURE 2 | Circulating antibodies response elicited in the nine breakthrough cases measured as IgG specific against the S1-RBD of SARS-CoV-2. Specific IgG antibodies against the S1-RBD of SARS-CoV-2 were evaluated in nine breakthrough cases that received two doses of CoronaVac. The figure shows the antibody titer in the serum samples obtained before administration of the first dose (pre-immune), before administration of the second dose (1st dose + 2 weeks or 1st dose + 4 weeks), 2 and 4 weeks after the second dose, and 2 and 4 weeks after the disease onset and a confirmed PCR result for SARS-CoV-2 (follow-up 1 and 2, respectively) and a confirmed PCR result for SARS-CoV-2. **(A)** shows the six volunteers enrolled in the 0–14 immunization schedule, and **(B)** shows the three volunteers enrolled in the 0–28 immunization schedule.

with the results obtained for IgG antibodies specific against the S1-RBD (**Figure 2A**). Notably, no neutralizing capacities were detected for the antibodies of Volunteer 4 (who displayed a moderate disease development) 2 or 4 weeks after the second dose, for both sVNT and cVNT (**Figures 3A, C**). All three cases in the 0–28 immunization schedule had detectable levels of neutralizing antibodies, by both sVNT and cVNT, 2 and 4 weeks after the administration of the second dose (**Figures 3B, D**). Noteworthy, Volunteer 7 (who developed severe symptoms) exhibited a very weak neutralizing capacity at these time points evaluated. As also seen for the circulating IgG antibodies specific against the S1-RBD, the neutralizing capacities of most volunteers increased drastically 2 and 4 weeks after the onset of disease symptoms, even for Volunteer 4, who exhibited no response after vaccination (**Figures 3A**–**D**).

IFN-γ Releasing by T Cells in Breakthrough Cases

To evaluate the cellular immune response elicited in these nine breakthrough cases, ELISPOT assays were performed as seen on **Figure 4** and **Supplementary Figure S3**. The number of spot-forming cells (SFC) positive for IFN-γ upon stimulation with MPs of peptides derived from SARS-CoV-2 were measured, as described in *Materials and Methods*. For most volunteers, upon stimulations with MPs containing 15-mer peptides (MP-S and MP-non-spike), SFC values measured in samples obtained 2 weeks after the administration of the second dose exhibited at least a two-fold increase as compared to those obtained before the administration of the first dose (**Figure 4A** for the 0–14 immunization schedule and **Figure 4B** for the 0–28 immunization schedule). Interestingly, Volunteer 6 showed no remarkable changes in the SFC values up to 4 weeks after the second dose, similar to that observed for Volunteer 9. SFC values increased for all volunteers (except Volunteer 2) 2 or 4 weeks after disease onset. Overall, SFC values obtained were higher when stimulating with MPs containing 15-mer peptides compared to those obtained when stimulating with MPs containing 9- to11-mer peptides (MP-CD8A and B) for both immunization schedules (**Figures 4A, C** for the 0–14 immunization schedule and **Figures 4B, D** for the 0–28 immunization schedule). Remarkably, Volunteer 6 displayed a good cellular response both after vaccination and infection, despite exhibiting a poor humoral response. The variation in SFC values for each volunteer after stimulation of MP-S and MP-non-spike and MP-CD8A and B is shown in **Supplementary Figure S3** and **Supplementary Tables 1**, **2**.

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

Immune Responses of Vaccine Breakthrough Cases as Compared to a Control Cohort

For the purpose of better understanding whether the immune response elicited after vaccination in breakthrough cases was an exclusive feature and a determining factor in the susceptibility to the further infection, we compared the humoral and cellular-mediated immune response of breakthrough cases with the response observed in a control group of individuals vaccinated with similar characteristics to the breakthrough population, but without manifestation of clinical symptoms related to COVID-19. Control cohort consisted of 18 subjects who received two doses of CoronaVac on similar dates to the breakthrough cases and shared demographic characteristics as detailed in **Supplementary Table 3**.

As observed in **Figure 5A**, breakthrough cases show neutralizing antibodies titers about two-fold lower than the control group for sVNT, with geometric mean titers (GMTs) of 9.5 (95% CI, 3.1–28.7) vs. 31 (95% CI, 17.8–53.2) and 13.7 (95% CI, 4.5–42.2) vs. 24 (95% CI, 14.2–38.9), 2 and 4 weeks after the second dose, respectively. In a similar way, the GMTs in the breakthrough group were approximately four-fold lower than those obtained by the control cohort for cVNT, 4.5 (95% CI, 2–10) vs. 18.7 (95% CI, 8.8–39.6) and 5.4 (95% CI, 2.5–11.6) vs. 28.5 (95% CI, 15–54.6), 2 and 4 weeks after the second dose, respectively. Importantly, these trends were sustained when titers of neutralizing antibodies from six additional breakthrough cases, which had data available for samples after vaccination, were added to the analysis (**Supplementary Figure S4**).

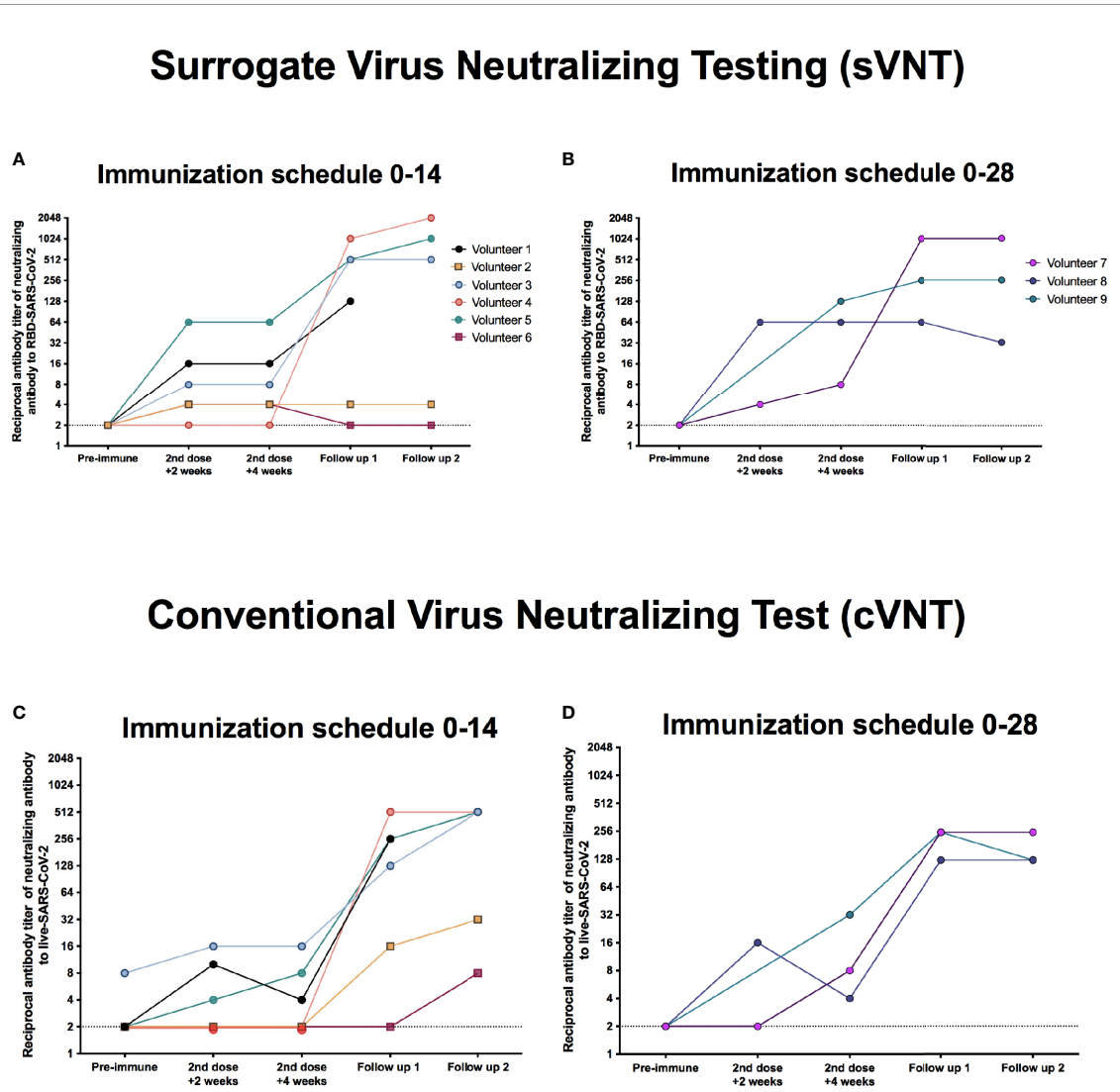

FIGURE 3 | Circulating antibodies exhibit varying neutralizing capacities in the nine breakthrough cases. Neutralizing antibodies were evaluated before administration of the first dose (pre-immune), 2 and 4 weeks after the second dose, and 2 and 4 weeks after the disease onset (follow-up 1 and 2, respectively). Two different techniques were used, a surrogate virus neutralization test (sVNT) based on the perturbation of the hACE2-spike protein–protein interaction mediated by antibodies, and a conventional virus neutralization test (cVNT) evaluating plaque and CPE reduction. **(A)** Neutralizing antibody titers detected by using the sVNT in six volunteers enrolled in the 0–14 immunization schedule. **(B)** Neutralizing antibody titers detected by using the sVNT in three volunteers enrolled in the 0–28 immunization schedule. **(C)** Neutralizing antibody titers detected by using the cVNT in six volunteers enrolled in the 0–14 immunization schedule. **(D)** Neutralizing antibody titers detected by using the cVNT in three volunteers enrolled in the 0–28 immunization schedule.

Conversely, we observed a better cellular response after stimulation with 15-mer MPs in the breakthrough cases than the control group at 2 weeks after the second dose administration, which decreased at 4 weeks after the second dose to lower levels than the control group. Regarding the 9- to 11-mer MPs stimulating (mainly $CD8^+$ T cells), a greater response was observed in the control group but only in approximately 50% of the individuals at 4 weeks after the second dose (**Figure 5B**).

In summary, these results show that detection of low levels of neutralizing antibodies after vaccination could be related to symptomatic infection; however, unknown underlying conditions must be affecting this susceptibility because low titers were also observed in some individuals belonging to the control group and high titers in the breakthrough group.

DISCUSSION

The use of different vaccines approved for emergency use due to the rapid spread of SARS-CoV2 has been key in stopping the uncontrolled progression of deaths worldwide. However, it has

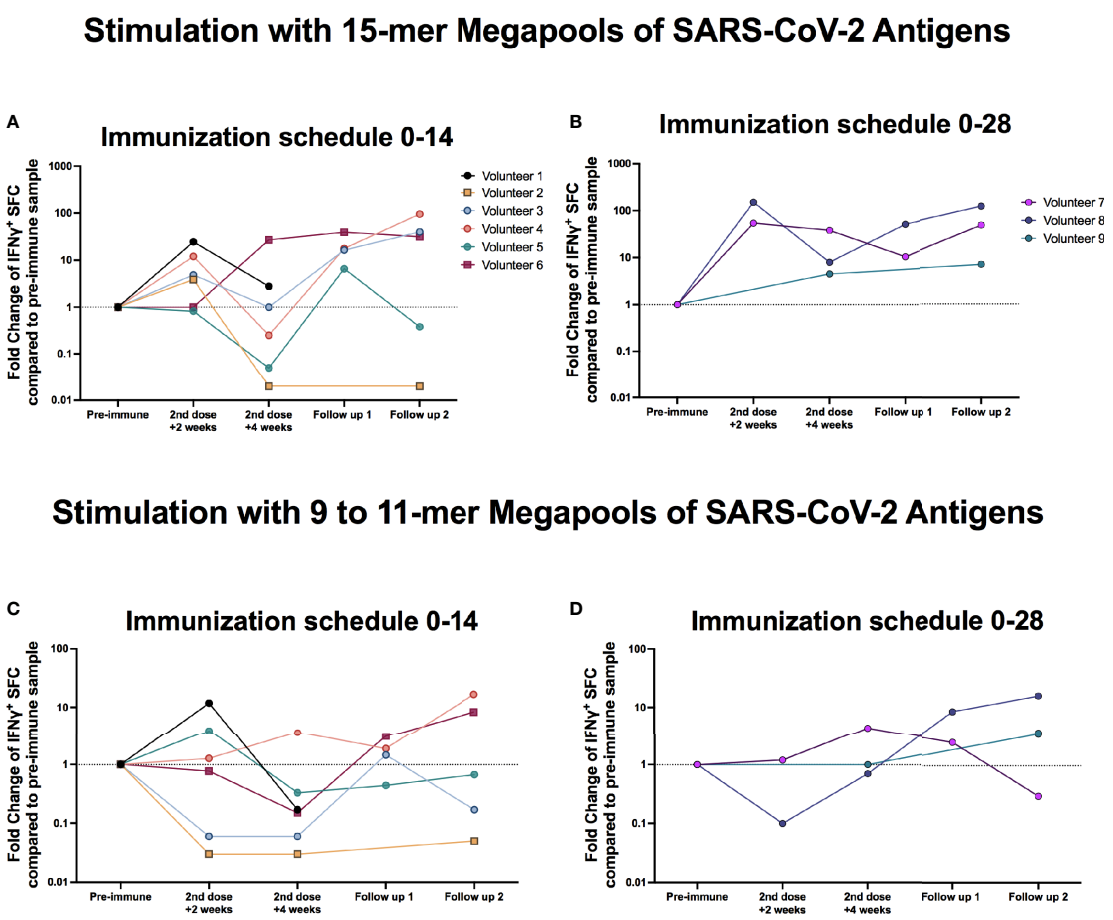

FIGURE 4 | The IFN-γ production by T cells from breakthrough cases after stimulation with MegaPools of SARS-CoV-2 peptides is heterogeneous. PBMCs from the nine breakthrough cases were obtained before administration of the first dose (pre-immune), 2 and 4 weeks after the second dose, and 2 and 4 weeks after the disease onset (follow-up 1 and 2, respectively) and evaluated by ELISPOT assays. Cells were stimulated for 48 h with two MPs containing several peptides from SARS-CoV-2 to induce the secretion IFN-γ by T cells. The number of spots-forming cells (SFCs) was evaluated. Data are shown as the fold increase regarding to the preimmune value for SFCs. **(A)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 15-mer peptides from SARS-CoV-2 of six volunteers enrolled at the 0–14 immunization schedule. **(B)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 15-mer peptides from SARS-CoV-2 of three volunteers enrolled at the 0–28 immunization schedule. **(C)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 9- to 11-mer peptides from SARS-CoV-2 of six volunteers enrolled at the 0–14 immunization schedule. **(D)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 9- to 11-mer peptides from SARS-CoV-2 of three volunteers enrolled at the 0–28 immunization schedule.

been reported that people with comorbidities can develop a more severe disease upon infection with SARS-CoV-2 (23). In this line, the efficacy of these vaccines can be impaired by the existence of previously described diseases or pathologies (24). In addition, the severity of the disease can be even more pronounced in the elderly, as they exhibit higher dysfunction in their immune system as compared to young people (25).

In this clinical trial, a total of 2,263 volunteers were vaccinated with two doses in two different immunization schedules. Out of all these volunteers, a total of 450 were part of the immunogenicity profile evaluation group. Here, we report the clinical outcome and immune response elicited by nine volunteers from the immunogenicity branch that were infected with SARS-CoV-2 and developed mild, moderate, or severe cases of COVID-19. Our results showed that the humoral and cellular immune response elicited by breakthrough CoronaVac cases was heterogeneous, and at least in these nine individuals, a correlate of infection was not evident. Yet, older people have a greater susceptibility to develop severe diseases as compared to younger people.

Of these nine volunteers, six exhibited some degree of overweight, and only one volunteer did not have any comorbidity. Two volunteers developed diseases that required hospitalization. Volunteer 7, a 69-year-old man, reported four comorbidities and required mechanical ventilation. Volunteer 4, a 62-year-old man, reported two comorbidities and required supplemental oxygen. Remarkably, in line with the results shown here, various publications have suggested that men are more

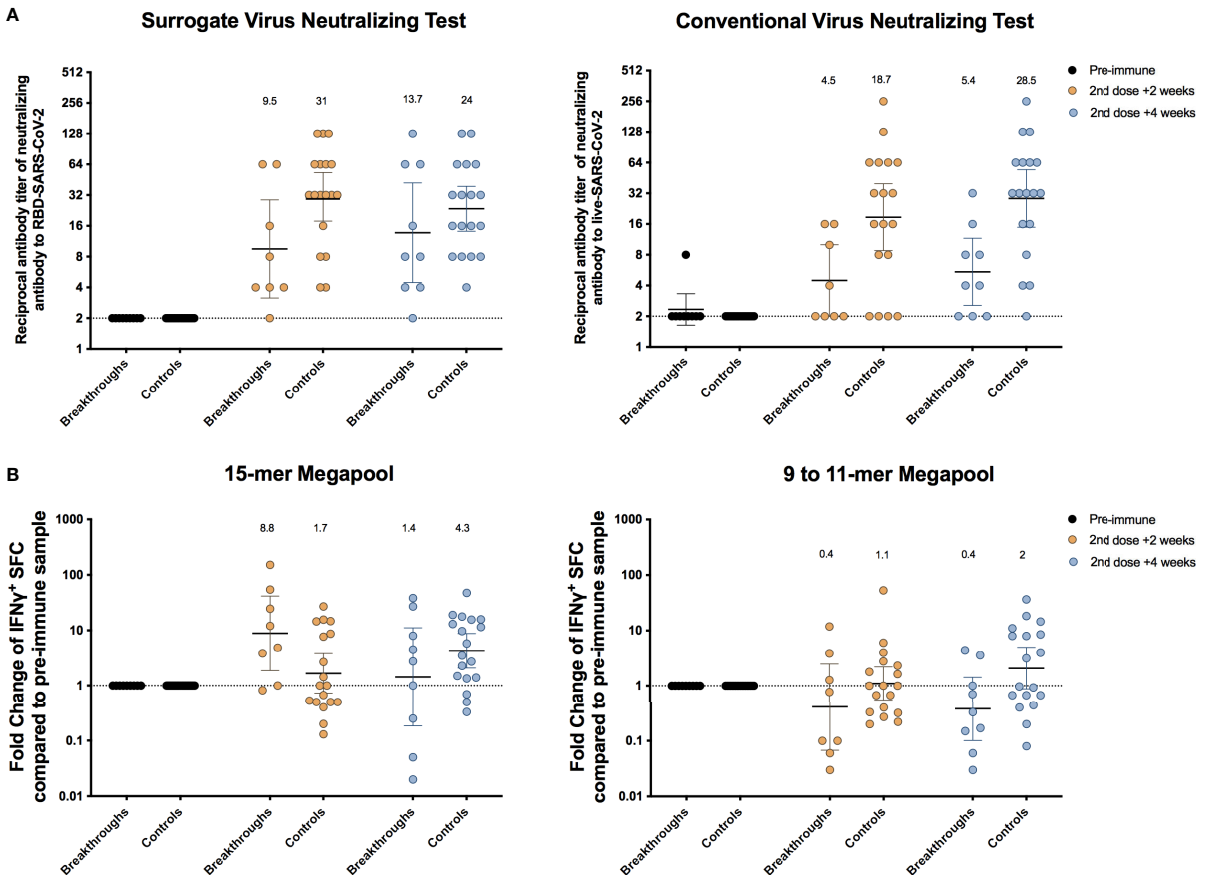

FIGURE 5 | Humoral and cellular immune responses of breakthrough cases as compared to a control cohort. A control cohort of 18 subjects who received two doses of the CoronaVac was selected by matching with breakthrough cases (2:1 ratio) according to the biological sex, range of age, and schedule of vaccination. **(A)** Titers of antibodies able to inhibit RBD-SARS-CoV-2 interaction with ACE2 receptor or surrogate virus neutralizing test (sVNT, left) and titers of neutralizing antibodies against infective SARS-CoV-2 or conventional virus neutralizing test (cVNT, right) detected in the breakthrough and control cohort. Serum samples were obtained before administration of the first dose (preimmune), 2 and 4 weeks after the second dose. The numbers above the spots indicate GMT, and error bars show the 95% CI of the GMT. **(B)** Fold change of IFN-γ^+ SFCs after stimulation of PBMCs with MPs containing 15-mer peptides (left) and 9- to 11-mer MPs (right) from SARS-CoV-2 proteome in the breakthrough and control cohort. PBMCs were obtained before administration of the first dose (preimmune), 2 and 4 weeks after the second dose. The numbers above the spots indicate geometric mean of the fold increase regarding to the preimmune sample, and error bars show the 95% CI. GMT, geometric mean titer; PBMCs, peripheral blood mononuclear cells; MPs, megapools.

prone to severe cases of COVID-19 and deaths than women, and this is even more pronounced in older populations (26, 27). Overweight and obesity are one of the most common comorbidities reported in critical patients suffering severe cases of COVID-19 (28). Furthermore, it has been reported that patients with elevated BMI exhibit more severe infection than patients with normal BMI (a high BMI is usually defined as ≥25) (29). This point is critical, as Volunteers 4 and 7 had a BMI of 28·0 and 29·3, respectively.

The particular bad evolution presented by Volunteer 7 could be partially explained by his underlying hypertension, and its corresponding treatment, which could induce an overexpression of angiotensin-converting enzyme 2 (ACE2), the receptor used by SARS-CoV-2 to infect target cells (30). Cardiac diseases have also been strongly associated with an increase in the susceptibility of SARS-CoV2 infection, the severity of COVID-19, and the susceptibility to death, as drugs used to control these illness may result in the overexpression of ACE2 in the heart (31, 32).

The hypothyroidism reported for Volunteer 4 has been related to increased susceptibility to severe COVID-19, as it affects the expression of ACE2 (33). Hypothyroidism may also be a factor predisposing the development of cardiac diseases, which increase the susceptibility of SARS-CoV-2 infection (33). As Volunteer 4 reported fewer comorbidities than Volunteer 7 (and therefore probably less risk factors to acquire SARS-CoV-2 and develop more severe COVID-19), a better prognosis would have been expected, which is in line with the information reported here.

Two volunteers out of the nine breakthrough cases did not exhibit a detectable immune response after immunization with CoronaVac. Volunteers 2 and 6 were younger than 60 years old

and were of different sex. Volunteer 2 was a male with overweight (BMI, 28.9) and several comorbidities such as hypothyroidism arterial hypertension, coronary heart disease, fatty liver disease and dyslipidemia. He also reported a medical history of several infectious diseases in his childhood and adulthood. The circulating antibodies of this volunteer showed a poor neutralizing capacity, and there was a practically null induction of IFN-γ-secreting T cells after both vaccination doses and even after infection with SARS-CoV-2. Despite this, the degree of the disease reported in this subject was mild, and he did not require hospitalization or oxygen assistance, but it is possible that innate immunity also played a key role in the protection of this individual or that antigen-specific adaptative immune responses were not detected, since they could be restricted to mucosae or lungs (34, 35). Volunteer 6 was a female with normal weight and comorbidities such as hypothyroidism. The circulating antibodies of this volunteer showed a poor neutralizing capacity, but unlike Volunteer 2, she developed a robust cellular response after 4 weeks of vaccination which was also increased after disease onset. Although the number of breakthrough cases between both immunization schedules are not balanced, it is important to note that Volunteer 2 and 6 were vaccinated in the 0–14 schedule, which has been reported to induce a lower seroconversion rate and GMTs than the 0–28 schedule (36). Interestingly, both volunteers had hypothyroidism as a common comorbidity, which could affect the induction of the immune response and produce a dysregulation of the immune system (37). In this line, more in-depth studies are required to understand which factors could be involved in these poor responses and how they could impact in the future with the appearance of new circulating variants of SARS-CoV-2.

Limitations of this study include the sample size and the focus on self-reporting to identify breakthrough vaccine infections. Asymptomatic infections were not discarded and could therefore be missed in the cohort chosen as control, which in turn may cause a misinterpretation of the results regarding the comparison with the immune response elicited by the breakthrough cases. Therefore, our conclusions are directed toward the correlation of protection to suffer a symptomatic infection. On the other hand, only in Volunteer 4 the Gamma variant was identified by molecular analysis, and these data remained unknown for the rest of the breakthrough cases analyzed (Volunteer 6, 7, and 9). Hence, we lack evidence to determine whether the frequency of breakthrough vaccine cases is related to community transmission of a particular variant, which, in the case of Chile, has been dominated by the SARS-CoV-2 variants Gamma and Lambda in recent months (38).

Despite the low number of breakthrough cases included in this report, our results provide a clear and extensive clinical and immune description of mild, moderate, or severe infections exhibited after full vaccination with CoronaVac and support previous evidence that a poor induction of neutralizing antibodies after vaccination could be correlated to a decrease in the vaccine efficacy (39–41). Furthermore, data presented here provide valuable information over the potential role that play the underlying comorbidities on the vaccine effectiveness, which could impair the ability of an individual to activate a robust immune response after vaccination, and increase the risk of severe COVID-19 in elderly people. This information could be helpful and timely support the need of a booster dose in susceptible individuals with underlying conditions after a specific time to increase its protection.

Although the information presented here must be interpreted with caution because the sample size is small to generalize, some strengths of our study are worth noting, such as the serial testing after vaccination and infection and the measurement of T-cell responses in addition to humoral response. Previous reports have been focused on viral sequence information or antibodies detection on samples obtained after the onset of symptoms (11, 12, 39, 42, 43). This new information could be the interest to the scientific community and health authorities due to the urgent need to understand the individual variables that predispose to breakthrough infections and further find a correlate of protection that has not been established to date for SARS-CoV-2 infections; yet, some studies suggest that the level of neutralizing antibody titers is highly predictive of immune protection (40, 41). In this regard, our serial sample data reveal some key features: first, older volunteers 4 and 7 who presented moderate and severe illness, respectively, displayed the weakest humoral response after vaccination, but conversely, they showed the highest level of neutralizing antibodies titers after infection. Notably, susceptibility to infection was irrespective of the immunization schedule, as one of them belonged to the 0–14 immunization schedule and the other one to the 0–28. Second, younger people could not be able to elicit a good humoral immune response after vaccination or subsequent infection, as shown by volunteers 2 and 6. These observations could be explained, at least in part, by the presence of some comorbidities in these individuals and highlighted the importance of combining clinical information along with immunogenicity and efficacy studies. Finally, individuals with evidence of neutralizing antibodies elicited by vaccination can also become sick, but this is more likely to course with a mild infection (Volunteers 1, 3, 5, 8, and 9). Importantly, we observed that the level of neutralizing antibodies in this breakthrough cohort was lower than that in controls without a confirmed SARS-CoV-2 infection, but it remains to be determined what titers of antibodies are needed to prevent infection.

On the other hand, since the approval for the emergency use of CoronoVac, the WHO has encouraged addressing the current knowledge gap about the vaccine efficacy through assessment and reporting of breakthrough infections by using neutralization and T-cell immunity assays (44). To our knowledge, this is the first time that cellular-mediated response is reported for breakthrough vaccine cases. Our results showed that breakthrough cases had a good T-cell response elicited after vaccination but that was more associated to $CD4^+$ than $CD8^+$ T cells. A similar response was observed after infection, with only a volunteer not responding (Volunteer 2). It is important to note that not only cellular response to spike protein was evidenced but also to others viral antigens, as shown after stimulation with the megapool R (**Supplementary Figure 3**). However, it is not clear whether both humoral and T-cells responses are needed for protection, and further studies are needed to address that issue.

In summary, vaccination with CoronaVac is effective, and vaccine breakthrough cases showed mainly mild symptoms of COVID-19, even in those who did not exhibit a potent humoral immune response, which could be possibly associated with different risk factors as overweight and other comorbidities that could impair the immune response induced upon immunization. While additional data have become available to draw more robust conclusions, this evidence and information could be useful to the countries that actually have implemented CoronaVac in their vaccination campaigns and to guide future vaccination program policies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité Ético Científico de Ciencias de la Salud UC, Pontificia Universidad Católica de Chile. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: AK, KA, SB, PG, JG-A, GZ, and WM. Visualization: AK, KA, SB, PG, JG-A, GZ, and WM. Methodology: RF and JM. Investigation: LD, NG, CI, FM-G, JS, BS, MU, RB-R, LG, GH-E, DM-T, GAP, MR, DR-P, OV, YV, MN, and ÁR. Funding acquisition: AK. Project administration: AK, KA, SB, and PG. Supervision: AK, KA, SB, and PG. Writing—original draft: LD, NG, JS, CI, and MU. Writing—review and editing: AK, KA, SB, and PG. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by The Ministry of Health, Government of Chile and the Confederation of Production and Commerce (CPC), Chile, through the funding of the CoronaVac03CL Study. The Millennium Institute on Immunology and Immunotherapy, ANID—Millennium Science Initiative Program ICN09_016 (former P09/016-F), supports SB, KA, PG, and AK. The Innovation Fund for Competitiveness FIC-R 2017 (BIP Code: 30488811-0) supports SB, PG, and AK. SINOVAC contributed to this study with the investigational vaccine and experimental reagents.

ACKNOWLEDGMENTS

We would like to thank the support of the Ministry of Health, Government of Chile; Ministry of Science, Technology, Knowledge, and Innovation, Government of Chile; and the Ministry of Foreign Affairs, Government of Chile and the Chilean Public Health Institute (ISP). We also would like to thank Rami Scharf, Jessica White, Jorge Flores, and Miren Iturriza-Gomara from PATH for their support in the experimental design and discussion. We also thank Daniela Weiskopf and Alejandro Sette for sharing the SARS-CoV-2 Megapools used in the ELISPOT assays and their assistance in T-cells stimulation experiments. We also thank Ana María Guzmán, Marcela Ferrés and Constanza Martínez from Escuela de Medicina, Pontificia Universidad Católica de Chile, for supporting in laboratory assays. We also thank the Vice Presidency of Research (VRI), the Direction of Technology Transfer and Development (DTD), the Legal Affairs Department (DAJ) of the Pontificia Universidad Católica de Chile. We are also grateful to the Administrative Directions of the School of Biological Sciences and the School of Medicine of the Pontificia Universidad Católica de Chile for their administrative support. Special thanks to the members of the CoronaVac03CL Study Team (list in the **Supplementary Appendix (SA)** for their hard work and dedication to the clinical study; the members of the Independent Data Safety Monitoring Committee (members in the SA) for their oversight, and the subjects enrolled in the study for their participation and commitment to this trial.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.742914/full#supplementary-material

Supplementary Figure 1 | Evaluation of anti-S1-RBD SARS-CoV-2 Ig-G antibodies through ELISA assays. Results are reported as the optical density value (OD_{450nm}) reached after two-fold serial dilutions, starting at 1:200. Samples were obtained before administration of the first dose (pre-immune), two and four weeks after the second dose, and two and four weeks after the disease onset (follow up 1 and 2, respectively). Dotted line indicates the cut-off for the serum dilution at 1:200. **(A–F)** Volunteers 1 to 6 belonging to the 0-14 immunization schedule. **(G–I)** Volunteers 7 to 9 belonging to the 0-28 immunization schedule.

Supplementary Figure 2 | Percentage of inhibition of hACE2-spike protein-protein interaction evaluated by a surrogate virus neutralization test (sVNT). Serum samples from nine volunteers were two-fold serially diluted starting to 1:2 and up to 4,096 for neutralizing antibodies detection. Samples were obtained before administration of the first dose (pre-immune), two and four weeks after the second dose, and two and four weeks after the disease onset (follow up 1 and 2, respectively). The dotted line represents the cut-off value at 30% of inhibition **(A–F)** Volunteers 1 to 6 belonging to the 0-14 immunization schedule. **(G–I)** Volunteers 7 to 9 belonging to the 0-28 immunization schedule.

Supplementary Figure 3 | T cells responses of breakthrough cases after stimulation with MPs composed of peptides from SARS-CoV-2 proteome. IFN-γ^+

SFCs of nine breakthrough cases. Data are shown as the fold increase regarding to the pre-immune value for SFCs **(A)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 15-mer peptides from the S protein of SARS-CoV-2. **(B)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 15-mer peptides from the proteome of SARS-CoV-2 excluding the S protein. **(C, D)** Fold change of IFN-γ^+ SFCs after stimulation with MPs containing 9 to 11-mer peptides from the SARS-CoV-2 proteome.

Supplementary Figure 4 | Neutralizing antibody titers of 15 breakthrough cases as compared to 18 vaccinated subjects with no evidence of symptoms associated with COVID-19. Serum samples of individuals were evaluated before vaccine administration (pre-immune), two and four weeks after the second dose. Neutralizing antibodies titers were determined by using **(A)** a surrogate virus neutralizing test and **(B)** a conventional virus neutralizing test. The numbers above the spots indicate the geometric mean titer (GMT) and error bars show the 95% CI of the GMT.

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

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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2.2 Países que optaram por vacinas de vírus inativado, como CoronaVac, estão mais protegidos contra variantes do SARS-CoV-2, aponta estudo espanhol

Um estudo realizado por pesquisadores da Universidade de Barcelona, na Espanha, concluiu que vacinas contra a Covid-19 elaboradas com vírus inativado, como é caso da CoronaVac vacina do Butantan e da farmacêutica chinesa Sinovac, conferem maior eficácia no médio e no longo prazo no controle da pandemia, na comparação com imunizantes feitos com outras tecnologias, devido a seu desempenho diante das variantes do vírus SARS-CoV-2.

Segundo Joan Serrano-Marín e Rafael Franco, autores do artigo "Two urgent needs in the battle against COVID-19: a classic-type vaccine and specific medication", publicado na plataforma de preprints OSF, as novas tecnologias de vacinas desenvolvidas em ritmo emergencial para o combate à pandemia, como RNA mensageiro e vetor viral de adenovírus, podem conferir proteção elevada frente à cepa original do SARS-CoV-2, mas tendem a perder eficácia à medida que vão emergindo novas variantes.

"As vacinas clássicas, como a CoronaVac, promovem a geração de um repertório mais amplo de anticorpos e respostas celulares. Ou seja, elas nos permitem neutralizar o vírus seguindo estratégias mais diversas. Prova disso é a situação positiva que vivem países como Chile, China e Uruguai, onde a principal vacina utilizada tem sido a CoronaVac", explicam Joan e Rafael em entrevista exclusiva para o Portal do Butantan.

Os imunizantes de vírus inativado contêm todas as partes do vírus morto. Isso pode gerar uma resposta imune mais abrangente que as das vacinas de RNA mensageiro ou que usam adenovírus como vetor viral, já que elas utilizam somente uma parte da proteína Spike (utilizada pelo SARS-CoV-2 para infectar as células).

O artigo sugere que a reinfecção e o colapso dos sistemas de saúde podem ocorrer em países que usam as vacinas de RNA mensageiro ou de adenovírus, embora a porcentagem da população vacinada seja alta – assim como aconteceu em Israel. A mesma tendência, ou seja, novas ondas pandêmicas após a vacinação em massa com vacinas de RNA/adenovírus, estaria sendo vista, de acordo com os pesquisadores, em vários países europeus e nos Estados Unidos.

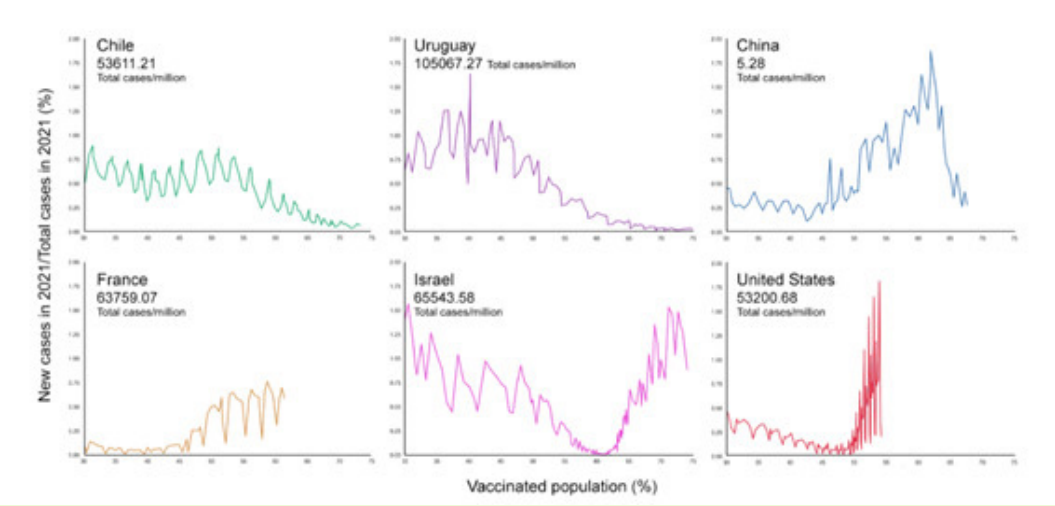
"A carga viral da variante delta é muito alta para vacinados e não vacinados. Em outras palavras, os vacinados continuarão infectando os vacinados e os não vacinados. A imunidade de rebanho, em termos gerais, é alcançada quando o número médio de infectados infecta menos de uma pessoa por infectado. Ou seja, é preciso reduzir drasticamente a transmissão. Conforme indicam os cálculos realizados, para o mesmo percentual de vacinados, a transmissão é extremamente menor nos países que utilizaram a CoronaVac como vacina principal", complementam Joan e Rafael.

Desempenho das vacinas de vírus inativado

Países como Estados Unidos, Israel e Reino Unido têm enfrentado um recrudescimento no número de casos de Covid-19, apesar dos altos índices de vacinação. O motivo é a chegada da variante delta (B.1.617.2, indiana), mais transmissível. É uma tendência oposta ao que se observa no Chile, Uruguai e China, que usaram a CoronaVac como principal imunizante.

Nos casos do Uruguai e do Chile, o aumento da porcentagem de vacinação da população com CoronaVac levou a uma redução considerável na proporção de novos casos. Em relação à China, os cientistas ressalvam que nem os aumentos nem as quedas são significativos, pois o total de 2.021 casos, medido por milhão de habitantes, é insignificante na comparação com os outros países (cinco novos casos por milhão de habitantes na China, contra 65.543 em Israel ou 53.200 nos Estados Unidos).

Para os pesquisadores, a administração da CoronaVac e outros imunizantes de vírus inativado é altamente desejável para a obtenção da imunidade coletiva devido ao amplo espectro de anticorpos que elas geram nos indivíduos vacinados, incluindo uma maior diversidade e quantidade de anticorpos neutralizantes e não neutralizantes, e sua maior capacidade de responder às possíveis mutações ou deriva genética de todas as proteínas do SARS-CoV-2.



"O maior número de estratégias imunológicas que as vacinas tradicionais induzem se deve principalmente ao fato de que, partindo do vírus completo, no caso da CoronaVac, o sistema imunológico é capaz de induzir um maior repertório de respostas, tornando esse processo mais eficaz. Isto não acontece com as vacinas modernas, de RNA mensageiro ou de adenovírus, todas elas concebidas para focar sua ação em única proteína do coronavírus, a proteína S, que também pode sofrer mutação quando o vírus sofre mutação", resumem Joan e Rafael.

Como funcionam as vacinas de vírus inativado

Cada dose de vacina de vírus inativado, cuja tecnologia é conhecida há mais de um século, é composta por trilhões de partículas do vírus em questão. Por serem inativadas, tais partículas são incapazes de provocar a doença em quem recebe o imunizante. Sua função é outra: estimular o sistema imune a reconhecer o vírus assim que entrar em contato com ele.

Como a CoronaVac contém o vírus SARS-CoV-2 inteiro inativado, o sistema imune produz anticorpos que reconhecem muitos antígenos (proteínas) do novo coronavírus. A proteína S é a principal delas, usada pelo SARS-CoV-2 para penetrar nas células humanas, mas não a única. O coronavírus conta ao todo com 29 proteínas, em sua grande maioria responsáveis por regular a multiplicação e a saída do vírus das células humanas. Sendo assim, uma variante que apresenta alteração da proteína S (mutação) deixa de ser reconhecida por vacinas específicas contendo somente a proteína S.

As vacinas modernas foram concebidas de modo a conferir ao sistema imune a habilidade de identificar a proteína S, estimulando assim a produção de anticorpos neutralizantes, que são as principais armas do nosso organismo no combate ao vírus. Já vacinas de modelo tradicional, como a CoronaVac, por conter o vírus inteiro, são capazes de estimular o sistema imune a reconhecer em maior ou menor grau todas as proteínas, disparando a produção tanto de anticorpos neutralizantes da proteína S, quanto de diversos outros relacionados às demais proteínas do arsenal viral.

Publicado em: 04/11/2021

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

Joan Serrano-Marín [1] and Rafael Franco [1,2,3,*]

[1] Dept. Biochemistry and Molecular Biomedicine. University of Barcelona, 08028 Barcelona. Spain.
[2] School of Chemistry. University of Barcelona, 08028 Barcelona. Spain.
[3] Network Center: Neurodegenerative diseases (CiberNed). Spanish National Health Institute Carlos III. 28034 Madrid. Spain.

* Correspondence: Rafael Franco, Dept. Biochemistry and Molecular Biomedicine. University of Barcelona. Diagonal 643. Prevosti Building. 08028 Barcelona. Catalonia. Spain; rfranco@ub.edu; rfranco1234@gmail.com; Tel.: +34-4021208 (R.F.)

Abstract: The COVID-19 pandemic has led to the development of vaccines against the causative virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The need for urgent release of anti-SARS-CoV-2 tools has motivated the approval of a new vaccines never used before for mass vaccination, some based on RNA (mRNA vaccines) and some using an adenoviral vector (AV vaccines). Despite high nominal efficacy, in some populations the actual numbers seem to be lower due to several factors that include new viral variants that scape from the immunological response elicited by the vaccines, which have led to new pandemic waves. In fact, the proportion of new cases has decreased in Countries using a classic-type vaccine (inactivated), CoronaVac. In the current August 2021 scenario there is a need to prevent infection, transmission and to diminish the symptoms of the disease by drug repurposing and/or development of ad hoc medication. This manuscript has two aims. On the one hand, it highlights the need to develop classic-type vaccines and to approve them in the US and in Europe. Without classic-type vaccines, herd immunity is unlikely to be achieved. On the other hand, the paper comments on different therapeutic approaches to reduce the severity of COVID-19 and the number of deaths.

Keywords: Vaccine booster, CoronaVac; Sputnik V; adenovirus; RNA vaccines; renin-angiotensin system; viral proteases.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been the worst pandemic since the so-called Spanish flu in 1918. The number of deaths and affected people around the world, in only two years, is incredibly high and the return to normal life is not expected anytime soon. As of today (August 10, 2021; https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---10-August-2021) the number of affected people is estimated to be >150 million and >3.5 million deaths, often with >10,000 occurring in a single day.

There is no approved drug/intervention to specifically fight the virus once a person is infected. Antibodies extracted from recovered or convalescent individuals may be useful (1–3), although there are doubts about their general efficacy and/or the correct protocol for use (4). Therefore, the first line of defense to stop pandemics is mass vaccination. The success in the fight against the coronavirus is based, mainly, on the speed with which the different vaccines have been developed, approved and produced. Vaccines aim to develop immunological mechanisms to stop infection, disease transmission and/or the worst consequences of infection. This is accomplished by challenging the immunological system with antigens made up of viral proteins. In the fight against SARS-CoV-2, the most successful option has been to combine new-technology vaccines including part of the nucleotide sequence coding for the spike protein. This makes sense, as the

spike is the protein that interacts with the main SARS-CoV-2 receptor on the target cell, namely angiotensin converting enzyme 2 (ACE2).

The production of the spike protein to be directly used in a vaccine is not an easy task. In fact, the spike S proteins of coronaviruses contain from 1104 to 1273 amino acids (5). Rapidly producing the huge amounts needed for the worldwide vaccination of hundreds, even thousands, of millions of people is a challenge that was never undertaken. An alternative option is to make the vaccine with a nucleic acid that encodes for the protein (in whole or in part). While it is difficult to produce and purify the protein *in vitro*, thus keeping its natural conformation and antigenicity, it is more feasible to produce the nucleic acids that encode for the protein. This approach has therefore been adopted with success in terms of efficacy against infection and production speed. Two types of nucleic acids have been used: RNA and DNA. In mRNA vaccines, the coding sequence is in the form of messenger RNA (mRNA), which enters the cells of vaccinated individuals and can be easily converted into the spike protein. To deliver the mRNA to the cells, a lipid-based encapsulation/nanoparticle can be used. In DNA vaccines, the DNA coding sequence for the spike protein can be delivered with viral vectors, like for instance those based on adenovirus (AV), which is a non-enveloped DNA virus. AVs were being developed as vaccines for diseases such as Ebola (6), but the COVID-19 pandemic has shifted the focus to the production and approval for emergency use of AV vaccines against SARS-CoV-2.

In terms of current vaccines using sequences coding for the spike protein and being administered worldwide, Pfizer and Moderna vaccines are based on RNA, whereas AstraZeneca, Johnson & Johnson and Sputnik V vaccines are based on AV, i.e. on DNA. At present (August 10) the ones approved in the European Union are those from Pfizer, Moderna, AstraZeneca, and Johnson & Johnson. In the United States, all except the AstraZeneca vaccine have obtained emergency use authorization. In other countries the vaccine developed in Russia, Sputnik V, is being tested with supposedly high efficacy rates and there are still doubts on its approval in the European Union. In China and some countries in South America, a classic type vaccine is the one that is mainly used. Looking at the whole picture one does not understand why in the EU and in the US no classic-type vaccine has been developed and approved by regulatory bodies. For decades classic-type vaccines have been developed using methods that have been successful in fighting a variety of diseases (7,8). Since the pioneering work of Louis Pasteur developing a vaccine against the rabies virus (See (9)), they have proven effective in the prevention of serious diseases caused by viruses (see WHO global vaccine Action plan: https://www.who.int/teams/immunization-vaccines-and-biologicals/strategies/global-vaccine-action-plan; accessed on August 16, 2021).

Benefits versus risks associated to new vaccines

First and foremost, the new mRNA and AV vaccines developed to fight COVID-19 are generally safe, at least in the short-term. However, due to the urgency to stop spreading SARS-CoV-2, they have been approved in less than one year after the outbreak of the SARS-CoV-2 pandemic. For one thing, possible long-term problems of vaccinated people due to a specific vaccine have not been empirically addressed. Even though, considering the preexistent bibliography, these effects are very unlikely to happen, this issue cannot be ignored considering the huge number of people receiving these vaccines. On the other hand, urgency has prevented the appearance of classic vaccines, which have shown in the past an impeccable efficacy and safety record (10,11). Accordingly, although mRNA/AV vaccines may be instrumental to achieving large numbers of short-term vaccinated people around the world, classic-type vaccines must also be considered. By August 2021, there are two classic-type vaccines approved for human use; both have been developed in China: Covilo or BBIBP-CorV (from Sinopharm) and CoronaVac (from Sinovac Research and Development) (https://www.who.int/es/news-room/q-a-detail/coronavirus-disease-(covid-19)-vaccines; accessed on August 16, 2021).

Despite the obvious benefits of reducing infections and deaths in vaccinated people, the risks must be brought to the table. The risks of thrombi for humans receiving the AstraZeneca or Johnson & Johnson vaccines are serious, but can be weighed against the risk-benefit assessment. Due to the high number of variables, it is difficult to reliably compare the percentage of cases with thrombus versus the total number of vaccinations with the overall risk of death in unvaccinated people. But it is reasonable to accept that the relatively low number of cases with thrombosis should not stop vaccination with AstraZeneca or Johnson & Johnson vaccines. However, caution should be exercised when these vaccines are administered to people taking medications in which one of the potential side effects is thrombus formation; the most obvious case is certain types of birth control pills. Another risk of the mRNA/AV vaccines is the possibility of integration of exogenous material into the DNA of host cells (12). AVs have been tested for decades as vectors in gene therapy and the problems of their use have led to the development of safer vectors such as adeno-associated viruses (see (13) for review).

The risk is seemingly lower in the case of mRNA vaccines, but it has been demonstrated that genetic material of SARS-CoV-2 can be converted into DNA that integrates into the human genome (12,14). The human genome does not include the gene for any typical reverse transcriptase, but it includes retrotransposons that can "move" using a copy and paste mechanism that requires a RNA intermediate. Accordingly, retrotransposon may act as instruments to convert RNA from viruses or mRNA vaccines into genomic DNA (12,14). One of the deciphered mechanisms is mediated by the LINE-1 retrotransposable element ORF2 protein (15,16). The human genome contains several full or truncated sequences of long interspersed element-1 retrotransposons and it is assumed that >80 of those elements can be transcribed; random integration of elements in the genome has been related to a variety of diseases (15,17,18). Interestingly, SARS-CoV-2 infection alters the usual dynamics of some transposable elements, such as LINEs, increasing their expression and, therefore, the probability of insertion of new transposable elements (19). Additionally, SARS-CoV-2 is not the only RNA virus with positive polarity (that is, that is directly transcribed by the host cell ribosomes) that has the capability of directly interacting retrotransposons; among others, Hepatitis C (16) or Sindbis (20) viruses may interact with transposons. In summary, the integration of exogenous genetic material into host genome may lead to risks, such as premature cell death or tumor cell growth, that cannot be addressed in the short term, i.e. before emergence use anti-COVID-19 vaccine approval.

The efficacy issue

The efficacy of a vaccine is not a direct measure of its capacity to avoid the symptoms of the COVID-19. In the case of the vaccines, efficacy cannot be measured as in the case of a drug for a disease, from diabetes to Alzheimer's. Efficacy of antidiabetic medication is measured in patients that take the drug and after some period of time the reduction in plasma glucose levels are measured. Few clinical parameters are needed, just the glycemia and the percentage of reduction that is considered as end point. If a 20% is selected, the efficacy is measured by the number of patients whose levels are reduced by more than 20% versus the total number of patients. In Alzheimer's disease the end point consists of increasing the score in a cognition test, for instance the mini-mental test (MMSE: Mini-Mental State Examination). The main parameters needed to test any anti-dementia medication are to select the range of scores of patients to recruit and to select the minimum expected score increase in the MMSE scale.

More parameters plus some ad hoc assumptions are needed for efficacy assessment of vaccines. First and foremost, vaccinated people does not have any disease. Then, it is not possible to assess efficacy by directly looking at whether or not vaccinated people have been cured or have fewer symptoms of the disease. The first assumption is that vaccinated individuals will have similar exposure to SARS-CoV-2 than non-vaccinated individuals (or placebo inoculated individuals). Fortunately, an ad hoc surrogate marker for vaccine efficacy is the level of IgGs in plasma, mainly

of neutralizing antibodies, i.e. antibodies that prevent infection. Unfortunately, in SARS-CoV-2 it is important to know the level of the IgGs but also the composition of IgGs. The serological quick tests have demonstrated that different COVID-19-suffering individuals produce different antibodies. In other words, quick tests, which nominally have >90% sensitivity, lead to false negatives, i.e. sensitivity may be >90% in one given infected population and may be far lower in another infected population. Plasma from convalescent patients show a mixture of anti-SARS-CoV-2 antibodies (21). Microfluidic devices have shown that humoral responses to coronavirus can elicit with a variety of antigen / antibody interaction affinities (22). To make thinks even more complicated, many of the vaccination schedules include two shots and this adds complexity to the estimation of the real preventive effect of anti-COVID vaccines. Taken together, it is almost impossible to estimate the efficacy of any vaccine with reliability. In addition, the neutralizing antibodies, i.e. those that impede infection, are unknown and/or may be neutralizing for a given strain of the virus but not for a different one. In practical terms, only the big pharma has the potential to enroll thousand individuals and to provide an efficacy estimates to apply for approval by regulatory bodies. Also, the efficacy data may vary from trial to trial, and or by adding more data if the trial is extended. It has been common for the companies developing the mRNA/AV vaccines to present, upon time, increases in the percentage of efficacy for the same vaccine. The poor efficacy values of classic-type is surely behind the decision to stop the development of some vaccines such as the TMV-083 (previously known as MV-SARS-CoV-2), which was developed by one of the most experienced institutes in the World, the Pasteur Institute (23) (see https://www.pasteur.fr/en/all-sars-cov-2-covid-19-institut-pasteur/research-projects/covid-19-vaccine-against-sars-cov-2-infection-using-measles-vector; accessed on April 19, 2021) and its partner company: Sanofi.

In summary, mRNA/AV vaccines have prevented deaths, but they have not been able to stop the spread of the virus and have favored the appearance of new variants. It is essential to have vaccines that not only prevent death, but also stop transmission and genetic shift/drift. In addition a very recent paper reporting clinical research with individuals vaccinated with RNA vaccines states: "*we document significant declines in antibody levels three months post-vaccination, and reduced neutralization of emerging variants*" (24).

The third dose issue

The use of vaccines that are not able to stop the transmission has contributed to selection of viruses with mutated forms of the spike protein. This issue was, among others, raised by Nobel Laureate Luc Montagnier. He doubted that vaccination to stop COVID-19 spread was convenient due to the appearance of new variants. No doubt vaccination has been instrumental to decrease the death toll, but novel SARS-CoV-2 variants have arisen that are able to lead to COVID-19 symptoms in vaccinated people. The current pandemic is due to a virus with a high transmission capacity, which means that a given individual may be exposed to the virus more than once and in relatively short periods of time. It is often forgotten that all people, vaccinated or not, may be infected by any SARS-CoV-2 variant. But mRNA/AV vaccines that use the sequence (DNA or RNA) of a given spike protein, may not be efficacious in attenuation infection/symptoms produced by new variants. In fact, more and more vaccinated people are being re-infected and able to infect close contacts. For instance, the AstraZeneca vaccine (ChAdOx1 nCoV-19) has shown a highly reduced efficacy, among others, against the B.1.351 variant. In summary, mRNA/AV vaccines have been useful but have led to new variants in a selection-escape fashion. In the search for convincing data to obtain vaccine approval, clinical trials with two injections were designed (with the exception of the Janssen vaccine). On the one hand, two shots surely lead to a higher production of anti-spike antibodies in serum and this may be convincing for regulatory bodies. On the other hand, two shots may be needed and/or convenient for viruses that do not have high mutation rates. However, two shots to combat a virus RNA that mutates

so rapidly is, quite likely, not the best option. Worse, here are chances of approval of a third shot of the same vaccine. Taken together, all available information and basic knowledge of the human immune system, indicates that a third dose with the same vaccine is not the best option. Fortunately, there is an alternative that, importantly, has already proven with high success, namely the use of a classic-type vaccine. By previous knowledge with this type of vaccines, the selection of new variants would be minimal and, in addition, "classical" vaccines lead to more efficient immunological tools, humoral and cellular, to fight SARS-CoV-2 via diverse components and not only via the spike protein.

Vaccination that allows viral escape by mutation will compromise the control of pandemics and the achievement of herd immunity. In reality, countries that are using mRNA/AV vaccines anticipate that herd immunity will not be achieved in such a scenario, complementary approaches should be sought (25). To combat the escape of the human immunodeficiency virus (HIV) by mutation, the so-called Highly Active Antiretroviral (HAART) or "triple" therapy was developed for acquired immunodeficiency syndrome (AIDS) patients. While one single drug was not efficacious to control the disease, the combination of three different compounds prevented mutations thus allowing disease control. The triple therapy consisted of inhibitors of two relevant components of HIV-1, the reverse transcriptase and the main viral protease (26,27). AIDS is now considered a chronic disease that produces few direct deaths. Currently, it is not possible to prevent the escape of SARS-CoV-2 by mutation using drugs, but the availability of different types of vaccines opens a window of opportunity. In the same way that a single drug is not effective for AIDS patients, a single vaccine can reduce the number of deaths, but it can allow a viral escape by mutation, a reduction in the effectiveness of the vaccine and an inability to achieve herd immunity. Accordingly, more shots of the very same vaccine will have a limited benefit in comparison with shots of a heterologous vaccine (28,29). More shots of the same vaccine may be detrimental on putting pressure to the virus thus selecting more infective viral particles. Recent developments in the anti-HIV-1 research field include the use of combining vaccines that, to combat the HIV-1 pandemic "*must induce responses capable of controlling vast HIV-1 variants circulating in the population as well as those evolved in each individual following transmission*" (30). In summary, despite the lack of a drug cocktail, a combination of different vaccines is emerging as a real alternative to effectively combat SARS-CoV-2. Obviously, the optimal treatment would not be to use vaccines directed against the same protein, that is, the SARS-CoV-2 spike protein. In European countries and in the US, all vaccines are directed against the spike protein. Should these countries approve vaccines of a different type (non-RNA-based, non AV-based) and/or directed against other viral components?

New cases after 30% population vaccination using new- or classic-type vaccines

Available data suggests that reinfection and collapse of emergency units at hospitals may occur in countries using the mRNA/AV vaccines even though the percentage of vaccinated population is high (31,32). Perhaps the main example is Israel that was among the quickest in vaccinating with mRNA/AV vaccines. The same trend, i.e. new waves after massive vaccination with mRNA/AV vaccines, has occurred in various European Countries and in the US. This trend is opposite in the only three countries that used the CoronaVac vaccine as the main vaccine (Figure 1).

Figure 1 shows the trend of new cases in three Countries mainly using CoronaVac and in three Countries using mRNA/AV vaccines. Despite alarms in Uruguay, it is clear that increasing the percentage of population vaccination with CoronaVac has led to a dramatic decrease in the proportion of new cases. Something similar has occurred in another Country mainly using CoronaVac, Chile. The data available for China suggests an increase followed by a sharp decrease, but it should be noted that neither the rises nor the falls are significant as total 2021 cases, measured per 1,000,000 inhabitants, are negligible in China compared to the other selected countries (5 in China versus 65,543 in Israel or 53,200 in the US, date: August

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

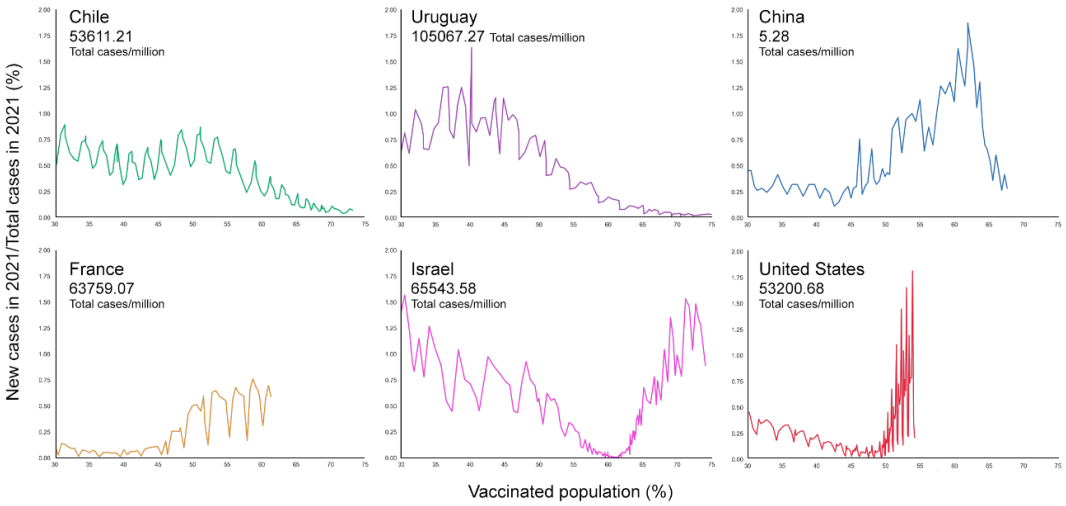


Figure 1. New COVID-19 cases versus percentage of vaccinated population. Data (retrieved until August 24, 2021) have been selected using 30% vaccinated population as threshold. Chile, Uruguay and China have mainly used CoronaVac vaccine. France, Israel and the US have used only mRNA/AV vaccines. The numbers below the name of the Country indicate total reported cases from the beginning of 2021. For comparison purposes the same axis, X and Y, were used in all graphics. A file with the data used construct the graphics, coming from repositories containing official data reported by the Countries (see "Data availability statement" below).

For statistical analysis we have considered 10 countries (US, Israel, Greece, Portugal, Ireland, Italy, Spain, United Kingdom, Denmark and France) that have not used CoronaVac but mRNA/AV vaccines, and the only three countries using CoronaVac as the main vaccine (>70% administrated doses at date August 24, 2021), China, Chile and Uruguay. Data were retrieved from a big data source, Github (https://github.com/owid/covid-19-data/tree/master/public/data), which is forged with COVID-19-related data in official webs such as in the Oxford COVID-19 Government Response Tracker or in independent global health research centers such as the Institute for Health Metrics and Evaluation at the University of Washington. The Excel file containing all data was directly downloaded from Github (https://covid.ourworldindata.org/data/owid-covid-data.xlsx; accessed (on August 24, 2021; see "Data availability statement" below). The interaction graphic was obtained using Statgraphics v. 18.1.14 from a general linear model analysis with type of vaccine (mRNA/AV or CoronaVac) as a qualitative factor, % vaccinated population as a quantitative factor and, as a dependent variable, the relative % positives in 2021 (which is the relation of new positives after reaching 30% of the vaccinated population and the total positives in 2021. The 30% threshold was set up because a lower percentage of vaccination has little effect on pandemic indicators). Although vaccination begun at the end of 2020 and the beginning of 2021, only data from 2021 were analyzed. To avoid interference due to differential public health decisions and differences in the timing and rate of vaccination in each country, no attempt was made to make comparisons between countries using similar vaccines. We have found a very significant correlation between the percentage of population receiving the mRNA/AV vaccination (full regime; two shots except for the Johnson & Johnson vaccine, which is administered in only one shot) and number of new cases after reaching 30% vaccination of the population in a given Country, namely cases in 2021 after reaching 30% vaccination versus total cases in 2021. The two lines (one for mRNA/AV viruses and another for CoronaVac) are of opposite slope, i.e. correlations are opposite when considering CoronaVac or the vaccines based in mRNA/AV. Whereas the ratio of cases after 30% vaccination increases with further vaccination with mRNA/AV vaccines, the ratio decreases in countries where CoronaVac

is used. In fact, statistical analysis shows significance for a differential trend using CoronaVac or mRNA/AV vaccines. The correlation was done using proportion of cases as quantitative variable and type of vaccine as qualitative variable. The significance holds if only three countries using the mRNA/AV vaccines are considered, i.e. considering data from 3 countries in both sets of data. The significance also holds taking out the data from China, whose management of the pandemic has been quite different to that in many other countries.

In summary, vaccination with mRNA/AV vaccines does not stop transmission, while in countries that use the CoronaVac vaccine, cases decrease with increasing population vaccination rate, suggesting effective neutralization that may eventually lead to herd immunity.

The need of a classic-type vaccine

A complete schedule of a mRNA/AV vaccine, two doses of Pfizer, Moderna or AstraZeneca, and one dose of the Johnson & Johnson vaccine, as many organizations define including The Pan American Health Organization/ World Health Organization (https://ais.paho.org/imm/IM_DosisAdmin-Vacunacion.asp), in 50% of the population has not eradicated the virus and, worse, new waves of infections have appeared. In our Country (Spain) we were, at the end of July 2021, in the mid of the fifth wave and there are officials stating (August 20) that the sixth wave is coming. In elderly houses in Catalonia (Spain) in which all residents are vaccinated (>90% with mRNA vaccines) there is a surge of new cases (August 2021; official data in: https://dadescovid.cat/?drop_es_residencia=1). This was not expected when vaccination started. Some of the reasons of having such unexpected scenario may be now figured out.

On the one hand, and apart from the reduction upon time of the antibody levels *(see above;* **The efficacy issue** section), it is known that significant amounts of mucosal IgA is associated with less viral transmission. Likewise, in all the viral infections studied to date, a higher proportion of IgA at the epithelial level reduces the risk of re-infection (33). Therefore, the production of IgAs is important to reduce (upon vaccination) re-infection and associated transmissibility (34). Not all vaccines have confirmed production of IgAs at the mucosal level; a recent publication reports IgAs secretion to human milk after shots of Pfizer's vaccine (35). This finding is important for preventing infection of the neonate, but the relevance in epidemiological terms is under question. Efficacious prevention of the infection requires production of aggregated, secretory, forms of IgA (SIgA), whose affinity for antigens is much higher than monomeric IgA (36). Therefore, one indicator of the effectiveness of a vaccine is the number of mucosal SIgAs and whether they are neutralizing or not (36). The few studies on this matter suggest that IgA production by mRNA/AV vaccines is, at the very least, very modest (37), and this seems to be one of the reason of low efficacy in reducing infection and transmission despite the high nominal values of efficacy in producing antibodies (33).

On the other hand, although it is commonly thought that the only antibodies capable of preventing infection are neutralizing antibodies, non-neutralizing antibodies are important irrespective of their later involvement in the viral replication cycle (38). In this sense, classic-type vaccines lead, by definition, to a more qualitative diverse repertoire of neutralizing and non-neutralizing antibodies than vaccines only based in producing IgG against a single viral protein.

The CoronaVac vaccine, developed by a Chinese company, Sinovac Research and Development, consists of inactivated SARS-CoV-2 and aluminum hydroxide as adjuvant. It has been among the first vaccines to be developed and at present is being tested in different countries (39). Only in China 1 million people was already vaccinated by the end of 2020 in a phase III clinical trial that started in November 2020. Fewer data about CoronaVac are available in English if compared with the huge amount of information available (in English) for the other vaccines. Although a direct comparison between classic-type vaccine and mRNA/AV vaccines is difficult to perform, some

reviews on this theme have recently appeared (see, for instance (39–41)). A recent paper compares data from 13 clinical trials of 11 different vaccines, taken both reports in English and in Chinese. The conclusion of the authors is that: "*Most of the COVID-19 vaccines appear to be effective and safe. Double-dose vaccination is recommended. However, more research is needed to investigate the long-term efficacy and safety of the vaccines and the influence of dose, age, and production process on the protective efficacy*" (42).

It is remarkable and far from being generally known by the population and by Western Health authorities that, CoronaVac lacks the serious side effects identified for RNA- and AV vaccines (43), namely, clot formation, Guillain-Barré syndrome, myocarditis, etc. Additionally, vaccine developers already have experience on controlling pandemics with inactivated vaccines, such as that caused by the poliovirus at the beginning of the 20th century, whose mutation rate is similar to that of SARS-CoV-2 (44,45) and whose basic reproduction number (R_0) throughout the pandemic was not different from that of the coronavirus (46,47). In summary, mRNA/AV vaccines have instrumental for the quickness in being approved and for the high nominal efficacy rate but classic-type vaccines are needed and the only one already developed shows that it should enter into the vaccination program to combat COVID-19 in all over the World.

Safety, tolerability and immunogenicity was successfully addressed in a first phase I/II trial in volunteers of the Suining County of Chinese Jiangsu province. One of the outputs of the study was the selection of 3 μg CoronaVac dose for phase III trials, which have been performed in different countries. Approval has been granted already in, among others, China (48), Brazil (https://www.reuters.com/article/us-health-coronavirus-brazil-coronavac-idUSKBN29R2GL; accessed April 23, 2021), Uruguay and Chile (https://www.ispch.cl/noticia/isp-autorizo-la-vacuna-coronavac-del-laboratorio-sinovac-life-sciences-co-ltd-para-uso-de-emergencia-en-el-pais/; accessed April 23, 2021).

Chile, which is a country of reference in anti-COVID-19 vaccination, is using the CoronaVac and the Pfizer vaccines in a 80:20 approximate proportion (80 CoronaVac, 20 Pfizer); the two vaccines are scheduled to be given as two injections. CoronaVac was approved in Chile after the results of a phase III clinical trials performed in the Country. It has been noticed that the efficacy in preventing productive infection, especially after the first shot is modest and comparable to that whose development was stopped by Pasteur/Sanofi, i.e. in the 50-60% range. Remarkably, this low level of efficacy does not result in poor performance and this has been proved by data obtained upon continuing vaccination schedules. The good COVID-19 data in Chile, which is due to the Pfizer and CoronaVac vaccines, strongly suggest that efficacy estimates are not enough to rule out a vaccine. There is strong evidence showing that despite low efficacy estimates, CoronaVac is achieving the key objective, which is to save human lives. Another phase III trial (PROFISCOV Study) was conducted between July 21 and December 16, 2020 in Brazil among healthcare professionals (49,50). The conclusion as posted in Elsevier's SSRN database is that the vaccine was "*efficacious against any symptomatic SARS-CoV-2 infections and highly protective against moderate and severe COVID-19*" (50).

Some of the advantages of vaccines that protect from infection despite having low nominal efficacy values and lower antibody titers than those elicited by mRNA/AV vaccines, may come for an appropriate engagement of T cell responses. The likelihood of requiring robust T helper cell responses to prevent COVID-19 infection has been suggested from a mouse study using recombinant spike proteins (51). In fact, based on previous experience with coronavirus, the risk of antibody-dependent potentiation (ADE) for anti-SARS-CoV-2 is significant, pointing to the need to develop vaccines that are less dependent on antibody production and more than T cell responses (52). In summary, both humoral and cellular responses are needed for an effective fight against this specific coronavirus. Surprisingly, there is evidence of negligible impact of SARS-CoV-2 variants on T-cell responses, i.e. variants that escape the action of antibodies are likely unable to cope with $CD4^+$ and $CD8^+$ T cell reactivity (53). In this sense, CoronaVac apart from

being safe and producing neutralizing antibodies against the receptor binding domain of the S1 spike protein, immunization induced the activation of T cells (when exposed to SARS-CoV-2 antigens) and the secretion of IFN-γ (54). A recent publication shows that one dose of CoronaVac is already effective against the spreading of the P-1 Brazilian variant of the virus (55).

The need of a specific anti-COVID-19 medication

Drugs used at the beginning of the pandemic, including antibiotics and human immunodeficiency virus protease inhibitors, were not at all effective. When noting that the most serious symptom derived from an imbalance in the immune response with exacerbation of the production of pro-inflammatory cytokines that aggravated the pneumonia, the treatment of choice consisted of glucocorticoids. Since vaccines have not been able to fully prevent infection and disease transmission, there is an urgent need to develop specific anti-COVID-19 drugs.

One interesting possibility is to target the renin-angiotensin system (RAS). The rationale is mainly based in the main SARS-CoV-2 receptor, angiotensin converting enzyme 2 (ACE2). This RAS member interacts with other RAS members such as angiotensin II receptors, which belong to the family of G protein-coupled receptors (GPCRs). GPCRs are very druggable and, in fact, are the target of about 40% of approved drugs worldwide. In addition, antagonists of angiotensin receptors are approved to combat hypertension. Accordingly, it would be informative to perform clinical research correlating the RAS status in with disease severity in COVID-19 patients. Parameters to consider are arterial blood pressure values, the use or not of anti-hypertensives and the type of anti-hypertensives, i.e. whether antihypertensives targeting RAS leads to a differential course of the disease compared with using other type of antihypertensives. In addition, targeting RAS members may lead to decrease in infection because RNA viruses need GPCRs to enter into cells and several RAS members are GPCRs and ACE2 interacts with some of those RAS GPCRs (see (56) and references therein). Often, the serious effects of SARS-CoV-2 infection that can eventually lead to death are due to an imbalance of the immune system in which macrophages play a key role (57). A hot topic in the immune system field is to find drugs able to produce M2 macrophages that, opposite to the M1 or proinflammatory macrophages, facilitate the resolution of inflammation. Accordingly, the discovery of targets to produce M2 macrophages is a promising approach to fight against COVID-19.

Soon after the beginning of the pandemics, a laboratory that has been for years involved in coronavirus research solved the structure of the main protease of SARS-CoV-2 (M^{pro} also known as $3CL^{pro}$) also designing specific inhibitors of the alpha-ketoamide type (58). These inhibitors are at the forefront of being used as specific anti-COVID-19 tools (59).

All over the world there are screening of several compound libraries to try to find inhibitors of viral infection. At present several target candidates have been proposed to manage SARS-CoV-2 infection but further research is needed to find the most promising ones in terms of druggability, efficacy and safety (60–62).

Data Availability Statement: Data used to build Figure 1 will be available upon request when the paper becomes published (data retrieved from repositories with official data on COVID-19 from all Countries).

Author Contributions: R.F. participated in conceptualization and wrote the first draft. J.S.M. did the search of the data and performed statistical analysis. Both authors have contributed to prepare the final version and approve submission.

Funding: Not applicable.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: Not applicable

Conflicts of Interest: The authors declare no conflict of interest.

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2.3 Estudo sugere que populações de países da América do Sul que usaram CoronaVac estão protegidas contra variantes gama e lambda

Um estudo de pesquisadores do Brasil e do Uruguai sugere que as populações dos países do sul da América do Sul estão mais protegidas contra as variantes regionais gama e lambda do vírus SARS-CoV-2. As conclusões estão em um artigo científico publicado na semana passada na plataforma de preprints MedRxiv. Segundo os pesquisadores, da Universidade da República, de Montevidéu, e da Fundação Oswaldo Cruz (Fiocruz), do Rio de Janeiro, a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, na condição de vacina inativada, contribuiu decisivamente para esse resultado.

De acordo com o estudo, Argentina, Brasil, Chile, Paraguai e Uruguai experimentaram ondas epidêmicas graves de Covid-19 no início de 2021, impulsionadas pela expansão das variantes gama e lambda. No entanto, a partir de junho, houve uma melhora nos indicadores da epidemia. Na 14ª semana epidemiológica, entre 4 e 10 de abril, foram registrados 21.141 óbitos por Covid-19, segundo o Painel Coronavírus do Ministério da Saúde. Foi a maior quantidade de mortes registrada em sete dias no ano inteiro. Já na 25ª semana epidemiológica, entre 20 e 26 de junho, o número de mortes havia se reduzido para 11.935. Desde então, o indicador continua caindo e, na última semana epidemiológica, entre 19 e 25 de setembro, o número de mortes por Covid-19 no Brasil foi de 3.692.

O estudo afirma que o uso generalizado da CoronaVac no sul da América do Sul foi não só eficaz para prevenir as formas graves da Covid-19, mas também conteve a disseminação das variantes regionais altamente transmissíveis. No Chile, 70% das vacinas aplicadas correspondem à CoronaVac; no Uruguai, 60%; no Brasil, 35%. Vale lembrar que até meados de maio, a vacina do Butantan respondia por cerca de sete a cada dez imunizantes aplicados.

Para investigar os resultados dos programas nacionais de vacinação e o impacto da infecção natural na transmissão viral dos países do Cone Sul, os pesquisadores analisaram a associação entre a mobilidade da população e o número efetivo de reprodução (Rt) – número médio de pessoas infectadas em um determinado momento por um indivíduo infectado introduzido em uma população parcialmente imune ou suscetível (ou seja, no início da epidemia).

As análises revelaram que, de janeiro a maio de 2021, a mobilidade da população na Argentina, Brasil, Chile, Paraguai e Uruguai esteve relacionada ao número efetivo de reprodução Rt. A partir de junho, no entanto, a taxa de transmissão viral começou a ser menor do que o esperado conforme os níveis de interação social. "O estudo sugere que as populações do Cone Sul da América do Sul provavelmente alcançaram o HIT [limiar condicional de imunidade de rebanho, em inglês herd immunity threshold] para conter a transmissão das variantes gama e lambda do SARS-CoV-2 por volta de meados de 2021", afirmam os pesquisadores. As análises dos cientistas indicam que o limiar de imunidade HIT para o vírus da Covid-19, na América do Sul, variou entre 29% na Argentina, 33% no Uruguai, 36% no Paraguai, 43% no Chile e 45% no Brasil.

Os pesquisadores sugerem que os níveis de imunidade natural elevados identificados nos países da América do Sul podem ser uma condição importante que esteja contribuindo para limitar a expansão da variante. Segundo informações dos especialistas, a contribuição desta imunidade é resultado da infecção natural associada à vacinação.

Publicado em: 04/10/2021

SARS-CoV-2 epidemic in the South American Southern cone: can combined immunity from vaccination and infection prevent the spread of Gamma and Lambda variants while easing restrictions?

Marcelo Fiori*† Gonzalo Bello‡ Nicolás Wschebor§ Federico Lecumberry¶
Andrés Ferragut‖ Ernesto Mordecki**

September 15, 2021

Abstract

All South American countries from the Southern cone (Argentina, Brazil, Chile, Paraguay and Uruguay) experienced severe COVID-19 epidemic waves during early 2021 driven by the expansion of variants Gamma and Lambda, however, there was an improvement in different epidemic indicators since June 2021. To investigate the impact of national vaccination programs and natural infection on viral transmission in those South American countries, we analyzed the coupling between population mobility and the viral effective reproduction number R_t. Our analyses reveal that population mobility was highly correlated with viral R_t from January to May 2021 in all countries analyzed; but a clear decoupling occurred since May-June 2021, when the rate of viral spread started to be lower than expected from the levels of social interactions. These findings support that populations from the South American Southern cone probably achieved the conditional herd immunity threshold to contain the spread of regional SARS-CoV-2 variants.

*Corresponding author: mfiori@fing.edu.uy
†Instituto de Matemática y Estadística "Rafael Laguardia", Facultad de Ingeniería, Universidad de la República, Uruguay
‡Instituto Oswaldo Cruz (FIOCRUZ), Rio de Janeiro , Brazil
§Instituto de Física, Facultad de Ingeniería, Universidad de la República, Uruguay
¶Instituto de Ingeniería Eléctrica, Facultad de Ingeniería, Universidad de la República, Uruguay
‖Facultad de Ingeniería, Universidad ORT, Uruguay
**Centro de Matemática, Facultad de Ciencias, Universidad de la República, Uruguay

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

notion as in a certain time SARS-CoV-2 incidence display sustained declines despite easing of lockdown restrictions, discontinuation of face mask use in open spaces and increase in population mobility [14, 15]

In the present article, we estimate the coupling between population mobility and the R_t of SARS-CoV-2 in the five South American countries from the Southern cone. Our analyses support that mobility data was highly correlated with the viral R_t in all South American countries analyzed between January and May, 2021; however, a clear decoupling between population mobility and viral transmissions was evident since May-June 2021. The mean estimated threshold of immune individuals (fully vaccinated pondered by vaccine effectiveness plus natural infected) necessary to produce such decoupling varies along the five countries from 29% to 45% and a discussion trying to understand these differences is provided. These findings also support the relevance of vaccination-induced herd immunity in South American countries with widespread use of the inactivated vaccine Coronavac.

2 Results

To analyze the potential correlation between social mobility and the spread of the SARS-CoV-2, we estimate the viral effective reproduction number R_t in every country based on mobility information provided by Google [16] during a time period of high viral transmission (see subsection 4.2). The resulting estimator, denoted as $\hat{R}_t$, was then correlated with the observed R_t estimated from the incidence data available in the Our World in Data (OWID) data base [1]. The correlation between $\hat{R}_t$ and R_t provides a measure of the value of social mobility as a predictor of viral transmissions in each country, while the ratio $\hat{R}_t/R_t$ provides a measure of the coupling between both indicators. In all five South American countries analyzed (Argentina, Brazil, Chile, Paraguay and Uruguay) we observed that during the first months of 2021, the estimated $\hat{R}_t$ was highly correlated (ρ^2 between 0.83 y 0.94) with the observed R_t about 1-2 weeks later and the ratio $\hat{R}_t/R_t$ was close to one (0.90-1.10) during the pre-vaccination and initial vaccination phases (Figure 1). We observed a high correlation between both estimators not only during the estimation period, but also during the beginning of the vaccination roll-out. These findings confirm that population mobility was a relevant driver of viral transmissions during early 2021 in all South American countries analyzed and revealed that, under a context of high community transmission, researchers can use the observed population mobility at a given time to infer the viral transmission dynamics without the typical lag of the observed R_t.

When we extended the estimation of the $\hat{R}_t$ during the vaccination roll-out period (with the same computed initial parameters), we observed a clear increase of the ratio $\hat{R}_t/R_t$ in all South American countries analyzed since late May and early June 2021, indicating that at a certain time the rate of spread of the virus started to be lower than expected from the levels of social interactions (Figure 1). We interpret such decoupling between population mobility and viral spread as a surrogate marker of conditional herd immunity, i.e. the achieved herd immunity conditioned to the social distancing policies and the circulating viral variants in each country. In order to test our method, we conducted a similar analysis in Israel, the first country to attain conditional vaccine-induced herd immunity. Our findings confirm that after a period of clear coupling between population mobility and viral transmission, a decisive increase of the ratio $\hat{R}_t/R_t$ was also observed at a certain time during vaccination roll-out in Israel (Figure A.1). The decoupling time, defined as the moment when the ratio $\hat{R}_t/R_t$ finally overcomes (i.e. the last time it crosses) the value 1.10, preceded the last peak of weekly reported cases and roughly coincides with the last day when the $R_t = 1$ in each country (Figure 1), indicating that the decoupling time was an early indicator of epidemic control.

The proportion of immunized population at the decoupling time could give us an idea of the conditional herd immunity threshold (HIT). In order to estimate the proportion of immune individuals around the decoupling time, we summed the estimated number of vaccine-immunized and natural-immunized individuals. The proportion of vaccine-immunized individuals was estimated from the number of fully vaccinated individuals adjusted by the estimated vaccine effectiveness (VE) in South America [17, 18], see also [19]. The number of infected people that acquired immunity through previous infection (cumulative infection) was estimated from the cumulative number of deaths assuming a constant (age adjusted) infection fatality rate (IFR) for each country (see subsection 4.1 and Table 1). The mean estimated HIT at the decoupling time varies along the countries from 29% in Argentina to 33% in Uruguay, 36% in Paraguay, 43% in Chile and 45% in Brazil, although confidence

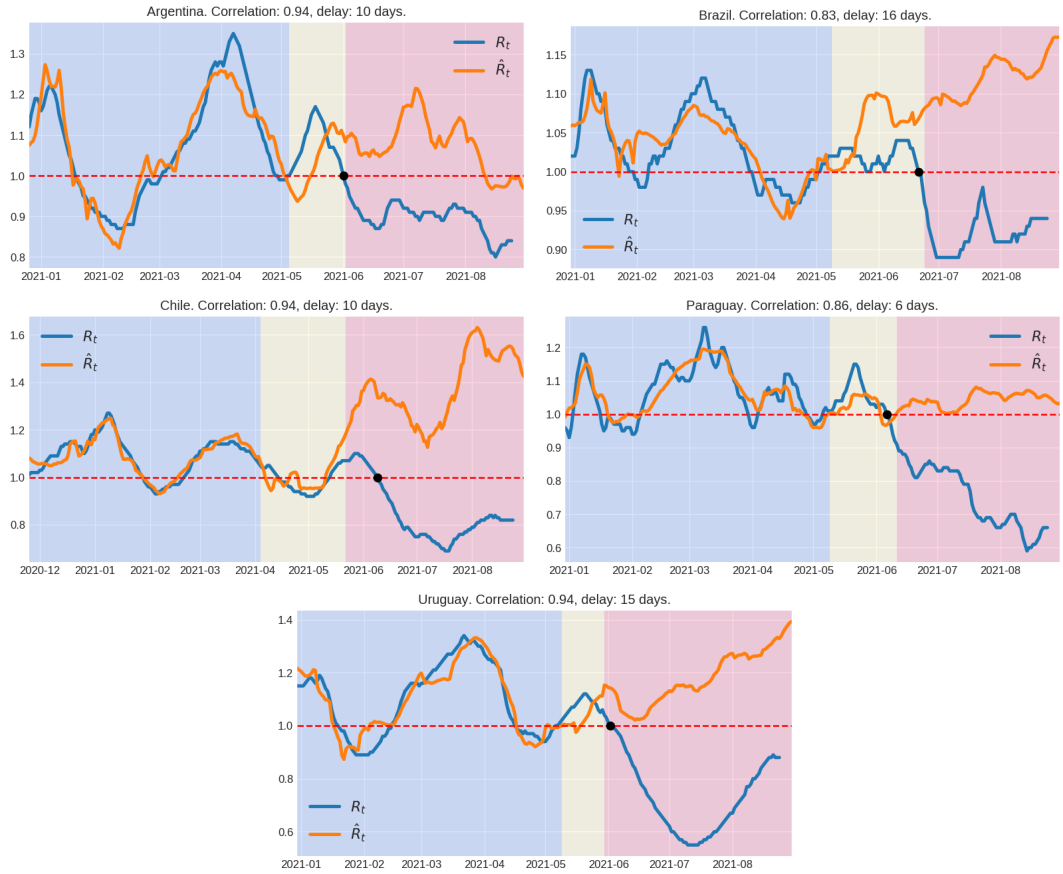

Figure 1: Viral effective reproduction number R_t and its estimation $\hat{R}_t$ using mobility information. Background colors indicate the following time periods: in blue, the time period used to fit the linear model (see Section 4.2), in yellow, the period after the fitting, but before the decoupling time, and in red after the decoupling point. The black dot corresponds to the last time the reproductive number was above one. The correlation corresponds to the period used to fit the model. The delay indicated is the time-shift between the mobility time series and R_t in order to maximize the correlation in the linear regression.

intervals were very large due to uncertainties in the IFR estimates (Table 1 and Figure 2). The HIT was reached by different proportions of natural infections and vaccination (Table 1). The estimated proportion of individuals that acquired immunity through vaccination (taking into account the VE) at the decoupling time was relatively high in Chile (29%) and Uruguay (24%), but very low in Brazil (9%), Argentina (5%) and Paraguay (1%). The estimated HIT in countries with widespread use of the inactivated vaccine Coronavac like Chile (43%) and Uruguay (33%) was similar to that estimated in Israel (42%) that only used the BNT162b2 (mRNA-based) vaccine (Figure A.2).

3 Discussion

All countries from the South America Southern cone (Argentina, Brazil, Chile, Paraguay and Uruguay) witnessed pronounced increases in daily SARS-CoV-2 cases and deaths during the firsts months of 2021 and a clear drop in relevant epidemic metrics (cases, deaths and R_t) from mid-2021 [1]. This study demonstrates that such epidemic control was preceded by a clear decoupling of viral transmissions from population mobility, consistent with the notion that those South American countries probably attained the HIT against SARS-CoV-2 variants Gamma and Lambda prevalent in the region, given some level of social dis-

Country	IFR	(VIN, ADV, RNA)	Dec-T	% Nat-Inf	% Vac	HIT
Argentina	0.67 (0.36-1.30)	(31.1, 64.7, 04.2)	Jun. 02	26 (13-48)	06	29 (17-52)
Brazil	0.59 (0.32-1.17)	(34.4, 48.1, 17.5)	Jun. 23	40 (20-74)	11	45 (25-79)
Chile	0.73 (0.40-1.43)	(71.1, 06.9, 22.0)	May 22	20 (10-37)	40	43 (34-60)
Paraguay	0.41 (0.23-0.83)	(11.6, 26.6, 61.8)	Jun. 11	35 (18-64)	02	36 (19-64)
Uruguay	0.90 (0.49-1.56)	(59.8, 01.6, 38.6)	May 29	13 (8-24)	29	33 (27-44)
Israel	0.65 (0.35-1.27)	(0,0,100)	Feb. 28	10 (5-19)	39	42 (37-51)

Table 1: IFR: infection fatality rate; VIN: percentage of virus inactivated vaccines; ADV: percentage of adenovirus vaccines; RNA: percentage of RNA vaccines [20, 21, 22, 23, 24, 25]; Dec-T: decoupling time; % Nat-Inf: percentage of population naturally infected at Dec-T; % Vac: percentage of the population fully vaccinated at Dec-T; HIT (herd immunity threshold): percentage of immunized population due to vaccines and natural infections at Dec-T. The vaccine effectiveness (VE) against SARS-CoV-2 infections was adjusted to 66% for VIN, 73% for ADV and 93% for RNA [17, 18].

tancing restrictions.

At the start of the pandemic, thresholds of 60-70% were given as estimates of herd immunity for SARS-CoV-2 [26]. Despite confidence intervals of HIT estimates were very large, mostly due to uncertainties in the IFR estimates, our analyses support that the conditional HIT for SARS-CoV in South America would be lower than 50%, ranging from 29% in Argentina to 45% in Brazil. Moreover, observe that these confidence intervals have a common range of $(34, 44) = 39 \pm 5$. A recent modeling study conducted in Stockholm, Sweden, also supports that this country reached the HIT against the original and Alpha variants of SARS-CoV-2 at 23% and 33% of seroprevalence, respectively [27]. The authors conclude that HIT for SARS-CoV-2, given limited social distancing restrictions, could be lower than initially estimated and that phenomena could be explained by population heterogeneity. By fitting epidemiological models that allow for heterogeneity in susceptibility or exposure to SARS-CoV-2 and given a basic reproduction number R_0 between 2.5 and 3, a recent study estimates that the HIT declines from over 60% to less than 10% as the coefficient of variation increases [28]. Another study estimate that in an age-structured community with mixing rates fitted to social activity, the HIT can be 43% if R_0 is 2.5 [29].

Our findings also support that the conditional HIT for SARS-CoV-2 in South America was attained through both natural and vaccinal immunity, with different relative proportions across countries. The extremely low proportion of vaccine-immune individuals in Paraguay (1%), Argentina (5%) and Brazil (9%) at decoupling time, suggest that conditional herd immunity in those countries was mostly attained by natural infections. Few studies estimated the proportion of infected individuals in South America after the large Gamma and Lambda epidemics in 2021, but some evidence from seroprevalence data support our estimations. A randomized study conducted in Paraguay between March to June 2021 gave a seroprevalence of 23.1% in Asunción and of 26.9% in the central region of the country [30] and a recent seroprevalence survey among adult individuals living in the largest Brazilian city of Sao Paulo also estimate a high proportion (45%: 39-51%) of individuals infected by SARS-CoV-2 [31].

At the other extreme, the relative proportion of vaccinal immunity at decoupling was highest in Chile (29%) and Uruguay (24%). CoronaVac accounted for most of vaccinations in Chile (75%) [32] and Uruguay (66%) [24] and the high incidence of SARS-CoV-2 in those countries during first months of vaccination roll-out raise concerns about the effectiveness of inactivated virus vaccines to control SARS-CoV-2 transmissions. Our results support that the widespread use of inactivated virus vaccines contributed to containing the spread of SARS-CoV-2 in Chile and Uruguay, despite abundant circulation of VOCs/VOIs and weak mitigation measures. Remarkably, the HIT at decoupling point in Chile (43%) and Uruguay (33%) was similar to the one estimated for Israel (42%), that mostly controlled the virus expansion through vaccination with BNT162b2. These findings are consistent with recent studies of vaccine effectiveness (VE) in Chile [17], Brazil [18] and Bahrain [33] that conclude that immunization with inactivated vaccines (CoronaVac and Sinopharm) was an effective strategy at mitigating the risk for transmissions of SARS-CoV-2 VOCs, although the perfor-

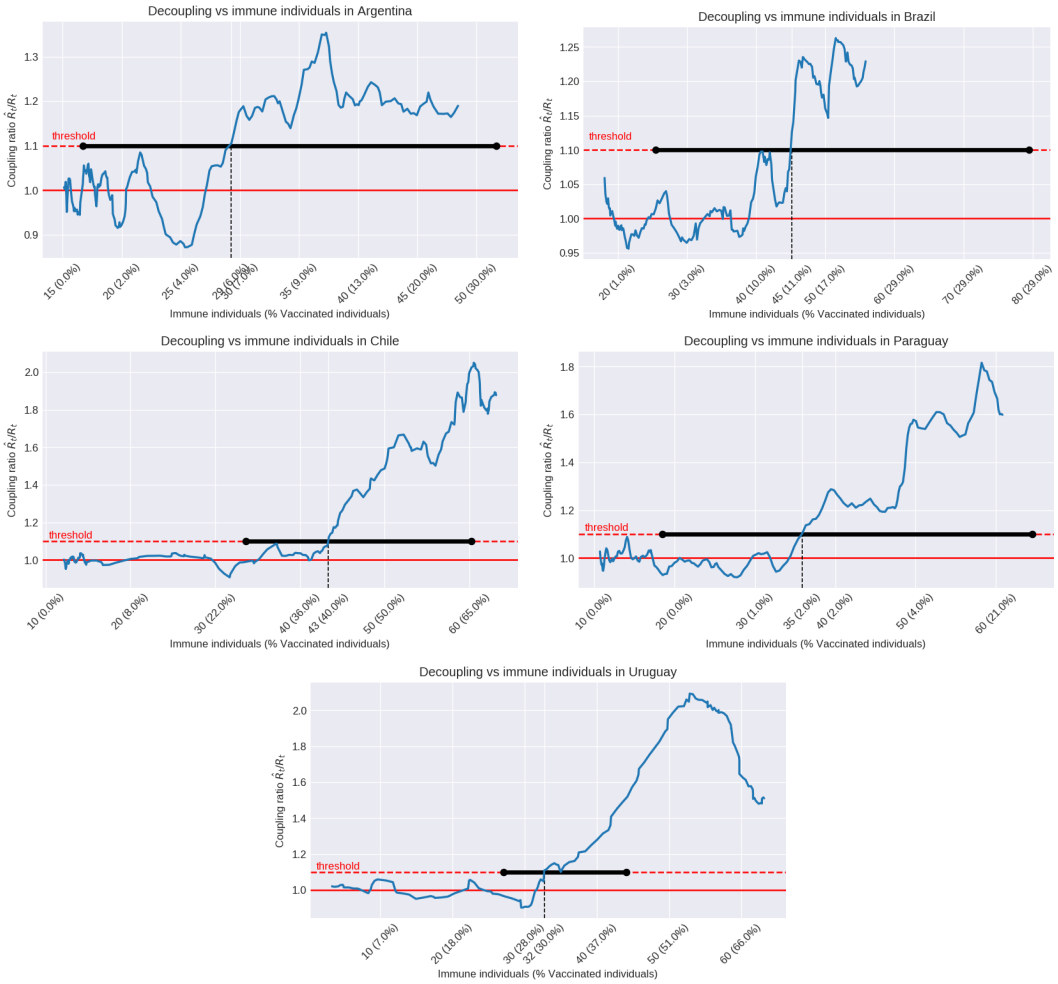

Figure 2: Coupling ratio $\hat{R}_t/R_t$ plotted with respect to the percentage of immune population. During the first months of 2021 the coupling ratio varies around 1, which corresponds to the periods where the R_t and $\hat{R}_t$ are in concordance in Figure 1. Immune population includes immunity achieved by vaccination (taking into account its effectiveness), and natural infection (see subsection 4.3). The percentage of people fully vaccinated is described as well. The coupling ratio crosses the threshold (decoupling point) at percentages of immune population that varies along the five countries from 29% in Argentina to 33% in Uruguay, 37% in Paraguay, 43% in Chile and 45% in Brazil. Confidence intervals are shown in horizontal black lines. They inherit the large uncertainty in the IFR estimation (see Table 1).

mance of BNT162b2 and adenovirus-based vaccines was superior.

The mean estimated HIT varied across South American countries and several factors may explain such variability. HIT will move upwards when more transmissible SARS-CoV-2 variants circulates in a population, but differences in the circulating SARS-CoV-2 variants do not explain variations among South American countries. Differences in the mean HIT were observed between countries where Gamma was the most prevalent variant like Brazil (45%), Paraguay (36%) and Uruguay (33%), and also between countries where Gamma and Lambda co-circulated at high prevalence like Chile (43%) and Argentina (29%) [2, 3, 4, 5]. Differences in vaccine platforms deployed in each country might also mod-

ulate the HIT at the decoupling time. Although we corrected the proportion of immune individuals according to the estimated VE and the proportion of each vaccine, we only considered immunity associated with fully vaccinated individuals. Previous studies, however, demonstrate some level of reduction of SARS-CoV-2 transmission after one dose of mRNA-based (46-58%), adenovirus-based (35%) and inactivated virus (16%) vaccines [17, 18, 34, 35]. Thus, we should expect that countries that used a higher proportion of mRNA-based and/or adenovirus-based vaccines like Argentina (69%) reached herd immunity at apparent lower thresholds that those that mostly used inactivated virus vaccines. Moreover, it should be stressed that Argentina had a very large proportion of individuals with a single dose at the decoupling point when compared to other countries in the region where second doses were administrated in a shorter period after first dose [1]. Notably, although Brazil also used an overall high proportion of mRNA-based and/or adenovirus-based vaccines (66%), most vaccinations during first months were of inactivated vaccines [18].

Reduction of SARS-CoV-2 transmission will also depend on the vaccination strategy (who is vaccinated and when). Vaccinations programs usually begin by elderly people and go on by gradually protecting the younger population [36]. Simulation studies indicate that prioritize vaccinating of high-risk groups will minimize the number of COVID-19-related hospitalizations and deaths in the short term, but vaccination of main transmission drivers (i.e. highly mobile working age groups) would be more effective at reducing the spread of the SARS-CoV-2 [37, 38]. Given enough vaccine supplies, vaccinating the adult population uniformly at random would thus be ideal to both prevent death and severe illness in high risk groups and to curb SARS-CoV-2 transmissions in the whole population. Uruguay developed an interesting vaccination strategy that prioritized vaccination of elderly populations ($\geq$ 70 years of age) with the BNT162b2 vaccine while highly mobile working age groups were simultaneously vaccinated with CoronaVac. This more homogeneous vaccination strategy across different age groups in Uruguay might partially explain the relative low HIT observed in this country. This may be related to the fact that, the decoupling effect due to vaccinations programs that we observe between mobility and the reproductive number is reached more abruptly than what could be expected from SIR-like models where all the population is treated homogeneously.

Our results support that proportion of immune population in South American populations attained a threshold enough to decoupling people mobility and viral dissemination and those countries could thus implement progressive relaxing of mitigation measures with relative safety. Such apparent herd immunity, however, was attained while maintaining moderate mitigation measures (social distancing, school closed, mask-wearing and other self-care behaviors). None of the countries analyzed have returned to the pre-pandemic levels of activity and it is unclear if current population immunity will halt the viral spread after removal of all mitigation measures. Long-term herd immunity could be also challenged by waning immunity and dissemination of more infectious SARS-CoV-2 variants [39]. Waning neutralizing antibodies might progressively reduce the population immunity level to below the critical HIT, while local evolution and/or introduction of SARS-CoV-2 variants that are more transmissible than those previous circulating will move the HIT upwards.

Both factors seems to have shaped the third epidemic wave in Israel [40, 41, 42, 43] Our study supports that after a transient period of decoupling in Israel, population mobility and viral transmissions were coupled again as Delta variant spread in both unvaccinated and fully-vaccinated individuals. It is unclear if the same phenomena could be observed in South America after introduction of Delta variant. First, herd immunity through natural infection seems to be less susceptible to waning immunity than by vaccination [44, 45, 46] and South American countries with a high natural immunity wall might be better prepared to limit the expansion of Delta variant than those with a large vaccine immunity wall. Second, hybrid immunity (natural infection plus vaccination) might provide longer lasting and stronger protection against infection than vaccine-induced immunity [47] and a high proportion of partial or fully vaccinated individuals in South America may be currently in this condition. Third, some South American countries like Chile, Uruguay and Brazil already started or approved the administration of a vaccine booster.

Our study has some important limitations: (i) difficulty to estimate precisely the IFR and consequently to have a precise estimate of the cumulative number of naturally infected people at decoupling point in each country; (ii) sub-reporting of SARS-CoV-2 deaths might underestimate the cumulative number of infections and thus the HIT; (iii) the assumption

that partially vaccinated people did not greatly contribute to reduce viral transmissions might have also underestimate the number of vaccine-immune individuals and the actual HIT; (iv) on the other hand, although we assumed some overlap between vaccinal immunity and natural immunity, the precise fraction of fully vaccinated individuals that were previously infected is unknown. Because of these limitations, the precise HIT estimated here should be interpreted with caution and should not be considered as general reference values for other countries.

In summary, our study supports that populations from the South American Southern cone probably achieved the conditional HIT to contain the further spread of SARS-CoV-2 variants Gamma and Lambda at around mid-2021. Presumed herd immunity was probably mostly attained by natural infection in Argentina, Brazil and Paraguay, and by a mixture of natural infections and vaccination in Chile and Uruguay. The widespread used of the Coronavac inactive viral vaccine in South America was not only effective to prevent the severe forms of COVID-19 disease but also has the potential to contain the community spread of highly transmissible SARS-CoV-2 regional variants. Inactivated SARS-CoV-2 vaccines, combined with other vaccines and mitigation measures, may thus represent a relevant tool to control the COVID-19 pandemic especially under the severe limitation of vaccine supplies faced by many countries around the world. Our findings stress that the herd immunity status might be rapidly lost if vaccine-induce neutralizing antibodies decrease over time and more transmissible SARS-CoV-2 variants are either introduced from abroad or evolved locally.

Acknowledgements

We are indebted to P. Bermolen, M. Cerminara, M. I. Fariello, F. Paganini, R. Radi, M. Reiris, A. Rojas de Arias and C. E. Schaerer, for useful discussion and support. Any errors are the responsibility of the authors.

4 Methods

4.1 Data and code availability

The SARS-CoV-2 incidence data, viral effective reproduction number R_t (also indicated as reproduction rate), confirmed deaths, vaccinated people, and other epidemiological indicators were retrieved from Our World in Data (OWID) [1]. Mobility index was estimated from the six indicators categories (retail and recreation, grocery and pharmacy, parks, transit stations, workplaces, and residential) provided by Google COVID-19 Community Mobility Reports [48]. For the sake of reproducible research, the code used to obtain all the results and figures is available at `https://github.com/marfiori/covid19-decoupling`.

4.2 Estimation of the viral effective reproduction number and decoupling time

As the correlations between the six different possible regressors are large, we construct indices that are more robust along time and different countries, to avoid overfitting. In order to do this, we choose for each country the three categories that give the best fit among all possible combinations. Although the categories may vary, the obtained fit quality is relatively robust over different time intervals. The six mobility time series were smoothed by averaging over a 14 days sliding window.

For each country, we selected a time period consisting of 75 days before the start of the vaccination campaign, and 55 days after, ending up with a 130-days period to carry out the estimation. Given a set of three mobility categories, we fitted a linear regression model to the viral effective reproduction number R_t, lagged a certain time period. This time shift was chosen as the lag that maximizes the correlation of the regression. This procedure was repeated for each combination of three categories among the six mobility measures provided by Google, and the combination achieving the best regression result was kept. It should be noted that, since the six categories are highly correlated, other combinations of three categories achieve similar fitting results, and therefore the chosen categories are not necessarily informative by themselves.

Using the coefficients obtained in this 130-days period, and rest of the mobility time series, we computed the predicted viral reproduction number $\hat{R}_t$. The procedure was tested using periods of different lengths for the estimation, and the results in the HIT are robust along the different experiments.

When population mobility and viral transmission are coupled, the coupling ratio $C_t = \hat{R}_t/R_t$ oscillates around one (0.90-1.10). Departing from a certain moment, the $\hat{R}_t$ becomes much higher than the R_t, re-

vealing the decoupling between population mobility and viral spread resulted. We defined the **decoupling time** D_t as the moment when the coupling ratio $C_t = \hat{R}_t/R_t$ definitely exceeds the value 1.10, i.e. the last crossing over 1.10.

4.3 Estimation of the IFR and immune population

As it is well known, the estimation of the infection fatality rate has been a hard task during all the pandemic. The cryptic circulation of the virus (due to asymptomatic infections) and different variants made that in fact this quantity varies along time and populations. Here we took into account the most relevant variable to compute it, that is the age structure of the population. We then took IFR by age taken from [49] and adjusted to the population pyramid of each of the considered countries [50]. Confidence intervals were calculated by considering the (very large) confidence intervals available from [49] and estimating the interval for the whole population as the weighted average of the positions for the maximum or minimum of the age-classes intervals. Only one exception was introduced: in the Uruguayan case, the confidence interval can be reduced because the IFR must be smaller than the Case Fatality Rate (CFR). Imposing this constraint the maximum possible value in the Uruguayan case is reduced (we obtained the CFR corresponding to July 31 from [1]) the other countries being unaffected. This IFR estimation was confirmed using an alternative methodology for the case of Uruguay, following [51], which led to similar results, but with slightly larger confidence intervals.

The percentage of immune population was computed considering the immunity achieved by vaccination (including its effectiveness), and natural infection. However, many people who gained immunity by natural infection, might have gotten vaccinated as well. In order to avoid the over estimation resulting from counting twice those subjects, we subtracted the intersection of these fractions, under the assumption that they are independent. Observe that this assumptions gives us a lower bound on the estimation of immune population.

For a given country, let us denote by FV the proportion of fully vaccinated people, by NI the proportion of people with immunity by natural infection, and by VE the vaccine effectiveness of the country, computed by combining the effectiveness of each vaccine type (VIN, ADV, RNA) using the proportion of vaccines used in the country (see Table 1). We assumed a perfect immunization due to natural infection. That is, we neglected in the present analysis the number of re-infections. Furthermore, let us denote by IM the estimation of the proportion of immunized population. Then, the computation described above is as follows:

$$IM = (FV - FV \cdot NI) \cdot VE + NI.$$

Here the product $FV \cdot NI$ accounts for the intersection of the populations, which is subtracted from the vaccinated population before the effectiveness factor is applied. As described through the text, the proportion of people with immunity by natural infection is inferred from the confirmed deaths, using the estimated IFR.

Observe that due to the vaccine effectiveness, the percentage of fully vaccinated people may by greater than the percentage of immunized population.

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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.

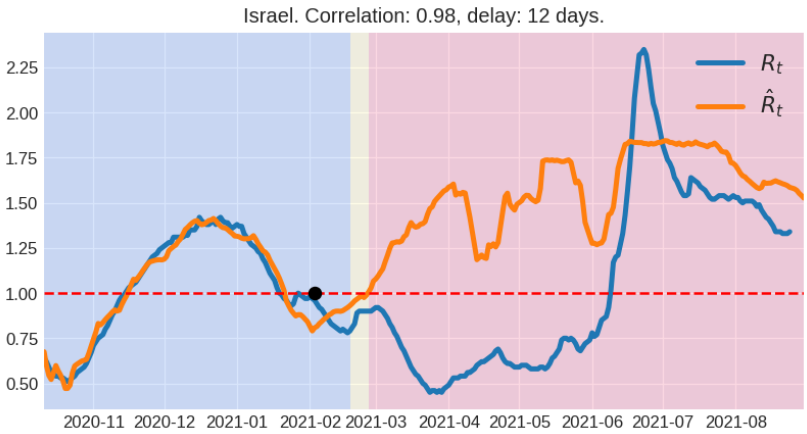


Figure A.1: Viral effective reproduction number R_t and its estimation $\hat{R}_t$ using mobility information. Background colors indicate the following time periods: in blue, the time period used to fit the linear model (see Section 4.2), in yellow, the period after the fitting, but before the decoupling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

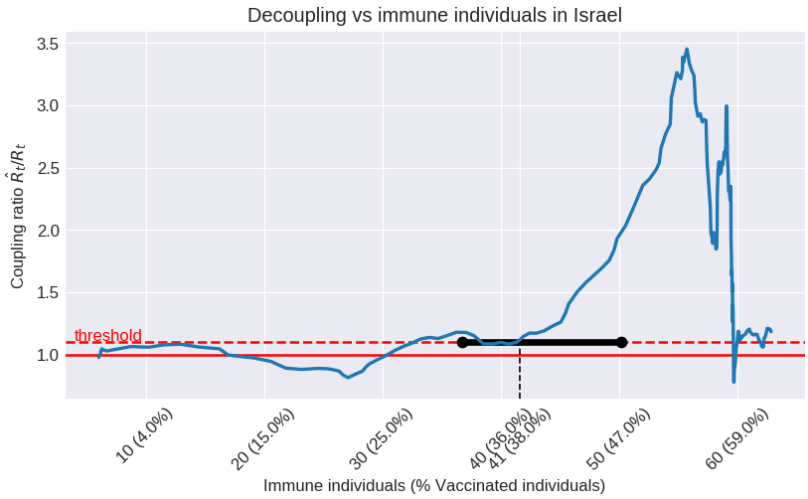


Figure A.2: Coupling ratio $\hat{R}_t/R_t$ plotted with respect to the percentage of immune population. During the first months of 2021 the coupling ratio varies around 1, which corresponds to the periods where the R_t and $\hat{R}_t$ are in concordance in Figure A.1. Immune population includes immunity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

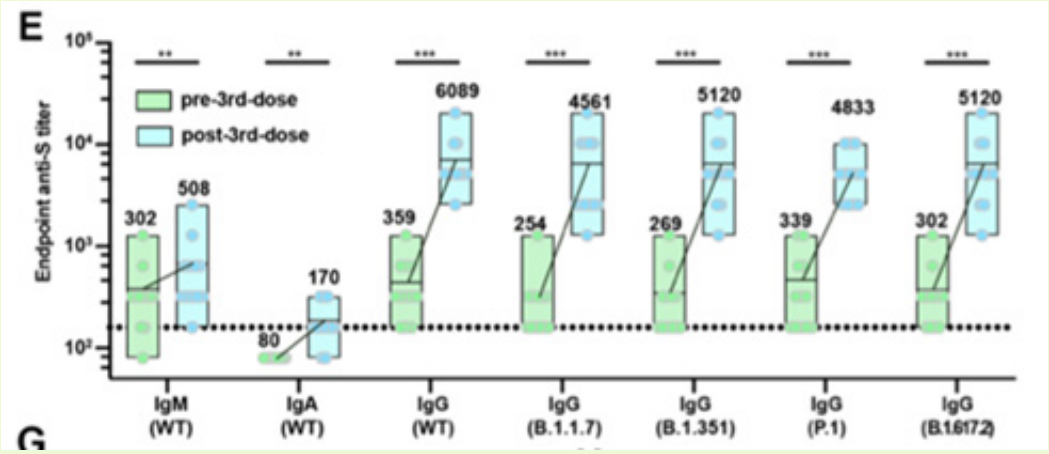
2.4 Dose de reforço da CoronaVac eleva em 17 vezes os níveis de anticorpos capazes de combater a variante delta do SARS-CoV-2, aponta estudo

Uma dose de reforço da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, aumenta em 17 vezes o nível de anticorpos neutralizantes contra a variante delta do vírus SARS-CoV-2 em quem já completou o esquema vacinal há seis meses. As conclusões estão no estudo A third dose of inactivated vaccine augments the potency, breadth, and duration of anamnestic responses against SARS-CoV-2, de pesquisadores da Academia Chinesa de Ciências, Universidade de Pequim, Faculdade de Medicina de Xangai e Sinovac, entre outras instituições, publicado na plataforma de preprints MerRxiv.

O estudo apontou que a dose de reforço da CoronaVac potencializa rapidamente e de forma robusta os níveis de anticorpos neutralizantes contra a proteína S, componente que o SARS-CoV-2 usa para invadir células humanas. Além de aumentar em 17 vezes a proteção contra a delta, a dose de reforço aumenta em 17 vezes o nível de anticorpos neutralizantes contra o vírus original (cepa de Wuhan); em 18 vezes contra a variante alfa; em 19 vezes contra a beta; e em 14 vezes contra a gama.

O estudo analisou amostras de plasma de 66 participantes, incluindo 38 voluntários que receberam duas ou três doses da vacina. A avaliação aconteceu quatro semanas após a administração da dose de reforço, sendo que esta foi aplicada seis meses após os participantes da pesquisa receberem a segunda dose.

O gráfico mostra o aumento do nível de anticorpos dos participantes da pesquisa, medidos imediatamente antes de tomarem a dose de reforço da CoronaVac (em verde), e passadas quatro semanas após a dose de reforço (em azul). São exibidos os resultados para o vírus original de Wuhan (WT, sigla para "wide type"), e para cada uma das quatro variantes de preocupação: alfa (B.1.1.7), beta (B.1.351), gama (P.1) e delta (B.1.617.2).



A CoronaVac já se mostrou eficaz contra a variante gama no estudo de efetividade Projeto S, realizado pelo Butantan no município paulista de Serrana. Por meio dele, 95% da população adulta foi vacinada com CoronaVac entre fevereiro e abril, quando a variante gama já era predominante no Brasil. A imunização coletiva fez os óbitos por Covid-19 despencarem 95%, as internações, 86%, e os casos sintomáticos, 80%.

Outra pesquisa realizada na China já apontava a eficácia da CoronaVac contra a variante delta. Um estudo do Centro de Controle e Prevenção de Doenças da província de Guangdong, feito durante um surto de Covid-19 causado pela delta, mostrou que a CoronaVac evitou o desenvolvimento de casos graves de Covid-19 e teve eficácia de 69,5% contra o aparecimento de pneumonias decorrentes da doença. O estudo envolveu 10.813 pessoas e foi realizado entre maio e junho de 2021

Publicado em: 13/09/2021

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

Authors: Kang Wang[1$], Yunlong Cao[3$], Yunjiao Zhou[2$], Jiajing Wu[4,$], Zijing Jia[1$], Yaling Hu[5$], Ayijiang Yisimayi[3], Wangjun Fu[1], Lei Wang[1], Pan Liu[1], Kaiyue Fan[1], Ruihong Chen[1,6], Lin Wang[5], Jing Li[5], Yao Wang[3], Xiaoqin Ge[5], Qianqian Zhang[2], Jianbo Wu[2], Nan Wang[1], Wei Wu[2], Yidan Gao[2], Jingyun Miao[7], Yinan Jiang[7], Lili Qin[7], Ling Zhu[1], Weijin Huang[5], Yanjun Zhang[9], Huan Zhang[8], Baisheng Li[8], Qiang Gao[5], Xiaoliang Sunney Xie[3]*, Youchun Wang[4]*, Qiao Wang[2]* and Xiangxi Wang[1,6]*

Affiliation:

[1] CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

[2] Key Laboratory of Medical Molecular Virology (MOE/NHC/CAMS), School of Basic Medical Sciences; Shanghai Institute of Infectious Disease and Biosecurity; Shanghai Medical College, Fudan University, Shanghai 200032, China

[3] Beijing Advanced Innovation Center for Genomics (ICG), Biomedical Pioneering Innovation Center (BIOPIC), School of life Science, Peking University, Beijing 100091, China

[4] Division of HIV/AIDS and Sex-transmitted Virus Vaccines, Institute for Biological Product Control, National Institutes for Food and Drug Control (NIFDC), Beijing 102629, China

[5] Sinovac Biotech Ltd, Beijing, China

[6] Guangzhou Laboratory, Guangzhou, Guangdong, China, 510320

[7] Acrobiosystems Inc, Beijing, China

[8] Guangdong Provincial Center for Disease Control and Prevention

[9] Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China

*Correspondence to: X.W. (Email: xiangxi@ibp.ac.cn) or Q.W. (Email: wangqiao@fudan.edu.cn) or Y.W. (Email: wangyc@nifdc.org.cn) or X.S.X. (Email: sunneyxie@biopic.pku.edu.cn)

[$] These authors contributed equally to this work.

Abstract: (~150 words)

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

One sentence summary

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

Main Text:

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

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

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

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

of 1: 10, with ~14% (3/22) and 16% (2/12) showing a near ineffectiveness in neutralizing B.1.351 *in vitro* (Fig. 1A). By contrast, only 1 out of 14 samples from the 3-dose vaccinees exhibited a weak neutralizing titer below 10 (Fig. 1A). Importantly, the 3-dose vaccinees showed over 2.5-fold higher neutralizing potency against B.1.617.2 than the convalescents and 2-dose vaccinees (Fig. 1A). The GMT NT_{50} values measured by a VSV-pseudovirus with the WT S were 840, 660 and 1,176 for convalescents, 2-dose and 3-dose vaccinees, respectively, which were 8-10-fold greater than those determined by live WT SARS-CoV-2 (Fig. 1A, 1B), confirming higher sensitivity of pseudovirus-based assays in determining neutralizing titers. In line with the results of live SARS-CoV-2 neutralization assay, the mean fold decrease in the neutralization of B.1.1.7 relative to the WT was 2.8-fold for convalescents, 2.2-fold for 2-dose vaccinees and 1.7-fold for 3-dose vaccinees (Fig. 1B). Similarly, plasma from convalescents, 2-dose and 3-dose vaccinees exhibited a 4.5-fold, 2.9-fold and 2.4-fold reduction, in NAb titers against P.1, respectively, when compared to the WT (Fig. 1B). These results reveal that a third-dose boost of inactivated vaccine leads to enhanced neutralizing breadth to SARS-CoV-2 variants, bolstering the potential to ward off VOCs effectively when compared to convalescent plasma. Of note, neither vaccination nor SARS-CoV-2 infection boosts distinct neutralizing potency against SARS-CoV, presumably due to the relatively far phylogenic relationship (Fig. 1B).

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

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

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

The molecular mechanism underlying these potent, broad and durative antibody

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

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

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

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

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

24 possessed cross-neutralization activity against all five SARS-CoV-2 VOCs (Fig. 2F and fig. S13). Among these, six RBD antibodies could cross-neutralize SARS-CoV, of which 2 exhibited more potent neutralization activity against SARS-CoV with IC_{50} values of 41 and 73 ng/ml. However, most of the NTD and S1/non-RBD-NTD NAbs lost their abilities to inhibit viral infection (Fig. 2F and fig. S13), indicative of higher variations for the NTD in VOCs. In comparison with NAbs from early convalescents, antibodies isolated from 3-dose vaccinees showed overall enhanced neutralizing potency and breadth to VOCs.

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

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

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

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

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

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

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

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

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

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

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

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

Acknowledgments: We thank Dr. Xiaojun Huang, Dr. Boling Zhu, Dr. Lihong Chen, Dr. Xujing Li and Dr. Gang Ji for cryo-EM data collection, the Center for Biological Imaging (CBI) in Institute of Biophysics for EM work and thank Dr. Yuanyuan Chen, Zhenwei Yang for technical help with BLI experiments. We also thank Prof. David Ho and Prof. Barton Ford Haynes for generous gift of the sequences of two reference mAbs. Work was supported by the Strategic Priority Research Program

(XDB29010000, XDB37030000), CAS (YSBR-010), National Key Research and Development Program (2020YFA0707500, 2018YFA0900801), Emergency Key Program of Guangzhou Laboratory, Grant No. EKPG21-09 and Beijing Municipal Science and Technology Project (Z201100005420017). Xiangxi Wang was supported by Ten Thousand Talent Program and the NSFS Innovative Research Group (No. 81921005). **Author contributions**: X.W., Q.W., Y.W., X.S.X and Y.C. conceived, designed and coordinated the experiments. Y.Z., A.Y., Y.W., Q.Z. and J-B.W. cloned and produced antibodies. K.W., Z.J., K.F., J.M., Y.J. and L.Q. expressed and purified all recombinant antigen proteins used in this manuscript. J-J.W., Y.H., L.W., J.L., X.G., Y-J.Z., H.Z. and B-S.L performed pseudovirus and authentic virus neutralization experiments. K.W., L.W., P. L., WJ.F, N.W and L.Z performed structural study. Y.C., Y.Z., W.W. and Y.G. prepared PBMCs and flow cytometry sorting. Y.C. and A.Y. performed 10X sequencing library construction. Z.J. and K.F performed BLI assay. Y.H. and Q.G recruited volunteers and coordinated the collection of blood samples. All authors analyzed data; X.W wrote the manuscript with input from all authors. **Competing interests**: All authors have no competing interests. **Data and materials availability**: Cryo-EM density maps of the SARS-CoV-2 S trimer in complex with XGv013 or XGv043, the SARS-CoV-2 S trimer in complex with XGv004, XGv030 and XGv016; the SARS-CoV-2 S trimer in complex with XGv026 and XGv046, and the SARS-CoV-2 S trimer in complex with XGv018, XGv038 and XGv42 have been deposited at the Electron Microscopy Data Bank with accession codes EMD-UUUU, EMD-WWWW, EMD-XXXX, EMD-YYYY and EMD-ZZZZ, respectively.

Figure legends

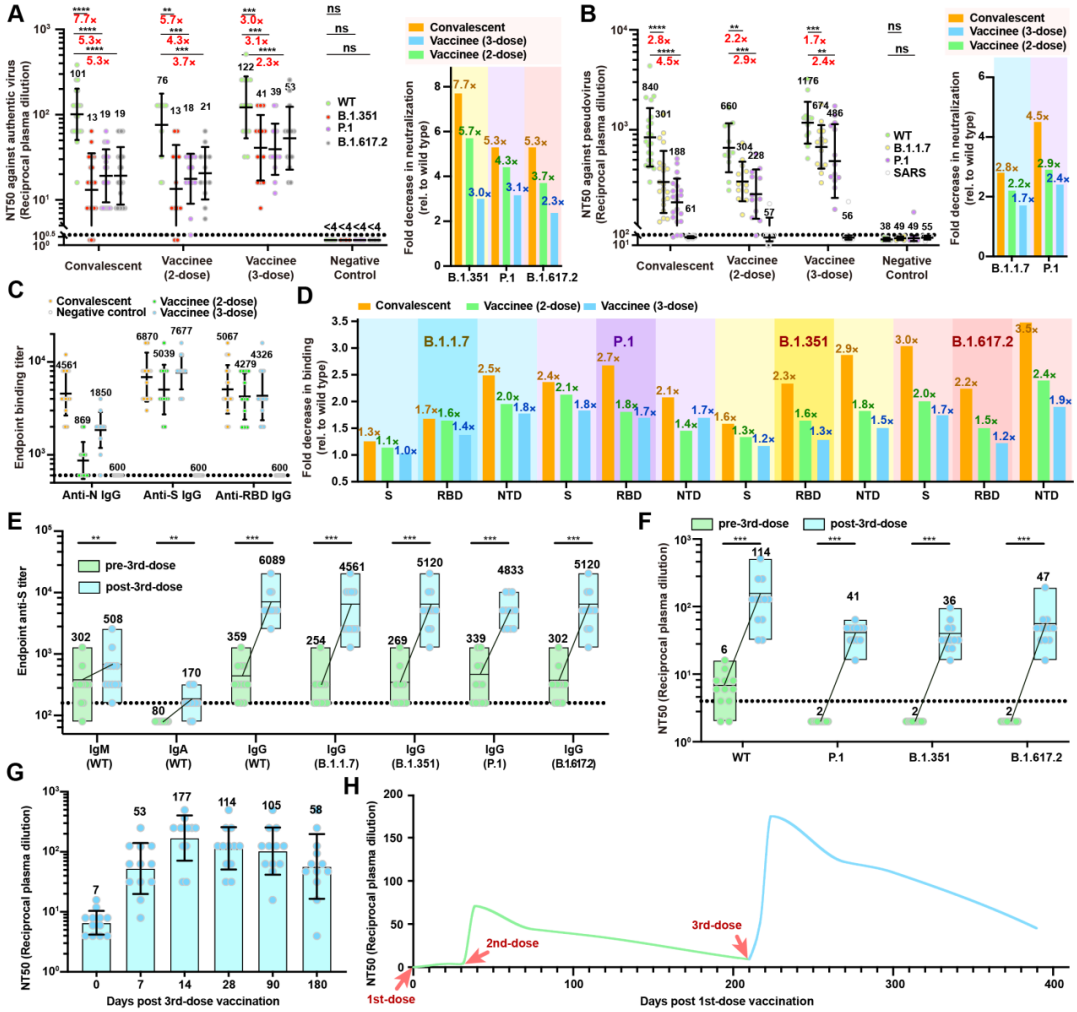


Fig. 1 A 3rd-dose booster of an inactivated vaccine elicits an expeditious and long-lasting recall antibody response

Plasma neutralizing activity evaluated by authentic SARS-CoV-2 **(A)** and pseudo-typed SARS-CoV-2 neutralization assays **(B)** Left: half-maximal neutralizing titer (NT_{50}) values for plasma from COVID-19 convalescents, 2-dose, 3-dose CoronaVac vaccine recipients (at week 4 after the last dose of vaccination) and negative controls (pre-COVID-19 historical control) against live SARS-CoV-2 WT, B.1.351, P.1 and B.1.617.2, and VSV-based SARS-CoV-2 pseudoviruses bearing WT or B.1.1.7 or P.1 S protein. Black bars and indicated values represent geometric mean NT_{50} values. Statistical significance was determined using the two-tailed Wilcoxon matched-pairs test. Experiments were repeated in triplicate. Dotted lines indicate the limit of detection. Right: fold decrease in neutralization for each variant relative to WT for

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

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

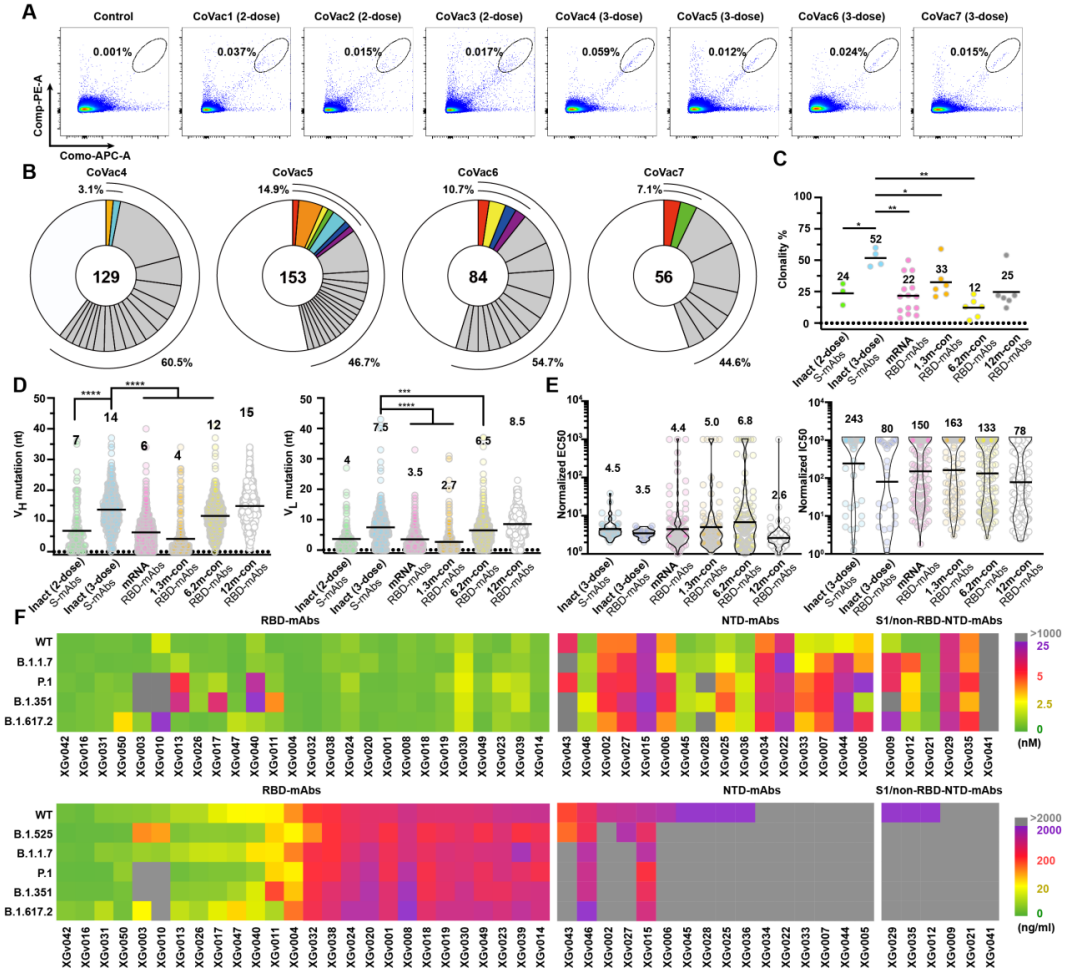
(D) Fold decrease in specific binding to the RBD, NTD and S for each variant over WT for each cohort of plasma samples as described above.

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

(F) Neutralizing titers against live SARS-CoV-2 WT, P.1, B.1.351 and B.1.617.2 for plasma from vaccinees before and 4 weeks after the 3rd-dose immunization. Black bars and indicated values represent geometric mean NT_{50} values.

(G) Longitudinal neutralizing titers of plasma from 3-dose vaccinees at days 0, 7, 14, 28, 90 and 180 post the 3rd-dose vaccination. The geometric mean NT_{50} values are labeled.

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



568

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.

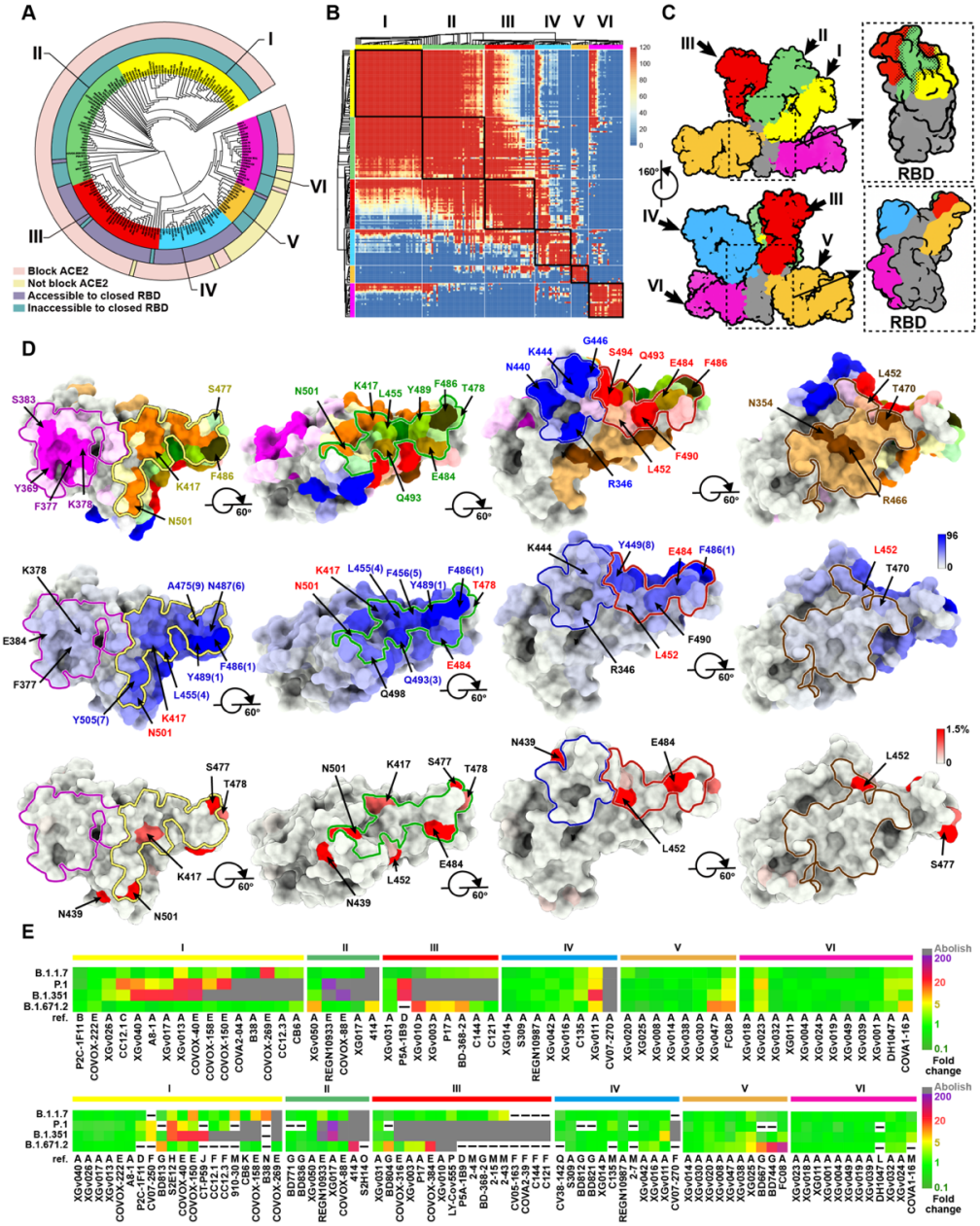
578 **(C)** Graph shows relative clonality among seven individuals who received 2-dose or
579 3-dose of inactivated vaccines. Relative clonality for COVID-19 convalescents
580 assayed at 1.3, 6.2 and 12 months after infection, as well as 2-dose mRNA vaccine
581 recipients (*2, 14, 18*), previously described by Michel's group, was compared. Black

horizontal bars indicate mean values. Statistical significance was determined using two-tailed t-test.

(D) Number of somatic nucleotide mutations in the *IGHV* (left) and *IGLV* (right) in antibodies from vaccinees, including 2-dose or 3-dose of inactivated vaccines and 2-dose of mRNA vaccines and COVID-19 convalescents assayed at 1.3, 6.2 and 12 months after infection (*2, 14, 18*).

(E) Normalized ELISA binding (EC_{50}) by antibodies isolated from the 3-dose inactivated and 2-dose mRNA vaccinees (ref) as well as COVID-19 convalescents to SARS-CoV-2 S trimer (left) and normalized pseudovirus neutralization activity (IC_{50}) (right) against SARS-CoV-2 assayed at 1.3, 6.2 and 12 months after infection (ref). Among these, eight antibodies reported by Michel's group were expressed and assessed for both binding by ELISA and pseudovirus neutralization activity for normalized comparison here. Black horizontal bars indicate mean values.

(F) BLI binding affinities (upper panel) and pseudo-typed virus neutralization (bottom panel) by antibodies isolated from the 3-dose vaccinees to circulating SARS-CoV-2 variants. Color gradient for upper panel indicates K_D values ranging from 0 (green), through 2.5 (yellow) and 5 (red) to 25 nM (purple). Gray suggests no/very limited binding activity (>1000 nM). Color gradient for bottom panel indicates IC_{50} values ranging from 0 (green), through 20 (yellow) and 200 (red) to 2000 ng/ml (purple). Gray suggests no/very limited neutralizing activity (>2000 ng/ml).



610

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

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

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

six antibody classes.

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

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

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

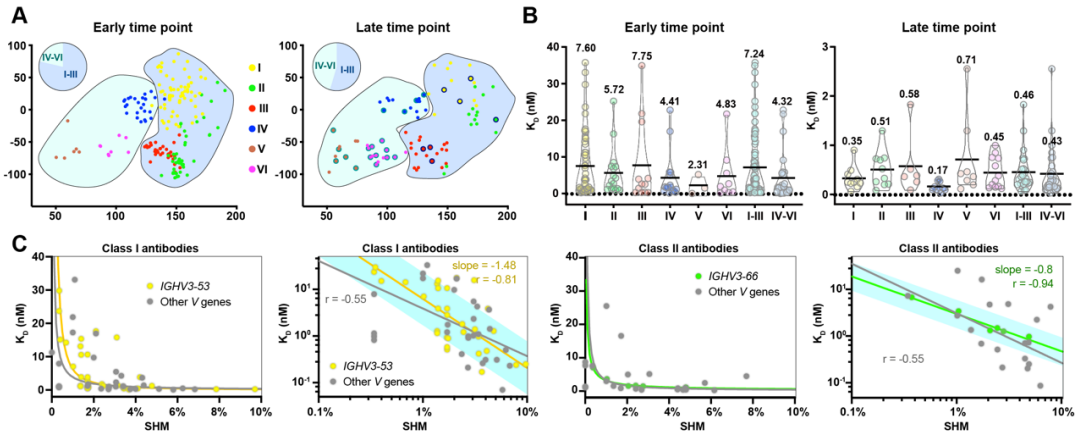


Fig. 4 Antibody evolution and affinity maturation

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

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

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

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2.5 Estudo mostra que CoronaVac é eficaz contra casos graves de Covid-19 causados pela variante delta

De acordo com um estudo feito por pesquisadores do Centro de Controle e Prevenção de Doenças da província de Cantão (Guangdong), na China, a CoronaVac, vacina do Butantan e da biofarmacêutica chinesa Sinovac contra a Covid-19, evita o desenvolvimento de casos graves de covid-19 causados pela variante delta do SARS-CoV-2 e tem eficácia de 69,5% contra o aparecimento de pneumonias decorrentes da doença.

As descobertas estão no artigo "Effectiveness of Inactivated COVID-19 Vaccines Against Covid-19 Pneumonia and Severe Illness Caused by the B.1.617.2 (Delta) Variant: Evidence from an Outbreak in Guangdong, China", que foi publicado na plataforma de preprints SSRN, vinculada à revista The Lancet, uma das mais prestigiosas publicações médicas do mundo. Esse é o primeiro estudo publicado sobre a eficácia das vacinas de vírus inativado, especialmente a CoronaVac/Sinovac, na prevenção de pneumonias e casos graves de Covid-19 causados pela variante delta.

Os pesquisadores concluíram que a imunização total com duas doses foi 69,5% eficaz para prevenir pneumonia, um dos desdobramentos mais graves da Covid-19: entre os não vacinados, houve 85 casos (1,44%); entre os vacinados com uma dose, 12 casos (1,42%); e entre os vacinados com duas doses, cinco casos (0,35%). Além disso, não foram registrados casos críticos entre os vacinados, indicando que os imunizantes analisados têm eficácia contra o desenvolvimento de casos graves de Covid-19 causados pela variante delta (entre os não vacinados, houve 19 casos graves ou críticos).

O estudo envolveu 10.813 pessoas e foi realizado em maio e junho de 2021, durante um surto da variante delta. Com exceção do grupo controle, os participantes haviam sido vacinados com uma das quatro vacinas de vírus inativado autorizadas para uso emergencial na China – a vacina da Sinovac (que no Brasil é chamada CoronaVac), as vacinas HB02 e WIV04, da Sinopharm, e a BICV, da Biokangtai.

Dos quase 11 mil voluntários, 5.888 (54,45%) não foram vacinados, 3.130 tomaram a primeira dose e 1.795 tomaram as duas doses. Entre os participantes que tomaram a primeira dose, 48,57% (2.392 pessoas) foram imunizadas com a vacina da Sinovac; entre os que receberam as duas doses, o indicador foi de 58,28% (1.046 pessoas).

O estudo foi feito com pessoas não vacinadas e vacinadas com uma ou duas doses porque quando o surto da variante delta começou em Cantão a imunização em massa ainda estava em andamento. Para a análise, os pesquisadores usaram dados de vigilância sanitária e de vacinação.

Sobre a variante delta

A variante delta do SARS-CoV-2 (B.1.617.2), identificada pela primeira vez na Índia em outubro de 2020, está sendo responsável pelo aumento do número de casos de Covid-19 inclusive em países onde a pandemia parecia controlada, como Israel e Reino Unido, devido à sua grande capacidade de transmissão. Cada indivíduo infectado com a variante delta pode transmitir o vírus para outras quatro a sete pessoas – ou seja, ela é 50% a 100% mais transmissível do que as demais cepas do SARS-CoV-2.

No Brasil, a variante dominante ainda é a gama (P.1, amazônica). De acordo com o boletim epidemiológico da Rede de Alerta das Variantes do SARS-CoV-2, coordenada pelo Butantan, no estado de São Paulo a gama é responsável por 89,82% dos casos, seguida da P.1.2 (4,22%), da alfa (B.1.1.7, britânica) e, em quarto lugar, da delta, com 0,54% dos casos. Em todo o Brasil, já foram registrados mais de 700 casos da variante delta, identificada em 14 estados e no Distrito Federal.

O Butantan já está estudando se a CoronaVac é efetiva contra a delta, iniciando a pesquisa pelo isolamento da variante.

Publicado em: 30/08/2021

Effectiveness of Inactivated COVID-19 Vaccines Against COVID-19 Pneumonia and Severe Illness Caused by the B.1.617.2 (Delta) Variant: Evidence from an Outbreak in Guangdong, China

Min Kang[1,2 #], MMed, Yao Yi[1#], MMed, Yan Li[1], PhD, Limei Sun[1], BM, Aiping Deng[1], MMed, Ting Hu[1], MMed, Jiayi Zhang[1], MMed, Jun Liu[1], MPAM, Mingji Cheng[1], MMed, Shen Xie[1], MMed, Min Luo[1], MMed, Jing Jiang[1], MMed, Yawen Jiang[3*], PhD, Shixing Tang[2*], PhD, Jianfeng He[1*], BSc

[1] Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, Guangdong, China

[2] School of Public Health, Southern Medical University, Guangzhou, Guangdong, China

[3] School of Public Health (Shenzhen), Sun Yat-sen University, Shenzhen, Guangdong, China

[#]These authors contributed equally to the submitted work.

[*]Corresponding author: Yawen Jiang, Room 215, Mingde Garden #6, Sun Yat-sen University, 132 East Outer Ring Road, Panyu District, Guangzhou, Guangdong, China. Email: jiangyw26@mail.sysu.edu.cn. Shixing Tang, No.1023-1063, Shatai South Road, Baiyun District, Guangzhou, China. Email: tamgshixing@smu.edu.cn. Jianfeng He, No.160, Qunxian Road, Panyu District, Guangzhou, China. Email: hjf@vip.sina.com.

Abstract

Background

Real-world evidence (RWE) of a vaccine supplements clinical trial data by providing information in populations differing from clinical trial populations, under different epidemiological situations, on alternative outcomes, or against different pathogen lineages. To date, RWE on inactivated COVID-19 vaccines against the highly transmissible SARS-CoV-2 B.1.617.2 (Delta) variant is limited, leaving an important gap in the evidence base of inactivated COVID-19 vaccines for use by immunization programs.

Methods

Between May and June 2021, an outbreak of the B.1.617.2 variant was discovered and traced in Guangdong, China. Before this outbreak, Guangdong province had started mass vaccination using inactivated vaccines approved by China's regulator for use in adults. Using surveillance and vaccination data from the outbreak, we assessed the real-world effectiveness of inactivated vaccines against pneumonia and severe illness caused by the B.1.617.2 variant. We enrolled 10813 subjects who were close contacts of laboratory-confirmed cases, categorizing them as an unvaccinated group, a partially vaccinated (1-dose) group, and a fully vaccinated (2-dose) group. We estimated relative risk (RR) and vaccine effectiveness (VE) of the vaccinated groups in relation to the unvaccinated group.

Findings

Unadjusted and adjusted VE of full vaccination against pneumonia were 77·7% (95% CI 45·1–90·9) and 69·5% (95% CI 42·8–96·3), respectively. Full vaccination was 100% effective against severe illness. Unadjusted and adjusted VE of partial vaccination against pneumonia were 1·4% (95% CI -79·7–45·9) and 8·4% (95% CI -47·6–64·4).

Interpretation

Full vaccination with inactivated vaccines is effective against pneumonia, severe, and critical illness caused by the B.1.617.2 variant. Effort should be placed to ensure full vaccination of target populations.

Funding

National Natural Science Foundation of China and Key-Area Research and Development Program of Guangdong.

Research in context

Evidence before this study

We searched the PubMed and medRxiv database for studies published from Jan 1, 2020 to Jul 22, 2021, with the combination of key words (vaccin OR immuniz OR immunis) AND (delta OR B.1.617.2) AND (effectiveness OR VE OR real-world) AND (sars-cov-2 OR COVID-19 OR COVID OR Severe Acute Respiratory Syndrome Coronavirus 2) to identify studies on the real-world effectiveness of COVID-19 vaccines against the B.1.617.2 variant. We excluded studies that were not conducted in humans or were not original studies. A retrospective cohort study in Scotland investigated the vaccine effectiveness (VE) of ChAdOx1 nCoV-19 and BNT162b2 vaccines against hospitalization associated with the B.1.617.2 variant. The study found that a vaccination status of at least 28 days after the first or second dose significantly reduced the risk of hospitalization by 62% (95% CI: 42-76). A test-negative case-control study in England, also examining ChAdOx1 nCoV-19 and BNT162b2 vaccines, found that both vaccines were effective against symptomatic infections of the B.1.617.2 variant (VE: 67·0% [95% CI, 61·3–71·8] and VE: 88·0% [95% CI, 85·3–90·1]). However, the magnitude of VE was reduced compared with that against the B.1.1.7 variant for both vaccines. A third study using Canadian data to investigate VE of

mRNA-1273, BNT162b2, and ChAdOx1 nCoV-19 vaccines against multiple types of variants suggested that full vaccination with BNT162b2 provided similar VE against symptomatic cases of the B.1.617.2 variant (87% [95% CI 64–95]) with that against the B.1.1.7 variant (89% [95% CI 86–91]). VE of full vaccination against symptomatic cases of the B.1.617.2 variant could not be estimated for the other two vaccines due to an absence of cases. We also identified studies that examined the effectiveness of inactivated vaccines. One study from Chile was identified, which found that inactivated vaccines were effective for the prevention of COVID-19 (65·9% [95% CI 65·2–66·6]) and COVID-19-related hospitalizations (87·5% [95% CI 86·7–88·2]). No evidence of VE of inactivated vaccines against the B.1.617.2 variant has been documented.

Added value of this study

An outbreak of the SARS-CoV-2 B.1.617.2 variant was discovered and traced in Guangdong, China, starting in late May 2021 and lasting through late June 2021. By analyzing data on vaccinated and unvaccinated individuals in mandatory, centralized quarantine identified through the tracing and management of the outbreak, we assessed real-world effectiveness of inactivated vaccines against pneumonia and severe illness caused by the B.1.617.2 SARS-CoV-2 variant. This variant is prevalent globally, yet the effectiveness of inactivated vaccines against the variant remains unknown. As such, evidence on the VE of inactivated vaccines against the B.1.617.2 variant represents an important addition to the knowledge base for policy-making in jurisdictions that have deployed mass vaccination using inactivated vaccines or are considering to do so. Our estimates of unadjusted and adjusted VE of full vaccination against pneumonia were 75·4% (95% CI 39·6–90·0) and 69·6% (95% CI 42·9–96·3), respectively. Full vaccination was 100% effective against severe illness.

Implications of all the available evidence

Our study provides strong evidence that full-series vaccination with inactivated COVID-19 vaccines reduce risk of pneumonia and severe illness from the B.1.617.2 variant. The evidence is consistent with VE studies of other COVID-19 vaccines against the B.1.617.2 variant. To ensure optimal protection of the population, mass vaccination campaigns should focus on completing the full two-dose series.

Introduction

Vaccination is considered an indispensable part of exit strategies from the COVID-19 pandemic.[1,2] Due to an unprecedented global effort to develop COVID-19 vaccines, several types of vaccines were approved in many jurisdictions by early 2021.[2-4] Among these, at least five were developed using whole-virus inactivation technology and have received partial or full approval in China and many other countries.[4-7] Due to their long shelf life without need for ultra-cold chain, inactivated vaccines are relatively easy to store and dispense.[8-10] Combined with their documented efficacy from randomized clinical trials (RCTs), inactivated vaccines may be a near-ideal candidate for mass immunization programs in low- and middle-income countries.[8,11,12]

Whereas RCTs are the gold standards to estimate efficacy, their results may be limited in generalizability due subject selection/exclusion criteria and implementation restrictions. Real-world evidence (RWE) supplements RCT data by providing insight on comparative effectiveness among populations excluded or insufficiently included in licensure RCTs, conducted under different settings and epidemiological situations, using alternative outcomes, or are against a different lineage of the pathogen.[13,14] To date, published RWE on COVID-19 vaccines has largely focused on mRNA vaccines, findings from which compare well with corresponding RCT results.[15-19] Similar evidence on inactivated vaccines remains

sparse. A real-world study in Chile assessed the effectiveness of CoronaVac, an inactivated vaccine used for mass vaccination in over 20 countries.[20] That study provided convincing evidence of the protective effect of CoronaVac against COVID-19.[20]

In late May 2021, an importation-related 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 previously lineages seen in China, posing substantial challenges for disease control.[21,22] The outbreak lasted from May 21 to June 18, 2021, during which 167 infected individuals were identified in clinical settings, during quarantine, or through community screenings. In addition to case identification, contact tracing continued through June 23, 2021. Before the start of this outbreak, China had already started to rapidly roll out mass immunization campaigns, with Guangdong province being one of the forerunners of vaccine deployment. Specifically, over 90 million doses were administered in Guangdong before mid-June 2021. Only inactivated vaccines were supplied in Guangdong by June 11, 2021. As such, the outbreak lent itself as 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 based on China's COVID-19 prevention and control policies, we were able to assess the real-world effectiveness of inactivated vaccines against pneumonia and severe illness caused by the B.1.617.2 variant. The vaccines we evaluated are approved and recommended by the World Health Organization; more than 2 billion doses of these vaccines have been administered globally.

Methods

Study population and design

We conducted a retrospective cohort analysis of all close contacts of infected individuals identified in the Guangdong outbreak. Close contacts were defined in accordance with national and provincial COVID-19 prevention and control protocols.[23] Briefly, close contacts were defined as all individuals who lived in the same household or stayed in the same public space without protection within close distance within up to four days before illness onset for symptomatic cases, or were identified by the first positive specimen for asymptomatic cases. All close contacts were traced, mandatorily quarantined in centralized managed facilities, and followed up with multiple RT-PCR tests, thereby comprising our study cohort as the outbreak was proceeding and being managed.

A total of 12 501 individuals were identified as cases or close contacts by public health authorities. All positive specimens were subject to whole-genome sequencing. Individuals were excluded if basic demographic information was missing, they received two doses of vaccine but less than 21 days apart, or were younger than 18 years.

Vaccination status

Vaccination histories were obtained by interviewing individuals and reviewing vaccination records. To determine vaccination status, the number of doses received and the time elapsed since the most recent dose were used to define the intervention groups. Based on vaccination history, individuals were assigned to a unvaccinated group, a partially vaccinated (1-dose) group, or a fully vaccinated (2-dose) group. The unvaccinated group consisted of individuals who did not receive any COVID-19 vaccines before their last known contact with a confirmed case. The partially vaccinated group consisted of individuals who received their first dose 21 days or earlier than the last known contact. Individuals who

received their second dose at least 14 days before the last known contact comprised the fully vaccinated group. Our primary analysis was a 3-group comparison. Those who received their first dose within 21 days (intermediate 1st-dose) or their second doses within 14 days (intermediate 2nd-dose) before the last known contact were excluded in the primary analysis to avoid ambiguity in definition. Categorization of the groups is illustrated in Figure 1. In an alternate comparison, the intermediate 2nd-dose group was pooled with the partially and fully vaccinated groups as a single intervention group with any vaccination. The intermediate 1st-dose group was excluded in the alternative comparison.

Outcomes

The two outcomes of interest were pneumonia and severe/critical illness associated with the B.1.617.2 variant of COVID-19. Severity was based on subjects' most serious manifestations during the follow-up period, per judgement of clinicians.

Characteristics and covariates

Epidemiological investigators collected information on basic sociodemographic characteristics, including age, sex, address, occupation, and contact frequency. These variables were used as covariates in subsequent analyses. Age was categorized as 18-34 years old, 35-49 years old, and 50 years old and above. Contact frequency was adjudicated by investigators as occasionally, sometimes, and frequently. Occupation may have been associated with vaccination status, in that professionals in occupations with relatively high likelihood of exposure were granted priority of vaccinating during early 2021, whereas community-dwelling individuals, including unemployed persons were allowed to receive their free vaccines later on. To reflect heterogeneity in the chances of vaccinating, we created indicators for people in the catering industry and for unemployed people. There were two streets that were epicenters of the outbreak. The numbers of cases in these two communities accounted for over 60% of all outbreak cases. As such, residents of these two streets could have experienced higher risks of exposure. Geographic area might affect vaccination status through distribution practices of vaccines and related preventive behaviors. Therefore, an indicator was created for each of the two epicenter streets and used as a covariate in addition to the sociodemographic variables.

Four types of inactivated vaccines have been distributed and administered in China: HB02 (by Sinopharm), WIV04 (by Sinopharm), CoronaVac (by Sinovac), and Biokangtai's inactivated COVID-19 vaccine (BICV).[4,12] Although not used as covariates in our analyses, we recorded and described the types of inactivated vaccines used by subjects.

Statistical analyses

Characteristics of subjects in each group were described using mean values (SD) and percentages, and tested using χ^2 tests and one-way analyses of variance (ANOVA). To estimate the unadjusted vaccine effectiveness (VE), the relative risks (RR) of each outcome was calculated in reference to the unvaccinated group and subtracted from one. In addition, multivariate logistic regressions were carried out to account for covariates that could potentially confound effect estimations. Adjusted odds ratios (OR) of logistic regressions were reported and used for inference of statistical significance. To estimate adjusted VE (aVE) from multivariate logistic regressions, we first calculated the adjusted relative risk (aRR) that equaled the ratio of the predicted event probability conditioned on being in each vaccination group in relation to that of being unvaccinated.[24] The aVE was then calculated as 1-aRR. We used aRRs to calculate aVEs because RRs are intuitively understandable for cohort studies and because ORs

consistently underestimated RRs for protection effects.[25] The standard errors of aRRs were estimated using the delta method, which is frequently used for nonlinear transformations of regression coefficients.[26] All analyses were conducted using Stata (version 16).

Sensitivity analysis

In a set of sensitivity analysis, vaccination status was defined based on the time since inoculation until the first report of the outbreak (May 21, 2021). In this sensitivity analysis, anyone who received their first dose and the second dose before May 7, 2021 were assigned to the partially vaccinated group and the fully vaccinated group, respectively. Unlike the base case, those who received vaccines after the initial outbreak were excluded. In addition, a between-dose window was not considered when determining vaccination status.

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 authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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 individuals had missing sociodemographic information, 15 had received two doses less than 21 days apart, seven were vaccinated with non-inactivated vaccines, and 1 467 individuals were less than 18 years old. Consequently, 10 813 subjects met all inclusion and no exclusion criteria and were further assigned to different groups of intervention based on their vaccination histories. The sample selection flowchart is displayed in Figure 2.

Of the 10 813 individuals that met inclusion but not exclusion criteria, 5 888 (54·45%) were unvaccinated, 2 287 (21·15%) had an intermediate 1st dose, 843 (7·80%) were partially vaccinated, 388 (3·59%) had an intermediate 2nd dose, and 1 407 (13·01%) were fully vaccinated (Table 1). Among the 4 925 first doses, 2 392 (48·57%) were HB02, 6 (0·12%) were WIV04, 2 526 (51·29%) were CoronaVac, and one (0·02%) was BICV. Among the 1 795 second doses, 745 (41·50%) were HB02, four (0·22%) were WIV04, 1 046 (58·28%) were CoronaVac, and none were BICV.

Across the five groups, age ($p<0·001$), contact frequency ($p<0·001$), living in Zhongnan Street ($p<0·001$), living in Baihedong Street ($p<0·001$), and occupation ($p<0·001$) were statistically significantly different, whereas sex was comparable ($p=0·184$). The unvaccinated group had the greatest mean age (48·03 years, SD: 18·09), the highest proportion of the age group of 50 years and older (46·42%), the highest proportion of occasional contact (41·41%), and the lowest proportion of frequent contact (2·65%). In addition, the unvaccinated group had a higher percentage of Baihedong Street residents (13·69%) than any other groups, whereas its percentage of Zhongnan Street residents (2·51%) was lower than that of the partially and fully vaccinated groups, but not of the intermediate 1st-dose and 2nd-dose groups. The unvaccinated group had a proportion of unemployed individuals (3.09%) that was only second to that of the partially vaccinated group and had the second lowest proportion of catering industry professionals (3·82%) - only surpassed by the fully vaccinated group. Characteristics of the groups are listed in Table 1.

Unadjusted VE estimates are shown in Table 2. The unvaccinated, partially vaccinated, and fully vaccinated groups had 85 (1·44%), 12 (1·42%), and 5 (0·35%) COVID-19 pneumonia cases,

respectively. As such, the RRs of partial and full vaccination were 0·986 (95% CI 0·541–1·797) and 0·223 (95% CI 0·091–0·549), which corresponded to VEs of 1·4% (95% CI -79·7–45·9) and 77·7% (95% CI 45·1–90·9). Any vaccination was associated with an RR of 0·525 (95% CI 0·323–0·852) and a VE of 47·5% (95% CI 14·8–67·7) for COVID-19 pneumonia.

There were no severe or critical cases among vaccinated individuals. By contrast, the unvaccinated individuals had 19 severe or critical cases. As such, the RRs and VEs were zero and 100% for both vaccinated groups, and the uncertainty could not be estimated.

The aVEs and aORs from multivariate logistic regressions are presented in Tables 2 and 3. Multivariate analyses of severe and critical cases could not be conducted. Based on aORs and aVEs, partial vaccination was not associated with statistically significantly different incidence of pneumonia from no vaccination. However, the aORs of full vaccination against pneumonia [0·25 (95% CI 0·09–0·68)] was significant. Consistent with the aORs, the aVEs of full vaccination against pneumonia [69·5% (95% CI 42·8–96·3)] were both significant. Any vaccination was effective against COVID-19 pneumonia [aVE: 40·2% (95% CI 11·0–69·5)] in multivariate analyses.

Table S1 (online supplementary materials) shows the sensitivity analyses. Full vaccination consistently had significant VE against both outcomes whereas partial vaccination did not.

Discussion

Our study evaluated the effectiveness of inactivated COVID-19 vaccines against COVID-19 pneumonia and severe and critical COVID-19 caused by the B.1.617.2 variant in a real-world setting. Using close contacts as study subjects, we showed that inactivated VE against the B.1.617.2 (Delta) variant was 70% for COVID-19 pneumonia and 100% for serious/critical COVID-19. Thus, we documented evidence of VE of inactivated COVID-19 vaccines against both outcomes among a fully vaccinated population, but not among a partially vaccinated population.

Our results were robust to an alternative design. Notably, our VE estimates against COVID-19 pneumonia and severe and critical COVID-19 were in line with RCT results and other real-world studies.[11,12,15,16,20] Our findings confirm that inactivated COVID-19 vaccines will be effective even when the B.1.617.2 variant is prevalent.

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 construct population immunity in spite of recent mutations of the virus. Third, the VE estimates against pneumonia and severe and critical cases calls for refreshed evaluations of strategies to manage the pandemic in the long term, and should be highlighted in future planning.

To our knowledge, this study adds unique contributions to the scientific literature. First, it expanded upon a previous study on the real-word effectiveness of inactivated vaccines by investigating multiple instead of one 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 (Delta) variant. Third, it is the first study that documented VE against clinical outcomes other than intermediate endpoints of COVID-19 in mainland China. By combining these features, the present study generated new evidence that helps informed decision-making.

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 made subgroup analyses not possible. Despite

these two limitations, we believe that our study provides useful insights on the effectiveness of vaccines and suggested that inactivated vaccines may be effective against COVID-19 pneumonia and severe and critical COVID-19 associated with the B.1.617.2 variant of COVID-19, if fully vaccinated.

Contributors

MK, YL, and JH conceptualised and coordinated the study. YJ contributed to the literature search. YJ and YY drafted the manuscript. MC, JL, AD, TH, YY, JZ, ML, and JJ contributed to the epidemiological investigation and collected the data. YY, YJ, and MK accessed, verified and analysed the data. All authors contributed to interpretation of results and revision of the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Individual-level data will not be made publicly available with this article. Requests for sharing of deidentified individual-level data for scientific research can be directed to MK (kangmin@yeah.net).

Acknowledgments

We thank Zhongjie Li, Zhijie An, Dan Wu, Ying Qin, Qianqian Liu, and Jing Wu for their valuable guidance and suggestions. This study was funded by the National Natural Science Foundation of China (82041030) and the Key-Area Research and Development Program of Guangdong Province (2019B111103001).

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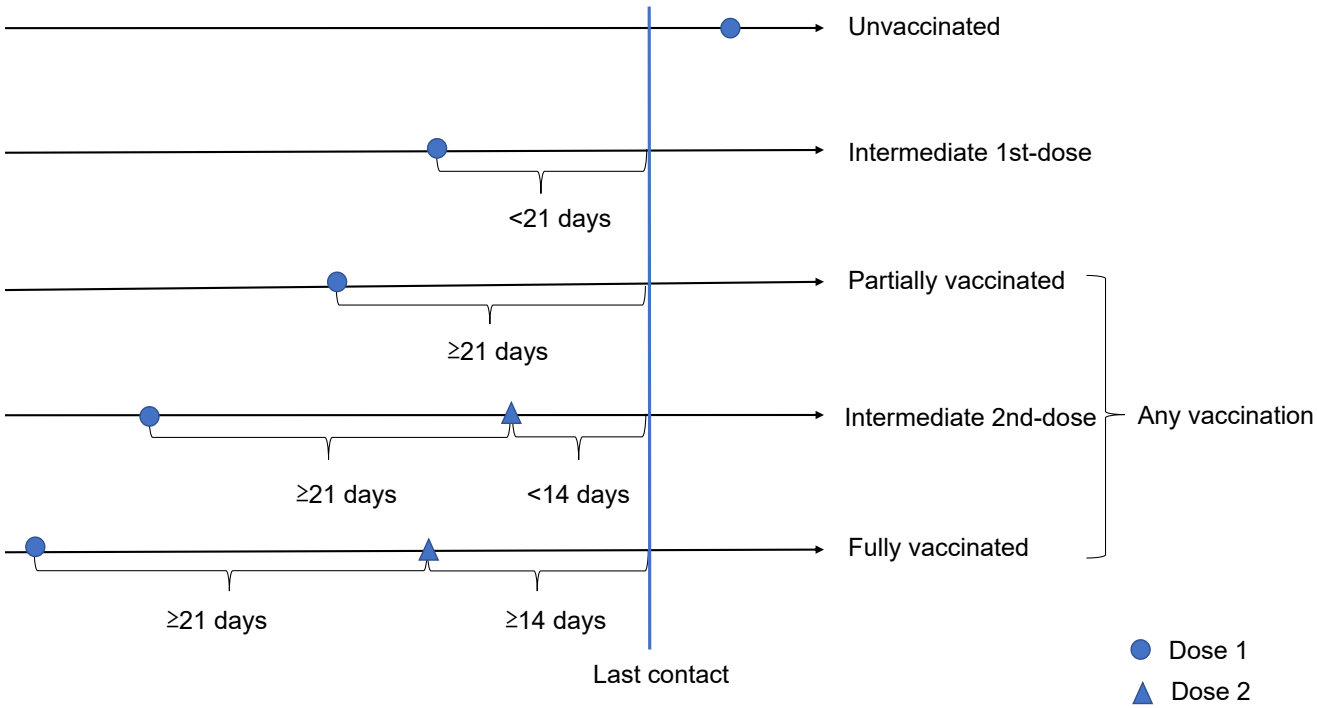
Figure legends

Figure 1. Definition of Different Vaccination status

Figure 2. Sample selection


Unvaccinated
Intermediate 1st-dose
<21 days
Partially vaccinated
≥21 days
Intermediate 2nd-dose
Any vaccination
≥21 days
<14 days
Fully vaccinated
≥21 days
≥14 days
Last contact
Dose 1
Dose 2

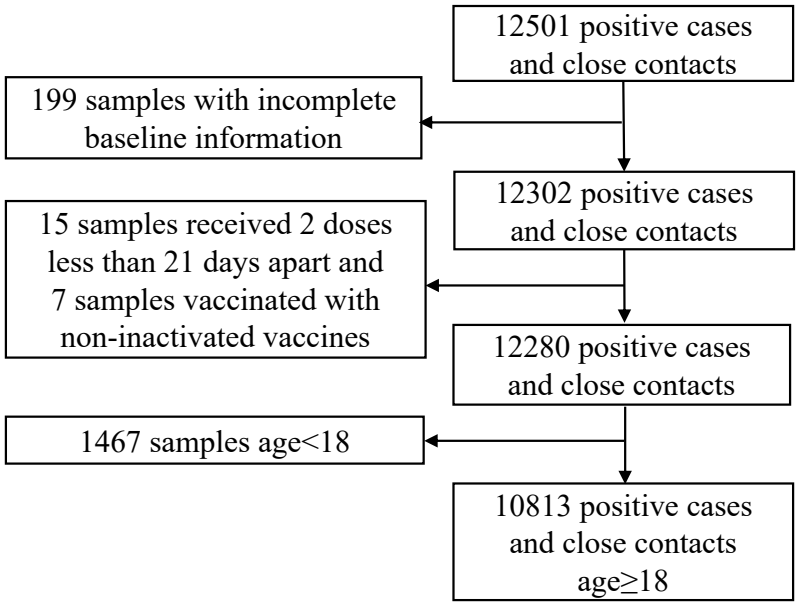

12501 positive cases and close contacts
199 samples with incomplete baseline information
12302 positive cases and close contacts
15 samples received 2 doses less than 21 days apart and 7 samples vaccinated with non-inactivated vaccines
12280 positive cases and close contacts
1467 samples age<18
10813 positive cases and close contacts age≥18

Table1. Characteristics by Vaccination status

Characteristics	No. (%)						
	Unvaccinated (N=5888, 54·45%)	**Intermediate 1st-dose (N=2287, 21·15%)**	**Partially vaccinated (N=843, 7·80%)**	**Intermediate 2nd-dose (N=388, 3·59%)**	**Fully vaccinated (N=1407, 13·01%)**	**Total (N=10813)**	***p* value**
Sex							
Male	3174 (53·91)	1198 (52·38)	454 (53·86)	189 (48·71)	772 (54·87)	5787 (53·52)	0·184
Female	2714 (46·09)	1089 (47·62)	389 (46·14)	199 (51·29)	635 (45·13)	5026 (46·48)	
Age, mean (SD)	48·03 (18·09)	38·31 (11·40)	38·53 (10·92)	38·76 (10·71)	39·30 (10·54)	43·77 (15·98)	<0·001
Age groups							
18–34 yr	1798 (30·54)	967 (42·28)	336 (39·86)	154 (39·69)	513 (36·46)	3768 (34·84)	<0·001
35–49 yr	1357 (23·05)	877 (38·35)	340 (40·33)	160 (41·24)	608 (43·21)	3342 (30·91)	
≥50 yr	2733 (46·42)	443 (19·37)	167 (19·81)	74 (19·07)	286 (20·33)	3703 (34·25)	
Contact frequency							
Occasionally	2438 (41·41)	880 (38·48)	327 (38·79)	134 (34·54)	496 (35·25)	4275 (39·54)	<0·001
Sometimes	3294 (55·94)	1343 (58·72)	473 (56·11)	234 (60·31)	829 (58·92)	6173 (57·08)	
Frequently	156 (2·65)	64 (2·80)	43 (5·10)	20 (5·15)	82 (5·83)	365 (3·38)	
Street							
Zhongnan	148 (2·51)	44 (1·92)	47 (5·58)	7 (1·80)	45 (3·20)	291 (2·69)	<0·001
Baihedong	806 (13·69)	130 (5·68)	81 (9·61)	30 (7·74)	141 (10·02)	1188 (10·99)	
Others	4934 (83·80)	2113 (92·39)	715 (84·81)	351 (90·46)	1221 (86·78)	9334 (86·32)	
Occupation							
Catering	225 (3·82)	186 (8·14)	48 (5·69)	22 (5·67)	34 (2·42)	515 (4·76)	<0·001
Unemployed/home	182 (3·09)	60 (2·62)	27 (3·20)	7 (1·80)	27 (1·92)	303 (2·80)	
Others	5481 (93·09)	2041 (89·24)	768 (91·10)	359 (92·53)	1346 (95·66)	9995 (92·44)	
First dose							

HB02	NA	1316 (57·54)	333 (39·50)	146 (37·63)	597 (42·43)	2392 (48·57)	<0·001
WIV04	NA	0 (0)	2 (0·24)	1 (0·26)	3 (0·21)	6 (0·12)	
CoronaVac	NA	970 (42·41)	508 (60·26)	241 (62·11)	807 (57·36)	2526 (51·29)	
BICV	NA	1 (0·04)	0 (0)	0 (0)	0 (0)	1 (0·02)	
Second dose							
HB02	NA	NA	NA	163 (42·01)	582 (41·37)	745 (41·50)	0·960
WIV04	NA	NA	NA	1 (0·26)	3 (0·21)	4 (0·22)	
CoronaVac	NA	NA	NA	224 (57·73)	822 (58·42)	1046 (58·28)	
BICV	NA	NA	NA	0 (0)	0 (0)	0 (0)	
Pneumonia							
Yes	85 (1·44)	16 (0·70)	12 (1·42)	3 (0·77)	5 (0·36)	121 (1·12)	0·001
No	5803 (98·56)	2271 (99·30)	831 (98·58)	385 (99·23)	1402 (99·64)	10692 (98·88)	
Severe/Critical							
Yes	19 (0·32)	0 (0)	0 (0)	0 (0)	0 (0)	19 (0·18)	0·003
No	5869 (99·68)	2287 (100)	843 (100)	388 (100)	1407 (100)	10795 (99·82)	

Notes: *p* value is obtained from chi-square tests or one-way analysis of variance, depending on whether the variable is categorical or continuous.

Table2. Vaccine effectiveness in preventing pneumonia, severe/critical cases by vaccination status

Outcomes	Vaccination status	N (%)	RR (95% CI)	Unadjusted VE (95% CI)	aVE (95% CI)
Pneumonia	Unvaccinated	85 (1·44)	Ref	-	-
	Partially vaccinated	12 (1·42)	0·986 (0·541–1·797)	1·4% (-79·7–45·9)	8·4% (-47·6%,64·4%)
	Fully vaccinated	5 (0·35)	0·223 (0·091–0·549)	77·7% (45·1–90·9)	69·5% (42·8%,96·3%)
	Any vaccination	20 (0·76)	0·525 (0·323–0·852)	47·5% (14·8–67·7)	40·2% (11·0%,69·5%)
Severe/ Critical	Unvaccinated	19 (0·32)	Ref	-	-
	Partially vaccinated	0 (0)	0 (NA)	100% (NA)	-
	Fully vaccinated	0 (0)	0 (NA)	100% (NA)	-
	Any vaccination	0 (0)	0 (NA)	100% (NA)	-

Notes: CI: confidence interval; VE: vaccine effectiveness; aVE: adjusted vaccine effectiveness.

Table 3. Adjusted odds ratios (aORs) from multivariate logistic regressions

Covariates	Pneumonia
Vaccination statuses (Ref: Unvaccinated)	
Partially vaccinated	0·90 (0·43–1·86)
Fully vaccinated	0·25** (0·09–0·68)
Sex (Ref: female)	0·44*** (0·28–0·69)
Age groups (Ref: 18–34 yr)	
35–49 yr	1·81 (0·80–4·12)
≥50 yr	4·25*** (2·06–8·79)
Occupation (Ref: Others)	
Catering	3·24 (0·94–11·15)
Unemployed/home	11·17*** (6·24–19·98)
Street (Ref: Others)	
Zhongnan	6·94*** (3·15–15·28)
Baihedong	11·89*** (7·31–19·35)
Contact frequency (Ref: Sometimes)	
Occasionally	1·51 (0·92–2·50)
Frequently	29·91*** (16·47–54·28)

Note: Adjusted odds ratios and 95% confidence intervals are shown.

***$p<0·001$，**$p<0·01$，*$p<0·05$.

3. É segura para gestantes e para os bebês

3.1 CoronaVac tem eficácia de 85% na prevenção de casos graves de Covid-19 em grávidas, mostra pesquisa

Uma pesquisa realizada por cientistas brasileiros e britânicos mostrou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, teve eficácia de 85% para evitar casos graves de Covid-19 entre gestantes brasileiras. O estudo foi publicado na última sexta (12) na plataforma de preprints SSRN, vinculada à revista The Lancet, e seus autores são da London School of Hygiene and Tropical Medicine, da Universidade Federal da Bahia, da Fundação Oswaldo Cruz, da Universidade de Brasília e da Universidade do Estado do Rio de Janeiro.

Segundo os pesquisadores, a eficácia do esquema completo de imunização com duas doses da CoronaVac foi de 85% para evitar casos graves de Covid-19, e de 75% na prevenção da progressão dos casos sintomáticos para a forma grave da doença. Nenhuma morte ocorreu entre as gestantes parcialmente ou totalmente imunizadas com a CoronaVac, enquanto que quatro óbitos seriam esperados se a mortalidade fosse a mesma do público não vacinado.

A população estudada foi a de todas as gestantes com sintomas de Covid-19, entre 18 e 49 anos, com registro de teste PCR realizado entre 15/3 e 3/10 de 2021, e registradas no Sistema de Notificação do Ministério da Saúde (e-SUS Notifica). Ao final da triagem, foram selecionados os dados de 19.838 gestantes, sendo que 7.424 (37,4%) haviam testado positivo para Covid-19, e 588 (7,9%) desenvolveram a forma grave da doença. No momento da extração dos dados, 83% das gestantes haviam recebido as duas doses da vacina, enquanto 17% haviam recebido apenas uma dose.

"Um regime completo de CoronaVac em gestantes foi eficaz na prevenção dos casos sintomáticos de Covid-19 e altamente eficaz na prevenção da forma grave da doença", salientaram os pesquisadores.

Em 17/1, o Ministério da Saúde iniciou a vacinação contra a Covid-19 com CoronaVac. Em 15/3, mulheres grávidas com comorbidades e em ocupações consideradas de alto risco tornaram-se elegíveis para receber a vacina. Em 26/4, a recomendação da imunização foi expandida para incluir todas as gestantes.

Publicado em: 17/11/2021

Artigo 17

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

Enny S. Paixao,[1,2*], Kerry LM Wong[1], Flavia Jôse Oliveira Alves[2], Vinicius de Araújo Oliveira[2,3,4] Thiago Cerqueira-Silva[3,4], Juracy Bertoldo Júnior[2,4] Tales Mota Machado[5], Elzo Pereira Pinto Junior[2], Viviane S Boaventura[4], Gerson O. Penna[6], Guilherme Loureiro Werneck[7,8], Laura C. Rodrigues[12], Neil Pearce[1], Mauricio L. Barreto [2,4], Manoel Barral-Netto[2,3,4]

1. London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK
2. Center of Data and Knowledge Integration for Health (CIDACS), Gonçalo Moniz Institute, Oswaldo Cruz Foundation , Salvador, Bahia, Brazil;
3. LIB and LEITV Laboratories, Instituto Gonçalo Moniz, Fiocruz, Salvador, Bahia, Brazil
4. Federal University of Bahia, Salvador, Bahia, Brazil;
5. Universidade Federal de Ouro Preto, Ouro Preto, Brazil;
6. Tropical Medicine Centre, University of Brasília, Fiocruz School of Government Brasília, Brazil (G O Penna PhD).
7. Instituto de Medicina Social, Universidade do Estado do Rio de Janeiro
8. Instituto de Estudos em Saúde Coletiva, Universidade Federal do Rio de Janeiro.

Abstract

Background
The effectiveness of Covid-19 inactivated vaccines in pregnant women is unknown. We estimated vaccine effectiveness (VE) of CoronaVac against symptomatic and severe Covid-19 and in preventing progression from symptomatic to severe Covid-19 in pregnant women in Brazil.

Methods
We conducted a test-negative design study in all pregnant women aged 18 to 49 years in Brazil, linking records of negative and positive SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) tests to national vaccination records. We also linked records of test positive cases with notification of severe, hospitalized or fatal Covid-19. Using logistic regression, we estimated adjusted odds and VE against symptomatic Covid-19 by comparing vaccine status in test positive (confirmed cases) to that in subjects with a negative test result. We also calculated the odds/VE against progression by comparing vaccine status in symptomatic cases to that in severe Covid-19 cases.

Findings
Of 19838 tested pregnant women, 7424 (37.4%) tested positive for Covid-19 and 588 (7.9%) had severe disease. Only 83% of pregnant women who received a first dose of CoronaVac completed the vaccination scheme. A single dose of the CoronaVac vaccine was not effective at preventing symptomatic Covid-19. Effectiveness of two doses of CoronaVac was 41% (95% CI 27.1- 52.2) against symptomatic Covid-19, 85% (95% CI 59.5-94.8) against severe Covid-19 and (75%; 95% CI 27.9- 91.2) in preventing progression to severe Covid-19 among those infected.

Interpretation
A complete regimen of CoronaVac in pregnant women was effective in preventing symptomatic Covid-19, and highly effective against severe illness in a setting that combines high disease burden and elevated Covid-19 related maternal deaths.

Research in Context

Evidence before this study

We searched PubMed for articles published "pregnant women" AND "vaccine" AND "SARS-CoV-2" AND “CoronaVac” AND “effectiveness” no results were found. Additionally, we repeated the search using "pregnant women" AND "vaccine" AND "SARS-CoV-2" AND “effectiveness”. Although pregnant women are at elevated risk of Covid-19 complications, they were excluded from most Covid-19 vaccine trials. The observational studies of vaccine effectiveness (VE) recently conducted were restricted to mRNA vaccines.

Added value of this study

This study observed that a single dose of the CoronaVac vaccine offered no protection against symptomatic Covid-19; a complete regimen of CoronaVac was 41% effective in preventing symptomatic Covid-19, and 85% effective in preventing severe Covid-19 disease; it was 75% effective in preventing severe outcomes in those who had been infected.

Implications of all the available evidence

A complete regimen of CoronaVac in pregnant women was effective in preventing symptomatic Covid-19, and highly effective against severe illness in a setting that combines high disease burden and elevated Covid-19 related maternal deaths.

Introduction

Cardiopulmonary and immune changes during pregnancy induce shifts in immune responses, increasing pregnant women's susceptibility to some infectious-related adverse outcomes.[1] Although pregnant women have higher a risk of Covid-19 complications, need intensive care and mechanical ventilation more often, and have higher fatality,[2] they were excluded from most Covid-19 vaccine trials.[3] There is considerable interest on establishing the safety and efficacy/effectiveness of Covid-19 vaccines in this population.[4] A number of observational studies of vaccine effectiveness (VE) were recently conducted[5,6,7,8], but those studying pregnant women were restricted to mRNA vaccines.[9,10,11,12,13]

Many low- and middle-income countries are conducting vaccination campaigns using CoronaVac,[5] an inactivated-virus vaccine; some countries, like Brazil, offer CoronaVac to pregnant women. On January 17, 2021, the Brazilian Ministry of Health initiated Covid-19 vaccination with two CoronaVac doses with two to four weeks interval between doses. The policy followed internationally agreed priorities.[14] On March 15, 2021, pregnant women with co-morbidities and in occupations considered, on balance, to be at high risk, became eligible to receive Covid-19 vaccine.[15] On April 26, this recommendation was expanded to include all pregnant women.[16] Although the exact figures for pregnant women are unclear, we anticipated that enough pregnant women would have been vaccinated to make it possible to evaluate vaccine effectiveness in pregnant women: Brazil combines a sufficient vaccine coverage (more than 50% of the population with two doses),[17] more than 21 million cases and 600,000 deaths (October 2021),[18] and a considerable number of maternal deaths.[19,20]

In this observational study of routine data in Brazil we estimated the VE of CoronaVac vaccine against symptomatic Covid-19 and in preventing progression from symptomatic to severe Covid-19 disease in pregnant women.

Methods

Objectives and study design

The primary objective of this study was to estimate VE of CoronaVac vaccine against symptomatic cases of Covid-19 in a test negative design (TND) in all pregnant women who had a RT-PCR test. We also estimated the effectiveness of vaccine the against developing severe Covid-19 (comparing severe, hospitalized or fatal Covid-19 with test negatives). As a further consistency check, we estimated VE against progression from symptomatic Covid-19 disease to severe Covid-19 (severe, hospitalized or fatal) by comparing the vaccine status of

those who developed severe disease with those who tested positive but did not develop severe disease.

Data sources

All data used was abstracted from 3 routinely collected sources: the national surveillance system for RT-PCR test for Covid-19 (e-SUS Notifica); the information system for severe acute respiratory illness (SIVEP-Gripe) and the national immunisation system (SI-PNI).

e-SUS Notifica: This database contains information on suspected cases of Covid-19 recorded in the country. It includes all positive and negative RT-PCR test results, and information on residence, demographic and clinical data of individuals, such as presence of comorbidities and pregnancy status (so we can identify women registered during pregnancy) and presence of symptoms, with acute respiratory diseases defined as presence of at least two of the following signs and symptoms: fever (even if referred), chills, sore throat, headache, cough, runny nose, loss or change to a sense of smell or taste.[21] Asymptomatic individuals with a positive RT-PCR test confirming by Covid-19 infection are registered but were not included in this study.

SIVEP-Gripe is the national registration for severe acute respiratory syndrome (SARS) in Brazil, created after the Influenza pandemic of 2009. In 2020, it was expanded to include Covid-19. All Covid-19 hospitalisations and deaths are meant to be registered in this system.[22] In SIVEP-Gripe, severe acute respiratory illness is defined as an individual with acute respiratory disease who presents dyspnea/respiratory discomfort, persistent pressure or pain in the chest, oxygen saturation less than 95% without oxygen, or cyanosis of the lips or face.[22] Individuals who died with severe acute respiratory illness independent of hospitalisation are also registered. By linking these data with e-SUS Notifica, we identified which pregnant women in e-SUSNotify with a positive RT-PCR test progressed to severe disease.

SI-PNI contains data on all vaccines administered in Brazil. Covid-19 vaccines are administered by health services and recorded in point-of-care applications.[23] From SI-PNI, we extracted information on which Covid-19 vaccine was received with dates of first and second doses. By linking these data with the data on pregnant women in the other files, we were able to determine: (i) which pregnant women who tested negative for Covid-19 had been vaccinated (ii) which pregnant women with confirmed symptomatic Covid-19 infections had been vaccinated and (ii) which pregnant women with severe Covid-19 associated severe case had been vaccinated. We assumed that pregnant women whose record did not link to a SI-PNI vaccination record were not vaccinated.

All data were extracted on October 05, 2021 and made available by the Brazilian Ministry of Health. The information technology bureau of the Brazilian Ministry of Health provided pseudo-anonymised data with a common unique identifier that were used to link individual-level records from the three databases (more details about linkage procedures are available at https://vigivac.fiocruz.br/).

Study population

All pregnant women with symptoms suggesting Covid-19, aged between 18 and 49 years in Brazil with a record of a RT-PCR test between March 15, 2021, and October 03, 2021, registered in e-SUS Notifica. Testing for Covid-19 in Brazil is accessible to anyone through the universal public health system (SUS). Subjects who received any Covid-10 Vaccine were excluded: ChAdOx1 nCoV-19 or Ad26.COV2.S (Janssen/Johnson & Johnson) because these are not indicated for pregnant women in Brazil and BNT162b2 because numbers of women with complete regimen were too small to allow evaluation given they were included in the Brazilian program more recently and the long interval between doses. So, the study is restricted to evaluating CoronaVac vaccine effectiveness. The population consisted of symptomatic pregnant women who were tested with RT-PCR for Covid-19 classified into 3 groups: RT-PCR test negative, RT-PCR test positive with Covid-19 symptoms and RT-PCR test positive with severe Covid-19. The study population in the TND included all symptomatic women with a RT-PCR irrespective of test result. For the nested case control study only women in the first study who had a positive RT-PCR test for Covid-19.

Definition of outcome, cases, and controls

In the TND, the primary outcome was a positive RT-PCR test in a symptomatic subject. Cases were defined as all symptomatic women in the study population with a RT-PCR test result from a respiratory sample collected within 10 days after the onset of symptoms and who did not have a positive RT-PCR test result in the preceding 90 days. We also conducted an additional analysis for the subgroup of cases with severe Covid-19, identified through notification to SIVEP-Gripe or with a register of hospitalization or death in e-SUS record. Controls were defined as all women in the study population with a negative RT-PCR test result, and no positive RT-PCR test in the previous 90 days or in the subsequent 14 days. The test date was defined as either the date of collecting a respiratory specimen or the date of the case registration (when the test date was missing).

As a further consistency check, we estimated VE against progression from symptomatic Covid-19 disease to severe Covid-19 (severe, hospitalized or fatal) by comparing the vaccine status of those who developed severe disease with those who tested positive but did not develop

severe disease. Cases were defined as all women with severe Covid-19, identified through notification to SIVEP-Gripe or with a register of hospitalization or death in e-SUS record. Controls were defined as all confirmed cases of Covid-19 in e-SUS not notified to SIVEP-Gripe and with no registration of hospitalisation nor deaths in e-SUS.

Exposure definition

The exposure studied was vaccination with CoronaVac. This was classified into partially vaccinated (≥14 days after the first dose and before receipt of the second dose at time of RT-PCR testing) and fully vaccinated (≥14 days after the second dose at time of RT-PCR testing). We also calculated effectiveness in the period <14 days since vaccination as the vaccine is expected to have no or limited effectiveness in the first 13 days since vaccination. This was used as a test as high effectiveness or increased risk during this period might serve as an indicator of unmeasured bias or confounding. The reference group for vaccination status was the women who did not received a first vaccine dose before the date of sample collection.

Covariates

A number of risk factors may be associated with both the likelihood of the exposure (i.e., receiving a vaccine) and the likelihood of receiving an RT-PCR SARS-CoV-2 test. These include age, ethnicity, comorbidities status, geography location, index of deprivation,[24] and time (reflecting changes in vaccination policy and disease circulation) and presence of a previous Covid-19 positive RT-PCR as this may both related with vaccination and the risk of a second Covid-19 infection. We extracted information on these potential confounders from the e-SUS Notifica.

Statistical analyses

The test negative design is a type of case-control study, in which the study population consist of the population tested, and controls are selected from those who have a negative test. [25] Accordingly, both the test negative design and the additional comparison of severe cases with non-severe cases were analysed using the standard methods for case-control studies.[25,26] Logistic regression was used to estimate the odds of vaccination with CoronaVac in RT-PCR test confirmed cases compared with those who tested negative, and the odds of vaccination in the severe cases compared to those who tested negative; finally, we also estimated the odds of progression from symptomatic to severe Covid-19, by comparing the odds of vaccination in the severe cases to that in the non-severe cases. Individuals only contributed their first positive test result from March 15, 2021 (when the vaccination programme was recommended for pregnant women nationally). Week of RT-PCR test was included in the regression models because of the variations over time in both Covid-19 incidence and vaccine delivery in Brazil.

We also adjusted for age (<20, 20-34, ≥35), ethnicity (white, mixed brown, black and others), presence of registered comorbidities, geography (region), index of deprivation (quintile). We estimated the VE as one minus the corresponding odds ratio (OR), obtained from a model including the described covariates, expressed as a percentage.

Data analyses were performed in Stata version 17.0.

This study analysed de-identified data and was approved by the National Ethics committee (CONEP) (CAAE registration no. 50199321.9.0000.0040).

Results

During the study period, 95,738 symptomatic suspected cases of Covid-19 among pregnant women were registered in the Brazilian surveillance system e-SUS Notify. Of those, 50,819 (53.1%) had an RT-PCR SARS-CoV-2 test, and the results were available for 30,947 (60.9%) samples. After exclusions, 19838 subjects were included in the analysis; 7424 (37.4%) were test-positive, and 12414 (62.6%) test-negative. Of the 7424 with a positive test, 588 (7.9%) were severe and 84 (1.1%) died (Figure1). Table 1 shows the characteristics of cases and controls.

Figure 2 shows the number of cases and controls by time since the first and second vaccination doses among vaccinated pregnant women. After the first doses of CoronaVac, the proportion of positive tests does not seem to change. Notably, 165 (16.6%) out of all women with a single dose of CoronaVac had not received a second dose after the recommended interval between doses (4 weeks).

The odds of testing positive among vaccinated women during the 13 days after the first dose, was 1.35 (95% CI 1.09 to 1.68) compared with those unvaccinated, indicating an unexpected small increase in risk of Covid-19 among the vaccinated during this initial period. VE among those receiving only the first dose with at least 14 days between the first dose and the date of RT-PCR) was low and not statistically significant 5.02 (95% CI -18.22- 23.69). The estimated adjusted VE in the fully vaccinated group against symptomatic Covid-19 was 41.0% (95% CI 27.1 to 52.2) (Table 2). The corresponding estimate for severe Covid-19 was 67.7 (95% CI 20.0-87.0) for those partially vaccinated and 85.4 (95% CI 59.4- 94.8) for fully vaccinated women (Table 3).

The estimated adjusted VE of CoronaVac against progression from symptomatic to severe Covid-19 was 67.4% (95% CI 17.7 to 87.1) among partially vaccinated pregnant women and 74.7% (95% CI 28.0 to 91.2) among fully vaccinated women (Table 3). No deaths occurred

among partially or fully vaccinated pregnant women when four would have been expected if mortality was the same as in unvaccinated.

Discussion

In this investigation of CoronaVac VE in pregnant women, we found that a single dose of the CoronaVac vaccine offered no protection against symptomatic Covid-19; two doses were 41% effective against symptomatic Covid-19 and 85% effective against severe Covid-19. Those who were fully vaccinated and went on to have symptoms had a 75% lower risk of progressing to severe Covid-19 than those unvaccinated. No deaths occurred among partially or fully vaccinated women, when 4 were expected. About 17% of vaccinated women did not get a second dose as prescribed by the time they were tested.

Although the findings from this study suggest that the complete CoronaVac vaccine regimen was effective against symptomatic Covid-19 among pregnant women, the magnitude of estimated effectiveness was lower than reported previously in studies in the general population conducted in Brazil,[8] Chile,[5] and Turkey.[27] Pregnancy promotes resistance to generating proinflammatory antibodies compared to non-pregnant women, suggesting that pregnant women may not respond to some vaccines as effectively.[28,29] We did not investigate biological mechanisms; further investigation is required to establish whether the lower effectiveness found is due to immunological changes during pregnancy. In contrast with other Covid-19 vaccines such as the BNT162b2 which confers protection after the first dose,[30] CoronaVac was effective against symptomatic Covid -19 only after a complete regimen. This was also found is in older people in Brazil.[31]

This study has strengths and limitations. As a strength, it used rich, routinely collected data from Brazil, recognised to be of high-quality.[32] By using the TND, we have minimised bias related to access to health care, the occurrence of symptoms and health-seeking behaviour. In most populations strong pressures have influenced who got tested for Covid-19. These biases can mean that those who get tested, and test positive for SARS-CoV-2 may not be a random sample of all cases in the population. The assumption that underlies the TND is that people who seek testing and manage to get tested would be influenced by similar pressures regardless of vaccine status and the test outcome,[26] thus biases will 'cancel out' and relatively unbiased estimates of effect can be obtained.[25,26]

However, as observational designs are vulnerable to confounding and bias. The fact that the risk of Covid-19 increased in vaccinated women in the 2 weeks after the first dose is not biological plausible and may be an indication of residual bias/confounding, which in this

case could lead to an underestimation of VE. A potential explanation for this would be if vaccinated subjects feel safer than unvaccinated subjects, such that unvaccinated subjects are more likely to seek testing for a symptom (not caused by Covid-19) that would not lead a vaccinated subject to test. This would result in a higher proportion of negative tests among the unvaccinated, leading to an apparent estimated increase in risk in the vaccinated, underestimating VE. Other potential explanations are that the process of vaccination itself increases the risk of infection, such travelling to or from a vaccination site, and finally, that after being vaccinated, believing themselves to be protected, women undergo a period of 2 weeks of contacts and reduced protective measures, leading to a peak of infection shortly after vaccination.

A limitation intrinsic to the use and availability of secondary data is the limited choice of covariates and the potential for misclassifying vaccine status due to linkage failure. Finally, we did not assess vaccination safety as data necessary for this assessment was not available. However, it is reassuring that CoronaVac contains an adjuvant that is commonly used in many other vaccines, such as against Hepatitis B and Tetanus, with a well-documented safety profile among pregnant women.[34] Previous evidence of safety of inactivated vaccines for other pathogens and using this adjuvant is reassuring.[34]

We note that an alarming 17% of the study sample with a single dose of CoronaVac did not take the second dose after the recommended maximum interval (4 weeks). This has important repercussions for public health authorities, highlighting the importance of actively searching those delaying the second doses and promoting opportunities to vaccinate these women during regular prenatal care appointments.

In conclusion, this study involved pregnant women in a setting that combines high disease burden and elevated Covid-19 related maternal related deaths. In this setting, we found that a complete regimen of CoronaVac was 41% effective in preventing symptomatic Covid-19, and 85% effective in preventing severe Covid-19 disease; it was 75% effective in preventing severe outcomes in those who had been infected.

Contributors
ESP, NP, MLB, MBN developed the study concept. VAO, TCS, JBJ, TMM, GP, MBN acquired, treated and linked the data. KLMW, FJOA, EPPJ, VO, GLW, LCR contributed to the data analyses and interpretation of results. VAO, KLMW vouched for the data analyses. ESP wrote the first draft. All authors decided to publish and revised the manuscript and approved the final version.

Declarations
We declare no competing interests. VO, VB, MB, and MB-N are employees from Fiocruz, a federal public institution, which manufactures Vaxzevria in Brazil, through a full technology transfer agreement with AstraZeneca. Fiocruz allocates all its manufactured products to the Ministry of Health for the public health service (SUS) use.

Funding
This study is part of the VigiVac Fiocruz program, partially supported by a donation from the "Fazer o bem faz bem" program. EPS is funded by the Wellcome Trust [Grant number 213589/Z/18/Z]. The funders had no role in study design, analysis, decision to publish or preparation of the manuscript.

This research was funded in part by the Wellcome Trust. For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

Acknowledgments
GLW, MLB, and MB-N are research fellows from CNPq, the Brazilian National Research Council.

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

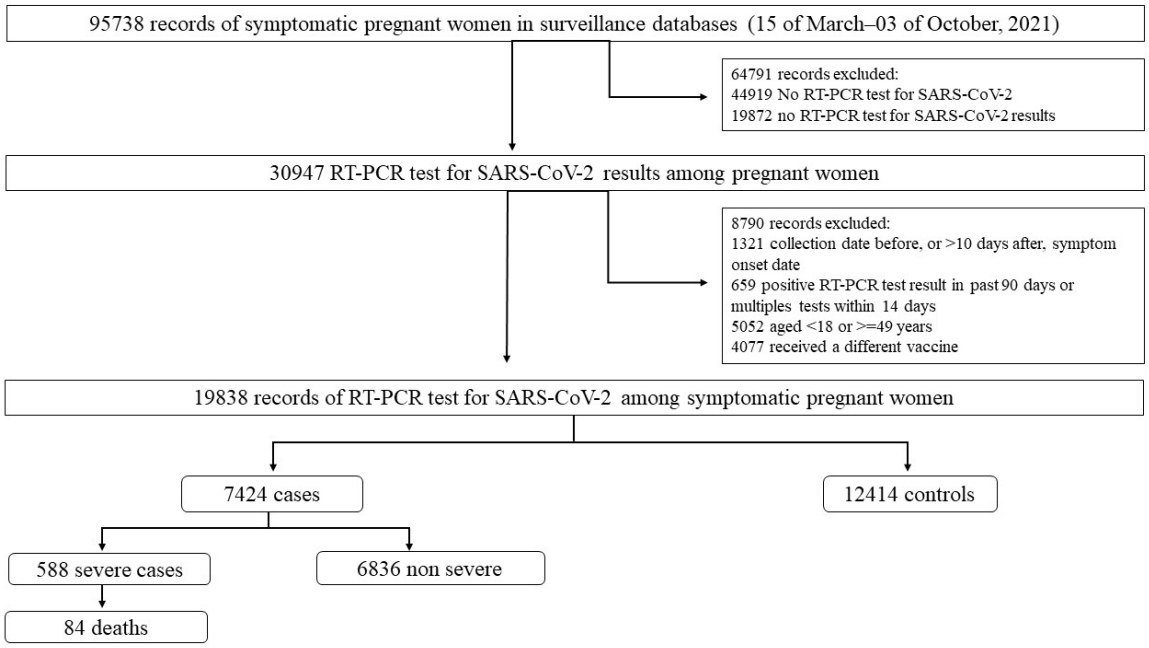

Figure 2: Number of cases and controls by interval since first and second vaccination

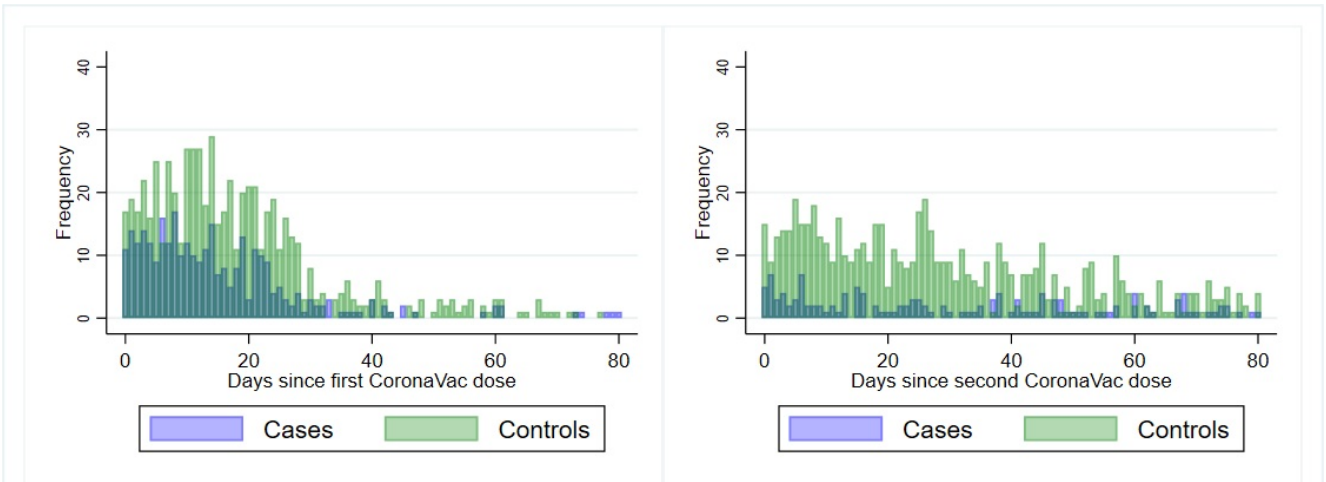

Table 1: Characteristics of cases and controls in pregnant women aged 18-49 years in Brazil.

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 Nº 1/2021-DAPES/SAPS/MS -** Vaccination for pregnant and postpartum women with comorbities	- Vaccination for pregnant and lactating women with comorbidities - Vaccine can be offered to pregnant and postpartum women without comorbidities after evaluating the risks and benefits, especially considering the professional activity performed by the woman.
26/04/2021	**NOTA TÉCNICA Nº 467/2021-CGPNI/DEIDT/SVS/MS -** Vaccination for pregnant and postpartum women without comorbidities	Phase I- Pregnant and postpartum women with comorbidities, regardless of age Phase II- Pregnant and postpartum women, regardless of comorbidities
14/05/2021	**NOTA TÉCNICA nº 627/2021-CGPNI/DEIDT/SVS/MS -** Temporary suspension of vaccination	- Temporary suspension of vaccination with the vaccine AstraZeneca/Oxford/Fiocruz in pregnant and postpartum women
19/05/2021	**NOTA TÉCNICA Nº 651/2021 - CGPNI/DEIDT/SVS/MS -** Continued vaccination in pregnant and postpartum women with comorbidities	- Vaccination of pregnant and postpartum women with comorbidities after benefit risk evaluation and medical prescription (Vaccines without viral vector -SINOVAC/Butantan or Pfizer-BioNTech BNT162b2) - Pregnant and postpartum women (including those without additional risk factors) who have already received the first dose of the AstraZeneca/Oxford/Fiocruz vaccine must wait for the end of the gestation and postpartum period (up to 45 days after delivery) for the administration of the second dose of the vaccine - Pregnant and postpartum women (including those without additional risk factors) who have already received the first dose of another COVID-19 vaccine that does not contain a viral vector (Sinovac/Butantan or Pfizer-BioNTech BNT162b2) should complete the regimen with the same vaccine at the usual intervals - Pregnant and postpartum women of other priority groups (health workers or other essential services workers, for example) may be vaccinated after an individual risk and benefit evaluation
06/07/2021	**NOTA TÉCNICA Nº 2/2021 - SECOVID/GAB/SECOVID/MS -** Continued vaccination in pregnant and postpartum women without comorbidities	- Vaccination of pregnant and postpartum women aged 18 years and over, regardless of risk factors - Pregnant of any gestational age - Needs for Medical evaluation and Prescription
23/07/2021	**NOTA TÉCNICA Nº 6/2021-SECOVID/GAB/SECOVID/MS** - Interchangeability between vaccines for pregnant and postpartum women who	- Vaccination of pregnant and postpartum women aged 18 years and over, regardless of risk factors - Pregnant of any gestational age

	took the oxford astrazeneca vaccine in the first dose	- Need for Medical evaluation and Prescription - To pregnant and postpartum women who received the first dose of the AstraZeneca/Fiocruz vaccine, at time of the second dose, preferably, the Pfizer-BioNTech BNT162b2 /Wyeth vaccine should be offered. If this immunising agent is not available locally, Sinovac/Butantan vaccine may be used

3.2 Proteção contra a Covid-19 gerada pela CoronaVac é passada aos bebês pelo leite materno das mães, aponta pesquisa

Um estudo recente feito pelo Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) aponta que lactantes que receberam a CoronaVac, vacina produzida pelo Instituto Butantan em parceria com a farmacêutica chinesa Sinovac, apresentam anticorpos contra Covid-19 no leite materno, capazes de proteger também os bebês, até quatro meses após a vacinação.

A pesquisa foi realizada com 20 funcionárias que foram imunizadas entre janeiro e fevereiro de 2021. Foram recolhidas nove amostras de leite no total: antes da imunização, quatro vezes depois da primeira dose e três vezes após a segunda dose, com intervalos de sete dias e quatro meses após a vacinação.

A pesquisa mostrou que os níveis de anticorpos do leite materno ainda estavam altos quatro meses após a vacinação. Os auges da produção de anticorpos se deram na segunda semana após a primeira dose e na quinta e na sexta semana após a segunda dose.

A imunização das lactantes e gestantes oferece proteção de duas formas: aos bebês ainda não nascidos, por meio da placenta, com anticorpos IgG, e por meio do leite materno, aos recém-nascidos, com anticorpos IgA.

De acordo com o Ministério da Saúde, cerca de 500 mil grávidas e puérperas com comorbidades já foram vacinadas contra a Covid-19 no Brasil. As gestantes se tornaram público prioritário da campanha de vacinação porque a taxa de letalidade da Covid-19 entre elas é muito maior que a média (10% para grávidas contra 2% da população em geral). Apenas duas vacinas são recomendadas para as gestantes, sendo uma delas a CoronaVac, por ter grande eficácia e um alto perfil de segurança.

Publicado em: 09/08/2021

CLINICS

EDITORIAL

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

Valdenise Martins Laurindo Tuma Calil[I,#], **Patricia Palmeira**[II,*,#], **Yingying Zheng**[III], **Vera Lúcia Jornada Krebs**[I], **Werther Brunow de Carvalho**[III], **Magda Carneiro-Sampaio**[II,III]

[I] Instituto da Crianca e do Adolescente, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR. [II] Laboratorio de Pediatria Clinica (LIM36), Departamento de Pediatria, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR. [III] Departamento de Pediatria, Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, BR.

Calil VMLT, Palmeira P, Zheng Y, Krebs VLJ, Carvalho WB, Carneiro-Sampaio M. CoronaVac can induce the production of anti-SARS-CoV-2 IgA antibodies in human milk. Clinics (Sao Paulo). 2021;76:e3185

*Corresponding author. E-mail: patricia.palmeira@hc.fm.usp.br
#Contributed equally as co-first authors.

To the Editor,

Human milk is the external secretion with the highest immunoglobulin A (IgA) concentrations, mostly produced in the lamina propria of mammary glands by plasma cells (1). The milk antibody repertoire is quite similar to the one observed in the blood; however, the levels of antibodies against enteric and respiratory pathogens are usually higher in the colostrum and mature milk than in the serum. Maternal immunization can elicit systemic immunoglobulin G (IgG) and mucosal IgA, IgM, and IgG responses that confer protection to the newborn infants (2,3,4).

During the current pandemic, milk anti-SARS-CoV-2-specific IgA antibodies have been found in 23.1% of 2,312 previously infected lactating women (5,6). In an Israeli prospective cohort, milk samples of 84 breastfeeding women were analyzed before immunization and then weekly for six weeks after immunization. All the mothers received two doses of the Pfizer-BioNTech vaccine 21 days apart (7). The levels of IgA antibodies were significantly elevated two weeks after the first dose, with 61.8% of the samples testing positive (86.1% at week 4—one week after the second dose, and 65.7% at week 6).

Here, we present data from an initial study on the presence of anti-SARS-CoV-2 IgA antibodies in human milk samples obtained from volunteers during the immunization process promoted by HC-FMUSP in January (17^{th}-21^{st}) and February (15^{th}-18^{th}), 2021. The preparation "CoronaVac" (an inactivated vaccine), produced by Sinovac Biotech Ltd. (China) and Instituto Butantan (Brazil), was administered to all healthy employees in two doses, four weeks apart. A total of 170 samples were collected. All the 20 milk donors were HC-FMUSP employees and were breastfeeding at the time of the first immunization phase and voluntarily donated 5-10 mL milk samples before the first dose and seven more samples weekly for three weeks after the second dose. Milk samples were collected four months after the first dose from 10 mothers to evaluate the persistence of SARS-CoV-2-specific IgA antibodies. Milk was collected by the donors themselves into sterile containers after careful local antisepsis with sterile water. Manual expression or milk pump were used for sample collection after rigorous handwashing. The milk was stored at home by the donor at -20ºC until delivery to the laboratory (LIM-36-ICr).

The study was approved by the Institutional Ethics Board (CAAE: 45565121.2.0000.0068), and written informed consent was obtained from all the participants. The levels of IgA antibodies that specifically bind the S1 domain of the spike protein (including RBD-Receptor Binding Domain) were semiquantitatively analyzed using the Euroimmun anti-SARS-CoV-2 S1 ELISA kit. The results were presented as the ratio of the optical density of the samples and the optical density of the calibrator (both read at 450 nm, using a reference wavelength of 620 nm), and ratios above 0.8 were considered positive. One-way ANOVA followed by Tukey's multiple comparison tests were used in the statistical analysis (GraphPad v.7.0 Software Inc., San Diego, CA, USA), and statistical significance was set at $p<0.05$.

No significant adverse reactions were reported in either the mothers or their babies. The mean maternal age was 35.6 (±3.2) years at the time of the first dose, with a mean nursing period of 11.2 (±8.7) months, quite similar to the Israeli study, which was 10.3 months (7).

Of the 20 mothers, 16 were COVID-negative at week 0 (Figure 1). Despite an increase in the mean levels of anti-SARS-CoV-2-specific IgA in the first two weeks after the first dose, significantly higher mean values were obtained only at weeks 5 and 6. Ten mothers presented specific IgA antibody levels above the seroconversion value at week 7 (21 days after the second dose). Among the ten mothers who donated a sample four months after the first dose, five still had specific IgA levels above the seroconversion value at that time. In our series, four mothers had COVID-19, of whom three presented high levels of anti-SARS-CoV-2 IgA antibodies in W0 (data not shown). One of them donated her milk four months after the first vaccine dose and still had high specific IgA levels (anti-SARS-CoV-2-specific IgA ratio=4.0).

No potential conflict of interest was reported.

Received for publication on May 26, 2021. **Accepted for publication on** June 10, 2021

DOI: 10.6061/clinics/2021/e3185

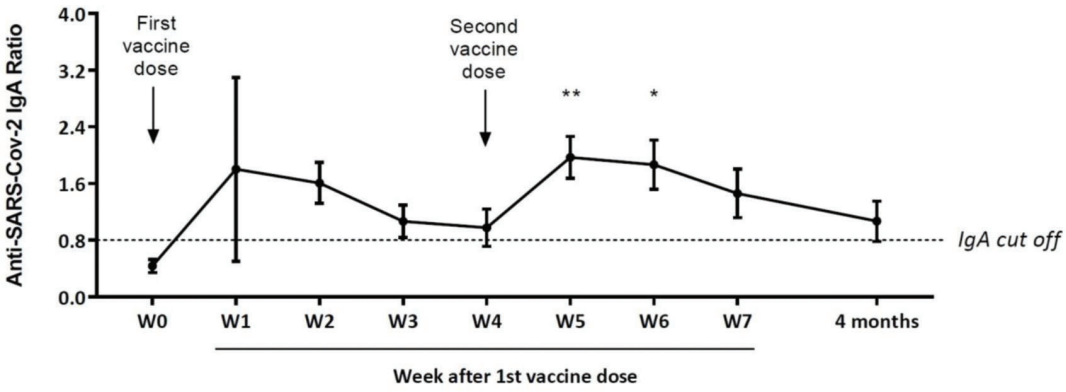

Figure 1 - Anti-SARS-CoV-2-specific IgA ratios (mean ± standard error) in milk samples collected over time (Weekly–W) from 16 healthy mothers previously COVID-negative after a 2-dose schedule of the CoronaVac vaccine (Sinovac Biotech Ltd., China). The last withdrawal was performed four months after the first dose in ten mothers. $^{**}p < 0.01$; $^{*}p < 0.05$.

This study strongly reinforces that mothers should continue breastfeeding their children after vaccination against SARS-CoV-2 and even after infection (5-7). As for other respiratory infections, maternal anti-SARS-CoV-2 immunization should protect infants with systemic IgG and milk IgA providing local mucosal defense, as demonstrated by Gray et al. (8) in a large group of pregnant and lactating women who received Pfizer-BioNTech vaccine where all cord blood and breastmilk samples presented specific IgG and IgA antibodies, respectively. Therefore, to analyze both the placental transfer of anti-SARS-CoV-2 IgG and production of IgA in early milk, we are planning an equivalent protocol with "CoronaVac" immunization during pregnancy involving the collection of maternal and cord blood, colostrum, and milk during the first two post-delivery months (3,4).

ACKNOWLEDGMENTS

We are grateful to all the participating mothers, to the press officer Markione de Santana Assis for logistical assistance during the research, nutritionists Analisa Gabriela, Zucchi Leite, and Renata Hyppolito Barnabe for assistance in the collection and transport of milk samples, and to FAPESP (grant 2014/50489-9) for Euroimmun anti-SARS-CoV-2 S1 ELISA kit acquisition.

AUTHOR CONTRIBUTIONS

Calil VMLT, Palmeira P, and Carneiro-Sampaio M contributed substantially to the study conception and design, data analysis and interpretation, manuscript writing and editing. Zheng Y was responsible for sample collection, laboratory, and statistical analyses. Krebs VLJ and Carvalho WB were responsible for revising the manuscript. All of the authors critically revised the manuscript and approved its final version.

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4. Protege indivíduos com comorbidades

4.1 CoronaVac traz níveis elevados de proteção para pessoas com HIV, indicam estudos do Brasil e da China

Dois estudos científicos publicados por pesquisadores do Brasil e da China evidenciam que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, é segura e capaz de gerar níveis elevados de proteção contra o SARS-CoV-2 em pessoas infectadas pelo vírus HIV, causador da AIDS.

O trabalho "Safety and Immunogenicity of CoronaVac in People Living with HIV", realizado por pesquisadores do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo e publicado em setembro na plataforma de preprints SSRN, avaliou a segurança e imunogenicidade da CoronaVac em 215 pessoas que vivem com HIV, na comparação com 296 pessoas sem imunossupressão conhecida. Todos os participantes receberam duas doses de CoronaVac com um intervalo de 28 dias.

Quatro semanas após a segunda dose da vacina, a porcentagem de participantes com positividade para anticorpos neutralizantes SC e NAb foi alta tanto para o grupo com HIV quanto no grupo controle. Nenhuma reação adversa séria foi relatada durante o estudo, seja entre pessoas com HIV ou nos participantes não imunossuprimidos.

No entanto, os pesquisadores encontraram diferenças nos parâmetros de imunogenicidade entre as pessoas com HIV. Os linfócitos T CD4 (células CD4) ajudam a coordenar a resposta imune, estimulando outras células imunes como os linfócitos B (células B) e T CD8 (células CD8) a combater a infecção. O vírus HIV enfraquece o sistema imunológico, destruindo as células CD4. Decorridos 69 dias da primeira dose da CoronaVac, os participantes com contagem de células T CD4 menor que 500 células/mm^3 tinham imunogenicidade mais baixa contra o vírus SARS-CoV-2 quando comparados aos membros do mesmo grupo com contagem maior ou igual a 500 células por mm^3.

A partir dessa análise, os pesquisadores concluíram que as pessoas com HIV e contagem maior ou igual a 500 células T CD4 por mm^3 tinham 2,26 vezes mais chances de apresentar positividade na atividade dos anticorpos neutralizantes

quando comparadas aos com contagem de células T CD4 por mm^3 menor que 500. Em relação aos participantes do grupo controle, esse indicador era 3,21 vezes maior.

"Nossos resultados mostraram que a CoronaVac tem imunogenicidade robusta em pessoas vivendo com HIV após um regime de duas doses, mas as respostas de anticorpos nesta população são um pouco mais baixas do que em indivíduos não imunossuprimidos", afirmam os autores. "Estratégias devem ser desenvolvidas para melhorar a imunogenicidade induzida por vacina entre as pessoas vivendo com HIV, especialmente no subgrupo com baixas contagens de células T CD4. Uma abordagem possível é usar uma dose de vacina de reforço ou mesmo administrar títulos de antígeno mais altos por dose de vacina", concluem eles.

Em outubro, outro estudo realizado por pesquisadores chineses e publicado na plataforma SSRN também trouxe evidências de que a CoronaVac é segura para pessoas vivendo com o vírus HIV, e que as pessoas deste grupo, quando totalmente imunizadas no esquema de duas doses da vacina do Butantan, podem alcançar níveis elevados de proteção contra o SARS-CoV-2, similares aos observados nos indivíduos HIV-negativos.

A Covid-19 e o HIV

Um relatório publicado em julho pelo Programa Conjunto das Nações Unidas sobre HIV/AIDS (UNAIDS) analisou mais de 168 mil pessoas hospitalizadas com Covid-19 em todo o mundo e concluiu que a incidência da forma mais grave da doença e o número de mortes intra-hospitalares eram maiores em pessoas que vivem com HIV, independentemente de idade, sexo e comorbidades. Estima-se que mais de 38 milhões de pessoas vivam com HIV em todo o mundo, sendo 1 milhão delas no Brasil.

Publicado em: 19/11/2021

Artigo 19

Safety and immunogenicity of CoronaVac in people living with HIV

Lucas Chaves Netto[1], Karim Yaqub Ibrahim[1], Camila de Melo Picone[1], Ana Paula Pereira da Silva Alves[1], Eliane Vieira Aniceto, Mariana Rodrigues Santiago[1], Patrícia da Silva Spindola Parmejani[1], Nadia E Aikawa[2], Ana C Medeiros-Ribeiro[2], Sandra G Pasoto[2], Emily F N Yuki[2], Carla G S Saad[2], Tatiana Pedrosa[2], Amanda Nazareth Lara[1], Carina Ceneviva[3], Eloisa Bonfa[2], Esper Georges Kallas[1]*, Vivian I. Avelino-Silva[1]*

1. Department of Infectious and Parasitic Diseases, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
2. Rheumatology Division, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil
3. Department of Pathology, Central Laboratory Division, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil Address: Av. Dr. Eneas Carvalho de Aguiar, 155 - 2º Andar - Cerqueira Cesar – CEP 01246-100 - Sao Paulo/SP – Brazil

*Equal contribution

Keywords: SARS-CoV-2; COVID-19; vaccine; HIV; immunogenicity; neutralizing antibodies; CoronaVac

Word count: 3,049

Abstract:

Background: People living with HIV (PLWH) may have a poor or delayed response to vaccines, mainly when CD4+ T cell counts are low. There are limited data concerning the safety and immunogenicity of COVID-19 vaccines in PLWH.

Methods: This prospective controlled study evaluated the safety and immunogenicity of the SARS-CoV-2 inactivated vaccine CoronaVac in PLWH compared with controls with no known immunosuppression. Immunogenicity was assessed with SARS-CoV-2 IgG seroconversion (SC), neutralizing antibodies (NAb) activity, and factor increase in IgG geometric mean titers (FI-GMT). We also investigated if levels of CD4+ T cell counts (< or ≥500 cells/mm^3) were associated with CoronaVac immunogenicity.

Findings: 511 participants (215 PLWH and 296 controls) were eligible for the immunogenicity analysis. At vaccine completion (D69), although the percentage of participants with SC and NAb positivity was high for both PLWH and controls, it was somewhat lower in PLWH. CD4+ T cell was identified as a relevant factor for immunogenicity, with lower SC and NAb positivity in PLWH with CD4+ counts <500 cells/mm^3 compared to those with ≥500 cells/mm^3. In a

multivariable logistic regression model for NAb positivity after a complete two-dose regimen adjusted for age and sex, compared with PLWH with a CD4+ T cell count <500/mm^3, those with CD4+ counts ≥500/mm3 had 2·26 times the odds of having positivity in NAb activity (95% CI 1·18-4·32; p=0·014), whereas controls had 3·21 times the odds of this outcome. No serious adverse reactions were reported during the study.
Interpretation: Immunogenicity following CoronaVac in PLWH seems robust but reduced compared with controls; PLWH with CD4+ counts <500/mm^3 are at increased risk for a blunted antibody response following vaccination.
Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); and B3 - Bolsa de Valores do Brasil.

Research in context:
Evidence before this study: Several studies have shown that people living with HIV (PLWH) may have a poor or delayed response to vaccines or even a reduced duration of immunogenicity following vaccination. So far, scarce data concerning safety and immunogenicity of COVID-19 vaccines in PLWH is available.
Added value of this study: This is the first controlled study addressing safety and immunogenicity of the SARS-CoV-2 inactivated vaccine CoronaVac in PLWH compared with controls with no known immunosuppression. At four weeks after the second vaccine dose, the percentage of participants with seroconversion and neutralizing antibodies positivity was high for both PLWH and controls. However, the study found significantly lower immunogenicity among PLWH compared to non-immunosuppressed participants. Moreover, PLWH with CD4+ T cell counts <500 cells/mm^3 had lower SARS-CoV-2 immunogenicity compared to PLWH with CD4+ T cell counts ≥500 cells/mm^3 and
Implications of all the available evidence: Strategies to improve vaccine-induced immunogenicity may be needed for PLWH. Data on clinical efficacy and real-life effectiveness studies are still lacking for this population.

Introduction:

Several vaccines have been implemented in clinical practice to prevent severe COVID-19 cases and related deaths. Brazil has been severely hit by the pandemic, with one of the highest rates of reported cases and deaths globally.[1] Up to September 2021, four vaccines have been implemented in Brazil; the ChAdOx1 by AstraZeneca and the CoronaVac by Sinovac and Butantan Institute have been more frequently used, followed by a more recent introduction of the single-dose Ad26.COV2.S by Janssen and the BNT162b2 by Pfizer and BioNTech . Compared to other COVID-19 vaccines, CoronaVac has logistical advantages in storage (requiring refrigeration only) and manufacturing technology. Mass vaccinations campaigns have already taken place in Turkey, Brazil, Chile, and Indonesia, with approval for emergency use in more than 20 low and middle-income countries.[2,3]

Several risk factors have been associated with poor outcomes among COVID-19 cases, including pulmonary, cardiac, and chronic renal conditions; older age; obesity; and immunosuppression such as solid organ transplants, recent chemotherapy, hematopoietic diseases, and HIV infection. Although large cohorts from United States, United Kingdom, and South Africa showed an increased risk of COVID-19-associated death among PLWH compared to HIV-uninfected individuals after adjustment for covariates[4], some observational and epidemiological data suggested no more significant risk, especially among PLWH with well-controlled HIV infection.[5] However, several studies demonstrate that PLWH may have a poor or delayed response to vaccines or even a reduced duration of immunogenicity following vaccination against *Pneumococcus sp*, Influenza, Hepatitis A and B[6], and Yellow Fever.[7]

So far, scarce safety data concerning PLWH vaccinated with COVID-19 vaccines is available, with only 0·6% and 0·5% representation of PLWH in clinical trials with the mRNA-1273 and BNT162b2 vaccines, respectively.[8,9] In a small cohort of 12 PLWH vaccinated with the mRNA vaccine, lower immunogenicity was observed among those with CD4+ T cell counts <200/mm^3.[9] There is also limited data regarding the use of ChAdOx1 in this population from a South African cohort (102 PLWH vs. 56 controls) and a subgroup analysis of a phase 2/3 study in England (54 PLWH), with no significant differences in immunogenicity.[10] There are, however, no data on the safety and immunogenicity of inactivated COVID-19 vaccines in PLWH to date.

This cohort study evaluated the safety and immunogenicity of the SARS-CoV-2 inactivated vaccine CoronaVac in PLWH compared with controls with no known immunosuppression.

Methods

Study design and population

In this prospective cohort nested within a large phase 4 vaccination protocol (clinicaltrials.gov #NCT04754698), PLWH aged >18 years regularly followed at the HIV/AIDS outpatient clinic at the University of São Paulo were invited to participate. We included adults with no known immunosuppression who received CoronaVac as controls. We excluded potential participants with a history of anaphylactic reaction to the vaccine components; acute febrile illness at vaccination; current hospitalization; a history of Guillain-Barre syndrome or demyelinating disease; previous vaccination with any SARS-CoV-2 vaccine; a history of vaccination with a live virus vaccine up to four weeks before enrolment, or an inactivated vaccine up to two weeks before enrolment; and a history of any blood product transfusion up to 6 months before enrolment. Participants with well-controlled comorbidities were included, but those reporting other types of immunosuppression or COVID-19 symptoms at the time of the first vaccine dose were excluded. Participants with positive results in baseline assessment of SARS-CoV-2 IgG or neutralizing antibodies (NAb) were also excluded from the analysis.

Study procedures

We collected demographic and clinical characteristics of study participants at baseline, and laboratory variables including last CD4+ T cell count and HIV viral load were extracted from medical charts. CoronaVac was administered in a twice-dose regimen 28 days apart, according to the manufacturer's recommendations.[11] CoronaVac (Sinovac Life Sciences, Beijing, China, batch #20200412) contains a β-propiolactone inactivated SARS-CoV-2 derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells - Vero 25 cells with aluminum hydroxide as an adjuvant. Single-use CoronaVac syringes containing 0·5 mL were administered intramuscularly in the deltoid area. Participants underwent blood collections immediately before each vaccine administration and four weeks after the second dose (D69). Serum samples were stored at -70˚C. In case of incident COVID-19 during the study period, the second vaccination was delayed by four weeks.

Immunogenicity evaluation

The immunogenicity evaluation comprised two serologic tests: a chemiluminescent immunoassay that measured IgG antibodies targeting S1 and S2 proteins in receptor binding domain (Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy), measured in AU/mL (Arbitrary Units) and a virus NAb detection assay SARS-CoV-2 sVNT Kit (GenScript, Piscataway, NJ, USA). Seroconversion (SC) was defined as a positive (≥15·0 AU/mL) serology for the IgG test. We also calculated IgG geometric mean titers (GMT) and 95% confidence intervals at all time points and the factor increase in GMT (FI-GMT) as the ratio of the GMT after vaccination to the GMT before vaccination. NAb activity was reported as percentages and categorized as positive when ≥30% as suggested by the manufacturer.[12] Immunogenicity tests were performed in samples collected at baseline (D0), immediately before the second vaccine shot (D28, intermediary assessment), and six weeks after the second vaccine dose (D69, final assessment).

Safety evaluation

The vaccine's local and systemic side effects were monitored using a standardized form and clinical evaluations at each study visit. Participants completed the standardized forms with solicited adverse reactions after each vaccine dose. Solicited local adverse reactions included pain, erythema, swelling, bruise, pruritus, and induration at the vaccine injection site. Systemic reactions included fever, malaise, somnolence, lack of appetite, sweating, nausea, vomit, diarrhea, abdominal pain, vertigo, tremor, headache, fatigue, myalgia, muscle weakness, arthralgia, back pain, cough, sneezing, coryza, runny nose, sore throat, shortness of breath, conjunctivitis, pruritus and skin rash.

Moderate and severe adverse events have been recorded from D0-D69 and classified as vaccine-related and unrelated. Participants with COVID-19 symptoms during the study period underwent a SARS-CoV-2 reverse transcriptase–polymerase-chain-reaction (RT-PCR) test in a nasal swab sample.

Statistical analysis:

We present the characteristics of study participants using descriptive statistics. Comparisons between PLWH and non-immunosuppressed controls were made using Mann-Whitney-Wilcoxon rank-sum tests for numeric variables and chi-squared or Fisher's exact tests for categorical variables. We generated categorical variables for age (<40; 40-49; 50-49; ≥60 years old), and $CD4^+$ T cell counts (<500; ≥500). A multivariable logistic regression model was used to assess the impact of HIV infection, and CD4+ T cell counts on the positivity of SARS-CoV-2 anti-S1/S2 IgG and NAb test following vaccination, adjusted for age and sex. We used the statistical software Stata 15·1 (StataCorp College

Station, TX: StataCorp LP) in all analyzes, with a two-tailed significance level of 0·05.

Ethical aspects

The national and local ethics committees approved the study. Each participant provided written informed consent before enrolment. Participant identifiable data remained confidential throughout the study.

The study sponsors had no role in study design, data collection, analysis, interpretation of data, writing of the report, or in the decision to submit the paper for publication

Results

Between February and March 2021, 776 consecutive participants were recruited, of whom 282 were PLWH and 494 non-immunosuppressed controls. Two participants from the control group were excluded after drop-out following the first vaccine dose. Additional 244 (31%) individuals were excluded from this analysis due to a positive IgG or NAb test at baseline (53 PLWH [19%] and 191 controls [39%]), and 19 individuals were excluded due to missing baseline results of IgG or NAb tests. The remaining 511 individuals comprised the study sample for the immunogenicity analysis (215 PLWH and 296 non-immunosuppressed controls). For the safety analysis, 465 participants completed the forms. A flowchart describing study participants is presented in Supplement Figure 1.

Demographic and clinical characteristics of study participants are presented in Table 1. Female participants comprised 85 (40%) of the PLWH and 187 (63%) of the non-immunosuppressed participants (p<0·001). PLWH were older than controls, with a median 54 years old (interquartile range [IQR] 45-60) and 48 years old (IQR 37-58), respectively (p<0·001).

The frequency of comorbidities was similar between PLWH and controls, except for a higher frequency of dyslipidemia (17% *vs*. 5%; p<0·001) and chronic kidney disease (2% *vs*. 0%; p 0·013) among PLWH.

We obtained CD4+ T cell counts of all 215 PLWH, with a median of 22 months from the last CD4+ T cell count measurement and study enrolment (IQR 11-33). CD4+ T cell counts were <500 cells/mm^3 for 64 (30%) participants and ≥500 cells/mm^3 for the remaining 151 (70%). Overall, 191 (89%) PLWH had undetectable (<50 copies/mL) viral load in at least three measurements before inclusion and were considered with viral suppression. The median time between the last HIV viral load assessment and study enrolment was two months (IQR 1-3).

SARS-CoV-2 vaccine immunogenicity: effect of HIV infection

Table 2 describes results of the immunogenicity assessment. In unadjusted analysis at vaccine completion (D69), the frequency of positive SARS-CoV-2 IgG SC and NAb positivity was high for both PLWH and non-immunosuppressed controls; it was significantly lower in PLWH (SC 91 vs. 97%, p<0·005; NAb positivity 70·7 vs. 84%, p<0·001). The FI-GMT and NAb activity were moderate and lower in PLWH compared to non-immunosuppressed controls [median FI-GMT 22·5 (IQR 10·9 – 41·1) vs. 31·8 (IQR 15 – 53·1), p<0·001; median NAb activity 46·1 (26·9 – 69·7) vs. 60·7 (39·8 – 79·9), p<0·001]. Of note, at the day of the second dose (D28), PLWH had lower percentages of SARS-CoV-2 IgG SC (19 vs. 39%, p<0·001), NAb positivity (19 vs. 39%, p<0·001), and lower levels of FI-GMT (2·3 vs. 4·6, p<0·001) and NAb activity (0 vs. 23.7%, p<0·001) compared to non-immunosuppressed controls.

SARS-CoV-2 vaccine immunogenicity: effect of CD4+ T cell counts among PLWH

In the final assessment (D69), PLWH with CD4+ T cell counts <500 cells/mm^3 had a lower immunogenicity compared to those with CD4+ T cell counts ≥500 cells/mm^3 [SC 82 vs. 94%, p=0·008; NAb positivity 59 vs. 76, p=0·001; median NAb activity: 41·6 vs. 49·9%, p=0·030]. At D28, PLWH with CD4+ T cell counts < or ≥500/mm^3 had comparable immunogenicity parameters (p>0·05) except for the NAb activity (0 vs. 23·7%, p=0·002; Table 2). Figure 1 shows the final SARS-CoV-2 NAb activity among PLWH with CD4+<500 cells/mm^3, CD4+≥500 cells/mm^3 and HIV-uninfected participants; the median final NAb activity was 41·6 % (IQR 20·8 – 64·6) among PLWH with <500 cells/mm^3; 49·9 % (IQR 30·6 – 73·1) for PLWH with ≥500 cells/mm^3; and 60·8 % (IQR 39·8 – 79·9) among HIV-uninfected participants.

Multivariable analysis for SARS-CoV-2 vaccine immunogenicity

Given the baseline differences between groups regarding sex and age distributions, we performed a multivariable logistic regression including HIV status and CD4+ T cell counts (< or ≥500/mm3), with age categories and sex as independent variables, and positivity in NAb at the final study assessment (D69) as the outcome.

The model showed that, compared with PLWH with a CD4+ T cell count <500/mm^3, those with CD4+ counts ≥500/mm3 had 2·26 times the odds of having a positive NAb after complete vaccination (D69) (95% CI 1·18-4·32; p=0·014), whereas HIV-uninfected individuals had 3·21 times the odds of this

outcome (95% CI 1·72-6·00; p<0·001). Female sex and age categories were not significantly associated with the odds of having a positive NAb (Table 3).

Vaccine safety

Information regarding adverse vaccine reactions was available for 189 PLWH and 296 non-immunosuppressed participants. Adverse events are detailed in Supplement Table 1, and the most frequently reported symptoms are presented in Figure 2. Most participants were asymptomatic after vaccination with the first (61%) and the second (68%) vaccine dose. Only mild adverse events were reported during the study. PLWH and non-immunosuppressed participants had no statistically significant differences in the occurrence of vaccine adverse events after the first dose, except for any local reactions (12% vs. 21% respectively; p=0·026) and sweating (5% vs. 1% respectively; p=0·005).
After the second shot, we found a higher frequency of adverse reactions among non-immunosuppressed participants, including nausea (2% *vs*. 6%; p=0·013), myalgia (4% *vs*. 8%; p=0·048), arthralgia (3% *vs*. 8%; p=0·048), shortness of breath (0 *vs*. 3%; p=0·016), and pruritus (0% *vs*. 3%; p=0·016) compared to PLWH.

Supplement Figure 1: Selection of study participants

Table 1: Demographic and clinical characteristics of participants eligible for immunogenicity analysis

	PLWH N=215	Non-immunosuppressed controls N=296	p-value
Age category (%)			
<40 years old	34 (16)	88 (30)	
40 – 49 years old	45 (21)	75 (25)	
50 – 59 years old	69 (23)	69 (23)	
>60 years old	64 (22)	64 (22)	
Median Age (IQR)	54 (45-60)	48 (37 – 58)	<0·001
Female sex, n (%)	85 (40)	187 (63)	<0·001
CD4+ category, cells/mm^3, n (%)			
CD4+ < 200	9 (4)	-	-
CD4+ 200 – 349	24 (11)	-	-
CD4+ 350 – 499	31 (14)	-	-
CD4+ ≥ 500	151 (70)	-	-
Median CD4+ count (IQR)	655 (458 – 900)	-	-
Viral suppression, n (%)	191 (89)	-	-
Median weeks between last CD4+ count and inclusion (IQR)	21 (10 – 33)	-	-
Comorbidities, n (%)			
Smoking	28 (13)	33 (11)	0·305
Hypertension	52 (24)	71 (24)	0·520
Diabetes	27 (13)	37 (13)	0·544
Cardiopathy	5 (2)	4 (1)	0·310
Dyslipidemia	37 (17)	15 (5)	<0·001
COPD	0	3 (1)	0·194
Asthma	5 (2)	10 (3)	0·338
Chronic kidney disease	5 (2)	0	0·013
Chronic liver disease	4 (2)	1 (<1)	0·103
Neoplasia	2 (1)	0	0·177
Previous stroke	5 (2)	0	0·013
Active tuberculosis	2 (1)	0	0·177

COPD: chronic obstructive pulmonary diseases

Table 2: Immunogenicity after one dose (D28) and two doses (D69) for PLWH, according to CD4+ counts category, and non-immunosuppressed controls

	HIV-uninfected N = 296	PLWH N = 215	*P*-value comparing PLWH and controls	PLWH CD4+ < 500 N = 64	PLWH CD4+ ≥ 500 N = 151	*P*-value comparing high and low CD4+
D69						
IgG levels (AU/mL)	75·2 (50·3 – 112)	48·7 (26·5 – 88·2)	<0·001	42·0 (22·9 – 68·9)	53·3 (30·2 – 92·4)	0·053
Seroconversion	265 / 274 (97%)	185 / 204 (91%)	0·005	51 / 62 (82%)	134/ 142 (94%)	0·008
FI-GMT	31·8 (16 – 53·1)	22·5 (10·9 – 41·1)	<0·001	19·3 (7·6 – 33·5)	23·0 (11 – 45)	0·120
NAb positivity	229 / 274 (84%)	143 / 202 (71%)	0·001	36 / 61 (59%)	107 / 141 (76%)	0·013
Percent NAb activity	60·7 (39·8 – 79·9)	46·1 (26·9 – 69·7)	<0·001	41·6 (20·8 – 64·6)	49·9 (30·6 – 73·1)	0·030
D28						
IgG levels (AU/mL)	10·4 (4·7 – 30·5)	5·1 (0 – 11·3)	<0·001	5·1 (0 – 7·9)	5·1 (0 – 12·3)	0·448
Seroconversion	114 / 295 (39%)	41 / 214 (19%)	<0·001	10 / 64 (15%)	31 / 150 (20%)	0·255
FI-GMT	4·6 (2·3 – 10·3)	2·3 (1·0 – 5·2)	<0·001	2·2 (1 – 3·8)	2·4 (1 – 6)	0·337
NAb positivity	112 / 289 (39%)	40 / 211 (19%)	<0·001	7 / 64 (11%)	33/147 (22%)	0·035
Percent NAb activity (%)	23·7 (0 – 39·6)	0 (0 – 27·3)	<0·001	0 (0 – 0)	23·7 (0 – 39·6)	0·002

Numeric variables are presented as medians and interquartile ranges; categorical variables are presented as frequencies and percentages; AU: arbitrary units; SC: seroconversion (positive IgG, ≥15AU/mL); NAb: Neutralizing antibody test (positive when ≥ 30%); FI-GMT: factor of increase – geometric mean titter

Figure 1: SARS-CoV-2 percentage neutralizing antibodies activity among persons living with HIV with CD4<500, CD4≥500, and non-immunosuppressed participants. Dots represent results from individual vaccines; whiskers indicate 25th, 50^{th,} and 75th percentiles.

Table 3: Multivariable logistic regression model for neutralizing antibody positivity after vaccination with a two-dose regimen of inactivated SARS-CoV-2 vaccine, according to HIV status and CD4+ T cell counts

	OR	95% CI	p-value
PLWH, CD4+<500 mm^3	Reference (1·00)	-	-
PLWH, CD4+≥500 mm^3	2·26	1·17 – 4·32	0·014
Non-immunosuppressed participants	3·21	1·72 – 5·99	<0·001
Female sex	1·17	0·73 – 1·85	0·510
Age category			
<40 years old	Reference (1·00)	-	-
40 – 49 years old	1·06	0·51 – 2·18	0·871
50 – 59 years old	0·77	0·40 – 1·56	0·512
>60 years old	0·55	0·28 – 1·07	0·082

PLWH: People living with HIV

Figure 2: Local (panel A) and systemic (panel B) adverse events after vaccination, according to vaccine dose and HIV infection status

Discussion

Here we present the findings of the first controlled study addressing the safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 among PLWH compared with non-immunosuppressed controls. No serious adverse reactions were reported during the study, either among PLWH or non-immunosuppressed participants. We found a few statistically significant differences with a higher occurrence of adverse reactions in the control group compared to PLWH. At four weeks after the second vaccine dose, the percentage of participants with SC and NAb positivity was high for both PLWH and controls. However, we found statistically significant differences in the immunogenicity parameters comparing PLWH and non-immunosuppressed participants in unadjusted analysis both after the first dose and after the second vaccine. In addition, at D69, PLWH with CD4+ T cell counts <500 cells/mm^3 had lower SARS-CoV-2 immunogenicity compared to PLWH with CD4+ T cell counts ≥500 cells/mm^3.

We observed a few differences between PLWH and non-immunosuppressed participants in baseline demographics and clinical characteristics. Female sex was more frequent among non-immunosuppressed controls, and PLWH were somewhat older. Both factors have been adjusted for in the multivariable model. Regarding comorbidities, the only significant differences were a higher frequency of dyslipidemia (17% *vs*. 5%) and chronic kidney diseases (2% *vs*. 0%) among PLWH. The higher occurrence of chronic non-communicable diseases in PLWH is a documented phenomenon.[13] Due to a low overall frequency, we did not include these variables as covariates in the multivariable model addressing immunogenicity. Our multivariable logistic regression model for NAb positivity at D69 adjusted for age and sex showed that non-immunosuppressed participants and PLWH with CD4+ T cell count ≥500/mm^3 had significantly higher odds of having a positive NAb compared to PLWH with CD4+ T cell count <500/mm^3.

Our results are consistent with previous knowledge on the immunogenicity elicited by vaccines among PLWH and patients with lower CD4+ T cell counts.[6] HIV infection is known to impair the immune system beyond the decrease of CD4+ T cell counts,[14] impacting various immunologic pathways resulting in immune activation, impaired humoral and cellular responses, and clinical

outcomes including a decreased immunogenicity to several vaccines. Studies have shown that vaccines such as the live attenuated Yellow Fever vaccine, inactivated tetravalent influenza and hepatitis A/B vaccines, pneumococcal (both polysaccharide [PPSV 23] and conjugated formulations [PCV10, PCV13]) and conjugated *Haemophilus influenzae* type B elicit a less robust immune response in PLWH compared with HIV-uninfected individuals regardless of antiretroviral treatment and CD4+ T cell counts. [7,15,16] Moreover, the vaccine-induced immune response seems to be particularly impaired in situations of advanced or uncontrolled HIV infection, with low CD4+ T cells (<200/mm^3) and detectable HIV viral load.[6] Studies also suggest that the vaccine-induced immunogenicity may wane more rapidly for this group of patients.[17]

Recent studies on the immunogenicity of COVID-19 vaccines in immunosuppressed patients suggest that the antibody response may be impaired in these populations. Medeiros-Ribeiro et al. published a phase IV controlled study assessing immunogenicity following CoronaVac among patients with autoimmune rheumatologic diseases and found a NAb positivity of 56% compared to 79% among controls.[18] Additional studies addressing other COVID-19 vaccines such as the mRNA Pfizer BioNTech also found a reduced antibody response in immunosuppressed patients such as chronic corticosteroid users,[19] patients under immunosuppressive drugs,[20] and solid organ transplant recipients.[21,22]

Our study had a few limitations. As seen in any observational study, groups were subject to imbalances in demographic and clinical characteristics. The older age and lower frequency of female sex among PLWH could partially explain the lower immune response to the inactivated SARS-CoV-2 vaccine, as older age has been associated with lower vaccine immunogenicity[23] and female sex was associated with higher vaccine immunogenicity and reactogenicity.[24] This imbalance could also partially explain the higher frequency of adverse reactions in the non-immunosuppressed group. We fit a multivariable logistic regression model including sex and age categories to adjust for these imbalances. Interestingly, in this model, sex and age categories had no statistically significant impact on final NAb positivity, whereas HIV status and CD4+ T cell count categories remained associated with final NAb positivity. Another limitation was the use of broad CD4+ T cell count categories due to the low number of participants with CD4+ T cell count<350/mm^3. As such, we were unable to explore the effect of lower levels of CD4+ T cells on vaccine immunogenicity. Other potential problems include the lack of recent CD4+ T cell count measurements for some PLWH, with a median of 22 months between the last

assessment and study enrolment. The current Brazilian HIV treatment guidelines recommend avoiding CD4+ T cell count measurements after HIV viral load becomes undetectable and CD4+ T cell counts are >350/mm^3. We believe this limitation is unlikely to impact our results significantly, as once antiretroviral therapy (ART) is initiated, the CD4+ T cell count tends to remain stable or increase progressively, and even after virologic failure, CD4+ counts take months or years to drop to pre-ART levels.[25]

PLWH are historically more vulnerable to complications of common viral respiratory diseases such as influenza[26] but the interaction between HIV and SARS-CoV-2 is still unclear. Although some observational and epidemiological data suggest no greater risk of detrimental outcomes of COVID-19 among PLWH, especially among those with well-controlled HIV infection,[5,27] there are a few other studies that show higher mortality in PLWH compared to HIV-uninfected individuals.[28] Interestingly, studies from different epidemiological contexts support that race and schooling are associated with greater mortality among PLHIV with SARS-CoV-2 infection,[29] and social issues may overtake immune dysfunctions as determinants of COVID-19 outcomes in this population.

Our results showed that CoronaVac has robust immunogenicity in PLWH after a two-dose regimen, but antibody responses in this population are somewhat lower than in non-immunosuppressed individuals. Strategies should be developed to improve vaccine-induced immunogenicity in PLWH, especially in the subgroup with low CD4+ T cell counts. One possible approach is using a booster vaccine dose or even administering higher antigen titers per vaccine dose. Such strategies are already utilized among PLWH, *e.g.*, in Hepatitis B vaccination.[30]

Although this is the first controlled study analysing COVID-19 inactivated vaccine-induced immunogenicity among PLHIV, data on clinical efficacy and real-life effectiveness studies are still lacking for this population, with limited data so far from big vaccine developers. More than 38 million people are estimated to be living with HIV worldwide, with almost 1 million cases living in Brazil. With such an overlay of these two pandemics, it is essential to reinforce strategies to mitigate the damage caused by the SARS-CoV-2 pandemic in the already vulnerable HIV population.

Acknowledgements: We thank the study participants for their voluntary contribution. This study was sponsored by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (no. 2015/03756–4 to N.E.A., S.G.P., C.A.S.

and E.B.; no. 2017/14352-7 to T.P.) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico (no. 305242/2019-9 to E.B.). Instituto Butantan supplied the study product and had no other role in the trial.

Author contributions:
EB, ACMR and EGK conceptualized the study. KYI, CMP, APPSA, EVA, MRS, PSSP, and ANL contributed with data collection and follow-up visits for PLWH. NEA, ACMR, SGP, EFNY, CGSS, TP, and CC contributed with data collection and follow-up visits for controls. VIAS performed statistical analysis. LCN, VIAS, EGK and EB wrote the manuscript. VIAS and LCN verified the underlying data. All author revised and approved the final version of the manuscript. All authors had full access to all the data in the study and accept responsibility to submit for publication.

Declaration of interests: EGK is the Principal Investigator for the CoronaVac phase 3 clinical trial at University of Sao Paulo. VIAS is the Principal Investigator for the Janssen COVID-19 vaccine phase 3 clinical trial at University of Sao Paulo.

Data sharing statement: De-identified, individual participant data, a data dictionary defining each field in the dataset, study protocol and statistical analysis plan will be made available to others after the publication of this manuscript, following approval of a proposal. Proposals should be directed to esper.kallas@usp.br; to gain access, data requestors will need to sign a data access agreement.

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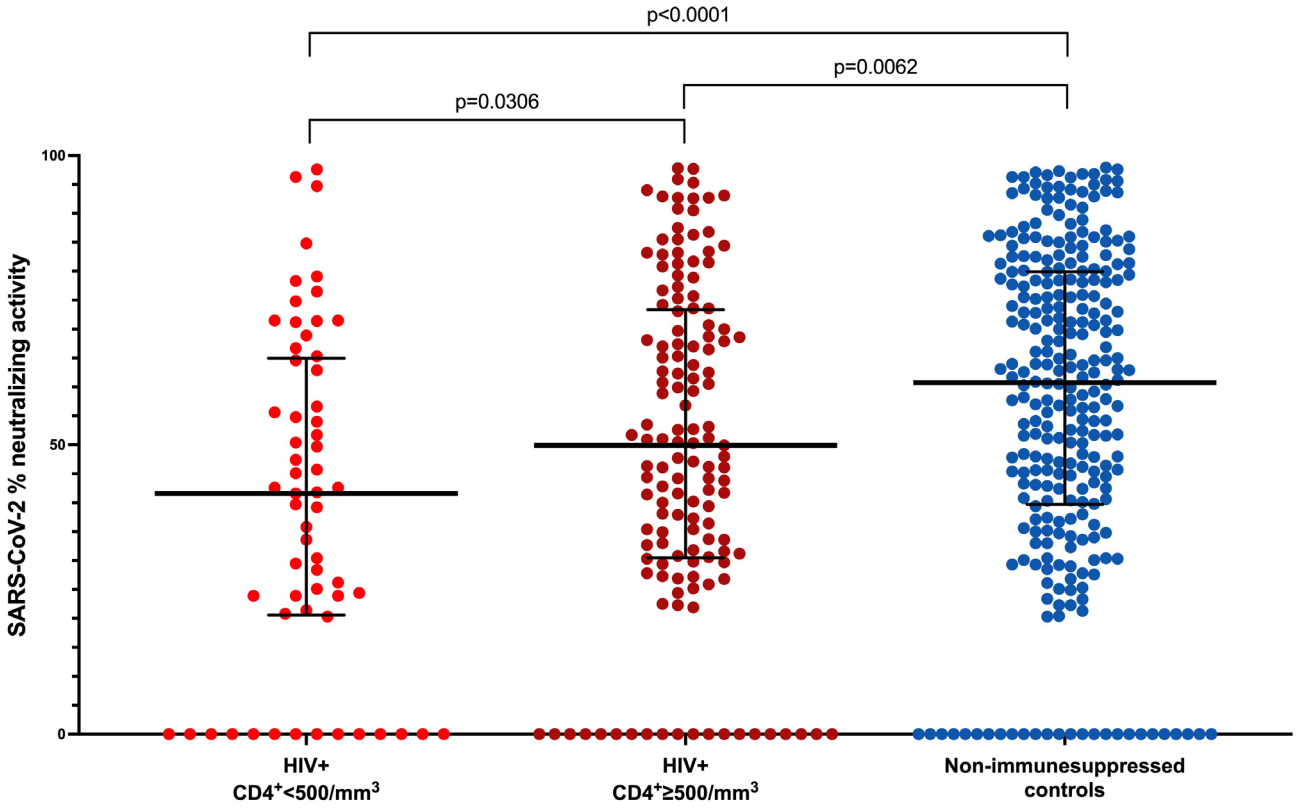
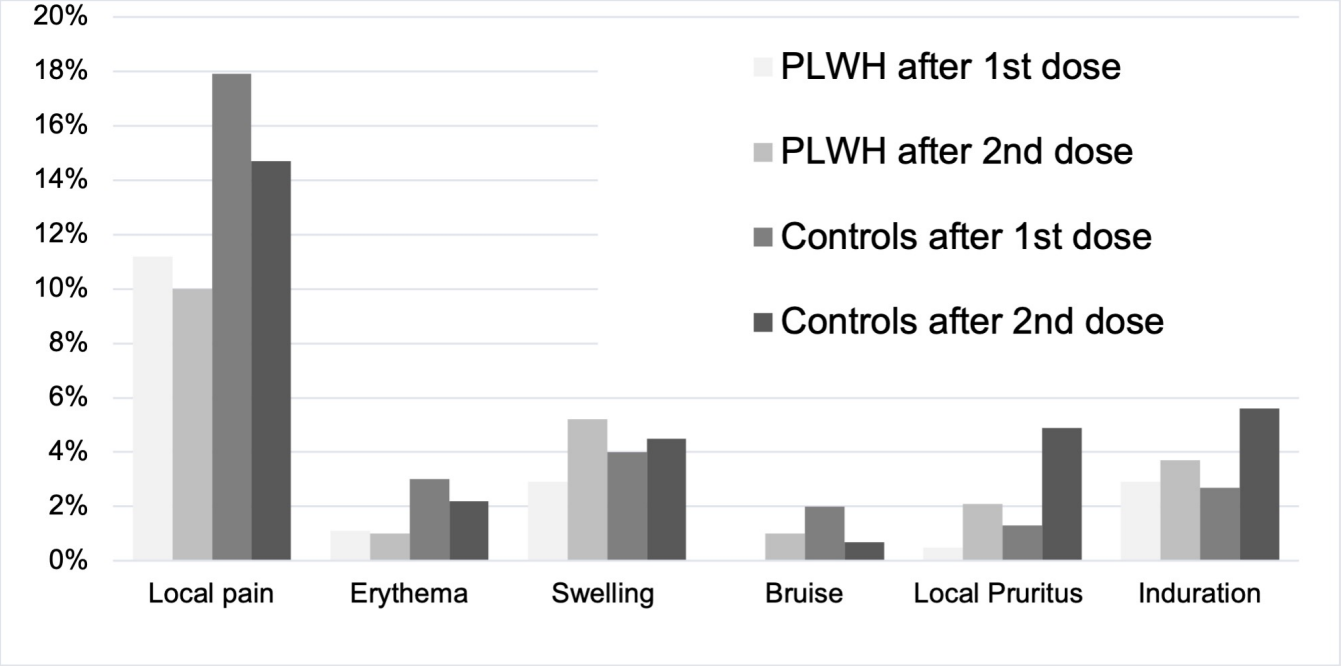

p<0.0001
p=0.0062
p=0.0306
SARS-CoV-2 % neutralizing activity
100
50
0
HIV+
CD4+<500/mm3
HIV+
CD4+≥500/mm3
Non-immunesuppressed
controls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PLWH after 1st dose
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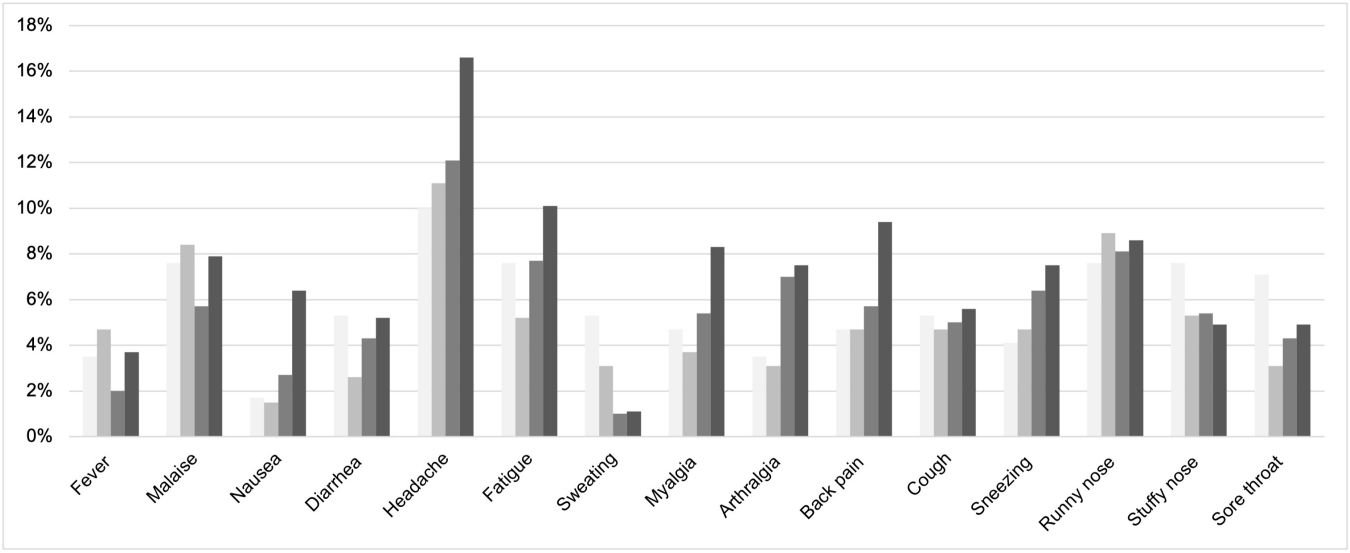
Controls after 2nd dose
Local pain
Erythema
Swelling
Bruise
Local Pruritus
Induration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Fever
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Nausea
Diarrhea
Headache
Fatigue
Sweating
Myalgia
Arthralgia
Back pain
Cough
Sneezing
Runny nose
Stuffy nose
Sore throat

Comparing immune responses to inactivated vaccines against SARS-CoV-2 between people living with HIV and HIV-negative individuals: a cross-sectional study in China

Xiaojie Huang[1*], Ph.D.; Ying Yan[2*], Ph.D.; Bin Su[1*], Ph.D.; Dong Xiao[3*], Maohe Yu[4], B.A.; Xia Jin[5], M. S.; Junyi Duan[1], B.A.; Xiangjun Zhang[6], Ph.D.; Shimin Zheng[7], Ph.D.; Yuan Fang[8], Ph.D.; Weiming Tang[9#], Ph.D.; Lunan Wang[2#], Ph.D.; Tong Zhang[1#], Ph.D.; Zixin Wang[10#], Ph.D.; Junjie Xu[11#], Ph.D.

Affiliations:

[1] Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, 100069, China.

[2] National Center for Clinical Laboratories, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing Hospital/National Center of Gerontology, 100044, China.

[3] Rainbow clinic of Beijing Jingcheng Skin Hospital, Beijing, 100101, China.

[4] Department of AIDS/STD Control and Prevention, Tianjin Centers for Disease Control and Prevention, Tianjin, 300011, China.

[5] AIDS Healthcare Foundation (AHF), Beijing, 100088, China.

[6] Department of Public Health, The University of Tennessee, Knoxville, Tennessee, 37996, USA.

[7] Department of Biostatistics and Epidemiology, East Tennessee State University, Johnson City, Tennessee, 70300, USA.

[8] Department of Early Childhood Education, The Education University of Hong Kong, Hong Kong, 200092, China.

[9] University of North Carolina Project-China, Guangzhou, Guangdong, 510095, China.

[10] JC School of Public Health and Primary Care, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, 666888, China.

[11] Clinical Research Academy, Peking University Shenzhen Hospital, Peking University, Shenzhen, 518036 China.

*, Co-first authors.

#, equal contribution as corresponding authors.

Corresponding author: Weiming Tang, Dermatology Hospital of Southern Medical University, Guangzhou, China; University of North Carolina Project-China, Guangzhou 510095, China. Email: Weiming_tang@med.unc.edu or;

Lunan Wang, National Center for Clinical Laboratories, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing Hospital/National Center of Gerontology, 100044, China. Email: lnwang@nccl.org.cn;

Tong Zhang, Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, 100069, China. Email: zt_doc@ccmu.edu.cn;

Zixin Wang, JC School of Public Health and Primary Care Faculty of Medicine, The Chinese University of Hong Kong, Room 508, School of Public Health, Prince of Wales Hospital, Shatin, NT, Hong Kong, 666888, China. Email: wangzx@cuhk.edu.hk;

Junjie Xu, Clinical Research Academy, Peking University Shenzhen Hospital, Peking University, Shenzhen, 518036 China. Email:xjjbeijing@gmail.com;

Running head: Comparing immune responses to inactivated vaccines against SARS-CoV-2 between people living with HIV and HIV-negative individuals.

Conflict of Interest Disclosures: The authors have no conflicts of interest to disclose.

Funding/Support: This work was supported by the Beijing Excellent Talent Plan (2018000021223ZK04) , Beijing Talent Project in the New Millennium (2020A35), and the National Institute of Mental Health of the National Institutes of Health under Award (R34MH119963).

Summary

Background There are concerns about the efficacy and safety of SARS-Cov-2 vaccines among People living with HIV (PLWH). We compared immunogenicity and safety of the inactivated SARS-CoV-2 vaccines (Sinopharm and Sinovac CoronaVac) between PLWH and HIV-negative individuals.

Methods PLWH and HIV-negative individuals aged 18-59 years who had received at least one dose of inactivated SARS-CoV-2 vaccine were recruited in two Chinese cities between April and June 2021. Participants completed a self-administered questionnaire collecting adverse events and background charactersitics. Venous blood samples were collected and tested for neutralizing antibody responses against authentic SARS-CoV-2, the total antibody specific to SARS-CoV-2, SARS-CoV-2 IgG antibody against the receptor-binding domain of the spike protein (S-IgG), and antigen-specific T-cell immune response level.

Findings A total of 129 PLWH and 53 HIV-negative individuals completed this study. Prevalence (P=0·19) and severity (P=0·13-0·77) of adverse events were similar among PLWH and HIV-negative individuals. The prevalence of seropositivity of neutralizing antibody, total antibody and S-IgG was 71·3%, 81·9% and 92·5% among fully vaccinated PLWH, which is similar to fully vaccinated HIV-negative individuals (P=0·07-0·48). Among all participants, PLWH had significantly lower neutralizing antibody, total antibody, S-IgG, and T-cell specific immune response levels compared to HIV-negative individuals, after controlling for types of vaccine, time interval between prime and second dose, time after receiving the second dose, and sociodemographics. PLWH who had a longer time since HIV diagnosis, completed the second dose for 15-28 days, and an interval between prime and second dose of ≥21 days had higher neutralizing antibody levels.

Intrepretation Inactivated SARS-CoV-2 vaccines are safe for PLWH. Fully vaccinated PLWH could achieve similarly high protection as HIV-negative individuals. Vaccination guidelines for PLWH should be developed.

Funding Beijing Excellent Talent Plan, Beijing Talent Project in the New Millennium, the National Institute of Mental Health of the National Institutes of Health under Award.

Keywords: People living with HIV; Inactivated SARS-CoV-2 vaccines; self-reported adverse events; neutralizing antibody responses against authentic SARS-CoV-2; total antibody specific to SARS-CoV-2; SARS-CoV-2 IgG antibody; antigen-specific T-cell immune response.

Introduction

Globally, about 38 million people are living with HIV [1]. Antiretroviral therapy (ART) could suppress viral replication, restore $CD4^+$ T-cell counts, rebuild immune function, and decrease morbidity and mortality among people living with HIV (PLWH) [2,3]. However, $CD4^+$ T-cell recovery is incomplete despite viral suppression in some PLWH [4]. The World Health Organization (WHO) confirmed that HIV infection is a significant independent risk factor for both severe SARS-CoV-2 cases at hospital admission and in-hospital mortality [5]. Both international health authorities and Chinese national guidelines recommend SARS-CoV-2 vaccination to PLWH regardless of their immune status [6-8].

PLWH is considered a priority group for vaccination in many countries [8]. However, there are concerns that PLWH might have a suboptimal response to SARS-CoV-2 vaccination. More imporntantly, less than 3% of the participants in the reported SARS-CoV-2 vaccine efficacy trials are PLWH, and the data for vaccine safety and immune response is insufficient [9-13]. The Novarax study showed the overall vaccine efficacy was higher when excluding PLWH from the analysis (increased from 49·4% to 60%) [13]. Most studies did not report vaccine efficacy specific for PLWH. Some studies have compared the safety and immunogenicity of mRNA (Pfizer BNT162b2 and Moderna mRNA-1273) or adenovirus vector (Oxford/AstraZeneca AZD1222) SARS-CoV-2 vaccines between HIV-negative individuals and PLWH with viral suppression and high $CD4^+$ T-cell levels (median around 700) [14-18]. These studies showed that SARS-CoV-2 vaccines were safe for PLWH, and there was no between-group difference in adverse events [14-18].

There are two inactivated SARS-CoV-2 vaccines manufactured by Chinese companies are approved for emergency use by the WHO (Sinopharm and Sinovac CoronaVac) [19,20]. More than three billion doses of these vaccines has been supplied to more than 40 countries [21]. No study compared PLWH and HIV-negative individuals regarding immunogenicity and safety of the inactivated SARS-CoV-2 vaccines. Such evidence is important to address COVID-19 vaccine hesitancy among PLWH or to implement boost dose for this group [22]. Previous findings on mRNA/adenovirus vector vaccines might not be applicable to PLWH receiving inactivated SARS-CoV-2 vaccines [14-18]. Moreover, it is unclear whether PLWH with lower $CD4^+$ T cell counts and detectable HIV viral load would have similar immunogenicity as HIV-negative individuals, as these PLWH were excluded by the aforementioned studies [14-18]. Furthermore, given the relatively short follow-up period in previous studies, there is no

consensus about the long-term immunogenicity to SARS-CoV-2 vaccines among PLWH [14-18].

This study aims to address these knowledge gaps by comparing the immunogenicity and adverse events between PLWH and HIV-negative individuals after vaccination. This study also investigated factors correlated with levels of neutralizing antibody responses against authentic SARS-CoV-2, the total antibody specific to SARS-CoV-2, SARS-CoV-2 IgG antibody against the receptor-binding domain (RBD) of the spike protein (S-IgG), and antigen-specific T-cell immune response among PLWH.

Methods

Study design

This cross-sectional study was conducted in two Chinese metropolitan cities (Beijing and Tianjin) conducted between April and June 2021. Participants included PLWH and HIV-negative individuals who have received at least one dose of inactivated SARS-Cov-2 vaccine.

Participants

The inclusion criteria for PLWH included: 1) aged 18-59 years, 2) willing to participate in the study activities, including survey and blood sample collection, and relevant laboratory testing, 3) having received at least one dose of inactivated SARS-CoV-2 vaccine (Sinovac CoronaVac or Sinopharm), and 4) having received HIV diagnosis confirmed by HIV-1/2 western blot assay. Exclusion criteria included: 1) presence of severe hearing loss, impaired vision, or intellectual disability observed by the interviewers, and 2) history of SARS-CoV-2 infection, major psychiatric illness (schizophrenia and bipolar disorder) or neurocognitive impairment based on clinician's assessment of their medical records. HIV-negative individuals shared the first three inclusion criteria and both exclusion criteria with PLWH. HIV serostatus was confirmed by Abbott ARCHITECT HIV Ag/Ab Combo assay.

Recruitment and data collection

Recruitment for PLWH was facilitated by two community-based organizations (CBOs), one in each city. These two CBOs have provided services to PLWH and HIV high-risk populations and worked closely with HIV clinical service providers. WeChat is the most commonly used social media application for the CBOs to communicate with PLWH clients. CBO staff posted the study recruitment information in the WeChat public accounts of their organizations. Interested PLWH contacted CBO staff through private WeChat messages, phone calls, and messages via other instant messaging applications. CBO staff screened

participants' eligibility, briefed them about the study purpose and procedures, assured them that identifiable information would be kept confidential, and refusal to participate would have no consequences. The recruitment of HIV-negative individuals was conducted in community hospitals. The hospital staff approached vaccinated individuals in their service records by telephone and invited them to participate.

PLWH and HIV-negative individuals interested in joining the study were invited to visit one of two clinics, one in each city. On-site, project staff obtained their written informed consent. All participants completed a 10-minute self-administered questionnaire on site. The STROBE checklist was adhered (see Appendix).

Blood sample collection and laboratory procedures

After completion of the survey, trained nurses collected two lithium heparin anticoagulated vacuum blood collection tubes (BD) of whole blood (10 ml), two EDTA anticoagulated vacuum blood collection tubes (BD) of whole blood (10 ml), and one SST blood collection tube of whole blood (5ml). One tube of lithium heparin salt anticoagulated whole blood and one tube of EDTA anticoagulated whole blood were placed at room temperature. They were assayed for T cell-specific immune response within 8 hours and $CD4^+$ T-cell count within 48 hours, respectively. The other three tubes of whole blood were centrifuged at 1300 relative centrifugal force (RCF) for 10 minutes, and the upper plasma/serum layers were transferred into lyophilized tubes of no less than 1·2 ml each, and were stored at -20°C for the detection of SARS-Cov-2 combined antibody and neutralizing antibody, as well as HIV viral load.

SARS-CoV-2 neutralizing antibody measurement. The neutralizing antibodies to authentic SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank number MT407649.1) were quantified using a micro cytopathogenic effect (CPE) inhibition assay with a minimum four-fold dilution as reported before [23]. The positive geometric mean titer (GMT) of the neutralizing antibodies to authentic SARS-CoV-2 was 8.

SARS-CoV-2 antigen/antibody combined testing. All samples were tested for total antibody and SARS-CoV-2 specific S-IgG antibodies using Chemiluminescence assay (CLIA) kits (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). The positive cut-off for the abovementioned tests was 1·0.

T-cell specific immune response. The T cell specific immune response was tested using the IFN-γ release assay (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.). Briefly, 1·5 ml of heparin blood was distributed into test tube containing specific SARS-CoV-2 S antigen (T tube), negative control tube (N tube), and positive control tube (P tube) within 8 hours.

The tubes were inverted and mixed 5 times, incubated in 37℃ for 20-24 hours. Then the plasma was collected after centrifuging at 3000 RCF for 10 minutes and detected for IFN-γ level. Level of T tube minus N tube, a value greater than 30 pg/ml was considered positive.

HIV viral load detection Viral load of PLWH was tested using HIV quantitative assay (Zhuhai Livzon Diagnostics Inc.). The limit of quantitation (LOQ) of this assay was 60 copies/ml.

$CD4^+$ cell count measurement. The assay was performed using flow cytometry testing methods (BD Biosciences, San Jose, CA, USA) in accordance with the China National Guideline for Detection of HIV/AIDS (version 2020) [24].

Background characteristics of the participants. All participants reported age, gender, and presence of chronic conditions. Characteristics related to HIV infection and SARS-CoV-2 vaccination were extracted from medical records.

Adverse events related to SARS-CoV-2 vaccination. A checklist was used to assess local adverse events (pain, redness, itch, swelling, induration, and skin rash in the arm where the shot was given) and systematic adverse events (fatigue, malaise, headache, dizziness, lethargy, joint pain or muscle ache, feverish, nausea, vomit, diarrhea, and others) within one month after receiving SARS-CoV-2 vaccines. Participants rated the severity the aforementioned adverse events (1=very mild, 2=mild, 3=moderate, 4=severe, and 5=very severe).

Sample size planning

Previous studies showed that the positive rate for SARS-CoV-2 neutralizing antibody was about 90% among HIV-negative individuals who received inactivated SARS-CoV-2 vaccines [23]. There was no data on seropositivity for SARS-CoV-2 neutralizing antibody among PLWH who received inactivated vaccines. Previous studies showed that the seroconversion rate of PLWH after inoculation of the hepatitis B vaccine ranged from 34% to 88% [25]. Therefore, we assumed 70% of vaccinated PLWH would be positive for SARS-CoV-2 neutralizing antibody. Using an allocation ratio of 2:1, a total of 102 PLWH and 51 HIV-negative individuals was required to detect a minimum between-group difference of 20% (90% versus 70%) in SARS-CoV-2 neutralizing antibody positive rate (α= 0·05, β= 0·10).

Statistical analysis

Chi-square tests were used to inspect the difference in background characteristics and adverse events related to SARS-CoV-2 vaccination between PLWH and HIV-negative individuals. Between-group differences in immunogenicity indicator levels (total antibody, neutralizing

antibody, S-IgG, and T-cell specific immune response) were tested using Mann-Whitney tests. We log transformed the immunogenicity indicator levels using the base of 10 to normalize the data. Multivariable linear regression models were performed to test the between-group difference in these indicators, after controlling for all background characteristics with $P<0{\cdot}05$ in between-group comparisons. Adjusted coefficients (B) were obtained. Moreover, same comparisons were performed between different subgroups of PLWH and HIV-negative individuals. Similar analyses on sero-positivity for these immunogenicity indicators was also performed. Among PLWH, linear regression models were used to inspect factors that were correlated with immunogenicity indicator levels. SPSS version 26.0 was used in all analyses, with two-tailed $P<0{\cdot}05$ was considered statistically significant.

Ethics approval

Written informed consent was obtained from all participants before their study participation in accordance with the Declaration of Helsinki. The Institutional Review Boards of Changzhi Medical College (RT2021002) and Beijing Youan Hospital Research Ethics Committee (No. 2021-031) approved this study.

Role of funding sources

This study was supported by the Beijing Excellent Talent Plan (2018000021223ZK04), the Beijing Talent Project in the New Millennium (2020A35), the National Natural Science Foundation of China (81772165 and 81974303 to B.S.), the National 13th Five-Year Grand Program on Key Infectious Disease Control (2017ZX10202102-005-003 to B.S.), and the China Primary Health Care Foundation-Youan Medical Development Fund (BJYAYY-2020PY-01 to B.S.), and the Beijing Key Laboratory for HIV/AIDS Research (BZ0089). Funders had no role in the design, data collection, analysis, interpretation of the study, or the preparation of the manuscript.

Results

Profiles of the participants

A total of 519 and 316 PLWH in Beijing and Tianjin were approached, 130 and 24 were screened to be eligible, and 110 (84·6%) and 19 (79%) completed the study. At the same period, 61 vaccinated HIV-negative individuals were approached, 8 (13·1%) refused to participate mainly due to logistic reasons, and 53 (86·9%) completed the study procedures.

Most PLWH received HIV diagnoses for more than one year (86%), and were on ART (97·7%). Over half of them had an undetectable viral load (58·1%), and the median $CD4^+$ T-cell count was 630·5 (IQR: 499·5, 848·8) (Table 1).

As compared to HIV-negative individuals, fewer PLWH were 50-59 years old (3·9% versus 17·0%, P=0·01) and female (0·8% versus 24·5%, P<0·001). More PLWH had chronic conditions (20·9% versus 0%, P<0·001), received Sinovac-CoronaVac (55·0% versus 30·2%, P<0·001) and only completed the prime dose (27·1% versus 3·8%, P<0·001). Receiving more than one type of vaccine was not observed. Among those who completed both doses, the time interval between the prime and second dose was shorter among PLWH than HIV-negative individuals were (median: 21 versus 27 days, P<0·001) (Table 1). These background characteristics were controlled when comparing immunogenicity indicators levels between PLWH and HIV-negative individuals.

SARS-CoV-2 vaccination adverse events

Among the participants, 45·0% of PLWH and 54·7% of HIV-negative individuals reported presence of any specific local and systematic adverse events. After controlling for significant background characteristics (i.e., age group, gender, presence of chronic conditions other than HIV, types of vaccine, time interval between prime and second dose, and time after receiving the second dose), there is no between-group difference in prevalence of any adverse events (AOR: 0·77, 95%CI: 0·31, 1·95, P=0·19). Most of the reported adverse events were very mild/mild (41-100% among PLWH and 62·2-100% among HIV-negative individuals). There was no between-group difference in the severity of these adverse events (P=0·13-0·77). (Table 2)

Subgroup analysis showed that PLWH did not have a higher prevalence of any adverse events when comparing with HIV-negative individuals, regardless of $CD4^+$ T-cell counts or HIV viral suppression status (Appendix 1).

Immunogenicity indicators level

The prevalence of seropositivity of neutralizing antibody, total antibody and S-IgG was 71·3%, 81·9% and 92·5% among fully vaccinated PLWH. Such prevalence is similar to that observed among fully vaccinated HIV-negative individuals (P=0·07-0·48). (Appendix 2). When comparing to HIV-negative individuals, PLWH had significantly lower levels of neutralizing antibody (adjusted B: -0·18, P=0·049), total antibody (adjusted B: -0·80, P<0·001), S-IgG (adjusted B: -0·31, P=0·002), and T-cell specific immune response (adjusted B: -0·64, P=0·002). Subgroup analyses showed that PLWH with detectable viral load (adjusted B: -0·29, P=0·047) or $CD4^+$ T cell counts <500 (adjusted B: -0·29, P=0·02)

had significantly lower neutralizing antibody levels. Such difference in neutralizing antibody level was not observed when comparing HIV-negative individuals with PLWH with undetectable viral load or $CD4^+$ T cell counts ≥500. In addition, PLWH had significantly lower levels of total antibody, S-IgG, and T-cell specific immune response regardless of $CD4^+$ T cell counts or HIV viral suppression. Neutralizing antibody levels among fully vaccinated PLWH did not lower than fully vaccinated HIV-negative individuals (adjusted B: -0·15, *P*=0·13) (Table 3 & 4).

Factors associated with immunogenicity indicator levels among PLWH

A longer time since HIV diagnosis was associated with higher neutralizing antibody and total antibody levels (2-5 years: adjusted B: 0·71 & 0·27; reference: ≤1 year). As compared to partially vaccinated participants, PLWH who completed the second dose for 15-28 days had higher neutralizing antibody levels (adjusted B: 0·30), while those who completed it for 15-56 days had higher total antibody (adjusted B: 1·00), S-IgG (adjusted B: 0·53), and T-cell specific immune response levels (adjusted B: 0·89-0·99). Compared to PLWH with a time interval of <21 days between the prime and second dose, those with an interval of 21-28 days and >28 days had higher neutralizing antibody (adjusted B: 0·37 & 0·36), total antibody (adjusted B: 1·22 & 1·28), and S-IgG levels (adjusted B: 0·43 & 0·53) (Table 5).

Discussion

Understanding the differences of immunoresponse between HIV negative and positive individuals is essential in planning the SARS-CoV-2 vaccination for PLWH. We found the levels of adverse events are comparable between PLWH and HIV-negative individuals. The prevalence of seropositivity of neutralizing antibody, the total antibody, and S-IgG were similarly high among fully vaccinated PLWH and HIV-negative individuals. However, PLWH had lower immunogenicity indicator levels than HIV-negative individuals after controlling for types of vaccine, time since receiving the prime dose, time interval between prime and second dose, and socio-demographics. Our findings filled the knowledge gap on the immune responses to SARS-CoV-2 vaccines among PLWH. It contributed critical evidence to policymaking and vaccination program planning for countries that mainly using inactivated SARS-CoV-2 vaccines.

Similar to studies on mRNA/adenovirus vector SARS-CoV-2 vaccines [14-18], there was no between-group difference in prevalence (*P*=0·19) or severity (*P*=0·13-0·77) of self-reported

adverse events. Most of the reported adverse events were very mild/mild among PLWH (41-100%). Therefore, inactivated SARS-CoV-2 vaccines are safe for PLWH.

Four immunogenicity indicator levels were significantly lower among PLWH at 0-14 days after receiving the second dose. PLWH might take longer to develop humoral and cellular immune responses to inactivated SARS-CoV-2 vaccines. Previous case reports observed a prolonged course of antibody development among PLWH infected with SARS-CoV-2 [26]. Similar to HIV-negative individuals and PLWH who received other SARS-CoV-2 vaccines, the studied immunogenicity indicators peaked at 15-56 days after the second dose among PLWH [14-18]. However, the peak levels of these indicators were lower among PLWH, especially for total antibody and S-IgG. A faster decline in immune responses were also observed among PLWH. All four immunogenicity indicators levels declined >56 days after receiving the second dose among PLWH, while these indicators remained stable among HIV-negative individuals even 84 days after the second dose. This study observed significantly lower total antibody and S-IgG levels among PLWH >56 days after the second dose. B-cell dysfunction caused by HIV gp120 binds directly to primary B-cell, and impaired cellular immunity caused by $CD4^+$ T cell depletion among PLWH might explain slower development, lower peak levels, and faster decline of both humoral and cellular immune responses to SARS-CoV-2 vaccines [27,28]. Such findings indicated that PLWH might need a boost dose after the initial doses, and might need it earlier than HIV-negative individuals do. Future studies with large sample size are needed to investigate long-term changes in these immunogenicity indicators among PLWH.

Neutralizing antibody plays an important role in SARS-CoV-2 clearance and is a key indicator for protection after vaccination [29]. We found that the seropositivity and levels of neutralizing antibody was similarly high among fully vaccinated PLWH and HIV-negative individuals. It implied that both groups obtained good protection against SARS-Cov-2 after the vaccination and PLWH should complete both doses of vaccination as required. Subgroup analysis showed that in line with studies using mRNA and/or adenovirus vector SARS-CoV-2 vaccines, PLWH with higher $CD4^+$ T-cell counts or undetectable viral load did not had significantly lower neutralizing antibody level than HIV-negative individuals [14-18]. However, PLWH with lower $CD4^+$ T-cell counts (<500) or detectable viral load had lower neutralizing antibody level. Such findings added knowledge to immune responses to SARS-CoV-2 vaccines among PLWH with severer immunodeficiency. PLWH with severer immunodeficiency should be encouraged to receive SARS-CoV-2 vaccines. In contrast to findings on other types of vaccines, our study observed significant lower total antibody, S-

IgG, and T-cell specific immune responses levels among PLWH compared to HIV-negative individuals. The difference could not be fully explained by the larger proportion of PLWH with low CD4+ T-cell counts or detectable HIV viral load in this study. These indicators were lower among PLWH regardless of their $CD4^+$ T-cell counts or HIV viral load. Future studies should compare PLWH's immunogenicity to different types of SARS-CoV-2 vaccines in order to determine the optimal choice for PLWH.

Compared to newly diagnosed PLWH, those who had been diagnosed for 2-5 years had higher neutralizing antibody and total antibody levels. It is possible that these PLWH had better functioning immune system after years of ART. It also highlighted the needs to further increase HIV testing coverage among key population to early identify HIV infection and link them to treatment and care. It will hence improve the effectiveness of SARS-CoV-2 vaccination for PLWH. Moreover, our results also suggested that, PLWH had a longer interval between the prime and second dose (21-28 days or >28 days) had significantly higher neutralizing antibody, total antibody and S-IgG levels compared to those with a shorter interval. Existing guidelines of SARS-CoV-2 vaccination for PLWH did not mention the optimal vaccination interval. Our findings suggested that future SARS-CoV-2 vaccination program for PLWH should consider a longer interval between doses. More research is needed to determine an optimal interval between doses for PLWH.

The study has several strengths. First, all participants underwent humoral and cellular immune responses analysis in this study. Second, this study included a diverse sample of PLWH with different $CD4^+$ T cell level and HIV viral load. It filled the knowledge gaps about immunogenicity to SARS-CoV-2 vaccines among PLWH with impaired functional immune system and poorer control of HIV. Third, impact of between-group difference in background characteristics on immunogenicity might be limited in this study, as background characteristics were controlled during the comparison. Furthermore, this is also one the first studies that assessed relationships between characteristics of PLWH and immunogenicity to SARS-CoV-2 vaccines.

This study also has some limitations. First, this was a cross-sectional study. Possible changes in immunogenicity indicator levels over time were unclear. Such study design cannot establish causal relationship as well. Second, we did not use matching to sample HIV-negative individuals according to PLWH's characteristics. There are significant between-group differences in socio-demographics, presence of other chronic conditions, and vaccination characteristics. We controlled these characteristics when comparing the between-group difference in immunogenicity. Third, PLWH was over-represented by male. However,

the impact of gender difference on immunogenicity might be limited, as previous studies did not show difference in immunogenicity between male and female [23]. Moreover, the presence and severity of adverse events were self-reported by participants and might be subject to recall bias. We were not able to compare the safety data with other studies that used clinician assessments.

Inactivated SARS-CoV-2 vaccines are safe for PLWH. Fully vaccinated PLWH could achieve similarly high protection as HIV-negative individuals. PLWH had significantly lower neutralizing antibody, total antibody, S-IgG, and T-cell specific immune response levels than HIV-negative individuals did. The immunogenicity indicator levels peaked 15-56 days after PLWH receiving the second dose. A longer time since diagnosis and a longer interval between the prime and second dose were correlated with better immune responses among PLWH. Future studies should compare PLWH's immunogenicity to different types of vaccines, assess immune responses in a longer term, and investigate the optimal interval between doses.

Contributors

All authors contributed to the conception of this study. XJH, WMT, and JJX developed the methodology. DX, YY, XJ, JYD, MHY, LNW, and JJX were responsible for site survey and coordination. XJH, BS, TZ, YY, LNW, and JJX were responsible for the laboratory testing and test result interpletation. WMT, ZXW, XJZ, SMZ, YF, and JJX wrote the original draft. All authors contributing to the reviewing and editing process. All authors agreed to submit the manuscript for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The individual participant data used in this analysis are available upon request. Requests should ne directed to the corresponding author, and need to sign a data access and confidentiality agreement.

Acknowledgments

The authors gratefully acknowledge the participation from HIV-negative individuals and PLWH who contribute to this research in the mainland of China. We are also grateful to local communities for the onsite recruitment and coordination.

414 **Table 1 Background characteristics of HIV-negative individuals and People living with HIV (PLWH) who had received at least one**
415 **dose of SARS-CoV-2 vaccine**

	People living with HIV (n=129)	HIV-negative individuals (n=53)	*P* values
Socio-demographics			
Age (years), n (%)			
18-29	39 (30·2)	14 (26·4)	
30-39	65 (50·4)	19 (35·8)	
40-49	20 (15·5)	11 (20·8)	
50-59	5 (3·9)	9 (17·0)	0·01
Median (IQR), range	34 (28, 38) (20-58)	34 (29, 47) (22-56)	0·15
Gender, n (%)			
Male	128 (99·2)	40 (75·5)	
Female	1 (0·8)	13 (24·5)	<0·001
Presence of chronic conditions other than HIV/AIDS			
No	102 (79·1)	53 (100·0)	
Yes	27 (20·9)	0 (0·0)	<0·001
Characteristics related to HIV infection			
Time since HIV diagnosis (years)			
≤1	18 (14·0)	N.A	N.A.
2-5	55 (42·6)	N.A	N.A.
6-10	35 (27·1)	N.A	N.A.
>10	21 (16·3)	N.A	N.A.
Viral load (cp/ml), n (%)			
Undetectable (≤60)	75 (58·1)	N.A.	N.A.
61-200	33 (25·6)	N.A.	N.A.
>200	21 (16·3)	N.A.	N.A.
CD4+ T cell count (cells/μL)			
<500	32 (24·8)	N.A.	N.A.
500-1,000	81 (62·8)	N.A.	N.A.
>1,000	16 (12·4)	N.A.	N.A.
Median (IQR), range	630·5 (499·5, 848·8) (78, 2650·35)	N.A.	N.A.
ART regimens			
TDF+3TC+EFV	60 (52·7)	N.A.	N.A.
TDF+3TC+LPV/r	5 (3·9)	N.A.	N.A.
AZT+3TC+LPV/r	3 (2·3)	N.A.	N.A.
AZT+3TC+NVP	2 (1·6)	N.A.	N.A.
AZT+3TC+EFV	8 (6·2)	N.A.	N.A.
Others	40 (31·0)	N.A.	N.A.
Not on ART	3 (2·3)	N.A.	N.A.
Information related to SARS-CoV-2 vaccination			

SARS-CoV-2 vaccination status			
Partially vaccinated	35 (27·1)	2 (3·8)	
0-14 days after fully vaccinated	15 (11·6)	8 (15·1)	
15-28 days after fully vaccinated	38 (29·5)	13 (25·5)	
29-56 days after fully vaccinated	26 (20·2)	21 (39·6)	
57-84 days after fully vaccinated	12 (9·3)	3 (5·7)	
>84 days after fully vaccinated	3 (2·3)	8 (15·1)	<0·001
Type of SARS-CoV-2 vaccine			
Sinopharm	58 (45·0)	37 (69·8)	
Sinovac-CoronaVac	71 (55·0)	16 (30·2)	<0·001
Time interval between the prime (1st) and second dose (among those who were fully vaccinated)	n=94	n=51	
<21 days	20 (21·3)	3 (5·7)	
21-28 days	58 (61·7)	40 (75·5)	
>28 days	16 (17·0)	10 (18·9)	0·043
Median (IQR), range	21 (21, 27) (14-59)	27 (21, 28) (14-83)	0·002

N.A.: not applicable.

418 **Table 2 Comparing self-reported local and systematic adverse events related to SARS-CoV-2 vaccination among People living with**
419 **HIV (PLWH) and HIV-negative individuals**

	People living with HIV (n=129)	HIV-negative individuals (n=53)	*P* values
	n (%)	n (%)	
Local adverse events			
Pain			
None	87 (67·4)	31 (58·5)	
Very mild	15 (11·6)	4 (7·5)	
Mild	16 (12·4)	11 (20·8)	
Moderate	11 (8·5)	7 (13·2)	
Severe	0 (0·0)	0 (0·0)	0·30
Any of above	42 (32·6)	22 (41·5)	0·25
Redness, itch, swelling, induration and/or skin rash			
None	124 (96·1)	50 (94·3)	
Very mild	0 (0·0)	1 (1·9)	
Mild	2 (1·6)	2 (3·8)	
Moderate	3 (2·3)	0 (0·0)	
Severe	0 (0·0)	0 (0·0)	0·21
Any of above	5 (3·9)	3 (5·7)	0·59
Systematic adverse events			
Fatigue, malaise, headache, dizziness, and/or lethargy			
None	107 (82·9)	43 (81·1)	
Very mild	5 (3·9)	3 (5·7)	
Mild	11 (8·5)	4 (7·5)	
Moderate	5 (3·9)	2 (3·8)	
Severe	1 (0·8)	1 (1·9)	0·94
Any of above	22 (17·1)	10 (18·9)	0·77
Joint pain and/or muscle ache			
None	119 (92·2)	45 (84·9)	
Very mild	4 (3·1)	1 (1·9)	
Mild	3 (2·3)	4 (7·5)	
Moderate	3 (2·3)	3 (5·7)	
Severe	0 (0·0)	0 (0·0)	0·23
Any of above	10 (7·8)	8 (15·1)	0·13
Fever			
None	122 (94·6)	52 (98·1)	
Very mild	2 (1·6)	0 (0·0)	
Mild	4 (3·1)	1 (1·9)	
Moderate	1 (0·8)	0 (0·0)	
Severe	0 (0·0)	0 (0·0)	0·69
Any of above	7 (5·4)	1 (1·9)	0·27
Nausea, vomit, and/or diarrhea			

None	129 (100·0)	52 (98·1)	
Very mild	0 (0·0)	0 (0·0)	
Mild	0 (0·0)	1 (1·9)	
Moderate	0 (0·0)	0 (0·0)	
Severe	0 (0·0)	0 (0·0)	0·12
Any of above	0 (0·0)	1 (1·9)	0·29
Other systematic side-effects			
None	127 (98·4)	53 (100·0)	
Very mild	2 (1·6)	0 (0·0)	
Mild	0 (0·0)	0 (0·0)	
Moderate	0 (0·0)	0 (0·0)	
Severe	0 (0·0)	0 (0·0)	0·36
Any of above	2 (1·6)	0 (0·0)	0·50
Any local and/or systematic adverse events	58 (45·0)	29 (54·7)	0·23

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Table 3 Levels of SARS-CoV-2 neutralizing antibody, total antibody, S-IgG, and T cell specific immune response among HIV-negative individuals and people living with HIV (PLWH) who had received at least one dose of SARS-CoV-2 vaccine

	Neutralizing antibody			Total antibody			S-IgG			T cell specific immune response		
	PLWH	HIV-negative	*P*	PLWH	HIV-negative	*P*	PLWH	HIV-negative	*P*	PLWH	HIV-negative	*P*
	GMT (95%CI)	GMT (95%CI)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Partially vaccinated	4·6 (4·0, 9·8)	5·6 (N.A.)	0·43	0·2 (0·02, 1·1)	2·1 (N.A.)	0·20	0·6 (0·3, 1·5)	3·99 (N.A.)	0·03	6·4 (0·2, 26·7)	36·2 (N.A.)	0·16
0-14 days after fully vaccinated	8·5 (4·0, 64·6)	31·6 (4·0, 257·0)	0·03	0·8 (0·03, 16·8)	104·8 (7·4, 279·5)	0·01	3·1 (1·1, 16·2)	11·9 (5·1, 55·5)	0·04	5·3 (0·1, 88·8)	413·6 (91·8, 575·5)	0·001
15-28 days after fully vaccinated	24·0 (4·0, 380·2)	23·4 (4·0, 64·0)	0·97	28·9 (7·4, 83·2)	40·3 (28·5, 71·6)	0·24	9·0 (4·6, 16·0)	13·9 (10·1, 32·0)	0·13	56·08 (19·6, 118·7)	91·54 (31·1, 227·4)	0·29
29-56 days after fully vaccinated	14·1 (4·0, 64·6)	20·9 (4·0, 190·5)	0·24	11·8 (5·7, 27·3)	42·7 (8·4, 74·9)	0·04	7·2 (4·5, 12·2)	9·6 (7·2, 21·9)	0·03	37·2 (6·4, 121·1)	63·6 (35·4, 182·1)	0·13
57-84 days after fully vaccinated	11·0 (4·0, 95·5)	26·3 (12·0, 64·0)	0·18	6·2 (0·5, 11·7)	33·4 (N.A.)	0·04	3·4 (1·4, 5·7)	10·5 (N.A.)	0·03	3·6 (0·1, 17·1)	205·5 (N.A.)	0·08
>84 days after fully vaccinated	6·3 (4·0, 8·0)	11·1 (4·0, 48·0)	0·50	3·0 (1·3, N.A.)	9·3 (4·0, 62·8)	0·15	3·8 (1·2, N.A.)	4·3 (2·9, 5·4)	0·31	18·3 (0·8, N.A.)	35·6 (13·5, 56·2)	0·41
Among all participants	11·0 (4·0, 95·5)	20·0 (4·0, 190·5)	0·001	5·6 (0·4, 25·2)	32·6 (8·4, 72·3)	<0·001	4·3 (1·2, 10·0)	9·6 (5·4, 18·9)	<0·001	18·7 (2·4, 77·9)	63·6 (36·0, 226·4)	<0·001
Among participants who were fully vaccinated	15·1 (4·0, 128·8)	20·9 (4·0, 190·5)	0·09	10·3 (2·3, 38·8)	33·4 (10·1, 73·0)	<0·001	6·8 (3·3, 12·1)	10·1 (6·5, 19·4)	0·007	30·6 (5·2, 103·2)	68·4 (36·1, 227·4)	0·001

P values were obtained by using Mann-Whitney tests.
N.A.: not applicable.

Table 4 Comparing immunogenicity indicator levels between different subgroups of people living with HIV (PLWH) and HIV-negative individuals

	Neutralizing antibody		Total antibody		S IgG		T cell specific immune response	
	Adjusted B (95%CI)	**P values**	**Adjusted B (95%CI)**	**P values**	**Adjusted B (95%CI)**	**P values**	**Adjusted B (95%CI)**	**P values**
Reference 1: HIV-negative individuals (n=53)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
PLWH (n=129)	-0·18 (-0·36, -0·001)	0·049	-0·80 (-1·15, -0·46)	<0·001	-0·31 (-0·51, -0·12)	0·002	-0·64 (-1·05, -0·23)	0·002
PLWH with CD4⁺ T cell counts<500 (n=32)	-0·29 (-0·58, -0·003)	0·047	-1·31 (-1·78, -0·84)	<0·001	-0·49 (-0·75, -0·22)	<0·001	-0·82 (-1·32, -0·32)	0·002
PLWH with CD4⁺ T cell counts≥500 (n=97)	-0·12 (-0·31, 0·07)	0·21	-0·65 (-1·01, -0·30)	<0·001	-0·26 (-0·47, -0·06)	0·01	-0·58 (-1·00, -0·17)	0·01
PLWH with detectable viral load (n=54)	-0·29 (-0·53, -0·05)	0·02	-1·15 (-1·62, -0·68)	<0·001	-0·50 (-0·77, -0·23)	<0·001	-0·75 (-1·26, -0·25)	0·004
PLWH with undetectable viral load (n=75)	-0·18 (-0·39, 0·03)	0·09	-0·71 (-1·06, -0·37)	<0·001	-0·26 (-0·45, -0·07)	0·008	-0·65 (-1·09, -0·22)	0·004
Reference 2: Fully vaccinated HIV-negative individuals (n=51)	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Fully vaccinated PLWH (n=94)	-0·15 (-0·35, 0·04)	0·13	-0·68 (-1·03, -0·33)	<0·001	-0·27 (-0·48, -0·07)	0·01	-0·61 (-1·00, -0·22)	0·002

Adjusted B: adjusted correlation coefficients, adjusted for background characteristics with significant between-group difference in Table 1 (age group, gender, presence of chronic conditions other than HIV, types of SARS-CoV-2 vaccine, time interval between prime and second dose, and SARS-CoV-2 vaccination status) .

Table 5 Factors associated with SARS-CoV-2 total antibody, neutralizing antibody, S-IgG, and T cell specific immune response levels among people living with HIV (PLWH) (n=129)

	Total antibody		Neutralizing antibody		S-IgG		T cell specific immune response	
	Unadjusted B (95%CI)	**Adjusted B (95%CI)**	**Unadjusted B (95%CI)**	**Adjusted B (95%CI)**	**Unadjusted B (95%CI)**	**Adjusted B (95%CI)**	**Unadjusted B (95%CI)**	**Adjusted B (95%CI)**
Socio-demographics								
Age (years)								
18-29	Ref		Ref		Ref		Ref	
30-39	-0·06		0·06		0·03		-0·07	
	(-0·57, 0·45)		(-0·14, 0·25)		(-0·26, 0·32)		(-0·51, 0·38)	
40-49	0·17		0·08		-0·09		-0·08	
	(-0·52, 0·86)		(-0·18, 0·35)		(-0·48, 0·31)		(-0·69, 0·53)	
50-59	-0·32		-0·03		0·03		-0·56	
	(-1·51, 0·87)	---	(-0·49, 0·43)	---	(-0·66, 0·71)	---	(-1·62, 0·49)	---
Gender								
Male	Ref		Ref		Ref		Ref	
Female	1·47		0·77		0·63		1·14	
	(-1·02, 3·97)	---	(-0·18, 1·73)	---	(-0·81, 2·06)	---	(-1·07, 3·34)	---
Presence of chronic conditions other than HIV/ADIS								
No	Ref		Ref		Ref		Ref	
Yes	0·12		-0·07		-0·01		-0·20	
	(-0·42, 0·66)	---	(-0·28, 0·14)	---	(-0·30, 0·32)	---	(-0·68, 0·28)	---
Characteristics related to HIV infection								
Years since HIV diagnosis (years)								
≤1	Ref	Ref	Ref	Ref	Ref		Ref	
2-5	0·55	0·71	0·21	0·27	0·21		0·46	
	(-0·12, 1·22)	(0·23, 1·19)**	(-0·05, 0·46)	(0·05, 0·48)*	(-0·18, 0·60)		(-0·14, 1·06)	
6-10	0·82	0·49	0·33	0·23	0·33		0·56	
	(0·10, 1·53)*	(-0·03, 1·00)†	(0·05, 0·60)*	(-0·01,0·46)†	(-0·09, 0·74)		(-0·08, 1·19)†	
>10	0·59	0·44	0·22	0·15	0·23		0·55	
	(-0·20, 1·38)	(-0·13, 1·05)	(-0·08, 0·53)	(-0·11, 0·20)	(-0·23, 0·69)	---	(-0·15, 1·26)	---
Viral load (cp/ml)								
Undetectable	Ref	Ref	Ref		Ref	Ref	Ref	Ref
61-200	-0·39	-0·24	-0·17		-0·33	-0·19	-0·15	-0·01
	(-0·89, 0·10)	(-0·61, 0·14)	(-0·36, 0·03)†		(-0·61, -0·05)*	(-0·40, 0·03)†	(-0·61, 0·30)	(-0·44, 0·42)
>200	-1·10)	-0·24	-0·23		-0·68	-0·24	-0·60	-0·27
	(-1·69, -0·51)***	(-0·69, 0·22)	(-0·47, 0·001)†	---	(-1·01, -0·34)***	(-0·50, 0·03)†	(-1·14, -0·06)*	(-0·78, 0·25)
CD4+ T cell count (cells/μL)								
<500	Ref		Ref		Ref		Ref	Ref
500-1,000	0·41		0·13		0·16		0·59	0·47
	(-0·10, 0·93)		(-0·07, 0·33)		(-0·14, 0·45)		(0·14, 1·04)*	(0·05, 0·89)*
>1,000	0·61		0·14		0·24		0·48	0·40
	(-0·15, 1·36)	---	(-0·15, 0·44)	---	(-0·20, 0·68)	---	(-0·18, 1·14)	(-0·22, 1·01)
On ART								
No	Ref		Ref		Ref		Ref	
Yes	0·47		0·12		0·13		0·34	
	(-0·99, 1·93)	---	(-0·44, 0·68)	---	(-0·71, 0·97)	---	(-0·95, 1·62)	---
SARS-CoV-2 vaccination								

SARS-CoV-2 vaccination status								
Partially vaccinated	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
0-14 days after fully vaccinated	0·68 (0·11, 1·25)*	N.A.	0·27 (0·02, 0·51)*	N.A.	0·49 (0·17, 0·80)**	N.A.	0·07 (-0·55, 0·69)	0·16 (-0·46, 0·78)
15-28 days after fully vaccinated	2·11 (1·68, 2·55)***	1·00 (0·43, 1·57)**	0·72 (0·54, 0·91)***	0·30 (0·04, 0·56)*	1·26 (1·02, 1·50)***	0·53 (0·20, 0·85)**	1·08 (0·61, 1·54)***	0·99 (0·50, 1·47)***
29-56 days after fully vaccinated	1·79 (1·32, 2·27)***	1·00 (0·43, 1·57)**	0·49 (0·28, 0·69)***	0·16 (-0·10, 0·41)	1·08 (0·82, 1·35)***	0·53 (0·20, 0·85)**	0·94 (0·42, 1·45)***	0·89 (0·37, 1·40)**
57-84 days after fully vaccinated	1·36 (0·74, 1·97)***	0·85 (0·15, 1·55)*	0·38 (0·12, 0·65)**	0·14 (-0·17, 0·45)	0·75 (0·40, 1·09)***	0·26 (-0·13, 0·65)	-0·07 (-0·74, 0·59)	-0·10 (-0·76, 0·57)
>84 days after fully vaccinated	1·31 (0·20, 2·41)*	0·29 (-0·83, 1·41)	0·15 (-0·33, 0·62)	-0·31 (-0·81, 0·19)	0·69 (0·07, 1·31)*	0·07 (-0·57, 0·70)	0·40 (-0·80, 1·60)	0·20 (-1·01, 1·41)
Type of SARS-CoV-2 vaccine								
Sinopharm	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
Sinovac-CoronaVac	0·71 (0·28, 1·13)**	0·26 (-0·07, 0·59)	0·23 (-0·07, 0·40)**	0·05 (-0·10, 0·20)	0·31 (0·06, 0·55)*	0·07 (-0·11, 0·25)	0·31 (-0·08, 0·69)	---
Time interval (days) between the prime and second dose								
<21 days	Ref	Ref	Ref	Ref	Ref	Ref	Ref	
21-28 days	1·22 (0·74, 1·69)***	0·93 (0·43, 1·43)***	0·42 (0·22, 0·64)***	0·37 (0·15, 0·59)**	0·67 (0·41, 0·94)***	0·43 (0·14, 0·71)**	0·52 (-0·02, 1·06)†	
>28 days	1·28 (0·67, 1·89)***	1·15 (0·53, 1·77)***	0·39 (0·12, 0·66)**	0·36 (0·09, 0·63)**	0·70 (0·35, 1·04)***	0·53 (0·18, 0·88)**	0·62 (-0·09, 1·32)†	
Not applicable (partially vaccinated)	-0·71 (-1·22, -0·19)**	-0·03 (0·63, 0·57)	-0·19 (-0·42, 0·03)†	-0·06 (-0·32, 0·21)	-0·47 (-0·76, -0·18)**	-0·21 (-0·54, 0·13)	-0·28 (-0·87, 0·31)	---

† *P*<0·10, * *P*<0·05, ** *P*<0·01.
Adjusted B: adjusted coefficients obtained from multivariate linear regression models using all significant variables as candidates.
---: P>0·05 in univariate analysis and was not considered in multivariate analysis.

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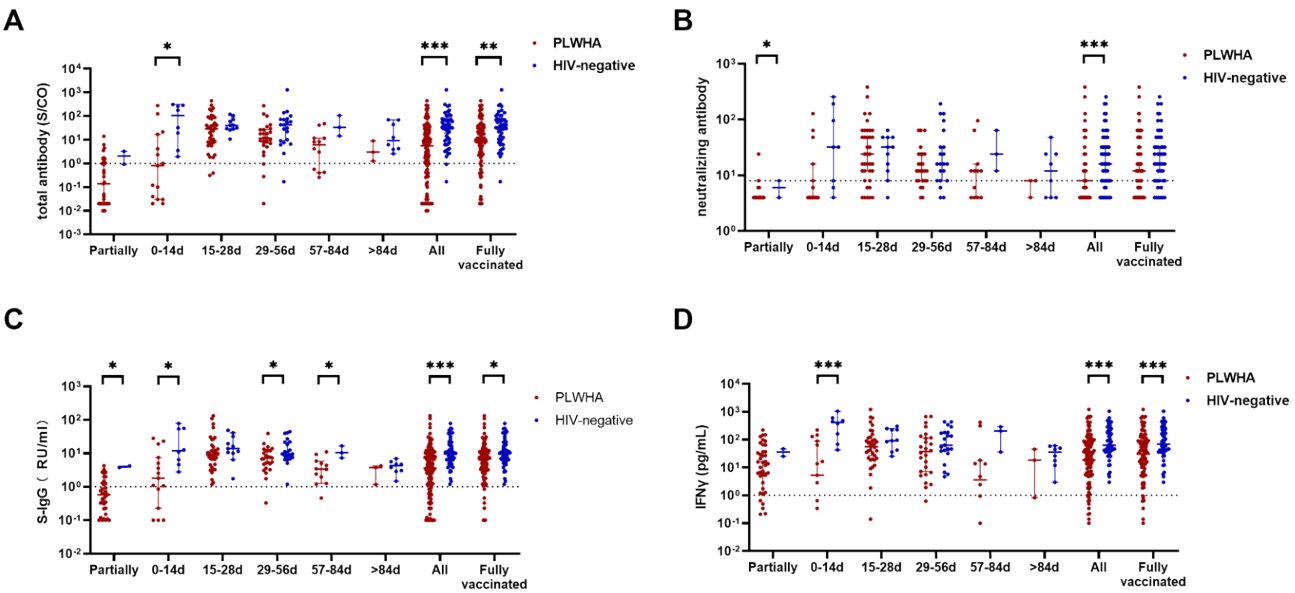

Figure 1. Levels of SARS-CoV-2 neutralizing antibody (A), total antibody (B), S-IgG (C) and T cell specific immune response (D) among HIV-negative individuals and People living with HIV (PLWH) who had received at least one dose of SARS-CoV-2 vaccine

4.2 CoronaVac produz anticorpos em 87% dos pacientes com hepatite B, mostra estudo chinês

A CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, gera alta proteção contra a Covid-19 em pacientes que convivem com a hepatite B sem causar reações adversas graves. A conclusão faz parte de um estudo publicado por pesquisadores chineses em artigo na revista Cellular & Molecular Immunology, do grupo Nature, no último dia 15. Segundo a pesquisa, após receber a segunda dose do imunizante os pacientes apresentaram uma taxa de soroconversão de 87,25% para anticorpos IgG, e de 74,5% para os anticorpos neutralizantes.

O trabalho Safety and immunogenicity of a SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B virus infection foi realizado por pesquisadores da Faculdade de Medicina da Universidade Huazhong de Ciência e Tecnologia, de Wuhan, na China, onde eclodiu a pandemia de Covid-19.

Participaram do estudo 284 pacientes com infecção crônica de hepatite B, sendo que 81 deles não haviam sido vacinados, 54 haviam tomado apenas a primeira dose da vacina, e 149 haviam completado o esquema vacinal de duas doses. Decorrido um mês após a primeira ou segunda dose, amostras de plasma foram coletadas e comparadas com as amostras dos não vacinados.

Enquanto a soropositividade para os anticorpos IgG e os anticorpos neutralizantes foi de 87,25% e 74,5%, respectivamente, os dados de reações adversas mostraram que quase todas foram leves, sendo que o sintoma mais comum foi dor no local da injeção seguida por sonolência. Apenas um paciente relatou febre no primeiro dia após a vacinação. Não foram observadas reações adversas graves mesmo nos 20 pacientes com casos mais sérios de infecção crônica de hepatite B (níveis anormais de alanina aminotransferase) ou nos dez pacientes com cirrose hepática.

Este é o primeiro estudo detalhado que analisa a segurança e imunogenicidade da CoronaVac em pacientes com infecção crônica de hepatite B. Estudos anteriores mostraram um risco aumentado de progressão para doença grave em pessoas com cirrose infectadas pelo vírus SARS-CoV-2.

Publicado em: 24/11/2021

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Safety and immunogenicity of a SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B virus infection

Tiandan Xiang[1,2,3], Boyun Liang[1,2,3], Hua Wang[1,2,3], Xufeng Quan[1,2], Shengsong He[1,2], Helong Zhou[1,2], Yongwen He[1,2], Dongliang Yang[1,2], Baoju Wang[1,2]✉ and Xin Zheng[1,2]✉

Cellular & Molecular Immunology; https://doi.org/10.1038/s41423-021-00795-5

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 infection, has become a major global public health threat. Although significant advances have been made in developing and applying different vaccines in clinical trials [1, 2], data are limited on the safety and efficacy of the inactivated vaccine in patients with chronic liver disease [3]. Recent studies have preliminarily described the safety and immunogenicity of SARS-CoV-2 vaccines in patients with nonalcoholic fatty liver disease and in liver transplant recipients [4, 5]. However, to date, there is no detailed information on the SARS-CoV-2 inactivated vaccine in patients with chronic hepatitis B (CHB) infection. It has been reported that CHB patients have impaired immune systems [6]. Hence, whether immunocompromised CHB patients within the different clinical stages can be safely vaccinated with the various types of SARS-CoV-2 vaccines and produce an effective immune response remains unclear. Our study aims to provide a comprehensive analysis from different clinical dimensions to characterize the safety and immunogenicity of SARS-CoV-2 inactivated vaccines (BBIBP-CorV, CoronaVac, or WIBP-CorV) within this specific patient population.

A total of 284 CHB patients who were unvaccinated ($n = 81$) or had completed the first ($n = 54$) or second dose ($n = 149$) of the vaccines were enrolled from March 23, 2021, to September 10, 2021 (Table S1). The median time post-vaccination was 33 (IQR, 24–48) days among the 149 completely vaccinated patients. Safety was evaluated by determined the overall incidence of adverse reactions via a standardized questionnaire. Moreover, plasma samples were examined for IgG antibodies against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (anti-S-RBD-IgG) and for neutralizing antibodies (NAbs). The complete methods regarding the study design and the statistical analysis are available in the Supplementary methods section.

The adverse reaction data were first analyzed in 149 completely vaccinated CHB patients. The overall incidence of adverse reactions within 7 days was 30.2% (Table S2), which was similar to that found in the phase 3 trials of CoronaVac in Turkey [2]. The most common side effect was injection-site pain (25.5%, 38/149), followed by drowsiness (3%, 3/149); only one patient reported fever on the first day after vaccination. Almost all of the adverse reactions were mild and self-resolved within a few days after vaccination. Serious side effects were not observed even in 20 CHB patients with abnormal alanine aminotransferase levels [61.5 (43–129) U/L] or 10 patients with compensated liver cirrhosis. The results demonstrated that SARS-CoV-2 inactivated vaccines had a favorable safety profile in CHB patients. Given that previous studies have shown an increased risk of progression to severe disease in COVID-19 patients with cirrhosis [7], the benefit of vaccination in compensated cirrhotic patients still outweighs the vaccine-related risk.

Next, we determined the immunogenicity of CHB patients who completed the two doses of the vaccination regimen. The seropositivity for anti-S-RBD-IgG and NAbs was 87.25% and 74.5%, respectively (Fig. 1A). The anti-S-RBD-IgG seropositivity of CHB vaccine recipients was similar to that in a clinical trial of CoronaVac in Turkey (89.7%) but much higher than the reported recently seropositivity of IgG antibodies to the spike protein (76%) in patients with chronic liver disease [5]. Both anti-S-RBD-IgG and NAb levels increased significantly to a higher level after completing the vaccination regimen (Fig. 1B, C, $P < 0.0001$). This finding indicates that SARS-CoV-2 inactivated vaccines can elicit an optimal antibody response even though some CHB patients may have pre-existing compromised immune function.

The seropositivity and antibody titers in CHB patients were further compared according to sex, age, antiviral therapy, and body mass index stratification (Fig. 1D, E). We found that younger patients (<40 y) had higher seropositivity for anti-S-RBD-IgG ($P < 0.05$), and female patients exhibited increased seropositivity for NAbs ($P < 0.05$). Recent clinical trials have also reported a similar trend: younger individuals and female vaccine recipients exhibited stronger humoral immune responses to vaccination [2]. Interestingly, the patients undergoing nucleos(t)ide analog therapy had a significantly higher NAb titer than those who were not ($P < 0.05$) (Fig. 1D, E). Long-term antiviral therapy can inhibit viral replication and facilitate the restoration of the impaired immune system by recovering the function of circulating dendritic cells, natural killer cells, or T cells, particularly nucleotide analogs that can induce the production of IFN-λ3 [6, 8]. These factors may account for the higher antibody titer in patients with antiviral therapy. Given that nucleos(t)ide analog therapy does not affect vaccine-induced immune responses, it should be continuously administered during vaccination to avoid negatively impacting CHB treatment.

[1]Department of Infectious Diseases, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. [2]Joint International Laboratory of Infection and Immunity, Huazhong University of Science and Technology, Wuhan, China. [3]These authors contributed equally: Tiandan Xiang, Boyun Liang, Hua Wang. ✉email: bjwang73@163.com; xin11@hotmail.com

Received: 22 September 2021 Accepted: 11 October 2021
Published online: 15 November 2021

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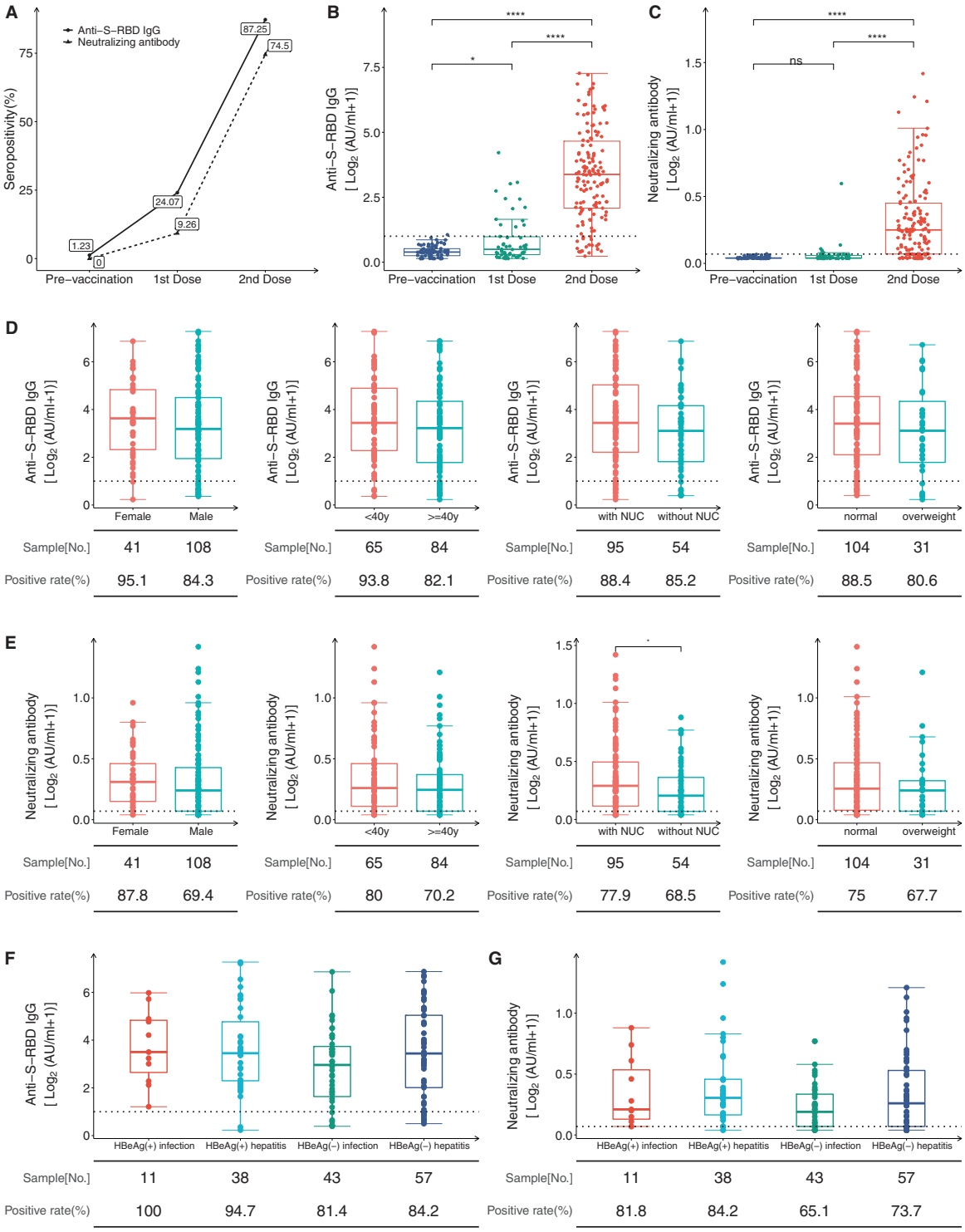

Fig. 1 Antibody responses following immunization with the inactivated vaccine in CHB patients. **A** The seropositivity of anti-S-RBD-IgG and NAbs in CHB patients. **B**, **C** Kinetics of the anti-S-RBD-IgG and NAb titers in vaccine-induced sera at different time points in CHB patients. Prevaccination, $n = 81$; 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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ACKNOWLEDGEMENTS

This study was supported by the Applied Basic and Frontier Technology Research Project of Wuhan (2020020601012233); the Science and Technology Key Project on Novel Coronavirus Pneumonia, Hubei Province (2020FCA002); the Fundamental Research Funds for the Central Universities (2020kfyXGYJ016 and 2020kfyXGYJ028); the National Science and Technology Major Project of China (2018ZX10302206, 2018ZX10723203, and 2017ZX10304402-002-005); the National Key R&D Program of China (2017YFC0908104); and the Innovation Team Project of the Health Commission of Hubei Province (WJ2019C003). The authors thank AiMi Academic Services and Diana Liu for English language editing. We wish to thank the assistance given by Jia Liu, Cheng Peng, Jun Wu, Juan Xu, Xuemei Feng, and Rong Zhang in patients enrollment and sample collection.

AUTHOR CONTRIBUTIONS

XZ, BJW, TDX, and BYL designed and conceived the study; TDX, BYL, and HW performed the experiments; TDX, BYL, HW, XFQ, HLZ, YWH, DLY, BJW, and XZ enrolled patients and acquired the data; BYL and HW analyzed the data and contributed to producing the charts; TDX drafted the manuscript; XZ and BJW revised the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41423-021-00795-5.

Correspondence and requests for materials should be addressed to Baoju Wang or Xin Zheng.

4.3 Estudo comprova a eficácia da CoronaVac contra a Covid-19 em pacientes com câncer

Um estudo realizado na Turquia e publicado no início de agosto na revista Future Oncology mostrou que a CoronaVac, vacina do Butantan e da biofarmacêutica chinesa Sinovac, é eficaz e gera proteção em relação à Covid-19 em pacientes em tratamento contra o câncer. Duas semanas após a aplicação da segunda dose do imunizante, houve soroconversão (ou seja, formação de anticorpos) em 63,8% das pessoas analisadas.

A taxa de imunogenicidade chegou a 100% nos pacientes que recebem apenas anticorpo monoclonal ou imunoterapia como medicação. Além disso, nenhum dos pacientes apresentou infecção por Covid-19 em um acompanhamento médio de 85 dias após completarem o esquema vacinal. O intervalo entre a aplicação das duas doses de CoronaVac foi de 28 dias.

Este é o primeiro estudo já publicado que analisa a eficácia da CoronaVac em pacientes oncológicos. As conclusões estão no artigo Immunogenicity and safety of the CoronaVac vaccine in patients with cancer receiving active systemic therapy, escrito por pesquisadores que trabalham em sete hospitais e duas universidades de Ancara.

A pesquisa foi realizada entre janeiro e abril de 2021 com 47 pacientes com tumores sólidos. Eles tinham, em ordem de frequência, câncer colorretal, câncer de mama, de pulmão, geniturinário, gástrico, de pâncreas, ginecológico, do trato biliar e do sistema nervoso central. A maioria dos pacientes foi diagnosticada com doença em estágio IV e recebia tratamento sistêmico paliativo. A idade média dos pacientes era de 73 anos, e nenhum deles havia tido contato com o vírus SARS-CoV-2.

Além da imunogenicidade, o estudo analisou a segurança da vacina. Após receberem a primeira e a segunda dose da CoronaVac, as taxas de efeitos adversos de qualquer grau entre os 47 pacientes analisados foram de 18,9% e 23,1%, respectivamente. Não foram observados efeitos adversos graves.

Os resultados do estudo turco se somam a outros artigos divulgados recentemente que também confirmam a eficácia da CoronaVac em pessoas imunossuprimidas, um público que possui maior dificuldade na defesa imunológica do organismo.

Uma pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) mostrou que pacientes com doenças reumatológicas autoimunes apresentaram um aumento de 70,4% no nível de anticorpos contra o vírus SARS-CoV-2 duas semanas após receberem a segunda dose da CoronaVac. Além disso, cientistas da Universidade Federal de São Paulo (Unifesp) e do Hemocentro de Ribeirão Preto da Universidade de São Paulo (USP) concluíram que 43% dos pacientes transplantados de rim analisados geraram anticorpos contra a Covid-19 15 dias após receberem a segunda dose da vacina.

Publicado em: 17/08/2021

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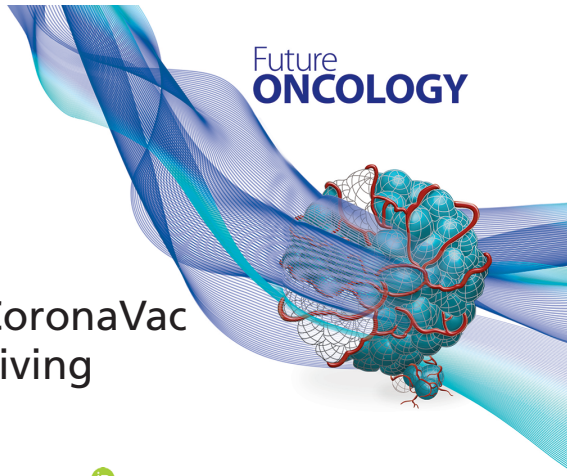

Immunogenicity and safety of the CoronaVac vaccine in patients with cancer receiving active systemic therapy

Cengiz Karacin*[,1,2], Tulay Eren[3], Esra Zeynelgil[3], Goksen Inanc Imamoglu[3], Mustafa Altinbas[3], Ibrahim Karadag[2], Fatma Bugdayci Basal[2], Irem Bilgetekin[2], Osman Sutcuoglu[4], Ozan Yazici[4], Nuriye Ozdemir[4], Ahmet Ozet[4], Yesim Yildiz[5], Selin Akturk Esen[6], Gokhan Ucar[6], Dogan Uncu[6], Bedia Dinc[7], Musa Baris Aykan[8], İsmail Erturk[8], Nuri Karadurmus[8], Burak Civelek[9], İsmail Çelik[10], Yakup Ergun[11], Mutlu Dogan[2] & Omur Berna Oksuzoglu[2]

[1]Department of Medical Oncology, Recep Tayyip Erdogan University Training & Research Hospital, Rize, Turkey
[2]Department of Medical Oncology, HSU Dr Abdurrahman Yurtaslan Oncology Training & Research Hospital, Ankara, Turkey
[3]Department of Medical Oncology, HSU Diskapi Yildirim Beyazit Training & Research Hospital, Ankara, Turkey
[4]Department of Medical Oncology, Gazi University, Ankara, Turkey
[5]Department of Infectious Diseases & Clinical Microbiology, Gazi University, Ankara, Turkey
[6]Department of Medical Oncology, Turkish Ministry of Health Ankara City Hospital, Ankara, Turkey
[7]Department of Medical Microbiology, Turkish Ministry of Health Ankara City Hospital, Ankara, Turkey
[8]Department of Medical Oncology, HSU Gulhane Training & Research Hospital, Ankara, Turkey
[9]Department of Medical Oncology, A Life Hospital, Ankara, Turkey
[10]Department of Preventive Oncology, Institute of Oncology, Hacettepe University, Ankara, Turkey
[11]Department of Medical Oncology, Batman Training & Research Hospital, Batman, Turkey
*Author for correspondence: Tel.: +90 312 339 0000; cengizkaracin@yahoo.com

Aim: To evaluate the immunogenicity and safety of the CoronaVac vaccine in patients with cancer receiving active systemic therapy. **Methods:** This multicenter, prospective, observational study was conducted with 47 patients receiving active systemic therapy for cancer. CoronaVac was administered as two doses (3 μg/day) on days 0 and 28. Antibody level higher than 1 IU/ml was defined as 'immunogenicity.' **Results:** The immunogenicity rate was 63.8% (30/47) in the entire patient group, 59.5% (25/42) in those receiving at least one cytotoxic drug and 100% (five of five) in those receiving monoclonal antibody or immunotherapy alone. Age was an independent predictive factor for immunogenicity (odds ratio: 0.830; p = 0.043). **Conclusion:** More than half of cancer patients receiving active systemic therapy developed immunogenicity.

Tweetable abstract: Immunogenicity developed with CoronaVac in 25 (59.5%) of 42 patients who received at least one cytotoxic drug and in all patients (n = 5) who received monoclonal antibody or immunotherapy alone.

First draft submitted: 12 May 2021; Accepted for publication: 22 July 2021; Published online: 3 August 2021

Keywords: cancer • chemotherapy • COVID-19 • immunogenicity • immunotherapy • monoclonal antibody • safety • tumors • vaccine

The coronavirus disease 2019 (COVID-19) pandemic has affected millions of people worldwide and caused more than 3 million deaths [1]. Advanced age and chronic disease are major risk factors for increased COVID-19 morbidity and mortality [2]. Cancer patients constitute a particular subgroup that needs more care because of delays in diagnostic and therapeutic processes during the pandemic leading to higher mortality rates [3,4]. Vaccines developed against COVID-19 have been promising for cancer patients as well as healthy individuals [5].

CoronaVac is an inactivated COVID-19 vaccine that has been shown to have immunogenicity, with vaccine-induced neutralizing antibodies to SARS coronavirus 2 (SARS-CoV-2) that can neutralize ten representative strains

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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	Docataxel	Y	0.87	N
3W	74	M	1	HT, CAD	Prostate	IV	Docetaxel	Y	0.64	N
3W	74	M	1	–	Gastric	IV	Docetaxel + cisplatin + 5-FU	Y	0.59	N
2W	80	M	1	–	Gastric	IV	FOLFIRI	Y	1.12	Y
2W	71	M	0	HT, CAD	Colon	IV	FOLFIRI + cetuximab	N	6.82	Y
2W	75	F	1	HT, DM	GBM	IV	Irinotecan + bevacizumab	N	>10	Y
2W	80	F	1	HT	Bladder	IV	Paclitaxel + carboplatin	Y	0.90	N
2W	73	M	1	DM	Colon	IV	FUFA + bevacizumab	N	1.58	Y
2W	69	M	0	–	Pancreas	IV	Gemcitabine 1–8	N	0.98	N
2W	80	F	1	HT	Colon	IV	FUFA + bevacizumab	N	5.29	Y
2W	71	M	1	DM, HT, COPD	Pancreas	IV	mFOLFIRINOX	Y	1.20	Y
2W	73	F	1	HT	Colon	IV	FOLFIRI	N	2.78	Y
2W	71	M	1	HT, DM	Colon	III	FUFA	N	6.31	Y
2W	72	M	1	Arrhythmia	Colon	IV	FOLFIRI + cetuximab	Y	9.15	Y
2W	78	M	1	Asthma	Pancreas	III	Gemcitabine	Y	1.66	Y
2W	74	M	1	HT, COPD	Gastric	III	FUFA	N	4.86	Y
2W	75	M	1	CAD	Colon	IV	FOLFIRI	Y	0.76	N
2W	72	F	1	–	Breast	IV	Gemcitabine	Y	0.98	Y
2W	72	M	0	HT	Bladder	IV	Gemcitabine + carboplatin	N	2.66	Y
2W	78	F	2	HT, DM	Endometrium	IV	Paclitaxel + carboplatin	N	0.86	N
2W	77	F	1	HT, COPD	Ovarian	IV	Gemcitabine	Y	0.05	N
2W	68	M	1	HT	Gastric	III	FLOT4	Y	1.05	Y
2W	65	M	1	HT, CAH	Rectum	IV	FOLFOX	N	4.42	Y
2W	77	F	2	HT, DM	Pancreas	IV	FOLFIRI	Y	>10	Y
2W	76	M	1	HT, DM, CAD	Biliary tract	IV	Gemcitabine + cisplatin	N	0.83	N
1W	73	M	1	–	Lung	IV	Paclitaxel	Y	1.05	Y
1W	77	M	1	CAH	Lung	IV	Irinotecan	N	0.19	N
1W	80	F	1	HT, DM, arrhythmia	Breast	III	Paclitaxel	Y	0.45	N
1W	66	F	0	–	Breast	II	Paclitaxel	N	0.97	N
1W	67	M	0	–	Rectum	IV	5-FU	N	>10	Y
1W	77	F	1	HT	Ovarian	IV	Paclitaxel + carboplatin	Y	7.20	Y
1W	70	M	0	–	Lung	III	Carboplatin	N	1.07	Y
C	73	F	1	–	Biliary tract	IV	Capecitabine	N	1.03	Y
C	73	M	1	Asthma	Colon	II	Capecitabine	N	1.59	Y
C	72	M	1	DM	Colon	II	XELOX	N	4.42	Y
C	73	M	2	–	Gastric	III	XELOX	N	0.80	Y
C	71	F	2	HT, DM	Rectum	IV	Capecitabine + cetuximab	N	0.05	N
C	76	M	1	–	Colon	IV	Capecitabine	N	0.95	N
IO	71	M	0	–	RCC	IV	Nivolumab	N	2.06	Y
IO	76	M	1	–	RCC	IV	Nivolumab	N	1.93	Y

1W: Cytotoxic drug or monoclonal antibody given each week; 2W: Cytotoxic drug or monoclonal antibody given every 2 weeks; 3W: Cytotoxic drug or monoclonal antibody given every 3 weeks; 5-FU: Fluorouracil; C: Cytotoxic drug given continuously orally; CAD: Coronary artery disease; CAH: Congenital adrenal hyperplasia; CHF: Congestive heart failure; COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; ECOG PS: Eastern Cooperative Oncology Group performance status; F: Female; FOLFIRI: Folinic acid, fluorouracil and irinotecan; FOLFOX: Folinic acid, fluorouracil and oxaliplatin; FUFA: Fluorouracil and folinic acid; FLOT4: fluorouracil plus leucovorin, oxaliplatin, and docetaxel; GBM: Glioblastoma multiforme; G-CSF: Granulocyte colony-stimulating factor; HT: Hypertension; IO: Immunotherapy given every 2 weeks; M: Male; mFOLFIRINOX: Modified folinic acid, fluorouracil, irinotecan and oxaliplatin; N: No; RCC: Renal cell carcinoma; TNM: Tumor, node, metastasis; XELOX: Capecitabine and oxaliplatin; Y: Yes.

Table 3. Univariate analysis of serological response rate.

	Seroconversion		p-value
	No	Yes	
Age (years), median (IQR)	75 (73–77)	72 (70–74)	0.031
Sex, n (%)			
Male	10 (34.5)	19 (65.5)	0.760
Female	7 (38.9)	11 (61.1)	
ECOG PS, n (%)			
0	3 (33.3)	6 (66.7)	0.249
1	10 (31.3)	22 (68.8)	
2	4 (66.7)	2 (33.3)	
Comorbidity, n (%)			
No	7 (50.0)	7 (50.0)	0.199
Yes	10 (30.3)	23 (69.7)	
TNM stage, n (%)			
II	1 (25.0)	3 (75.0)	0.767
III	3 (30.0)	7 (70.0)	
IV	13 (39.4)	20 (60.6)	
Treatment, n (%)			
Palliative	13 (41.9)	13 (58.1)	0.252
Other	4 (25.0)	12 (75.0)	
Treatment group, n (%)			
1W	3 (42.9)	4 (57.1)	NA
2W	7 (31.8)	15 (68.2)	
3W	4 (57.1)	3 (42.9)	
C	3 (50.0)	3 (50.0)	
IO	0 (0)	2 (100)	
Monoclonal AB only	0 (0)	3 (100)	
G-CSF, n (%)			
No	8 (26.7)	22 (73.3)	0.072
Yes	9 (52.9)	8 (47.1)	

1W: Cytotoxic drug or monoclonal antibody given each week; 2W: Cytotoxic drug or monoclonal antibody given every 2 weeks; 3W: Cytotoxic drug or monoclonal antibody given every 3 weeks; AB: Antibody; C: Cytotoxic drug given continuously orally; ECOG PS: Eastern Cooperative Oncology Group performance status; G-CSF: Granulocyte colony-stimulating factor; IO: Immunotherapy given every 2 weeks; IQR: Interquartile range; NA: Not applicable; TNM: Tumor, node, metastasis.

Table 4. Multivariate analysis of serological response.

	OR	95% CI	p-value
Comorbidity	2.937	0.729–11.833	0.130
G-CSF	0.468	0.116–1.881	0.284
Age	0.830	0.693–0.994	0.043

G-CSF: Granulocyte colony-stimulating factor; OR: Odds ratio.

systemic therapy [15]. In the same study, it was stated that immunogenicity decreased independently of treatment in patients with chronic lymphocytic leukemia. In another study evaluating 167 patients with chronic lymphocytic leukemia, the immunogenicity rate was found to be 39.5% with the BNT162b2 mRNA COVID-19 vaccine [16]. In a study by Massarweh *et al.* that included patients with solid organ tumors or hematological malignancies receiving active systemic therapy, it was shown that the mean antibody level detected after vaccination (BNT162b2 mRNA) was lower than that seen in healthy individuals [17].

In previous influenza vaccine studies, it has been shown that the immunogenicity rate may be lower in immunosuppressive patients compared with healthy individuals [9]. Adjuvant and high-dose vaccines are beneficial for increasing immunogenicity in seasonal influenza vaccines in immunosuppressive patients. It was also shown in a meta-analysis that the immunogenicity of the influenza vaccine was lower in cancer patients, who constituted

Table 5. Local and systemic reactions after first and second vaccine doses.

	First dose			Second dose		
	Any grade	Grade 1	Grade 2	Any grade	Grade 1	Grade 2
Total, n (%)	9 (18.9)	7 (14.7)	2 (4.2)	11 (23.1)	8 (16.8)	3 (6.3)
Local reaction, n (%)						
Pain at injection site	2 (4.2)	2 (4.2)	0 (0)	3 (6.3)	3 (6.3)	0 (0)
Swelling	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Itchiness	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Erythema	0 (0)	0 (0)	0 (0)	2 (4.2)	0 (0)	2 (4.2)
Systemic reaction, n (%)						
Fever	1 (2.1)	1 (2.1)	0 (0)	1 (2.1)	1 (2.1)	0 (0)
Myalgia	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)
Fatigue	2 (4.2)	0	2 (4.2)	5 (10.5)	4 (8.4)	1 (2.1)
Headache	1 (2.1)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)

the immunosuppressive group, compared with healthy individuals [9,18]. In the VACANSE study in which the immunogenicity of the H1N1v vaccine was evaluated in patients with solid organ tumors receiving active systemic treatment, it was reported that a single dose of the vaccine did not provide sufficient immunogenicity [10]. However, the immunogenicity might have increased had the vaccine been administered in two doses. Similarly, the fact that immunogenicity was lower in the authors' study compared with studies using healthy individuals raised the question of whether administration of a booster CoronaVac vaccine dose may increase the immunogenicity rates; this needs further clinical trials.

With aging, many molecular changes – called immunosenescence – occur in the immune system [19]. This dysregulation in the elderly immune system causes a decrease in the immune response obtained with vaccines. Considering that advanced age is a significant risk factor for COVID-19 morbidity and mortality, elderly patients have been given priority for vaccination against COVID-19 in many countries, including the authors' [20]. One of the concerns in the vaccination of elderly patients is immunogenicity sufficiency. The CoronaVac phase I and II trial, which was conducted with elderly volunteers, showed that the vaccine developed an immunogenicity profile comparable to that seen with young adults, without any serious adverse events [7]. The authors' study showed that the only independent factor affecting immunogenicity in multivariate analysis was age ($p = 0.043$). As mentioned, immunogenicity decreases with increasing age. This point might have also contributed to the lower immunogenicity rate seen with the CoronaVac vaccine in the authors' elderly cancer patients on active cancer treatment.

In the authors' study, the cumulative rate of possible vaccine-related side effects observed after two doses of the CoronaVac vaccine was 32%. Toxicity rates were reported to be 33 and 20% in the 3-μg cohorts of the Phase I and II CoronaVac trials, which were conducted with younger and elderly healthy volunteers, respectively [6,7]. The fatigue rate in the authors' study was higher than that seen in other CoronaVac trials (14.7 vs <10 and 3%). The higher fatigue rate in the authors' patients might have been related to cancer diagnosis and its active treatment during vaccination. Similar to the CoronaVac Phase I and II trials, no serious vaccine-related adverse events were observed in the authors' study.

Some researchers have hypothesized that the vaccine could hypothetically lead to an exaggerated immune response in immunotherapy recipients [21]. However, in a study evaluating short-term safety in 134 patients who received immunotherapy and the BNT162b2 mRNA COVID-19 vaccine, it was reported that there was no increase in immunotherapy-related immune side effects [22]. In the authors' study, only two patients received imunotherapy, and they did not experience any side effects. The median interval between the vaccine and the start of the previous immunotherapy cycle was 7 days in both patients.

This study did not have a validation cohort, which was a strong limitation. The study population also consisted of elderly patients, which was another limitation. Lower immunogenicity rate in the geriatric population irrespective of vaccination is a well-known finding, so it should be kept in mind that the study results do not reflect immunogenicity with vaccination in young cancer patients receiving active systemic therapy. It is a fact that the development of immunogenicity alone does not mean absolute protection from COVID-19 infection. Despite a median follow-up period of 85 days, the authors note that this is not long enough to comment on whether the vaccine has a long-term protective effect against COVID-19 infection. Another limitation was that cellular immunity, which has a

preventive effect against COVID-19 infection, was not evaluated in this study. Comorbidities and active cancer treatment modalities might be confounding factors in the evaluation of 'real' vaccine-related side effects. Therefore, it has been stated that the side effects were 'probably' related to the vaccine. The low number of patients and absence of a control group are another limitation of the study. Despite these limitations, to the best of the authors' knowledge, this study was the first to evaluate the efficacy and safety of the CoronaVac vaccine in cancer patients undergoing active systemic cancer treatment with chemotherapy, monoclonal antibody or immunotherapy.

Conclusion

Immunogenicity developed with two doses of the CoronaVac vaccine (3 μg/day days 0 and 28) in more than half of the patients with solid organ tumors undergoing active systemic cytotoxic chemotherapy.

Future Perspective

The fact that vaccination rates do not reach the targeted levels worldwide and virus mutations show that our fight against COVID-19 will continue in the coming years. There is a need for studies investigating more effective vaccination programs in cancer patients receiving active systemic therapy.

Summary points

- This prospective observational multicenter study was conducted with 47 patients with solid organ tumors receiving active systemic therapy to evaluate the immunogenicity and safety of the CoronaVac vaccine in patients with solid organ tumors receiving active systemic therapy (cytotoxic chemotherapy, monoclonal antibody, immunotherapy).
- Evaluation of vaccine immunogenicity was the primary outcome of the study; the secondary outcome was determining the vaccine's safety.
- The median patient age was 73 (range: 64–80), and 61.7% were male. Immunogenicity developed in 25 (59.5%) of 42 patients who received at least one cytotoxic drug and in all patients (n = 5) who received monoclonal antibody or immunotherapy alone.
- In univariate analysis, patients who had immunogenicity were younger, with a median age of 72 years (p = 0.031), whereas the median age of those who had no seroconversion was 75 years.
- Immunogenicity developed in 47.1% of those who were administered granulocyte colony-stimulating factor and 73.3% of those who were not administered granulocyte colony-stimulating factor (p = 0.072).
- In multivariate analysis, the only independent predictive factor affecting immunogenicity was patient age (odds ratio: 0.830; 95% CI: 0.693–0.994; p = 0.043).
- After the first and second doses of the vaccine, side effect rates of any grade were 18.9 and 23.1%, respectively, and there were no serious (grade 3 or 4) side effects or toxic deaths.
- Immunogenicity developed with two doses of the CoronaVac vaccine (3 μg/day days 0 and 28) in more than half of the patients with solid organ tumors undergoing active systemic cytotoxic chemotherapy.

Author contributions

C Karacin contributed to study concept, study design, data analysis and interpretation and manuscript writing. T Eren, E Zeynelgil, G I Imamoglu, M Altinbas, I Karadag, F B Basal, I Bilgetekin, O Sutcuoglu, O Yazici, N Ozdemir, A Ozet, Y Yildiz, S A Esen, G Ucar, D Uncu, B Dinc, M B Aykan, I Erturk, N Karadurmus, B Civelek and I Celik contributed to enrolling patients and interpreting data. M Dogan contributed to enrolling patients and revising the manuscript. Y Ergun contributed to study concept, study design and manuscript writing. O B Oksuzoglu contributed to study concept and revising the manuscript.

Acknowledgments

The authors thank all patients who participated in the study. Special thanks to Aydanur Sargın, Pinar Karacin, Arif Hakan Önder, Hatice Yiğit, Zeynep Sipahi Karslı, Ayşe Ocak Duran.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The study protocol was approved by the ethics committee of the HSU Dr Abdurrahman Yurtaslan Oncology Training & Research Hospital, and the study was undertaken in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent. Special permission for this study was obtained from the Ministry of Health.

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4.4 *CoronaVac aumenta em 70% anticorpos contra Covid-19 em pacientes imunossuprimidos, afirma estudo do HC*

Pacientes com doenças reumatológicas autoimunes apresentaram um aumento de 70,4% no nível de anticorpos contra o vírus SARS-CoV-2 duas semanas após receberem a segunda dose da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19. Além de aumentar a soroconversão dos pacientes imunossuprimidos, a CoronaVac também elevou em 56,3% a quantidade de anticorpos neutralizantes.

As conclusões são de um estudo realizado pelo Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) com 910 pessoas e estão descritas no artigo Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial, divulgado nesta sexta (30) na publicação científica Nature Medicine.

O resultado é extremamente positivo porque mostra que a CoronaVac não só é bem aceita pelo organismo de pacientes imunossuprimidos (que têm mais dificuldade para produzir anticorpos), como gera um alto nível de anticorpos de defesa e neutralizantes. A pesquisa do HC mostra não só que a CoronaVac é segura nesse público, como também eficaz.

"Trata-se do maior estudo já realizado no mundo com pacientes imunossuprimidos de doenças reumatológicas", afirma a diretora clínica do HCFMUSP, Eloisa Bonfá. "O acréscimo no nível de anticorpos é muito relevante e mostra que a CoronaVac conferiu uma proteção importante entre os imunossuprimidos", completa.

Outro dado que atesta a segurança da CoronaVac é a ausência de reações adversas nos vacinados. "Não tivemos nenhum caso de efeito colateral grave ou moderado entre os pacientes, mesmo sabendo que isso poderia ser esperado entre imunossuprimidos. Só tivemos efeitos colaterais leves. A CoronaVac é uma vacina altamente segura", assinala Eloisa.

De acordo com a diretora do hospital, os 910 pacientes imunossuprimidos participantes da pesquisa foram vacinados em dois dias. Pouco depois da segunda dose, quando os anticorpos ainda estavam em produção, houve 33 casos de Covid-19; 40 dias depois, esse número havia caído para seis casos.

Por que esse resultado é tão relevante?

O resultado de soroconversão (capacidade de produzir anticorpos) da CoronaVac nos pacientes imunossuprimidos do HCFMUSP é surpreendente, especialmente na comparação com o grupo controle, formado por pessoas sem deficiências de imunidade. O nível de anticorpos de defesa gerados nos imunossuprimidos foi de 70,4%, enquanto no grupo controle foi de 95%; já o nível de anticorpos neutralizantes foi de 56,3% nos imunossuprimidos, e de 79,3% no grupo controle.

Pessoas com doenças reumáticas autoimunes são geralmente tratadas com corticoides combinados com imunossupressores. Ou seja, seus tratamentos costumam envolver medicações que atuam justamente para reprimir o sistema imunológico, impedindo que ele atue de forma a agravar a doença autoimune.

A consequência disso é que imunossuprimidos têm uma menor capacidade de produzir anticorpos. Por isso, seus organismos são mais suscetíveis a contrair doenças infecciosas, como é o caso da Covid-19, e evoluir para casos graves. Antes da pesquisa do HCFMUSP, esse público estava impedido de tomar a vacina e só podia contar com medidas ainda em desenvolvimento, como o soro anti-Covid.

As doenças reumatológicas autoimunes compreendem diversas síndromes, como miosite autoimune, fasciíte eosinofílica, doença mista do tecido conjuntivo, policondrite recidivante, síndrome de Sjögren, lúpus eritematoso sistêmico e esclerodermia.

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Publicado em: 03/08/2021

ARTICLES
https://doi.org/10.1038/s41591-021-01469-5
nature medicine

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Immunogenicity and safety of the CoronaVac inactivated vaccine in patients with autoimmune rheumatic diseases: a phase 4 trial

Ana C. Medeiros-Ribeiro[1,6], Nadia E. Aikawa[1,2,6], Carla G. S. Saad[1], Emily F. N. Yuki[1], Tatiana Pedrosa[1], Solange R. G. Fusco[1], Priscila T. Rojo[1], Rosa M. R. Pereira[1], Samuel K. Shinjo[1], Danieli C. O. Andrade[1], Percival D. Sampaio-Barros[1], Carolina T. Ribeiro[1], Giordano B. H. Deveza[1], Victor A. O. Martins[1], Clovis A. Silva[2], Marta H. Lopes[3], Alberto J. S. Duarte[4], Leila Antonangelo[4], Ester C. Sabino[3,5], Esper G. Kallas[3], Sandra G. Pasoto[1] and Eloisa Bonfa[1]✉

CoronaVac, an inactivated SARS-CoV-2 vaccine, has been approved for emergency use in several countries. However, its immunogenicity in immunocompromised individuals has not been well established. We initiated a prospective phase 4 controlled trial (no. NCT04754698, CoronavRheum) in 910 adults with autoimmune rheumatic diseases (ARD) and 182 age- and sex-frequency-matched healthy adults (control group, CG), who received two doses of CoronaVac. The primary outcomes were reduction of ≥15% in both anti-SARS-CoV-2 IgG seroconversion (SC) and neutralizing antibody (NAb) positivity 6 weeks (day 69 (D69)) after the second dose in the ARD group compared with that in the CG. Secondary outcomes were IgG SC and NAb positivity at D28, IgG titers and neutralizing activity at D28 and D69 and vaccine safety. Prespecified endpoints were met, with lower anti-SARS-Cov-2 IgG SC (70.4 versus 95.5%, $P < 0.001$) and NAb positivity (56.3 versus 79.3%, $P < 0.001$) at D69 in the ARD group than in the CG. Moreover, IgG titers (12.1 versus 29.7, $P < 0.001$) and median neutralization activity (58.7 versus 64.5%, $P = 0.013$) were also lower at D69 in patients with ARD. At D28, patients with ARD presented with lower IgG frequency (18.7 versus 34.6%, $P < 0.001$) and NAb positivity (20.6 versus 36.3%, $P < 0.001$) than that of the CG. There were no moderate/severe adverse events. These data support the use of CoronaVac in patients with ARD, suggesting reduced but acceptable short-term immunogenicity. The trial is still ongoing to evaluate the long-term effectiveness/immunogenicity.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected millions of people around the world[1]. Brazil is among those countries with the highest numbers of confirmed cases of, and deaths from, SARS-CoV-2 (refs. [1,2]), with >430,000 deaths registered and approximately 15 million cases as of May 2021 (ref. [1]). A second infection wave was driven by the Gamma coronavirus variant[3], which is considered to be 2.5-fold more contagious than the original strain[4] and possibly associated with a higher risk for hospitalization and intensive care unit admission in patients younger than 60 years of age[5]. This second peak in March and April 2021 resulted in more than double the reported coronavirus disease 2019 (COVID-19) cases of the first peak in 2020 (ref. [6]). Vaccines are therefore essential in regard to reducing COVID-19 mortality and morbidity.

Although phase 3 clinical trials results are still being consolidated in China, Hong Kong, Indonesia, Brazil, Chile, Philippines and Turkey[7], CoronaVac, an inactivated virus vaccine against SARS-CoV-2, has received emergency use approval by the World Health Organization (WHO) in several countries, including three of the six most populated in the world—Brazil, China and Turkey—which are important for the global control of this disease. At the time of this submission, CoronaVac has accounted for approximately 75% of the vaccines administered in Brazil. It can be kept refrigerated[8], a great advantage for deployment in developing countries. In addition, the more traditional technology using the whole virus may have the benefit of a broader immune response compared to the other vaccine platforms using only the Spike protein. This may be relevant for control of SARS-CoV-2 variants containing mutations in the Spike protein, which have been documented in Brazil[3,9]. Cross-reactive humoral immune responses against the Gamma and Zeta variants were achieved in healthy volunteers vaccinated with CoronaVac in a phase 3 clinical trial conducted in Brazil[10,11].

However, the reported 50.7% efficacy in prevention of mild COVID-19 in the phase 3 clinical trial[10] raises concerns about the immunogenicity of CoronaVac in immunosuppressed patients, who number millions, including those with autoimmune diseases, neoplasia, transplant recipients and those living with human immunodeficiency virus (HIV) among other groups, with an estimated prevalence in the United States of 2.7% of the population[12]. A recent letter reported a greatly reduced anti-Spike antibody response after two doses of SARS-CoV-2 mRNA 1273 or BNT162b2 vaccination in solid organ transplant recipients[13,14]. Previous studies on COVID-19 vaccine immunogenicity in patients with ARD have suggested slightly reduced humoral responses, but have been

[1]Rheumatology Division, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [2]Pediatric Rheumatology Unit, Instituto da Criança, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [3]Infectious Disease Department, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [4]Central Laboratory Division, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [5]Instituto de Medicina Tropical, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil. [6]These authors contributed equally: Ana C. Medeiros-Ribeiro, Nadia E. Aikawa. ✉e-mail: eloisa.bonfa@hc.fm.usp.br

limited by the absence of a control group, small numbers of patients with ARD, and the fact that neutralizing antibodies have not necessarily been assessed[15–19]. In addition, most earlier studies evaluated immunogenicity following messenger RNA vaccines and thus CoronaVac immunogenicity in immunocompromised individuals remains unclear[13–19]. Importantly, immunocompromised patients are at high risk for infectious diseases due to immune dysregulation and treatment regimens. In addition, they may fulfill criteria for prioritization in the context of limited vaccine supply, since COVID-19 severity is associated not only with highly prevalent comorbidities in these patients but also with disease activity[10–24]. Moreover, an immunocompromised state was reported to be associated with prolonged SARS-CoV-2 shedding[25], reduced SARS-CoV-2 virus clearance and enhanced viral genomic evolution[26], emphasizing the relevance of the vaccine for this group of patients in reducing transmission and preventing the emergence of new variants.

In this context, the present study aimed to prospectively evaluate the immunogenicity (anti-SARS-CoV-2 IgG and neutralizing antibodies) and safety of CoronaVac in a large cohort of patients with ARD compared with an age- and sex-frequency-matched control group without these conditions and with no immunosuppressive therapy. As an exploratory outcome, we further checked for incident symptomatic cases, as confirmed by real-time reverse transcriptase–PCR (RT–PCR) for SARS-CoV-2 and the presence of variants of concern (VOC) (Gamma, Alpha and Beta lineages).

Results

Study design and participants. This phase 4 prospective controlled clinical trial (CoronavRheum clinicaltrials.gov no. NCT04754698) was conducted at a single tertiary center in Brazil.

The primary outcome was humoral immunogenicity, assessed by two coprimary endpoints: a minimum of 15% reduction in SC rates of anti-S1/S2 SARS-CoV-2 IgG and the presence of NAb 6 weeks after administration of the second vaccine dose (D69) in patients with ARD compared to controls, based on a previous study of primary vaccination with the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of patients with ARD[27].

Secondary immunogenicity outcomes were: anti-S1/S2 IgG seroconversion and presence of NAb at D28 (after vaccine first dose); geometric mean titers of anti-S1/S2 IgG and their factor increase in geometric mean titer (FI-GMT) at D28 and D69; and median (interquartile range, IQR) neutralizing activity of NAb at D28 and D69. Another secondary outcome was safety related to the vaccine doses. Exploratory outcomes were factors associated with anti-SARS-CoV-2 IgG SC and NAb positivity at D69, and incident COVID-19 case evaluation for a total of 80 days (from day of vaccination (D0) to 10 days after the second dose (D39) and thereafter for the following 40 days (from D40 to D79)).

A total of 1,418 patients with ARD were invited to join the study, but 225 were excluded according to established criteria: acute febrile illness/symptoms of suspected COVID-19 on the day of vaccination or with real-time RT–PCR-confirmed COVID-19 <4 weeks before D0 ($n=24$); demyelinating disease ($n=1$); previous vaccination with any COVID-19 vaccine ($n=25$); inactivated virus vaccine up to 2 weeks before D0 ($n=1$); individuals who did not consent to participate in the study ($n=161$); and hospitalization for general reasons ($n=13$). Subsequently, 542 healthy adult controls were invited but 50 individuals refused to participate. The remaining 1,193 patients with ARD and 492 controls received the first dose of CoronaVac, but 232 (19.4%) patients with ARD and 191 (38.8%) controls had positive baseline IgG serology and/or NAb and were thus excluded from this analysis. The remaining 961 patients with ARD and 301 controls with negative serology were then frequency matched in a 5/1 ratio (five ARD/one control) by age (maximal variation ± 5 years) and sex, with 910 patients with ARD and 182 healthy adults (CG) comprising the final study groups

Table 1 | Baseline characteristics of patients with ARD and CG

	ARD ($n=910$)	CG ($n=182$)	*P* value
Demographics			
Current age (years)	51 (40–60)	50 (41–60)	0.985
Female sex	700 (76.9)	140 (76.9)	>0.999
Caucasian race	482 (53.0)	82 (45.1)	0.051
Comorbidities			
Systemic arterial hypertension	400 (44.0)	55 (30.2)	0.001
Diabetes mellitus	106 (11.6)	28 (15.4)	0.161
Dyslipidemia	246 (27.0)	14 (7.7)	<0.001
Obesity	295 (32.4)	58 (31.9)	0.954
Chronic cardiomyopathy	52 (5.7)	3 (1.6)	0.024
Chronic renal disease	44 (4.8)	0	0.001
Current smoking	84 (9.2)	21 (11.0)	0.461
Chronic obstructive pulmonary disease	13 (1.4)	2 (1.1)	>0.999
Asthma	36 (4.0)	6 (3.3)	0.673
Interstitial lung disease	78 (8.6)	0	<0.001
Pulmonary hypertension	13 (1.4)	0	0.142
Hematologic disease	3 (0.3)	0	>0.999
Hepatic disease	39 (4.3)	0	0.001
Current cancer	8 (0.9)	0	0.365
Stroke	34 (3.7)	0	0.004
Current tuberculosis	2 (0.2)	0	>0.999
HIV	0	0	-
ARD			
Chronic inflammatory arthritis (RA, axSpA, PsA)	451 (49.6)	-	-
Other ARD (SLE, primary vasculitis, SSc, pSSj, IIM, PAPS)	459 (50.4)	-	-
Current therapy			
Prednisone	348 (38.2)	-	-
Prednisone dose, mg	5 (5–10)	-	-
Prednisone ≥20 mg day^{-1}	32 (3.5)	-	-
Hydroxychloroquine	269 (29.6)	-	-
Sulfasalazine	73 (8.0)	-	-
Immunosuppressive drugs	573 (63.0)	-	-
Methotrexate	229 (25.2)	-	-
Leflunomide	130 (14.3)	-	-
Mycophenolate mofetil	119 (13.1)	-	-
Azathioprine	109 (12.0)	-	-
Tofacitinib	19 (2.1)	-	-
Cyclophosphamide	10 (1.1)	-	-
Tacrolimus	10 (1.1)	-	-
Cyclosporine	9 (1.0)	-	-
Biologic therapy	321 (35.3)	-	-
TNFi	138 (15.2)	-	-
Abatacept	51 (5.6)	-	-
Tocilizumab	50 (5.5)	-	-
Belimumab	30 (3.3)	-	-
Secukinumab	29 (3.2)	-	-
Rituximab	19 (2.1)	-	-
Ustekinumab	5 (0.5)	-	-

Results are expressed as median (IQR) and *n* (%). Continuous data were compared using the Mann–Whitney *U*-test, and categorical variables with the chi-square or Fisher's exact test, as appropriate, always as two-sided analyses.

Table 2 | Seroconversion rates at D28 and D69; anti-SARS-CoV-2 S1/S2 IgG titers before (D0) and after the first (D28) and second dose (D69) of CoronaVac vaccination in patients with ARD and CG

	SC		GMT (AU ml^{-1})			FI-GMT	
	D28	D69	D0	D28	D69	D0 to D28	D0 to D69
ARD, $n = 859$	161 (18.7)	605 (70.4)	2.2 (2.2–2.3)	5.1 (4.7–5.5)	27.0 (24.7–29.5)	2.3 (2.1–2.5)	12.1 (11.0–13.2)
CG, $n = 179$	62 (34.6)	171 (95.5)	2.3 (2.1–2.4)	10.3 (8.5–12.5)	67.0 (59.8–74.9)	4.6 (3.9–5.4)	29.7 (26.3–33.5)
P (ARD versus CG)	<0.0001	<0.0001	>0.9990	<0.0010	<0.0010	<0.0010	<0.0010

SC is defined as post-vaccination titer ≥15 AU ml^{-1} by indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG. Frequencies of SC are presented as number (%), and were compared using a two-sided chi-square test between ARD and CG at prespecified time points (D28 and D69). IgG antibody titers and FI-GMT are expressed as geometric means with 95% CI. Data regarding IgG titers were analyzed using ANOVA with repeated measures and two factors (two groups (ARD versus CG) at three time points (D0, D28 and D69)), followed by Bonferroni's multiple comparisons at ln-transformed data (Supplementary Table 1). The behavior of IgG titers was different for ARD and CG groups between D28 and D69: mean titers increased at each time point for ARD and CG ($P < 0.001$). FI-GMT values were compared using the Mann–Whitney *U*-test for intergroup comparisons in ln-transformed data at prespecified time points (D28 and D69). All analyses were two-sided.

Table 3 | Frequency of NAb and median percentage of neutralizing activity in positive cases, after the first (D28) and second dose (D69) of CoronaVac vaccination in patients with ARD in comparison to CG

	D28		D69	
	Subjects with positive NAb, *n* (%)	Neutralizing activity (%) median (IQR)	Subjects with positive NAb, *n* (%)	Neutralizing activity (%) median (IQR)
ARD, $n = 859$	177 (20.6)	42.6 (35.8–60.4)	484 (56.3)	58.7 (43.1–77.2)
CG, $n = 179$	65 (36.3)	45 (34 .5–71.1)	142 (79.3)	64.5 (48.4–81.4)
P (ARD versus CG)	<0.0001	0.4900	<0.0001	0.0130

Frequencies of subjects with positive NAb are expressed as number (%). Positivity for NAb was defined as neutralizing activity ≥30% (cPass sVNT Kit). Data were compared using a two-sided chi-square test between ARD and CG at prespecified time points (D28 and D69). Percentage of neutralizing activity among subjects with positive NAb is expressed as median (IQR). Data were compared using a two-sided Mann–Whitney *U*-test for comparison between ARD and CG, at prespecified time points (D28 and D69).

(Extended Data Fig. 1). Enrollment and vaccination occurred on the same day for each participant. The first subject was enrolled and vaccinated on 9 February 2021 and the last participant was enrolled and vaccinated on 24 February 2021. The majority ($n = 1{,}017$, 93.1%) of patients and controls were recruited and vaccinated on 9 or 10 February 2021, with no differences between the ARD and CG groups (92.7 versus 95.1%, $P = 0.261$). Patients and controls were followed until D79 after the first vaccine dose (D0) for analysis of immunogenicity and incident cases in this study. The trial is no longer recruiting, but it is still ongoing for long-term effectiveness and immunogenicity.

Patients with ARD had the following disease diagnoses: chronic inflammatory arthritis (CIA) ($n = 451$, 49.6%), rheumatoid arthritis (RA) ($n = 256$, 28.1%), axial spondyloarthritis (axSpA) ($n = 106$, 11.6%) or psoriatic arthritis (PsA) ($n = 89$, 9.8%) and other systemic ARD ($n = 459$, 50.4%), systemic lupus erythematosus (SLE) ($n = 232$, 25.5%), primary vasculitis ($n = 66$, 7.3%), primary Sjögren's syndrome (pSSj) ($n = 42$, 4.6%), systemic sclerosis (SSc) ($n = 41$, 4.5%), idiopathic inflammatory myopathies (IIM) ($n = 41$, 4.5%) and primary antiphospholipid syndrome (PAPS) ($n = 37$, 4.1%) (Table 1). The control group ($n = 182$, CG) included hospital cleaning and general maintenance services workers ($n = 109$, 59.9%), health professionals ($n = 45$, 24.7%) and hospital administrative services employees or their relatives ($n = 28$, 15.4%).

The ARD and CG groups had comparable median ages (51 versus 50 years, $P = 0.985$) and enrollment of females (76.9 versus 76.9%, $P > 0.999$) (Table 1). Frequencies of comorbidity were higher in ARD, particularly systemic arterial hypertension (44.0 versus 30.2%, $P = 0.001$), dyslipidemia (27.0 versus 7.7%, $P < 0.001$), interstitial lung disease (8.6 versus 0%, $P < 0.001$), cardiomyopathy (5.7 versus 1.6%, $P = 0.024$) and chronic renal disease (4.8 versus 0%, $P = 0.001$) (Table 1). A total of 348 (38.2%) patients with ARD were receiving ongoing treatment with prednisone and 573 (63.0%) were using immunosuppressive drugs. Of those patients treated with immunosuppressive drugs, 25.2% were using methotrexate, 14.3% leflunomide, 13.1% mycophenolate mofetil, 12% azathioprine and <3% others. Of those 321 (35.3%) patients were being treated using biologic therapies, 15.2% were using tumor necrosis factor inhibitor (TNFi), 5.6% abatacept, 5.5% tocilizumab, 3.3% belimumab, 3.2% secukinumab and <3% others (Table 1).

For the primary outcome analysis of immunogenicity, we excluded 38 (4.2%) participants (35 patients with ARD and three CG participants) with real-time RT–PCR-confirmed COVID-19 after either the first or second dose of vaccine until D69, and 16/910 (1.5%) patients who did not attend the final visit (D69), including two deaths not related to COVID-19.

Primary immunogenicity outcomes. Humoral response parameters in the remaining 859 patients with ARD and 179 controls, all with negative anti-SARS-CoV-2 S1/S2 IgG antibodies and NAb pre-vaccination, are shown in Tables 2 and 3.

The study met the primary outcomes, defined as a minimum of 15% reduction in anti-S1/S2 SARS-CoV-2 IgG SC and in the presence of NAb in patients with ARD compared to CG at 6 weeks (D69) after the second dose. Analysis of the SARS-CoV-2 S1/S2 IgG response at D69 revealed a lower SC rate in patients with ARD (70.4 versus 95.5%, $P < 0.001$). Similarly, NAb positivity was lower in patients with ARD compared to controls (56.3 versus 79.3%, $P < 0.001$).

Secondary outcomes. Secondary immunogenicity outcomes defined by anti-SARS-CoV-2 IgG SC at D28, as well as IgG GMT and FI-GMT at D28 and D69, are presented in Table 2 and Fig. 1. SARS-CoV-2 cPass virus NAb positivity at D28 and median activity of NAb at D28 and D69 were also secondary outcomes (Table 3).

A minority of participants in both groups developed anti-SARS-CoV-2 IgG antibodies after the first dose (D28), with a lower frequency and level in patients with ARD compared to CG (161 (18.7%) versus 62 (34.6%), $P < 0.001$) and FI-GMT (2.3 (95% confidence interval (CI) 2.1–2.5) versus 4.6 (95% CI 3.9–5.4), $P < 0.001$). The SC rates doubled after the second vaccine dose, with an

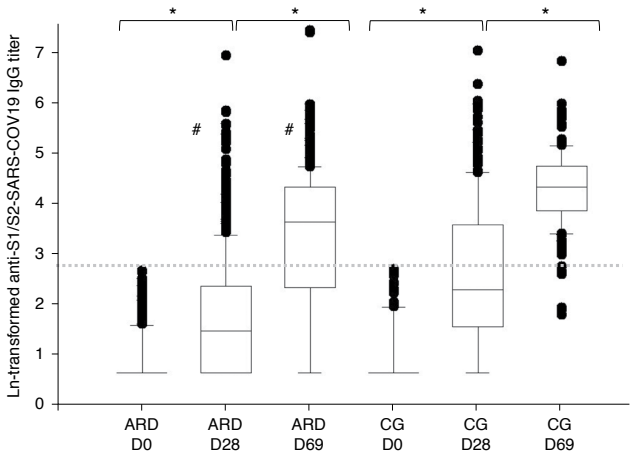

Fig. 1 | Anti-SARS-CoV-2 S1/S2 IgG titers of patients with ARD and subjects in CG at D0, D28 and D69. Box plots show the distribution of ln-transformed IgG titers over time. Data for each group (ARD, $n=859$ and CG, $n=179$) are presented at each time point as box plots: central values within boxes correspond to median (50th percentile, or Q2); the range between the lower (25th percentile, or Q1) and upper (75th percentile, or Q3) bounds of the boxes is the IQR. Whiskers represent scores outside IQR and ends in maximum (higher "calculated value" = Q3 + 1.5 x IQR) and minimum (lower "calculated value" = Q1 - 1.5 x IQR). Spots are outliers above the maximum or under the minimum values. The minimum possible value is 0.64 (ln 1.9, the value attributed to IgG titers ≤3.8 AU ml^{-1}). Data regarding IgG titers were analyzed using ANOVA with repeated measures and two factors (two groups (ARD versus CG), at three time points (D0, D28 and D69)), followed by Bonferroni's multiple comparison of ln-transformed data (Supplementary Table 1). Tests were always two-sided. The mean behavior of the ln-transformed IgG titers was different in ARD and CG groups at D28 ($P<0.001$) and D69 ($P<0.001$). Mean titers increased at each time point for ARD and CG ($^{*}P<0.001$). At D28 and D69 evaluations, patients with ARD presented lower mean titers than CG ($^{\#}P<0.001$). ARD and CG were comparable only at D0 ($P>0.999$). Dotted line denotes the cut-off level for positivity (ln 15 AU ml^{-1} = 2.71 by Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG).

increase of more than fivefold in GMT (FI-GMT) for both groups (Table 2 and Fig. 1).

According to Bonferroni's multiple comparison, the mean behavior of the neperian logarithm (ln)-transformed IgG titers was different in the ARD and CG groups between D28 and D69 ($P<0.001$). Mean IgG titers were similar at D0 in both groups ($P>0.999$) and increased at each time point for ARD and CG ($P<0.001$). At the D28 and D69 evaluations, patients with ARD presented lower mean titers than CG ($P<0.001$) (Table 2, Fig. 1 and Supplementary Table 1).

Analysis of the dynamics of NAb detection showed that after the first dose (D28), a minority of participants had positive antibodies and patients with ARD had lower frequencies (177 (20.6%) versus 65 (36.3%), $P<0.001$), but with similar median (IQR) activity (42.6% (35.8–60.4) versus 45% (34.5–71.1), $P=0.490$) compared with CG (Table 3). At D69, lower median (IQR) neutralization activity (58.7% (43.1–77.2) versus 64.5% (48.4–81.4), $P=0.013$) was observed.

Vaccine tolerance and safety. Vaccine safety analysis, another secondary outcome, is illustrated in Table 4. No moderate/severe adverse events (AEs) related to the vaccine were reported. After the first dose, the most frequently reported vaccine reactions in patients with ARD and CG were pain at the injection site (19.8 versus 17.0%, $P=0.388$), headache (20.2 versus 11.0%, $P=0.003$) and somnolence (13.6 versus 10.4%, $P=0.243$). Overall reactions were more frequently reported in patients with ARD than CG (50.5 versus 40.1%, $P=0.011$), including arthralgia (13.5 versus 6.0%, $P=0.005$), back pain (9.8 versus 4.9%, $P=0.037$), malaise (9.5 versus 4.4%, $P=0.026$), nausea (6.1 versus 2.2%, $P=0.032$) and sweating (5.6 versus 1.1%, $P=0.007$). After the second dose, patients with ARD reported less local itching (2.7 versus 5.5%, $P=0.047$) and more sweating (5.3 versus 1.1%, $P=0.010$) (Table 4).

Factors associated with lower anti-SARS-CoV-2 IgG SC and NAb positivity in patients with ARD. We also analyzed factors associated with anti-SARS-CoV-2 IgG SC and NAb positivity as exploratory outcomes (Table 5). Patients with negative anti-SARS-CoV-2 IgG after two doses of CoronaVac (D69) were of older age ($P<0.001$), with a higher frequency of females (81.9 versus 74.7%, $P=0.023$) compared to those with positive anti-SARS-CoV-2 IgG. Non-seroconverters used the following therapies more often: prednisone (55.9 versus 31.1%, $P<0.001$) and prednisone ≥20 mg day^{-1} (5.5 versus 2.6%, $P=0.037$); immunosuppressants (81.9 versus 54.5%, $P<0.001$), particularly methotrexate (34.6 versus 21.7%, $P<0.001$) and mycophenolate mofetil (24.4 versus 7.9%, $P<0.001$); and biologic therapy (44.1 versus 32.2%, $P=0.001$), especially abatacept (11.4 versus 3.3%, $P<0.001$) and rituximab (4.3 versus 1.3%, $P=0.006$) (Table 5). Multivariate logistic regression analysis (Supplementary Table 2) was performed using as dependent variables SC or the presence of NAb at D69 (primary endpoint), and as independent variables those with $P<0.2$ in the univariate analysis presented in Table 5. This analysis revealed that age ≥60 years (odds ratio (OR) = 0.51; 95% CI 0.36–0.74, $P<0.001$), prednisone (OR = 0.40; 95% CI 0.28–0.56, $P<0.001$), methotrexate (OR = 0.42; 95% CI 0.29–0.61, $P<0.001$), mycophenolate mofetil (OR = 0.15; 95% CI 0.09–0.24, $P<0.001$), TNFi (OR = 0.41; 95% CI 0.26–0.64, $P<0.001$), abatacept (OR = 0.24; 95% CI 0.13–0.46, $P<0.001$) and rituximab (OR = 0.34; 95% CI 0.13–0.93, $P=0.036$) were associated with the absence of SC in patients with ARD (Supplementary Table 2).

Similarly, patients with negative NAb after complete vaccination (D69) were older (52 (43–62) versus 49 (39–59) years, $P<0.001$) than those with positive NAb. Patients with negative NAb at D69 were more frequently ≥60 years of age (32.5 versus 22.5%, $P=0.001$) and using prednisone (49.3 versus 30%, $P<0.001$), immunosuppressants (72.5 versus 55%, $P<0.001$), including methotrexate (30.4 versus 21.7%, $P=0.004$) and mycophenolate mofetil (17.9 versus 8.9%, $P<0.001$) or biologic therapy (41.3 versus 31.4%, $P=0.003$), including abatacept (8.0 versus 3.9%, $P=0.011$) and rituximab (4.0 versus 0.8%, $P=0.002$) (Table 5). Multivariate analysis identified age ≥60 years (OR = 0.65; 95% CI 0.46–0.91, $P=0.011$), prednisone (OR = 0.48; 95% CI 0.35–0.65, $P<0.001$), methotrexate (OR = 0.67, 95% CI 0.47–0.95, $P=0.024$), mycophenolate mofetil (OR = 0.33; 95% CI 0.21–0.53, $P<0.001$) and rituximab (OR = 0.28; 95% CI 0.09–0.87, $P=0.028$) as associated with the absence of neutralizing activity in patients with ARD (Supplementary Table 2).

COVID-19 incident cases. For the analysis of incident cases, another exploratory outcome was used—participants were followed during strictly equivalent time periods of 40 days before and after full vaccination: from D0 to D39 and from D40 to D79. Therefore, the evaluation period for incident cases was extended to 10 days (D79) after the final immunogenicity analysis (D69). A total of 39 incident symptomatic, RT–PCR-confirmed COVID-19 cases among patients with ARD and CG were observed during the evaluation periods, with no significant difference between groups (4.0 versus 1.6%, $P=0.186$). The frequency of cases occurring

Table 4 | Adverse events following CoronaVac vaccination in patients with ARD and CG

	After vaccine first dose			After vaccine second dose		
	ARD ($n = 909$)	CG ($n = 182$)	*P* value	ARD ($n = 893$)	CG ($n = 181$)	*P* value
No symptoms	450 (49.5)	109 (59.9)	0.011	545 (61.0)	118 (65.2)	0.293
Local reactions (at the injection site)	213 (23.4)	36 (19.8)	0.284	154 (17.2)	32 (17.7)	0.888
Pain	180 (19.8)	31 (17.0)	0.388	125 (14.0)	30 (16.6)	0.368
Erythema	25 (2.8)	5 (2.7)	0.998	23 (2.6)	3 (1.7)	0.602
Swelling	43 (4.7)	12 (6.6)	0.294	45 (5.0)	10 (5.5)	0.787
Bruising	28 (3.1)	6 (3.3)	0.878	23 (2.6)	2 (1.1)	0.232
Pruritus	28 (3.1)	4 (2.2)	0.637	24 (2.7)	10 (5.5)	0.047
Induration	56 (6.2)	4 (2.2)	0.032	41 (4.6)	12 (6.6)	0.248
Systemic reactions	392 (43.3)	61 (33.5)	0.014	298 (33.4)	56 (30.9)	0.526
Fever	25 (2.8)	5 (2.7)	0.998	23 (2.6)	7 (3.9)	0.336
Malaise	86 (9.5)	8 (4.4)	0.026	80 (9.0)	15 (8.3)	0.772
Somnolence	124 (13.6)	19 (10.4)	0.243	83 (9.3)	15 (8.3)	0.668
Lack of appetite	37 (4.1)	7 (3.8)	0.888	37 (4.1)	7 (3.9)	0.864
Nausea	55 (6.1)	4 (2.2)	0.032	58 (6.5)	13 (7.2)	0.734
Vomiting	14 (1.5)	1 (0.5)	0.488	11 (1.2)	2 (1.1)	>0.999
Diarrhea	56 (6.2)	9 (4.9)	0.527	56 (6.3)	12 (6.6)	0.857
Abdominal pain	44 (4.8)	7 (3.8)	0.562	43 (4.8)	10 (5.5)	0.688
Vertigo	64 (7.0)	9 (4.9)	0.302	46 (5.2)	9 (5.0)	0.921
Tremor	22 (2.4)	1 (0.5)	0.155	20 (2.2)	2 (1.1)	0.562
Headache	184 (20.2)	20 (11.0)	0.003	130 (14.6)	33 (18.2)	0.209
Fatigue	99 (10.9)	14 (7.7)	0.196	95 (10.6)	22 (12.2)	0.550
Sweating	51 (5.6)	2 (1.1)	0.007	47 (5.3)	2 (1.1)	0.010
Myalgia	81 (8.9)	10 (5.5)	0.128	78 (8.7)	17 (9.4)	0.776
Muscle weakness	68 (7.5)	7 (3.8)	0.077	68 (7.6)	11 (6.1)	0.470
Arthralgia	123 (13.5)	11 (6.0)	0.005	93 (10.4)	13 (7.2)	0.184
Back pain	89 (9.8)	9 (4.9)	0.037	77 (8.6)	19 (10.5)	0.420
Cough	63 (6.9)	8 (4.4)	0.206	57 (6.4)	12 (6.6)	0.902
Sneezing	75 (8.3)	9 (4.9)	0.127	87 (9.7)	18 (9.9)	0.933
Coryza	75 (8.3)	13 (7.1)	0.616	76 (8.5)	17 (9.4)	0.701
Stuffy nose	52 (5.7)	8 (4.4)	0.474	55 (6.2)	11 (6.1)	0.967
Sore throat	67 (7.4)	7 (3.8)	0.084	60 (6.7)	11 (6.1)	0.751
Shortness of breath	29 (3.2)	6 (3.3)	0.941	23 (2.6)	6 (3.3)	0.576
Conjunctivitis	12 (1.3)	0	0.235	9 (1.0)	2 (1.1)	>0.999
Pruritus	33 (3.6)	3 (1.6)	0.253	39 (4.4)	6 (3.3)	0.519
Skin rash	9 (1.0)	3 (1.6)	0.433	14 (1.6)	0	0.090

Results are presented as *n* (%) and compared with the chi-square or Fisher's exact test, as appropriate, always as two-sided analyses.

between D0 and D39 (until 10 days after the second dose) was higher compared to D40–D79 (33/1,092 (3.0%) versus 6/1,057 (0.6%), $P < 0.0001$). Four patients with ARD were hospitalized (<10 days after the second dose) and none died from COVID-19. There was no hospitalizations or deaths associated with COVID-19 in the CG. Eighteen symptomatic participants with RT–PCR-confirmed COVID-19 were genotyped in our service; 83.3% of infections were due to Gamma variants, 5.6% to Alpha and 11.1% to other variants. SARS-CoV-2 genotyping could not be performed in the remaining 21 symptomatic participants because they were unable to attend our center due to the long traveling distance involved, and therefore their samples were collected for RT–PCR at an independent laboratory near to their home.

Finally, we considered environmental factors that could influence SARS-CoV-2 infection risk in those participants who answered the targeted questions about their exposure. Patients with ARD reported higher adherence to social isolation 69.5 versus 21.7%, $P < 0.001$) with lower household contact with infected people (4.6 versus 15.5%, $P = 0.0001$) and lower use of public transportation (47.7 versus 81.7%, $P < 0.001$) compared to CG. The numbers of people living in the same home were comparable in both groups (median of two).

Discussion

Vaccination of immunosuppressed patients, who were excluded from phase 3 vaccine trials, is of the utmost importance since

Table 5 | Baseline characteristics of patients with ARD with and without SC for anti-SARS-CoV-2 S1/S2 IgG antibodies and with and without NAb after two doses of CoronaVac vaccination

	ARD patients without SC ($n = 254$)	ARD patients with SC ($n = 605$)	*P* value	ARD patients without NAb ($n = 375$)	ARD patients with NAb ($n = 484$)	*P* value
Demographics						
Current age (years)	53 (45–63)	49 (39–59)	<0.001	52 (43–62)	49 (39–59)	<0.001
Age ≥60 years	89 (35)	142 (23.5)	<0.001	122 (32.5)	109 (22.5)	0.001
Female sex	208 (81.9)	452 (74.7)	0.023	293 (78.1)	367 (75.8)	0.427
Caucasian race	144 (56.7)	312 (51.6)	0.170	213 (56.8)	243 (50.2)	0.055
ARD						
CIA	126 (49.6)	304 (50.2)	0.864	200 (53.3)	230 (47.5)	0.091
Other ARD	128 (50.4)	301 (49.8)		175 (46.7)	254 (52.5)	
Current therapy						
Prednisone	142 (55.9)	188 (31.1)	<0.001	185 (49.3)	145 (30.0)	<0.001
Prednisone dose (mg)	5 (5–10)	5 (5–10)	0.926	5 (5–10)	5 (5–10)	0.731
Prednisone ≥20 mg day^{-1}	14 (5.5)	16 (2.6)	0.037	15 (4)	15 (3.1)	0.476
Hydroxychloroquine	72 (28.3)	182 (30.1)	0.611	98 (26.1)	156 (32.2)	0.052
Sulfasalazine	10 (3.9)	61 (10.1)	0.003	24 (6.4)	47 (9.7)	0.081
Immunosuppressive drugs	208 (81.9)	330 (54.5)	<0.001	272 (72.5)	266 (55)	<0.001
Methotrexate	88 (34.6)	131 (21.7)	<0.001	114 (30.4)	105 (21.7)	0.004
Leflunomide	37 (14.6)	84 (13.9)	0.793	57 (15.2)	64 (13.2)	0.409
Mycophenolate mofetil	62 (24.4)	48 (7.9)	<0.001	67 (17.9)	43 (8.9)	<0.001
Azathioprine	31 (12.2)	69 (11.4)	0.739	40 (10.7)	60 (12.4)	0.433
Tofacitinib	3 (1.2)	15 (2.5)	0.301	10 (2.7)	8 (1.7)	0.304
Cyclophosphamide	2 (0.8)	7 (1.2)	>0.999	3 (0.8)	6 (1.2)	0.739
Tacrolimus	4 (1.6)	6 (1.0)	0.493	4 (1.1)	6 (1.2)	0.815
Cyclosporine	4 (1.6)	4 (0.7)	0.245	6 (1.6)	2 (0.4)	0.085
Biologic therapy	112 (44.1)	195 (32.2)	<0.001	155 (41.3)	152 (31.4)	0.003
TNFi	45 (17.7)	86 (14.2)	0.193	63 (16.8)	68 (14.0)	0.266
Abatacept	29 (11.4)	20 (3.3)	<0.001	30 (8.0)	19 (3.9)	0.011
Tocilizumab	12 (4.7)	33 (5.5)	0.661	23 (6.1)	22 (4.5)	0.300
Belimumab	13 (5.1)	17 (2.8)	0.093	16 (4.3)	14 (2.9)	0.277
Secukinumab	2 (0.8)	26 (4.3)	0.006	7 (1.9)	21 (4.3)	0.043
Rituximab	11 (4.3)	8 (1.3)	0.006	15 (4.0)	4 (0.8)	0.002
Ustekinumab	1 (0.4)	4 (0.7)	>0.999	2 (0.5)	3 (0.6)	0.869

Results are expressed as median (IQR) and *n* (%). Continuous data were compared using the Mann–Whitney *U*-test, and categorical variables with the chi-square or Fisher's exact test, as appropriate, always as two-sided analyses. SC defined as positive serology (IgG titer ≥15 AU ml^{-1}) for anti-SARS-CoV-2 S1/S2 IgG antibodies after vaccination (Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG). Positivity for NAb defined as neutralizing activity ≥30% (cPass sVNT Kit).

patients with ARD have an increased risk of hospitalization for severe COVID-19 (refs. [21,24]). In this large prospective study of an inactivated SARS-CoV-2 vaccine in patients with ARD, CoronaVac demonstrated a good safety profile with no serious/moderate AEs related to the vaccine. The vaccine was immunogenic in patients with ARD, but at lower levels when compared to the CG. Controlling the groups for age was essential, since SC may be lower in the older population[10], and this differentiates the current trial from earlier studies[15–18].

We prospectively included a large population of patients with ARD representing eight systemic diseases fulfilling their respective classification criteria, and followed all participants with scheduled face-to-face appointments, telephone, smartphone instant messaging and email contacts, which allowed a more precise monitoring of vaccine-induced AEs in this population. Tolerance and safety are a relevant concern for patients with ARD, since they have an intrinsic risk for thrombosis[28], a rare complication reported for some of the new COVID-19 vaccines[29], and autoimmune/autoinflammatory manifestations, a problem with adjuvanted vaccines in this already predisposed population[30]. Similar to previous results from CoronaVac trials in healthy populations[31], most vaccine-related AEs were mild with pain at the injection site being the most frequently reported. Interestingly, vaccine-related AEs, particularly systemic symptoms, were much less frequent in both ARD and CG than those reported with mRNA vaccines[32,33]. These data confirm the previously reported safety profile of CoronaVac[11], and extend this finding to a large group of immunocompromised patients. Data on disease activity were not available due to the study design, with approximately 93% of participants vaccinated in a single center over 2 days, and therefore the influence of this factor on CoronaVac immunogenicity remains to be determined. The lack of assessment of vaccine T cell responses was another limitation of the present study[34,35].

The exclusion of seropositive participants and those with COVID-19 during the study period allowed a more accurate evaluation of the immunogenicity of CoronaVac. In addition, there was no difference in blood sample collection timing between the two groups because most participants received vaccine in the same timeframe, precluding the possible confounding nonlinear relationship between the elapsed time and the vaccine. We observed lower CoronaVac immunogenicity in patients with ARD, although within the immunologic response standards (SC rates and GMT) established by the European Medicine Agency and the Food and Drugs Administration recommendations for Emergency Use Authorization of pandemic vaccines[36,37]. The 70% SC rate was comparable to that obtained against the pandemic influenza A /H1N1 inactivated vaccine (approximately 63%)[27], but lower than those reported for the SARS-CoV-2 mRNA vaccine in a very small ARD population[17] and in a study with patients predominantly using cytokine inhibitors and with limited representation of systemic diseases[16]. There was a substantial increase in immune response parameters, including anti-SARS-CoV-2 IgG titers and SC and NAb positivity rates, only after the second dose, reinforcing the importance of the full vaccination schedule for optimal vaccine immunogenicity, particularly in the ARD group. Similar to the anti-SARS-CoV-2 IgG antibody response, the frequency of mean inhibitory neutralizing activity against SARS-CoV-2 (56.4%) was reduced compared to controls and that reported after SARS-CoV-2 mRNA vaccination[15,16]. Again, the second dose was essential to achieving the maximum response for both groups, with a lower neutralization activity in ARD than in CG after the two vaccine doses. A recent report including 53 patients with RA who had received mRNA vaccines also emphasized the importance of a second dose to improve immunogenicity[38].

The profile of tertiary hospital patients evaluated in this trial, with a high frequency being treated with immunosuppressive/glucocorticoid/biological therapies, probably contributed to the reduced humoral response observed in the ARD group. In fact, 63% were on immunosuppressive therapy and more than one-third on prednisone and biologics. Of note, these three groups of drugs were identified as independent variables that negatively impact both anti-SARS-CoV-2 IgG and neutralizing antibodies following vaccination. Among the immunosuppressive drugs, methotrexate and mycophenolate mofetil had the greatest negative impact on immunogenicity whereas abatacept and rituximab were the most negative among those treated with biologics. This finding is in line with other studies in patients with ARD and on other COVID vaccines[15,17,18,39] although these earlier reports did not control for age, which may limit the strength of the conclusions that can be drawn regarding the impact of these drugs[18]. Specifically for CoronaVac, these data added new information since another small trial found rituximab to be the only drug associated with low seropositivity after complete vaccination in immunocompromised patients[19]. We also found a detrimental effect of TNFi therapy solely on anti-S1/S2 IgG response, contrasting with a recent study of patients with ARD[16]. However, our findings require further investigation since most patients with CIA under TNFi were also being treated with methotrexate, which itself was associated with reduced humoral responses in the present trial.

Although not the main objective of this study, these data also provide preliminary evidence of the short-term efficacy of CoronaVac in prevention of symptomatic COVID-19 cases. An extension period of observation (up to 12 months) for incident cases is already in progress. Importantly, the majority of patients with ARD and CG were all vaccinated at the same epidemiological week over a 2-day period, providing a unique setting of comparable influence of the ongoing local SARS-CoV-2 infection rates. Remarkably, the 45% increase in COVID-19 cases in Sao Paulo occurred from mid-March through to the end of April, coinciding with the study period between D40 and D79 (>10 days after the second dose)[40]. In this 40-day interval in which vaccine immunity is already expected, the frequency of COVID-19 cases was notably lower than in the previous 40 days after the first vaccination (D0–D39). The unanticipated overall similar frequency of SARS-CoV-2 infection in patients with ARD, a known vulnerable immunosuppressed population, compared to CG during the study period may be explained by the higher adherence to social isolation and lower household contact with infected people, as well as by reduced use of public transportation among patients. It may also be related to high exposure due to the professions of the majority of CG. The small number of new RT–PCR-confirmed COVID-19 cases during the observation period hampers, however, a definitive conclusion on the role of vaccine efficacy. The Gamma variant was the dominant strain amongst incident cases, in line with the virologic surveillance in the region, where Gamma represented 90% of all sequenced samples in the state in late April 2021 followed by Alpha and Beta as the other VOC[41].

In conclusion, this study provides evidence of safety and reduced, but acceptable, short-term immunogenicity of an inactivated SARS-CoV-2 vaccine in the ARD population. The impact of this diminished humoral response on long-term vaccine effectiveness is already ongoing, and it will also shed light on the persistence of CoronaVac-elicited immune responses and the need for a vaccine booster.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-021-01469-5.

Received: 22 May 2021; Accepted: 15 July 2021;
Published online: 30 July 2021

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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 Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil (no. CAAE: 42566621.0.0000.0068). Written informed consent was obtained from all participants before enrollment, including an agreement for sharing of source data following publication of this manuscript, with indirect identifiers. There was no participant compensation.

Study design. This phase 4 prospective controlled clinical trial (CoronavRheum clinicaltrials.gov, no. NCT04754698) was conducted at a single tertiary center in Brazil.

Patients and controls. Patients with ARD and ≥18 years of age from the Outpatient Rheumatology Clinics at our center were included, with the following diagnoses: RA[42], SLE[43], axSpA[44], PsA[45], primary vasculitis[46,47], pSSj[48], SSc[49], IIM[50] and PAPS[51].

After confirmation of participation by patients with ARD, CG were invited, with frequency matching by age (up to ±5 years difference) and sex, using an Excel program for random selection of participants (one control/five patients). None of these were previously vaccinated in the hospital's regular campaign. ARD diagnosis, use of immunosuppressive drugs and HIV infection were exclusion criteria for CG, whereas other well-controlled medical conditions were allowed in the CG group (Extended Data Fig. 1). None of the patients included in this analysis held medications to improve vaccine response.

Overall exclusion criteria were: history of anaphylactic response to vaccine components; acute febrile illness or symptoms compatible with COVID-19 at vaccination; Guillain–Barré syndrome; decompensated heart failure (class III or IV); demyelinating disease; previous vaccination with any SARS-CoV-2 vaccine; history of live virus vaccine up to 4 weeks previously; inactivated viral vaccine up to 2 weeks previously; history of having received blood products up to 6 months before the study; individuals who did not agree to participate in the study; hospitalized patients; and prevaccination positive COVID-19 serology and/or NAb (for immunogenicity analysis) (Extended Data Fig. 1).

After receiving the first vaccine dose, participants with RT–PCR-confirmed COVID-19 were excluded from the immunogenicity analysis but included in the evaluation of incident cases.

Vaccination protocol. The vaccination protocol for patients with ARD and GC consisted of a two-dose schedule of the COVID-19 vaccine. The first dose (with blood collection) was given for most participants on 9–10 February 2021 (D0), the second dose (with blood collection) on 9–10 March 2021 (D28) and a final blood collection on 19 April 2021 (D69) at the Hospital Convention Center. Incident COVID-19 cases were assessed for a further 10 days until D79. This protocol was delayed by 4 weeks for participants with incident COVID-19 during the study. Ready-to-use syringes loaded with CoronaVac (Sinovac Life Sciences, batch no. 20200412), consisting of 3 μg in 0.5 ml of β-propiolactone-inactivated SARS-CoV-2 (derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells—Vero 25 cells) with aluminum hydroxide as an adjuvant, were administered intramuscularly in the deltoid area.

Primary and secondary outcomes. The primary outcome was humoral immunogenicity assessed by two coprimary endpoints: the presence of anti-S1/S2 SARS-CoV-2 IgG and the presence of NAb 6 weeks after the second vaccine dose (D69).

Secondary immunogenicity outcomes were: anti-S1/S2 IgG seroconversion and the presence of NAb at D28 (after vaccine first dose); geometric mean titers of anti-S1/S2 IgG and their factor increase in GMT (FI-GMT) at D28 and D69; and median (IQR) neutralizing activity of NAb at D28 and D69.

A further secondary outcome was safety related to the vaccine doses. Additionally, factors associated with anti-SARS-CoV-2 IgG SC and NAb positivity and incident COVID-19 case evaluation were exploratory outcomes.

Samples for immunogenicity evaluation. To assess these outcomes, blood samples (20 ml) from all participants were obtained at D0 (baseline, immediately before first vaccine dose), D28 (immediately before the second dose) and D69 (6 weeks after the second dose). Sera were stored in a freezer at −70 °C.

Anti-SARS-CoV-2 S1/S2 IgG antibodies. A chemiluminescent immunoassay was used to measure human IgG antibodies against proteins S1 and S2 in the receptor-binding domain (RBD) (Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG, DiaSorin). SC rate was defined as positive serology (≥15.0 UA ml^{-1}) after vaccination, taking into consideration that only patients with prevaccination negative serology were included. GMT and 95% CIs of these antibodies were also calculated at all time points, attributing the value of 1.9 UA ml^{-1} (half of the lower limit of quantification, 3.8 UA ml^{-1}) to undetectable levels (<3.8 UA ml^{-1}). FI-GMT is the ratio of GMT after vaccination to that before, with growth measured in titers. These values are also presented and compared as geometric means and 95% CIs.

SARS-CoV-2 cPass virus NAb. The SARS-CoV-2 sVNT Kit (GenScript) was utilized according to the manufacturer's instructions. This analysis detects circulating NAb against SARS-CoV-2 that block the interaction between the RBD of the viral Spike glycoprotein with the angiotensin-converting enzyme 2 cell surface receptor. Tests were performed on ETI-MAX-3000 equipment (DiaSorin). Samples were classified as either "positive" (inhibition ≥30%) or "negative" (inhibition <30%), as suggested by the manufacturer[52]. The frequency of positive samples was calculated at all time points. Medians (IQR) of the percentage of neutralizing activity, for positive samples only, were calculated at all time points.

Vaccine AEs and incident cases of COVID-19. Safety was rigorously followed by the National Research Ethics Council, and all serious AEs were classified as either vaccine related or not related. In addition an independent Data Safety Monitoring Board, comprising vaccine-prominent experts, periodically reviewed and evaluated the study protocol. Patients and control groups were advised to report any side effects of the vaccine; to this end, they received on D0 (first dose) and D28 (second dose) a standardized diary for recording of local and systemic manifestations. Local manifestations included local pain, erythema, swelling, bruising, pruritus and induration at the vaccine site. Systemic reactions included fever, malaise, somnolence, lack of appetite, nausea, vomiting, diarrhea, abdominal pain, vertigo, tremor, headache, fatigue, myalgia, muscle weakness, arthralgia, back pain, cough, sneezing, coryza, stuffy nose, sore throat, shortness of breath, conjunctivitis, pruritus and skin rash. Vaccine AE severity was defined according to the WHO definition[53].

Environmental factors associated with high risk of exposure to SARS-CoV-2 were recorded from all participants, including adherence to social isolation, number of people living in the same house, household contact with infected people and use of public transportation.

Additionally, to evaluate incident COVID-19 cases (exploratory outcome), all patients with ARD and controls were instructed to communicate any manifestation associated or not with COVID-19 by telephone, smartphone instant messaging or email. Our medical team was divided to provide a proper follow-up for the assigned group of patients/controls including the need for medical care, hospitalizations, severity of infections, sick days and treatment. Participants with suspicion of COVID-19 were instructed to seek medical care near their residence and, if recommended, to come to our tertiary hospital to undergo a RT–PCR test for SARS-CoV-2 or make an in-person visit. If tertiary care was required, the participant was transferred to a referenced hospital. The standardized diary of AEs was carefully reviewed with each participant on the day of the second dose (D28) and at the last visit (D69). COVID-19 incident cases were followed for 40 days (from D0 to 10 days after the second dose (D39)) and thereafter for the following 40 days (from D40 to D79).

Study data were collected and managed using REDCap electronic data capture tools (10.5.0, 2021 Vanderbilt University) hosted at our Institution[54,55].

RT–PCR for SARS-CoV-2 and analysis of VOC. Clinical samples for SARS-CoV-2 RT–PCR consisted of naso- and oropharyngeal swabs, using a laboratory-developed test[56]. All participants with positive test results were invited to collect samples at our hospital, and these materials were further analyzed for VOC. RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions. For rapid access of VOC, we performed two real-time PCR protocols in parallel. Romano et al.[57] used two sets of probes to detect NSP6 Δ 106–108, which encodes a protein that participates in the viral replication process and allows the differentiation of ancestral variants from Alpha, Beta and Gamma VOC. The protocol of Vogels et al. uses a multiplex quantitative RT–PCR (RT–qPCR) assay that targets three regions (N1, ORF1a Δ3675–3677 and Spike Δ69–70 primer) and facilitates differentiation of Alpha VOC from Beta and Gama VOC, and from ancestral variants[58]. To confirm the results, we sequenced the virus using a combination of targeted multiplex PCR amplification and a portable nanopore sequencing MinION platform (Oxford Nanopore Technologies)[3,58]. In brief, complementary DNA was synthesized with random hexamers and the Protoscript II First Strand cDNA synthesis Kit (New England Biolabs). Whole-genome multiplex PCR amplification was then conducted using the ARTIC network SARS-CoV-2 V3 primer scheme. Multiplex PCR products were purified using AmpureXP beads (Beckman Coulter), and quantification was carried out using the Qubit dsDNA High Sensitivity assay on the Qubit 3.0 (Life Technologies). Samples were then normalized (10 ng per sample), DNA fragments were barcoded using the EXP-NBD104 (refs. [59,60]) and EXP-NBD114 (ref. [61]) Native Barcoding Kits (Oxford Nanopore Technologies) and pooled. Sequencing adapter ligation was performed using the SQK-LSK 109 Kit (Oxford Nanopore Technologies). Sequencing libraries were loaded onto an R9.4.1 flow-cell (Oxford NanoporeTechnologies) and sequenced using MinKNOW v.20.10.3 (Oxford Nanopore Technologies).

Symptomatic participants who were unable to come to our center to collect the RT–PCR kit were instructed to go to an independent laboratory near their home.

Statistical analysis. Sample size calculation was based on the previous 15% reduction in SC rate after first vaccination with the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of patients with ARD[36]. In expectation of

SC rates of 63% in the ARD patient cohort and 78% in the control group, and considering an alpha error of 5% and power of 80% in a 5/1 ratio to include more patients with ARD, the minimum sample required would be 445 patients with ARD and 89 healthy subjects, sex controlled and of similar age. In expectation of a higher SC rate of 98% for this vaccine[28], such sample size had a power >99% to detect a 15% reduction in SC of patients with ARD. Due to the peak of the ongoing pandemic in Brazil during the vaccination period, we invited additional patients and controls, expecting a high incidence of previously infected people and a high rate of infection.

Categorical variables are presented as number (percentage) and compared using the chi-square or Fisher's exact test, as appropriate. Only for patients with ARD, multivariate logistic regression analyses were performed using as dependent variables SC or the presence of NAb at D69 (primary endpoints), and as independent variables those with $P < 0.2$ in each univariate analysis.

Continuous general data are presented as medians (IQR) and compared using the Mann–Whitney *U*-test for intergroup comparison. Continuous data regarding anti-S1/S2 serology titers are presented as geometric means (95% CI); their comparisons were performed using repeated-measures analysis of variance (ANOVA) with two factors (two groups (ARD and CG) at three time points (D0, D28 and D69)), followed by Bonferroni's multiple comparisons in ln-transformed data.

Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences, v.20.0 (IBM-SPSS for Windows 20.0).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All background information on controls and clinical information for patients with ARD in this study are included in the Source data provided with this paper (https://figshare.com/s/0a8921e7422a4fb8436f). Requests for sera sharing will need approval from the Hospital das Clinicas da Universidade de Sao Paulo´s review board and the National Research Ethics Council and a Material Transfer Agreement, which typically requires about 1 month. The SARS-CoV-2 sequences are available on GISAID (http://www.gisaid.org) (nos. EPI_ISL_2894869–2894885). An account (free registration) on GISAID is needed to obtain access to sequences. Additional correspondence and requests for materials should be addressed to the corresponding author (E.B.).

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Acknowledgements

We thank the contribution of the Central Laboratory Division, Registry Division, Security Division, IT Division, Superintendence, Pharmacy Division and Vaccination Center for their technical support. We also thank the volunteers for participating in the three in-person visits of the protocol and for handling the biological material, and those responsible for the follow-up of all participants. This study was sponsored by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (no. 2015/03756–4 to N.E.A., S.G.P., C.A.S. and E.B.; no. 2017/14352-7 to T.P., no. 2018/09937-9 to V.A.O.M., no. 2020/11677-5 to G.B.H.D.; and no. 2019/21173-7 to C.T.R.) and from Conselho Nacional de Desenvolvimento Científico e Tecnológico (no. 305242/2019-9 to E.B.; no. 304984/2020-5 to C.A.S.; no. 305556/2017-7 to R.M.R.P.; and no. 303379/2018-9 to S.K.S.). Instituto Butantan supplied the study product and had no other role in the trial.

Author contributions

A.C.M.-R., N.E.A., C.G.S., E.F.N.Y., T.P., S.G.P., E.G.K. and E.B. conceived and designed the study, participated in data collection and analysis and supervised clinical data management, writing of the manuscript and revision of the manuscript. S.G.R.F. and P.T.R. organized and supervised blood collection and vaccination. A.J.S.D. and L.A. supervised serum processing, SARS-CoV-2-specific antibody ELISA/neutralization assays and SARS-CoV-2 RT–PCR. A.C.M.-R., N.E.A., C.G.S., E.F.N.Y., T.P., S.G.P., E.B, S.R.G.F., P.T.R., R.M.R.P., S.K.S., D.C.O.A., P.D.S.-B., C.T.R., G.B.H.D., V.A.O.M. and C.A.S. collected epidemiological and clinical data and assisted with the identification of SARS-CoV-2 infection and follow-up of patients. M.H.L. organized and supervised the vaccination protocol. E.C.S. performed SARS-CoV-2 genotyping of positive RT–qPCR samples and screening of variants of concern. All authors helped to edit the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-021-01469-5.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-021-01469-5.

Correspondence and requests for materials should be addressed to E.B.

Peer review information: *Nature Medicine* thanks Dawn Teare, Philip Robinson and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Saheli Sadanand was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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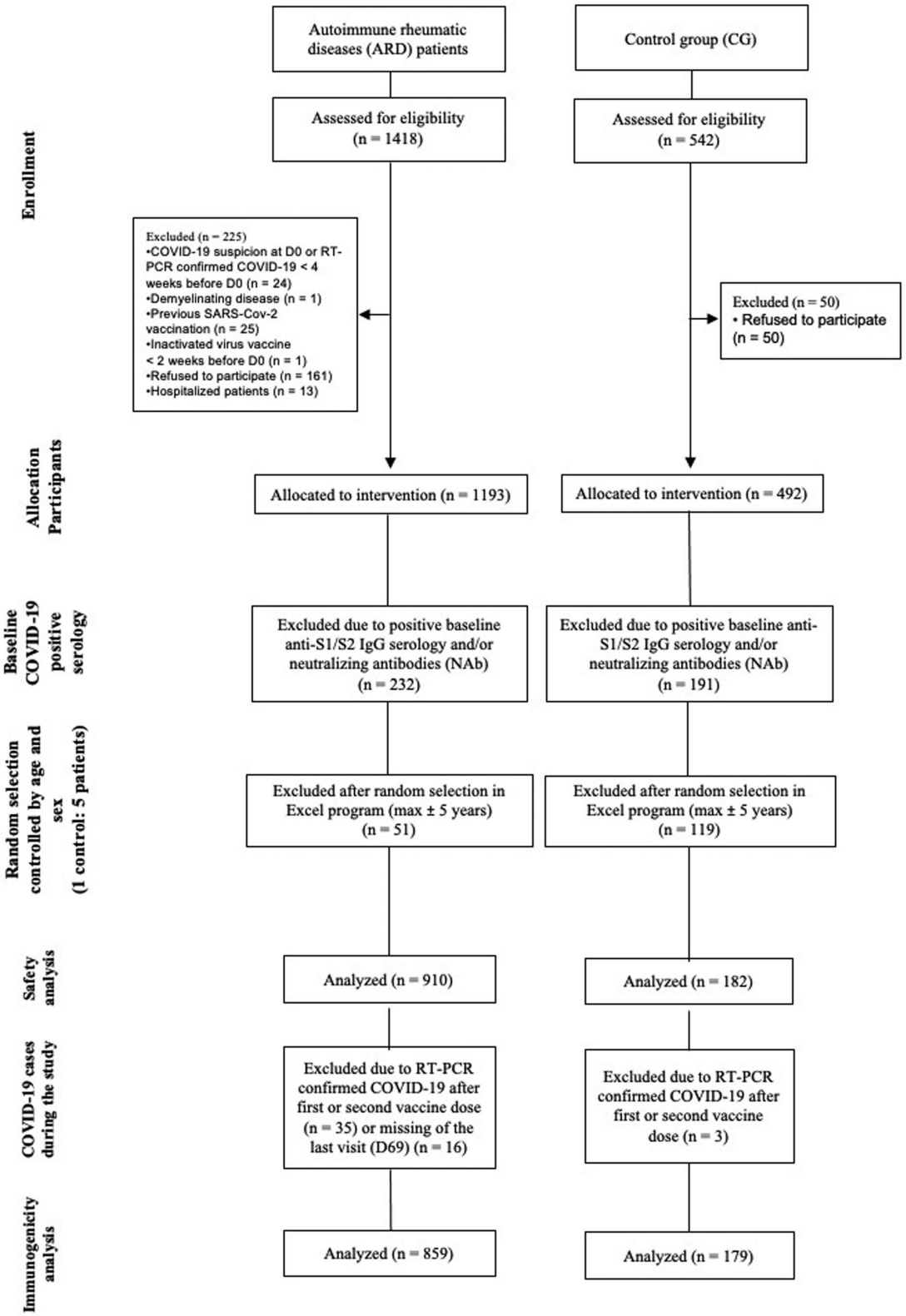

Extended Data Fig. 1 | Trial Design. The diagram depicts the enrollment and analysis of participants in the ARD and CG groups. Reasons for exclusions are provided.

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Last updated by author(s): Jul 15, 2021

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☐	☒ 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
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Software and code

Policy information about availability of computer code

Data collection	The analyzed data were extracted from the patients' electronic medical records (PRONTMED) and Study data were collected and managed using REDCap electronic data capture tools (10.5.0 - © 2021 Vanderbilt University) hosted at our Institution. Data collection for ELISA was performed using Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy. Neutralizing antibodies were performed on the ETI-MAX-3000 equipment (DiaSorin, Italy). No custom software codes have been developed.
Data analysis	All statistical analyses were performed using Statistical Package for the Social Sciences version 20.0 (IBM-SPSS for Windows. 20.0. Chicago, IL, USA)

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
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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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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size calculation was based on the previous 15% reduction of seroconversion rate after primo vaccination with the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of ARD patients35. Expecting seroconversion rates of 63% in the ARD patient's cohort and 78% in the control group, considering an alpha error of 5% and power of 80%, in 5 : 1 ratio in order to include more ARD patients, the minimum sample required would be 445 ARD patients and 89 healthy subjects, sex-matched and with similar ages.
Data exclusions	All safety and immunogenicity data were included in the study. No data were excluded from the analyses.
Replication	This is an ongoing human trial and therefore there was still no attempt of replication.
Randomization	This was an observational study with no randomized intervention. All participants (patients and controls) received the same vaccine, without experimental groups.
Blinding	This phase 4 prospective controlled observational study with no randomized intervention. All participants (patients and controls) received the same vaccine, without placebo group. Therefore, blinding was not performed.

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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

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	Male and female individuals with autoimmune rheumatic disease and volunteers (control group) ≥ 18 anos. The 910 patients with ARD and 182 controls included in immunogenicioty analysis had comparable median ages [51 (40-60) vs. 50 (41-60) years, p=0.985] and female sex (76.9% vs. 76.9%, p>0.999). Three hundred and forty-eight (38.2%) patients were receiving ongoing treatment with prednisone, median dose 5 (5-10) mg/day, 573 (63.0%) were using immunosuppressive drugs [methotrexate (25.2%), leflunomide (14.3%), mycophenolate mofetil (13.1%), azathioprine (12%) and others less than 3% each] and 321 (35.3%) were under biologic therapy.
Recruitment	Autoimmune rheumatic disease (ARD) patients from the Outpatient Rheumatology Clinics at Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil. Control Group (CG) were invited, matching by gender and sex (up to ± 5 years differences). None of them were previously vaccinated in the hospital's regular campaign. Well-controlled medical conditions were allowed in the CG, except ARD, use of immunosuppressive drugs or HIV infection. Overall exclusion criteria were: history of anaphylactic response to vaccine components, acute febrile illness or symptoms compatible to COVID-19 at vaccination, Guillain-Barré syndrome, decompensated heart failure (class III or IV), demyelinating disease, previous vaccination with any SARS-Cov-2 vaccine, history of live virus vaccine up to four weeks before, virus vaccine inactivated up to two weeks before, history of having received blood products up to six months before the study, individuals

who did not accept to participate in the study, hospitalized patients, and pre-vaccination positive COVID-19 serology and/or neutralization antibodies.
All statistical analyses took into account the frequency matching, with exclusion of non-matched subjects. Immunogenitics analysis also excluded incident COVID-19 cases and patients who did not attend the final visit, composing the final sample of 859 patients with ARD and 179 CG. In the logistic regression model (Supplementary Table 2, only with patients with ARD), the age was included in the model using the cut-off > 60 years. This model intended to highlight the known importance of older age in vaccine response.

Ethics oversight

The protocol was conducted according to the Declaration of Helsinki and local regulations and approved by the National and Institutional Ethical Committee of Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil (CAAE: 42566621.0.0000.0068). Written informed consent was obtained from each participant before enrollment.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ClinicalTrials.gov Identifier: NCT04754698
Study protocol	The protocolo has been submitted.The full trial can be assessed at ClinicalTrials.gov
Data collection	The study was conducted at a single tertiary center in Brazil. Enrollment and vaccination occurred in the same day for each participant. The first subject was enrolled and vaccinated on Feb 9th, 2021 and the last participant was enrolled and vaccinated on February 24th, 2021. The vaccination protocol for patients with ARD and controls consisted of a two-dose schedule of the COVID-19 vaccine. The first dose with blood collection was given for most of participants on February 9-10th 2021 (D0), the second dose with blood collection on March 9-10th 2021 (D28) and the last blood collection on April 19th 2021 (D69) at the Hospital Convention Center. Incident COVID-19 cases were assessed for another 10 days to D79. During 2 consecutive days of the 2021 epidemiological week 6th, all ARD patients and CG received the 1st CoronaVac dose, repeated at a 2-dose schedule after 28 days. Blood samples were collected from all participants for quantitative serological testing for SARS-CoV-2. The primary outcome was seroconversion rate (SC) at 6 weeks after the 2nd dose. Geometric meantitles (GMT) and factor increase in GMT (FI-GMT) were also calculated. ARD patients and CG were evaluated using standardized vaccination and COVID-19 symptom diaries, 3 face-to-face visits, and 24-hs available phone, whatsapp and e-mail contact. Symptomatic cases were tested by RT-PCR for SARS-CoV-2 and a subgroup of positive samples were evaluated for the presence of variants of concerns (Gamma, Alpha and Beta lineages).
Outcomes	Immunogenicity and safety of the CoronaVac vaccine in ARDs patients. Primary Outcome Measure: presence of ≥30% of neutralizing activity of SARS-CoV-2 antibodies and seroconversion rate of anti-SARS-Cov-2 IgG antibodies. Secondary outcome: safety of CoronaVac in a large cohort of ARD patients compared with age- and sex-matched controls without these conditions. Incident symptomatic cases confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for SARS-CoV-2 and the presence of variants of concerns (Gamma, Alpha and Beta lineages).

4.5 CoronaVac ajuda a melhorar imunidade em pacientes transplantados, afirma estudo da Unifesp e USP

Um estudo realizado por pesquisadores do Instituto Butantan, da Universidade Federal de São Paulo (Unifesp) e do Hemocentro de Ribeirão Preto da Universidade de São Paulo (USP) mostrou que 43% dos pacientes transplantados de rim geraram anticorpos contra a Covid-19 15 dias após receberem a segunda dose da CoronaVac (ou seja, apresentaram soroconversão). O resultado indica que a vacina do Butantan e da farmacêutica chinesa Sinovac tem desempenho nesse público levemente superior ao de dois outros imunizantes, que utilizam a tecnologia de RNA mensageiro, e geraram anticorpos em pouco mais de 30% dos casos, segundo estudos.

Esses dados mostram a importância da vacina também para todos os imunossuprimidos que, assim como os transplantados e as pessoas com doenças autoimunes, possuem maior dificuldade na defesa imunológica do organismo.

"Toda vacina é menos eficaz em quem é transplantado por causa do uso das medicações contra a rejeição ao transplante. Isso acontece com os imunizantes contra hepatite B, gripe, pneumonia, e também com a vacina contra o coronavírus", explica o principal autor do artigo e professor titular da área de transplantes da Escola Paulista de Medicina da Unifesp, José Medina.

O trabalho foi desenvolvido no Hospital do Rim e seus resultados preliminares foram divulgados em artigo na revista Transplantation, a principal publicação mundial da área de transplantes. A pesquisa foi realizada entre 20 e 28 de março de 2021 com 3.354 pacientes transplantados renais entre 30 e 69 anos, que haviam realizado o transplante há mais de 30 dias, não apresentavam caso anterior de Covid-19 e completaram o esquema vacinal de duas doses da CoronaVac com intervalo de 28 dias.

"Como o número de transplantados é muito pequeno dentro da população em geral, assim que a maioria das pessoas estiver vacinada cairá a circulação do coronavírus, protegendo também os transplantados", afirma Medina. As taxas de soroconversão entre os transplantados renais após a primeira e segunda doses da CoronaVac alertam para a necessidade de manutenção das medidas de proteção individual, como usar máscara, evitar aglomerações e higienizar sempre as mãos.

Os receptores de transplante renal foram incluídos no calendário nacional de vacinação contra a Covid-19 no público prioritário com comorbidades a partir de abril, em função das elevadas taxas de mortalidade associadas ao SARS-CoV-2 nessa população (de até 30%).

As conclusões do estudo da Unifesp se somam a outra pesquisa realizada pelo Hospital das Clínicas da USP, na qual 1000 pacientes com doenças reumatológicas (também imunossuprimidos) foram vacinados com a CoronaVac. A imunização gerou uma resposta imune moderada nos pacientes: o acompanhamento pré e pós vacina mostrou 33 casos de Covid-19 antes da vacinação e apenas seis casos após a imunização.

A eficácia da CoronaVac foi comprovada no Brasil por meio de um estudo com 13.060 voluntários, todos profissionais da saúde, população altamente exposta à Covid-19. Os resultados finais do estudo clínico de fase 3 demonstraram que a eficácia geral do imunizante pode chegar a 62,3% quando o intervalo entre a primeira e a segunda dose da vacina é de 21 a 28 dias. Os dados foram divulgados na plataforma de preprints da revista The Lancet e estão em processo de revisão por pares.

Publicado em: 28/07/2021

Transplantation Publish Ahead of Print

DOI: 10.1097/TP.0000000000003901

Clinical Impact, Reactogenicity, and Immunogenicity After the First CoronaVac Dose in Kidney Transplant Recipients

José Medina-Pestana, PhD,[1] Marina Pontello Cristelli, PhD,[1] Laila Almeida Viana, MD,[1] Renato Demarchi Foresto, MD,[1] Lucio R. Requião-Moura, PhD,[1] Helio Tedesco-Silva, PhD,[1] Dimas Tadeu Covas, PhD[2,3]

[1] Nephrology Division, Hospital do Rim, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil.

[2] Instituto Butantan, São Paulo, Brazil.

3 Center for Cell-based Therapy (CTC), Regional Blood Center of Ribeirão Preto, University of São Paulo, São Paulo, Brazil.

Funding: No funding

Disclosure: The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

Author contributions

J.M.-P., M.P.C., L.A.V., H.T.-S., and D.T.C. participated in the research design; J.M.-P., M.P.C., L.A.V., R.D.F., L.R.R.-M., and H.T.-S. participated in the writing of the paper; and M.P.C., L.A.V., and H.T.-S. participated in data analysis.

Corresponding author: Marina Pontello Cristelli Nephrology Division, Hospital do Rim, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil. Postal address: Borges Lagoa, 960, São Paulo, Brazil 04038-002 E-mail address: ninacristelli@yahoo.com.br Telephone number: +551155768000

Introduction

In phase-3 trial, inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life-Sciences, Beijing, China) was associated with 71.1% seroconversion at least 14-days after the 2nddose, showing 50.7% efficacy against symptomatic COVID-19 among healthcare workers.[1] Currently this vaccine has been approved for emergency use in 24 countries, including Brazil, where the national vaccination program was launched on 01/Jan/2021 following the age criterion.

Kidney transplant recipients have shown 20-30% COVID-19-associated fatality rates,[2] have been excluded in vaccine trials, and had no early priority for vaccination. Therefore, this single-center, prospective, 12-month follow-up study was designed to assess clinical impact, reactogenicity, and immunogenicity of CoronaVac.

Materials and Methods

Between March 20th and 28th 2021, 3354 patients aged 30-69 years, >30 days of transplantation and no previous COVID-19 received standard two-dose schedule of CoronaVac (3µg each dose, 28 days apart). Patients were scheduled to receive the vaccine on 2 consecutive weekends, with approximately 800-900 patients per day, from 7 a.m. to 7 p.m. All communication resources were used to reach them within 3 weeks before the vaccination day (telephone call, SMS text messages, WhatsApp messages). Workstations were set up at the outpatient clinic and patients were admitted in groups of 30 persons to: a) obtain general information regarding COVID-19, the clinical study, the vaccine, and preventive measures; b) inform consent discussion and signature; c) registration of the patient in the electronic medical records of the institution; d) blood sampling for serology followed by vaccination, and a reminder of the scheduled second dose. All employees of the institution were invited to participate in the vaccination campaign, and more than 300 professionals volunteered for the activity, including students from different universities.

The study was approved by the local ethics committee, registered at ClinicalTrials.gov, NCT04801667, and all patients signed an informed consent-form. At day 28, a prespecified questionnaire was obtained to capture adverse reactions to the vaccine or newly diagnosed SARS-CoV-2 infection. Sample-size for the immunogenicity cohort (942 patients seronegative for IgG anti-SARS-CoV-2 before first dose) was calculated using the age distribution and seroconversion rate (71%) of the phase-3 study,[1] with 95%CI and an absolute error of 10%. Antibody response at day 28 was assessed using the AdviseDx SARS-CoV-2 IgG II assay (Abbot Laboratories, Il, USA). Values >50 arbitrary units (AUs)/mL were considered positive.[3]

Results

Characteristics and outcomes of the study population (n=3354) are in Table 1. They were predominantly male, median age of 52 (interquartile range, IQR 44-60) years, low prevalence of diabetes mellitus, and median time posttransplant of 7 (IQR 3 – 12) years. Seroprevalence of IgG anti-SARS-CoV-2 nucleocapsid protein at D0 was 3.6%, and these seropositive patients at the time of vaccine were excluded for the analysis of the antibody responses. Among the seronegative patients at D0, there were 1012 individuals randomly selected for the immunogenicity analysis. The other patients did not have any testing performed after the vaccination. After the first vaccine dose, 61 (1.8%) patients had COVID-19 confirmed by RT-PCR or antigen-test at a median time of 12 (IQR 8-16) days. Of them, 44 (72%) required hospitalization and 16 (26%) died 14-49 days after the first vaccine dose.

The most common adverse-reaction was local pain/tenderness (11%). Systemic symptoms occurred in 5% or less of the patients; no severe adverse reaction was observed. There was only one episode of acute cellular rejection (Banff IB) 6 days after vaccination in a patient with documented nonadherence that showed partial recovery of renal function after treatment with methylprednisone and anti-thymocyte globulin. Seroconversion 28 days after the 1st dose was 15.2% (95%CI 12.9%-17.5%), median IgG value of 477 AUs/mL (IQR 123-1705). Patients over 60-years and combined kidney-pancreas-transplants had lower seroconversion than those younger than 60-years and isolated kidney-transplants.

Discussion

The potential advantage of the traditional inactivated vaccines, the induction of a broader polyclonal immune response,[4] was not associated with a higher seroconversion rate compared to the newer RNA-based COVID-19 vaccines.[5] In this ongoing prospective study, there was no obvious clinical impact after the first dose, as demonstrated by the 26% lethality rate, similar to that of unvaccinated kidney transplant recipients.[2] CoronaVac vaccine was safe, but seroconversion after the first dose was low, similar to what was reported to the RNA-based vaccines. The elderly showed even lower rates of seroconversion. These findings support the need for maintaining individual protection measures, even after the 1st dose of the vaccine.

Acknowledgments

This work would not be possible without the provision of the vaccines, coordinated by Ricardo Palacios, MD, PhD and Roberta Piorelli, MD from Instituto Butantan, the volunteer work of almost 300 students and professionals of the Hospital do Rim and other medical institutions in São Paulo during vaccination, and the valuable contribution of our biochemist Elizabeth França Lucena, BA, who conducted all the laboratory analysis.

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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)			
Sore throat	65 (2)			
Fever	39 (1)			
Serologic status before vaccination, n (%)				
Negative	3182 (95)	942	-	-
Positive	122 (4)	0	-	-
Indeterminate	50 (1)	0	-	-
Serologic status after the 1st dose, n (%)				
Negative (<50 AUs/mL)	-	799 (85)		
Positive[a]	-	143 (15); 95% CI, 13%–17%		
30–60 y, n (%)		134 (16); 95% CI, 14%–19%		
>60 y, n (%)		9 (8); 95% CI, 3%–13%		

[a]$P = 0.026$ for comparison between the two-age range.

AZA, azathioprine; COVID-19, coronavirus disease 2019; CSA, cyclosporine; IgG, immunoglobulin G; IQR, interquartile range; MPA, mycophenolate; mTORi, mammalian target of rapamycin inhibitors; Pred, prednisone; TAC, tacrolimus.

4.6 Pacientes com doenças reumáticas autoimunes que já tiveram Covid-19 podem estar protegidos com uma única dose de CoronaVac, sugere estudo

Um estudo publicado na revista The Lancet Rheumatology na última sexta (3) mostrou que uma única dose de CoronaVac, vacina do Butantan e da Sinovac, pode ser suficiente para promover uma resposta imune robusta em pacientes com doenças reumáticas autoimunes que foram previamente infectados pelo SARS-CoV-2. A pesquisa foi conduzida no Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (FMUSP).

De acordo com a pesquisa, 95% dos 157 pacientes que já tinham contraído Covid-19 e foram imunizados com a CoronaVac produziram uma quantidade média expressiva de anticorpos IgG após a primeira dose. Após a segunda dose, o indicador saltou para 98% dos voluntários.

Os pesquisadores também analisaram 471 indivíduos com doenças reumáticas que nunca tinham tido contato com o coronavírus. A imunização completa com as duas doses da vacina nesse grupo induziu a produção de anticorpos em 75% dos participantes.

Participaram do estudo 1.193 pacientes e 492 controles. Após seleção aleatória de amostras, foram analisadas 942 pessoas (157 com sorologia positiva e 471 com sorologia negativa). Ambos os grupos também contaram, cada um, com 157 indivíduos controles.

Os pesquisadores coletaram amostras sanguíneas dos voluntários imediatamente antes da primeira dose (dia zero), antes da segunda dose (dia 28) e decorridos 69 dias da primeira dose (ou 40 dias da segunda dose).

Memória imunológica

Os resultados do artigo da USP apoiam outras pesquisas feitas com indivíduos com doenças reumáticas autoimunes com sorologia positiva e negativa para Covid-19, que mostram que vacinas de RNA mensageiro e adenovírus induzem o mesmo padrão de resposta imune observado no estudo com a CoronaVac. "Um possível mecanismo que explica essa resposta robusta em quem já teve Covid-19 está relacionado às células B de memória pré-existentes, porque a exposição recorrente é conhecida por gerar respostas mais extensas do que uma infecção primária", apontam os autores do artigo.

Publicado em: 03/12/2021

Immunogenicity and safety of two doses of the CoronaVac SARS-CoV-2 vaccine in SARS-CoV-2 seropositive and seronegative patients with autoimmune rheumatic diseases in Brazil: a subgroup analysis of a phase 4 prospective study

Nadia E Aikawa, Leonard V K Kupa, Sandra G Pasoto, Ana C Medeiros-Ribeiro, Emily F N Yuki, Carla G S Saad, Tatiana Pedrosa, Ricardo Fuller, Samuel K Shinjo, Percival D Sampaio-Barros, Danieli C O Andrade, Rosa M R Pereira, Luciana P C Seguro, Juliana M L Valim, Filipe Waridel, Ana Marli C Sartori, Alberto J S Duarte, Leila Antonangelo, Ester C Sabino, Paulo Rossi Menezes, Esper G Kallas, Clovis A Silva, Eloisa Bonfa

Summary

Background We aimed to examine the immunogenicity pattern induced by the inactivated SARS-CoV-2 vaccine CoronaVac (Sinovac Life Sciences, Beijing, China) in SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases compared with seropositive controls, seronegative patients with autoimmune rheumatic diseases, and seronegative controls.

Methods CoronavRheum is an ongoing, prospective, controlled, phase 4 study, in which patients aged 18 years or older with autoimmune rheumatic diseases, and healthy controls were recruited from a single site (Rheumatology Division of Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo) in São Paulo, Brazil. Participants were vaccinated with two doses of CoronaVac (intramuscular injection, 3 μg in 0·5 mL of β-propiolactone inactivated SARS-CoV-2) on day 0 and on day 28. Blood samples were taken pre-vaccination on day 0, day 28, and also on day 69. For this subgroup analysis, participants were defined as being SARS-CoV-2 seropositive or seronegative prevaccination via anti-SARS-CoV-2 spike (S)1 or S2 IgG (cutoff of 15·0 arbitrary units [AU] per mL) or neutralising antibody titres (cutoff of ≥30%) and were matched for age and sex, via convenience sampling, in a 1:3:1:1 ratio (seropositive patients to seronegative patients to seropositive controls to seronegative controls). The primary outcomes were rates of anti-SARS-CoV-2 S1 and S2 IgG seropositivity and SARS-CoV-2 neutralising antibody positivity at day 28 and day 69 and immunogenicity dynamics assessed by geometric mean titres (GMTs) of IgG and median neutralising activity in seropositive patients with autoimmune rheumatic diseases compared with seronegative patients and seropositive and seronegative controls. We assessed safety in all participants randomly selected for this subgroup analysis. This study is registered with ClinicalTrials.gov, NCT04754698, and is ongoing for long-term immunogenicity evaluation.

Findings Between Feb 4 and Feb 8, 2021, 1418 patients and 542 controls were recruited, of whom 1685 received two vaccinations (1193 patients and 492 controls). After random sampling, our immunogenicity analysis population comprised 942 participants, of whom 157 were SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases, 157 were seropositive controls, 471 were seronegative patients, and 157 were seronegative controls; the median age was 48 years (IQR 38–56) and 594 (63%) were female and 348 (37%) were male. For seropositive patients and controls, an increase in anti-SARS-CoV-2 S1 and S2 IgG titres (seropositive patients GMT 52·3 [95% CI 42·9–63·9] at day 0 *vs* 128·9 [105·6–157·4] at day 28; seropositive controls 53·3 [45·4–62·5] at day 0 *vs* 202·0 [174·8–233·4] at day 28) and neutralising antibody activity (seropositive patients 59% [IQR 39–83] at day 0 *vs* 82% [54–96] at day 28; seropositive controls 58% [41–79] at day 0 *vs* 92% [79–96] at day 28), was observed from day 0 to day 28, without further increases from day 28 to day 69 (at day 69 seropositive patients' GMT was 137·1 [116·2–161·9] and neutralising antibody activity was 79% [57–94]); and seropositive controls' GMT was 188·6 [167·4–212·6] and neutralising antibody activity was 92% [75–96]). By contrast, for seronegative patients and controls, the second dose was required for maximum response at day 69, which was lower in seronegative patients than in seronegative controls. GMTs in seronegative patients were 2·3 (95% CI 2·2–2·3) at day 0, 5·7 (5·1–6·4) at day 28, and 29·6 (26·4–33·3) at day 69, and in seronegative controls were 2·3 (2·1–2·5) at day 0, 10·6 (8·7–13·1) at day 28, and 71·7 (63·5–81·0) at day 69; neutralising antibody activity in seronegative patients was 15% (IQR 15–15) on day 0, 15% (15–15) at day 28, and 39% (15–65) at day 69, and in seronegative controls was 15% (15–15) at day 0, 24% (15–37) at day 28, and 61% (37–79) at day 69. Neither seronegative patients nor seronegative controls reached the GMT or antibody activity levels of seropositive patients at day 69.

Interpretation By contrast with seronegative patients with autoimmune rheumatic diseases, seropositive patients have a robust response after a single dose of CoronaVac. Our findings raise the possibility that the reduced immunogenicity observed in seronegative patients might not be the optimum response potential to SARS-CoV-2 vaccination, and therefore emphasise the importance of at least a single booster vaccination in these patients.

Lancet Rheumatol 2021

Published Online December 3, 2021 https://doi.org/10.1016/S2665-9913(21)00327-1

See Online/Comment https://doi.org/10.1016/S2665-9913(21)00392-1

For the Portuguese translation see Online for appendix 1

Rheumatology Division (N E Aikawa PhD, L V K Kupa PhD, S G Pasoto PhD, A C Medeiros-Ribeiro PhD, E F N Yuki PhD, C G S Saad PhD, T Pedrosa PhD, R Fuller PhD, S K Shinjo PhD, P D Sampaio-Barros PhD, D C O Andrade PhD, Prof R M R Pereira PhD, L P C Seguro PhD, J M L Valim MD, F Waridel, Prof C A Silva PhD, Prof E Bonfa PhD), **Pediatric Rheumatology Unit, Instituto da Criança e do Adolescente** (N E Aikawa, Prof C A Silva), **Department of Infectious and Parasitic Diseases** (A M C Sartori PhD, Prof E G Kallas), **and Central Laboratory Division** (Prof A J S Duarte, L Antonangelo PhD), **Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Instituto de Medicina Tropical** (E C Sabino PhD), **and Department of Preventive Medicine** (Prof P R Menezes), **Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil**

Correspondence to: Prof Eloisa Bonfa, Rheumatology Division, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo 01246-903, Brazil **eloisa.bonfa@hc.fm.usp.br**

Funding Fundação de Amparo à Pesquisa do Estado de São Paulo, Conselho Nacional de Desenvolvimento Científico e Tecnológico, and B3-Bolsa de Valores do Brasil.

Introduction

In June, 2021, WHO recommended the emergency use of the inactivated SARS-CoV-2 vaccine, CoronaVac (Sinovac Life Sciences, Beijing, China),[1] which has shown a high level of protection against COVID-19-related hospitalisation and death in the Chilean population.[2] As of Aug 1, 2021, only a quarter of the Brazilian population had received two doses of a SARS-CoV-2 vaccine and CoronaVac accounted for approximately 38% of all SARS-CoV-2 vaccines administered in Brazil.[3]

Previously, we have shown a seroconversion rate of 70·4% with two doses of CoronaVac in adults with autoimmune rheumatic diseases without previous SARS-CoV-2 infection, compared with 95·5% in controls, with a diminished frequency of COVID-19 incident cases after vaccination.[4]

New SARS-CoV-2 variants are emerging and vaccine supply is still restricted worldwide. Therefore, improving strategies to maximise vaccine coverage and enhance immunogenicity is crucial, especially in immunosuppressed populations. A few recent reports, including some preprints, have shown that antibody responses to the first dose of mRNA-based SARS-CoV-2 vaccines in people with previous laboratory-confirmed SARS-CoV-2 infection were similar to or exceeded those found in individuals without previous infection after the second dose,[5–10] raising the possibility of allocating vaccine to other at-risk groups.

However, data are scarce on immune responses to SARS-CoV-2 vaccines in the context of previous SARS-CoV-2 infection in patients with autoimmune rheumatic diseases; a population known to have reduced virus clearance and to be prone to genomic evolution.[11] It is crucial to investigate whether immunogenicity of previous SARS-CoV-2 infection in this population might surpass that of patients without previous SARS-CoV-2 infection who have received two doses, or if humoral response will be limited by an intrinsic defect of these patients' immune system or immunosuppressive treatment, as previously described.[12,13] A study in patients with autoimmune diseases showed that a single dose of mRNA-based or adenovirus-based SARS-CoV-2 vaccine in those with previous SARS-CoV-2 infection could elicit antibody responses similar to two vaccine doses in patients without previous infection, with seroconversion in the vast majority of patients on any immunosuppressive treatment.[14] However, the small sample size of the seropositive group, heterogeneous schedules for blood collection, and the absence of serial samples hampered a definitive conclusion on the kinetics of humoral response.[14] Understanding antibody kinetics is even more relevant in the context of the approval of a third

Research in context

Evidence before this study

Pre-existing immunity for COVID-19 affects vaccine response and might allow a change in the current vaccination guidelines, allowing for increased vaccine availability. We searched PubMed for publications between Dec 1, 2020, and Aug 27, 2021, for studies published in English on COVID-19 vaccines in patients with autoimmune rheumatic disease, using the terms "seropositive" AND ("vaccination" OR "vaccine") AND ("COVID-19" OR "SARS-CoV-2") AND ("autoimmune" OR "rheumatic"). Few reports suggested that one dose of mRNA-based SARS-CoV-2 vaccine could elicit a large antibody response in SARS-CoV-2 seropositive individuals, with no further increase in antibody response after the second dose. However, we found no studies with data for inactivated SARS-CoV-2 vaccines and little information on patients with autoimmune rheumatic diseases, in whom immunogenicity is known to be reduced. Moreover, only few studies have focused on immunological analysis of neutralising antibodies, which are relevant in immune protection against SARS-CoV-2 infection.

Added value of this study

This study provides the first evidence that previous exposure to SARS-CoV-2, independent of symptoms, in patients with autoimmune rheumatic diseases results in distinct dynamics of antibody response (measured via anti-SARS-CoV-2 spike antibody titres and neutralising antibody activity) to an inactivated SARS-CoV-2 vaccine (CoronaVac; Sinovac Life Sciences, Beijing, China) compared with patients without previous exposure. Our study expands on previous reports in healthy individuals and a small sample of seropositive patients with autoimmune rheumatic diseases immunised with mRNA-based or adenovirus-based SARS-CoV-2 vaccines, in that seropositive patients showed a robust boost in antibody response after the first dose of inactivated vaccine, independent of their underlying disease or treatment. No further increase in response was observed between the first and second dose, and the antibody response remained up to 6 weeks after the second dose.

Implications of all the available evidence

The CoronaVac vaccine presents distinct kinetics of immune response in seropositive patients with autoimmune rheumatic diseases compared to seronegative patients. Our finding raises the possibility that the reduced immunogenicity observed in seronegative patients might not represent the optimum response potential and suggest that these patients might benefit from booster doses.

SARS-CoV-2 vaccine dose for immunocompromised individuals in some countries.[15]

To add to this knowledge, we assessed the dynamics of antibody production induced by the inactivated CoronaVac vaccine in patients with autoimmune rheumatic disease who were SARS-CoV-2 seropositive and those who were SARS-CoV-2 seronegative compared with SARS-CoV-2 seropositive and seronegative controls.

Methods

Study design and participants

This is a retrospective subgroup analysis of a large ongoing prospective, controlled, phase 4 study (CoronavRheum) of immunogenicity and safety of two doses of the inactivated SARS-CoV-2 vaccine CoronaVac in patients with autoimmune rheumatic diseases[4] being conducted in a single site (Rheumatology Division of Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo) in São Paulo, Brazil, to assess the dynamics of response to this SARS-CoV-2 inactivated vaccine in patients with autoimmune rheumatic diseases who are seropositive for SARS-CoV-2-specific antibodies at baseline compared with those who are seronegative at baseline and with controls.

For the main trial, patients with autoimmune rheumatic diseases from our outpatient rheumatology clinics in São Paulo, Brazil, were consecutively invited to participate in the study if they were aged 18 years or older and if they fulfilled the classification criteria for one of the following autoimmune rheumatic diseases: rheumatoid arthritis, systemic lupus erythematosus, spondiloarthritis, vasculitis, primary Sjogren's syndrome, systemic sclerosis, systemic autoimmune myopathies, and primary antiphospholipid syndrome. Additionally, hospital services workers, health professionals, and hospital administrative service employees or their relatives without autoimmune rheumatic disease and not taking immunosuppressive therapy were recruited to comprise the healthy control group. Exclusion criteria were in accordance to our previous report.[4] Key exclusion criteria were history of anaphylactic response to vaccine components, acute febrile illness or symptoms compatible with COVID-19 at vaccination, decompensated heart failure (class III or IV), demyelinating disease, previous vaccination with any SARS-CoV-2 vaccine, history of live virus vaccine up to 4 weeks before enrolment, receipt of inactivated virus vaccine up to 2 weeks before enrolment, patients who were being treated in hospital for any reason, and not providing consent to participate.

The study protocol was approved by the National and Institutional Ethical Committee (CAAE: 42566621.0.0000.0068) and written informed consent was obtained from all participants.

Procedures

The CoronaVac COVID-19 vaccine (batch number 20200412, Sinovac Life Sciences, Beijing, China) used in this study was supplied by the Instituto Butantan (São Paulo, Brazil). Patients and controls were vaccinated in a two-dose schedule, via intramuscular injection with 3 μg of vaccine in 0·5 mL of β-propiolactone inactivated SARS-CoV-2. The first dose and blood collection were done for most participants on Feb 9–10, 2021 (day 0), the second dose with blood collection was done on March 9–10, 2021 (day 28), and the last blood collection was done on April 19, 2021 (day 69) at the hospital convention center. For this subgroup analysis, incident COVID-19 cases were assessed from day 0 to day 79.

Laboratory tests were done at the central laboratory division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (supervised by AJSD and LS). Human IgG antibodies against the SARS-CoV-2 spike (S) 1 and S2 proteins were measured using a chemiluminescent immunoassay (Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). The lower limit of quantification of the assay was 3·8 UA/mL and seropositivity was defined as anti-SARS-CoV-2 (S1/S2) IgG of more than 15·0 UA/mL. For titres below the limit of quantification, a value of 1·9 UA/mL was assigned.

A SARS-CoV-2 neutralising antibody assay was done using the cPass SARS-CoV-2 neutralisation antibodies detection kit (GenScript, Piscataway, NJ, USA). Results are expressed as positive or negative neutralising antibodies according to the manufacturer recommended cutoff of percentage signal inhibition (≥30% inhibition).[16] Medians and IQRs of the percentage of neutralising activity were calculated at all timepoints (at day 0, day 28 and day 69), attributing the value of 15% (half of positive inhibition cutoff) to undetectable levels (<30%).

The study was monitored by independent vaccine experts, who comprised the Data Safety Monitoring Board. Local and systemic vaccine-related adverse effects were carefully reviewed with each participant at in-person visits on day 28 and day 69, as previously reported.[2] Vaccine adverse effect severity was ranked according to WHO definitions.[17] 24 h access to the medical team was available to all participants, including telephone contacts, email, and WhatsApp messages for safety support, from day 0 until day 69.

All participants completed a standardised questionnaire to assess their history of SARS-CoV-2 infection at baseline (appendix 2 p 8). Reports of any previous positive RT-PCR test were requested. Social risk factors associated with increased risk of exposure to SARS-CoV-2 were also registered by all participants. Incident cases were defined as new cases of symptomatic SARS-CoV-2 infection, confirmed with RT-PCR between day 0 and day 79.[4] All positive samples tested at our site were further characterised for variants of concern at the same hospital. RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions, as previously described.[18]

For this subgroup analysis, seronegative and seropositive patients with autoimmune rheumatic disease and

See Online for appendix 2

seropositive and seronegative controls were selected from the main cohort. Patients with pre-vaccination positive COVID-19 serology (ie, anti-S1 or S2 IgG or neutralising antibodies) were classified as being seropositive patients or controls and those with pre-vaccination negative COVID-19 serology were classified as seronegative patients or controls.

Outcomes

The primary outcomes were rates of anti-SARS-CoV-2 S1 and S2 IgG seropositivity and SARS-CoV-2 neutralising antibody positivity at day 28 and day 69 and immunogenicity dynamics were assessed by median neutralising activity (ie, activity of neutralising antibodies) and by geometric mean titres (GMTs) of anti-SARS-CoV-2 S1 and S2 IgG and median neutralising antibody activity in SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases compared with seronegative patients and seropositive and seronegative controls.

Secondary outcomes were the influence of previous (ie, prevaccination) symptomatic versus asymptomatic SARS-CoV-2 infection ascertained by RT-PCR or rapid antigen test on vaccine-induced antibody response, antibody dynamics in patients who had symptomatic SARS-CoV-2 infection within the past 3 months (inclusive) versus more than 3 months previously, and vaccine safety.

Exploratory outcomes were prevalence of RT-PCR positive test results among participants (ie, COVID-19 incident cases), analysis of variants of concern, and analysis of infection severity and of social risk factors associated with exposure to SARS-CoV-2.

We did post-hoc analyses of demographic and disease-specific factors associated with anti-SARS-CoV-2 S1 and S2 IgG seropositivity and neutralising antibody positivity at day 28 in seropositive patients, and comparison of vaccine-induced anti-SARS-CoV-2 antibody seropositivity between previously asymptomatic patients and seronegative patients.

Statistical analysis

All treatment groups in this subgroup analysis were selected via convenience sampling from the large phase 4 prospective cohort CoronavRheum.[4] Seronegative and seropositive patients with autoimmune rheumatic disease and seropositive and seronegative controls were selected from the main cohort, in a 1:3:1:1 ratio, matched for age (up to 5 years difference) and sex using an in-house program run on Excel (Microsoft 2018) for random selection of individuals in each category.

We present categorical variables as n (%), continuous variables as median (IQR), and anti-SARS-CoV-2 S1 and S2 IgG serology titres as geometric means (95% CI). We did statistical comparisons between groups using the χ^2 test or Fisher's exact test for categorical variables and Student's *t* test or the Mann-Whitney *U* test for continuous variables. We transformed anti-SARS-CoV-2 S1 and S2 IgG titre data in natural logarithm(ln) before analysis, and we describe the values of ln(IgG) titres and neutralising antibodies according to groups (seropositive and seronegative patients with autoimmune rheumatic diseases and seropositive and seronegative controls) and at each assessment timepoint (day 0, day 28, and day 69). We compared ln-transformed anti-SARS-CoV-2 S1 or S2 IgG titres and neutralising antibody activity between groups and between timepoints (day 0, day 28, and day 69) using generalised estimating equations with normal marginal distribution (for IgG titres) and gamma distribution (for neutralising antibodies) and identified binding function assuming first order autoregressive correlation matrix between time-points. We did Bonferroni multiple comparisons to identify differences between groups and timepoints.

The primary outcomes and post-hoc analysis of factors associated with anti-SARS-CoV-2 S1 and S2 IgG seropositivity and neutralising antibody positivity at day 28 were assessed in all participants who were selected as part of random sampling. Secondary outcomes were assessed in all participants who received vaccine, before random sapling. We assessed incident case surveillance in all participants of CoronavaRheum of data cutoff (April 29, 2021) from day 0 to day 79. Participants with RT-PCR-confirmed previous SARS-CoV-2 infection between day 0 and day 69 were excluded from the immunogenicity analyses, but were included in incident case surveillance (from day 0 to day 79).

We assessed vaccine safety among all the participants who were randomly selected for this subgroup analysis. We did this by analysing reports of any vaccine side-effect and the reviewing the standardised diary completed by the participants, including local and systemic manifestations. Vaccine-related adverse effects were carefully reviewed with each participant at in-person visits on day 28 and day 69.

We did all analyses using the IBM-SPSS for Windows (version 22.0) and we made graphs of mean profiles and SEs using the Microsoft-Excel 2010 software. The tests were performed with a significance level of 5%. This study is registered with ClinicalTrials.gov, NCT04754698

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Instituto Butantan supplied the study product and had no other role in the trial.

Results

Between Feb 4 and Feb 8, 2021, 1418 patients and 542 controls were recruited to CoronavRheum, of whom 1193 patients and 492 controls attended three study visits that occurred on Feb 9–10, 2021 (day 0), on March 9–10, 2021 (day 28), and on April 19, 2021 (day 69), and received two doses of inactivated SARS-CoV-2 vaccine on days 0 and 28. Of the 1685 participants who received both doses of CoronaVac, 86 were excluded from further analyses because they became infected with SARS-CoV-2 during the

study or did not have available data for analysis (figure 1). After applying the exclusion criteria and random sampling, the final study groups for this immunogenicity analysis comprised 942 participants, of whom 157 were SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases, 157 were seropositive controls, 471 were seronegative patients with autoimmune rheumatic diseases, and 157 were seronegative controls (figure 1).

In the analysable population, the median age was 48 years (IQR 38–56) and 594 (63%) were female and 348 (37%) were male. Participant groups were comparable with regards to baseline age, sex, and ethnicity distribution (table 1) . A shorter disease duration was observed in SARS-CoV-2 seropositive patients with autoimmune rheumatic disease than in seronegative patients (p=0·011; table 1). Disease and treatment distributions were similar between seropositive and seronegative patients (table 1).

A high proportion of seropositive patients and controls had anti-SARS-CoV-2 S1 or S2 IgG seropositivity at day 28 (149 [95%] of 157 *vs* 155 [99%] of 157; p=0·10) and these proportions remained high at day 69 (154 [98%] *vs* 157 [100%]; p=0·25) with comparable seropositivity rates at both timepoints (table 2). In the seropositive patient and control groups we also observed high proportions of participants with neutralising antibody positivity at day 28 (138 [88%] *vs* 151 [96%]; p=0·0067), which was sustained at day 69 (141 [90%] *vs* 155 [99%]; p=0·0005); although, a lower proportion of patients were neutralising antibody positive than controls.

A distinct pattern was detected for seronegative patients with autoimmune rheumatic diseases, with a low proportion of patients having anti-SARS-CoV-2 S1 or S2 IgG seropositivity (99 [21%] of 471) and neutralising antibody positivity (108 [23%]) at day 28, and the second dose was required to obtain moderate proportions with

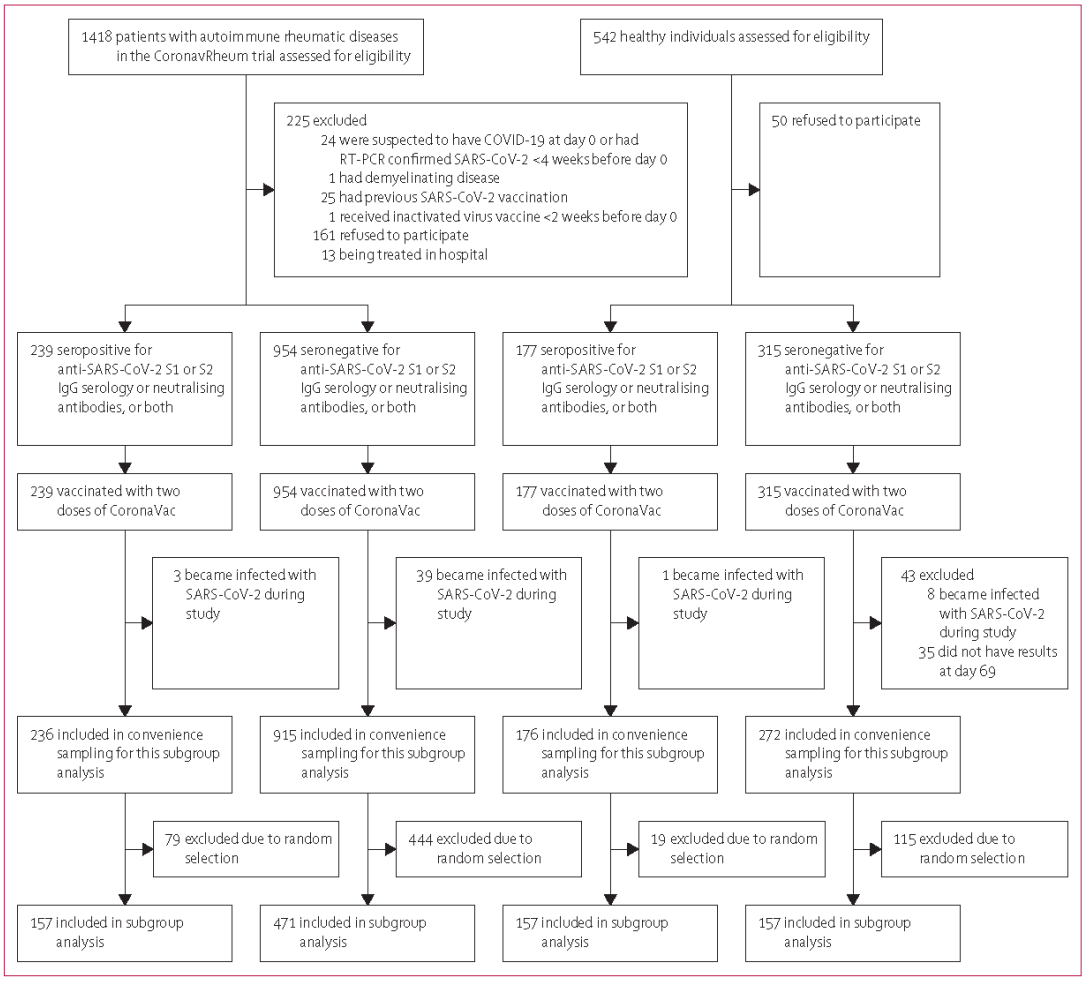

Figure 1: Study profile
S=spike.

	SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases (n=157)	SARS-CoV-2 seronegative patients with autoimmune rheumatic diseases (n=471)	SARS-CoV-2 seropositive controls (n=157)	SARS-CoV-2 seronegative controls (n=157)	p value
Demographic data					
Age, years					
Median	48 (38–57)	48 (38–56)	48 (36–56)	48 (38–57)	0·98
>65	4 (3%)	12 (3%)	4 (3%)	7 (4%)	>0·999
At diagnosis	33 (22–43)	30 (22–40)	..	..	0·11
Disease duration, years	12 (7–19)	14 (8–22)	..	..	0·011
Sex	..	..	..	..	>0·999
Female	99 (63%)	297 (63%)	99 (63%)	99 (63%)	..
Male	58 (37%)	174 (37%)	58 (37%)	58 (37%)	..
Race	..	..	..	..	0·12
White	78 (50%)	234 (50%)	58 (37%)	76 (48%)	..
African-Latin American	76 (48%)	226 (48%)	95 (61%)	74 (47%)	..
Asian	1 (1%)	7 (1%)	4 (3%)	4 (3%)	..
Indigenous Brazilian	2 (1%)	4 (1%)	0	3 (2%)	..
Clinical data					
Autoimmune rheumatic disease					
Rheumatoid arthritis	39 (25%)	125 (27%)	..	..	0·68
Axial spondyloarthritis	32 (20%)	80 (17%)	..	..	0·34
Psoriatic arthritis	16 (10%)	56 (12%)	..	..	0·56
Systemic lupus erythematosus	37 (24%)	115 (24%)	..	..	0·83
Systemic vasculitis	10 (6%)	32 (7%)	..	..	0·85
Systemic autoimmune myopathy	6 (4%)	20 (4%)	..	..	>0·999
Systemic sclerosis	7 (4%)	13 (3%)	..	..	0·29
Primary Sjögren's syndrome	6 (4%)	16 (3%)	..	..	0·80
Primary antiphospholipid syndrome	4 (3%)	13 (3%)	..	..	>0·999
Current therapies					
Hydroxychloroquine	44 (28%)	127 (27%)	..	..	0·80
Sulfasalazine	20 (13%)	45 (10%)	..	..	0·26
Prednisone	47 (30%)	182 (39%)	..	..	0·050
Dose, mg per day	6 (5–10)	5 (5–10)	..	..	0·21
Immunosuppressive drugs	94 (60%)*	296 (63%)	..	..	0·51
Methotrexate	44 (28%)	135 (29%)	..	..	0·88
Leflunomide	18 (11%)	57 (12%)	..	..	0·83
Mycophenolate mofetil	16 (10%)	55 (12%)	..	..	0·61
Azathioprine	15 (10%)	49 (10%)	..	..	0·76
Other†	8 (5%)	19 (4%)	..	..	0·57
Biologic agent	53 (34%)	174 (37%)	..	..	0·47
TNF inhibitor	27 (17%)	81 (17%)	..	..	>0·999
Abatacept	5 (3%)	20 (4%)	..	..	0·56
Secukinumab	11 (7%)	21 (4%)	..	..	0·21
Other‡	10 (6%)	49 (10%)	..	..	0·13

Data are n (%) or median (IQR). p values are calculated using data across all groups where possible, and only between the seropositive and seronegative patients for rheumatic disease characteristics. Categorical variables were compared between groups using the χ^2 test or Fisher's exact test and all continuous variables were compared using the Mann-Whitney *U*. *Sums to more than the patient numbers provided because seven patients were taking more than one immunosuppresive drug. †Cyclophosphamide, cyclosporin, tacrolimus, and tofacitinib. ‡Tocilizumab, rituximab, belimumab, and ustekinumab.

Table 1: **Baseline demographic and clinical characteristics of SARS-CoV-2 seropositive and seronegative patients with autoimmune rheumatic diseases and seropositive and seronegative controls**

anti-SARS-CoV-2 S1 or S2 IgG seropositivity (353 [75%]) and neutralising antibody positivity (289 [61%]) at day 69. Likewise, seronegative controls also needed two doses to reach a moderate response at day 69 (proportion with IgG seropositivity was 57 [36%] of 157 at day 28 and 150 [96%] at day 69; neutralising antibody positivity was

56 [36%] at day 28 and 128 [82%] at day 69; table 2). The proportion of seronegative patients who had a response was significantly lower than among seropositive patients at day 28 (p<0·0001) and day 69 (p<0·0001). Also, the proportion of seronegative controls with IgG seropositivity and neutralising antibody positivity was lower than among seropositive patients at day 28 (p<0·0001) but not at day 69 (p=0·34), and the proportion who had neutralising antibody positivity was lower at day 28 (p<0·0001) and day 69 (p=0·036; table 2).

Seropositive patients and controls had similar vaccine-induced antibody dynamics, with substantial increases from day 0 to day 28 and no further increase from day 28 to day 69 (table 3, figure 2; appendix 2 pp 2–3).

We observed changes from day 0 to day 28 in seronegative patients for anti-SARS-CoV-2 S1 or S2 IgG GMTs (from 2·3 arbitrary units [AU]/mL [95% CI 2·2–2·3] to 5·7 [5·1–6·4]; table 3, figure 2 [data presented as ln(IgG)]) and for neutralising antibody activity (15% [IQR 15–15] to 15% [15–15]; table 3; appendix 2 pp 2–3). A substantial increase was seen in anti-SARS-CoV-2 S1 or S2 IgG GMTs from day 28 to day 69 for seronegative patients (from 5·7AU/mL [95% CI 5·1–5·4] to 29·6 AU/mL [26·4–33·3]). A similar increase was observed for neutralising antibody activity from day 28 to day 69 (15% [IQR 15–15] to 39% [15–65]; table 3; appendix 2 pp 1–2). Seronegative controls had a similar pattern, with minor increases after the first dose and substantial increases after the second dose for both anti-SARS-CoV-2 S1 or S2 IgG GMTs and neutralising antibody activity (table 3; appendix 2 pp 1–2). Significantly lower proportions of seronegative patients had IgG seropositivity and neutralising antibody positivity at day 28 and day 69 than did seronegative controls (table 2).

In line with these findings, when the groups were compared at different timepoints, seropositive patients and controls had similar IgG titres at day 0 (p>0·999) and day 69 (p=0·41) but titres were higher in seropositive controls at day 28 (p=0·0080; table 3). For neutralising antibody activity, the values were similar at day 0 (p>0·999), day 28 (p=0·119), and day 69 (p=0·300; table 3). By contrast, seropositive patients had significantly higher values than seronegative patients at all timepoints for IgG GMTs and neutralising antibody activity (table 3). Seropositive patients also had significantly higher IgG GMTs and neutralising antibody activity than did seronegative controls at all timepoints (table 3; appendix 2 pp 1–2).

In a post-hoc analysis, we found no significant associations between demographic data and specific autoimmune rheumatic diseases and therapies and anti-SARS-CoV-2 S1 or S2 IgG seropositivity and neutralising antibody positivity in the seropositive patient group at day 28 (appendix 2 p 3).

We assessed the effect of previous symptomatic versus asymptomatic SARS-CoV-2 infection on vaccine-induced response. Of 157 seropositive patients with autoimmune rheumatic diseases, 43 had no confirmation of previous acute infection by RT-PCR or rapid antigen test and therefore they were excluded from this analysis. The remaining 114 patients with a previous symptomatic RT-PCR or rapid antigen test confirmed COVID-19 were included. 41 (36%) of 114 had a previous symptomatic infection and 73 (64%) had a previous asymptomatic infection. We found significantly higher levels anti-SARS-CoV-2 S1 or S2 IgG GMTs on day 0 in the symptomatic group than in the asymptomatic group (75·1 AU/mL [95% CI 55·4–101·8] *vs* 39·0 AU/mL [28·0–54·3]; p=0·010) and thereafter similar levels after each vaccine dose (figure 3A). Neutralising antibody activity responses showed the same pattern, with higher day 0 neutralising activity in the previously symptomatic group than in the previously asymptomatic group (74% [IQR 47–88] *vs* 53% [37–75]; p=0·042) but similar levels at day 28 (p=0·12) and day 69 (p=0·20; figure 3B). At day 69, the comparison of previously asymptomatic patients with seronegative patients revealed significantly higher IgG seropositivity (71 [97%] of 73 *vs* 353 [75%] of 471; p<0·0001) and neutralising antibody positivity (66 [90%] *vs* 289 [61%]; p<0·0001) in previously asymptomatic seropositive patients than in seronegative patients (post hoc). IgG and neutralising antibodies positivities were also higher in previously asymptomatic seropositive patients than in seronegative patients at day 0 (p<0·0001) and at day 28 (p<0·0001; data not shown).

	Anti-SARS-CoV-2 S1 or S2 IgG seropositivity			Neutralising antibody positivity		
	Day 0	Day 28	Day 69	Day 0	Day 28	Day 69
SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases (n=157)	140 (89%)	149 (95%)	154 (98%)	135 (86%)	138 (88%)	141 (90%)
SARS-CoV-2 seropositive controls (n=157)	149 (95%)	155 (99%)	157 (100%)	140 (89%)	151 (96%)	155 (99%)
SARS-CoV-2 seronegative patients with autoimmune rheumatic diseases (n=471)	0	99 (21%)	353 (75%)	0	108 (23%)	289 (61%)
SARS-CoV-2 seronegative controls (n=157)	0	57 (36%)	150 (96%)	0	56 (36%)	128 (82%)
p value						
Seropositive patients vs seropositive controls	0·061	0·10	0·25	0·39	0·0067	0·0005
Seropositive patients vs seronegative patients	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Seropositive patients vs seronegative controls	<0·0001	<0·0001	0·34	<0·0001	<0·0001	0·036
Seronegative patients vs seronegative controls	>0·999	<0·0001	<0·0001	>0·999	0·0016	<0·0001

Data are n (%). Positivity for anti-SARS-CoV-2 S1 or S2 IgG was defined as post-vaccination titre of ≥15 AU/mL. Positivity for neutralising antibodies was defined as a neutralising activity ≥30%. Frequencies of seropositivity were compared using the χ^2 test.

Table 2: **Anti-SARS-CoV-2 S1 or S2 IgG and neutralising antibody seropositivity rates at baseline and after the first (day 28) and second (day 69) doses of CoronaVac vaccination**

	Anti-SARS-CoV-2 IgG S1 or S2 IgG GMT, AU/mL (95% CI)			Median neutralising activity of neutralising antibodies, % (IQR)		
	Day 0	Day 28	Day 69	Day 0	Day 28	Day 69
SARS-CoV-2 seropositive patients with autoimmune rheumatic diseases (n=157)	52·3 (42·9–63·9)	128·9 (105·6–157·4)	137·1 (116·2–161·9)	59 (39–83)	82 (54–96)	79 (57–94)
SARS-CoV-2 seropositive controls (n=157)	53·3 (45·4–62·5)	202·0 (174·8–233·4)	188·6 (167·4–212·6)	58 (41–79)	92 (79–96)	92 (75–96)
SARS-CoV-2 seronegative patients with autoimmune rheumatic diseases (n=471)	2·3 (2·2–2·3)	5·7 (5·1–6·4)	29·6 (26·4–33·3)	15 (15–15)	15 (15–15)	39 (15–65)
SARS-CoV-2 seronegative controls (n=157)	2·3 (2·1–2·5)	10·6 (8·7–13·1)	71·7 (63·5–81·0)	15 (15–15)	24 (15–37)	61 (37–79)
p value						
Seropositive patients vs seropositive controls	>0·999	0·0080	0·41	>0·999	0·119	0·300
Seropositive patients vs seronegative patients	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001
Seropositive patients vs seronegative controls	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	0·010
Seronegative patients vs seronegative controls	>0·999	<0·0001	<0·0001	>0·999	<0·0001	<0·0001

Proportion of neutralising activity of neutralising antibodies are expressed as median (IQR) and anti-SARS-CoV-2 S1 or S2 IgG antibody titres are expressed as GMTs with 95% CIs. The minimum possible value for neutralising activity is 15% (attributed for values of <30%). AU=arbitrary units. GMT=geometric mean titre.

Table 3: **Geometric mean titres of anti-SARS-CoV-2 S1 or S2 IgG and median percentage of neutralising activity and before (day 0) and after the first (day 28) and second (day 69) doses of CoronaVac vaccination**

The median of elapsed time after SARS-CoV-2 infection in symptomatic patients was 81 days (IQR 8–395) before vaccination. Antibody dynamics in patients with symptomatic infection less than or equal to 3 months (n=21) and more than 3 months (n=20) before vaccination were similar for IgG GMTs and neutralising antibody activity, with a significant increase from day 0 to day 28 (≤3 months only for IgG [p=0·038]; >3 months both IgG [p<0·0001] and neutralising antibodies [p=0·0040]) with no further increase from day 28 to day 69 (≤3 months: IgG p=0·92 and neutralising antibodies p=0·64; >3 months: IgG p=0·55 and neutralising antibodies p=0·49; data not shown).

The inactivated SARS-CoV-2 vaccine CoronaVac was well tolerated, with only mild adverse events reported (appendix 2 pp 5–6). Most adverse events were reported at higher frequencies among seropositive patients than among seronegative patients and seropositive and seronegative controls, particularly abdominal pain (p=0·026) and tremor (p=0·0040) after the first vaccine dose. After the second dose, vaccine injection erythema (p=0·022) and induration (p=0·023) were also more frequently reported by seropositive patients than the other groups. (appendix 2 p 5–6). Among all participants in CoronavRheum as of data cutoff (April 29, 2021), incident cases of SARS-CoV-2 infection confirmed with RT-PCR from day 0 to day 79 were less often observed in seropositive patients than in seronegative patients (three [1%] of 239 *vs* 39 [4%] 954; p=0·031). Eight cases of SARS-CoV-2 infection were reported between day 38 (10 days after complete vaccination) and day 79 (seven among seronegative patients with autoimmune rheumatic diseases and one in a seropositive patient). Regarding infection severity among these cases, seronegative and seropositive patients had a similar frequency of hospital admissions for COVID-19 (one [33%] of three *vs* five [13%] 39; p=0·378) and mechanical ventilation (one [33%] *vs* zero; p=0·071). SARS-CoV-2 genotyping could not be done for all symptomatic participants because 24 participants could not attend our centre for testing and instead had a PCR test for suspected SARS-CoV-2 infection at an external site. Among the 18 samples analysed for variants of concern, 16 (89%) had the gamma (P.1) variant, one (6%) had the alpha (B.1.1.7) variant, and one (6%) had a distinct variant.

Further analysis of incident RT-PCR-confirmed COVID-19 cases in seronegative patients with and without seroconversion after full vaccination (from 10 days after vaccine second dose to day 79) showed no difference between both groups (six [1%] of 707 *vs* one [<1%] of 247; p=0·68).

In the convenience sampled population, the analysis of social risk factors associated with exposure to SARS-CoV-2 showed that suspected COVID-19 contact in close relatives was significantly higher among seropositive patients (70 [45%] of 157) than among seronegative patients (92 [20%] of 471; p<0·0001) and seronegative controls (33 [21%] of 157; p<0·0001), but similar to among seropositive controls (57 [36%] of 157; p=0·035; appendix 2 p 7). Adherence to social quarantine was lower in seropositive controls (25 [16%]) and seronegative controls (35 [22%]) than among seropositive patients (98 [62%]), whereas use of public transportation was less frequent in patients (86 [55%] of seropositive patients and 221 [47%] of seronegative patients) than among controls (130 [83%] of seropositive controls and 121 [77%] seronegative controls; appendix 2 p 7).

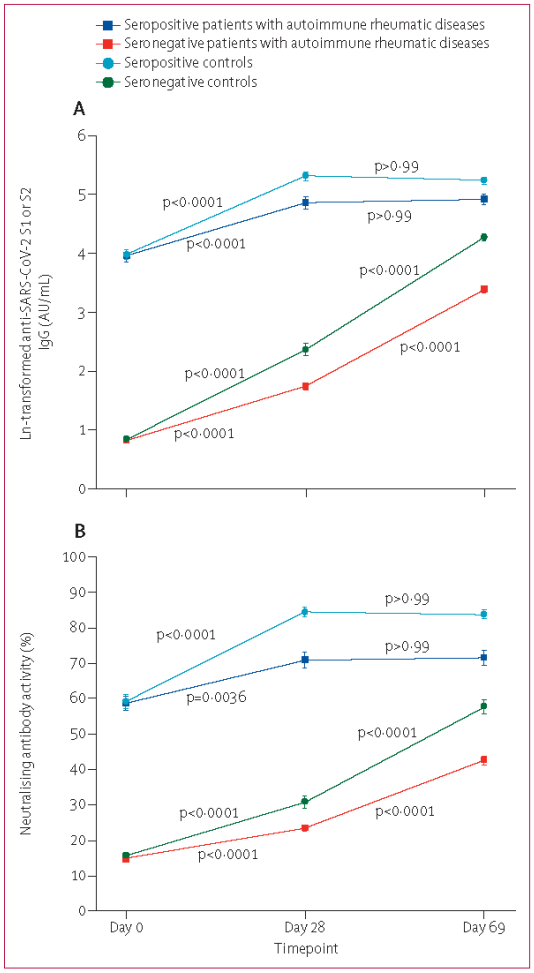

Figure 2: **Anti-SARS-CoV-2 S1 or S2 IgG GMTs (A) and neutralising antibody activity (B) before (day 0) and after the first (day 28) and second (day 69) doses of CoronaVac**

Datapoints are mean values, with error bars showing SD. The minimum possible value for anti-SARS-CoV-2 S1 or S2 IgG is 0·64 (ln 1·9, the value attributed IgG titres of ≤3·8 AU/mL) and for neutralising activity is 15% (attributed for values of <30%). Data are also shown after Bonferroni's multiple comparison in the appendix (pp 2–3). Tests were always two-sided. AU=arbitrary units. GMT=geometric mean titre. S=spike.

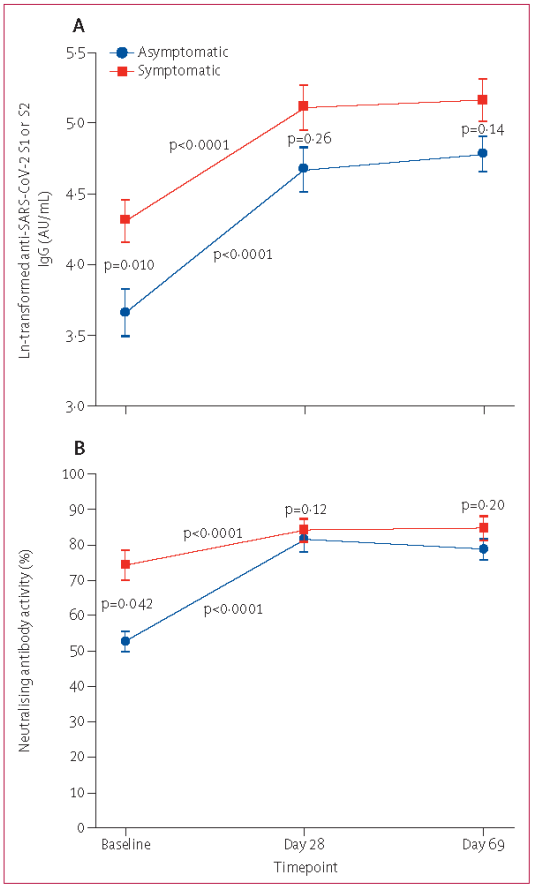

Figure 3: **Anti-SARS-CoV-2 S1 or S2 IgG GMTs (A) and neutralising antibody activity (B) before (day 0) and after the first (day 28) and second (day 69) doses of CoronaVac in seropositive patients with autoimmune rheumatic diseases who had symptomatic infection (n=41) versus asymptomatic infection (n=73)**

Datapoints are means with error bars showing SDs. The minimum possible value for anti-SARS-CoV-2 S1 or S2 IgG is 0·64 (ln 1·9, the value attributed IgG titres of ≤3·8 AU/mL) and for neutralising activity is 15% (attributed for values of <30%). AU=arbitrary units. GMT=geometric mean titre. S=spike.

Discussion

Here we provide the first evidence that previous exposure to SARS-CoV-2, with or without symptoms, results in distinct dynamics of antibody response in a large population of seropositive and seronegative patients with autoimmune rheumatic diseases and controls immunised with an inactivated SARS-CoV-2 vaccine, CoronaVac. Seropositive patients developed a robust response that plateaued between the first and second dose, whereas seronegative patients had moderate antibody production only after two doses of vaccine.

The criterion of positive pre-vaccination immune response that we used, which was independent of symptoms or RT-PCR positivity, offered a broader definition of SARS-CoV-2-exposure.[19] In fact, serological detection is a more precise estimation of previous SARS-CoV-2 infection because asymptomatic infection can account for 40–50% of cases.[20]

Our findings support those of a previous small study in seropositive patients with autoimmune rheumatic diseases showing that mRNA-based and adenovirus-based SARS-CoV-2 vaccines induced high and similar IgG responses, with a substantial increase after the first dose, and no further increase after a second dose.[14] We found here that, in a larger population, the same response occurred with an inactivated vaccine in an immunosuppressed population. The possible underlying mechanism for this robust response is related to pre-existing memory B cells, because recurrent exposure is

known to recall responses to a greater extent than the primary response.[6] In line with these findings, previous reports on an mRNA-based SARS-CoV-2 vaccine have already found that one dose of vaccine was sufficient to increase both cellular and humoral immune responses in healthy individuals who have recovered from COVID-19.[5,7,21,22]

Although patients with autoimmune rheumatic diseases have reduced vaccine immunogenicity, not only to SARS-CoV-2 infection[1,4] but also to other vaccines (eg, for H1N1 influenza),[23] our study provides convincing evidence that patients who have been exposed to SARS-CoV-2 respond adequately to an inactivated SARS-CoV-2 vaccine independent of intrinsic immunological defects or therapy. This finding is of great relevance for individuals who are immunocompromised because the presence of anti-SARS-CoV-2 S1 or S2 antibodies after infection was associated with a considerable reduction of the risk of COVID-19 in health-care workers.[24]

Supporting this result, we observed the same kinetics for neutralising antibody activity in seropositive patients and controls, with a peak reached after the first dose in both groups without further increase after the second dose, and with both groups achieving levels of approximately 70–80%. This immune response in seropositive patients with autoimmune rheumatic disease contrasts with the lower neutralising antibody activity observed in seronegative patients after two doses of same the vaccine[4] and it was also higher than in the seronegative controls. This observation is relevant because of the reported correlation between serum neutralising antibody titres and protection from SARS-CoV-2 infection in human and animal models.[25] Notably, the mRNA-based vaccine BNT162b2 (BioNTech–Pfizer) elicited an increase in anti-SARS-CoV-2 S1 and S2 antibody response after two doses in seropositive healthy individuals (20 times higher than in seronegative individuals)[5] compared with what we observed after vaccination with CoronaVac after two doses; an approximately five times higher antibody response in seropositive patients and controls than in seronegative patients.

Previous studies in patients with autoimmune rheumatic diseases have shown effects of immunosuppressive therapy on antibody production after inactivated virus-based, mRNA-based, and adenovirus-vector-based SARS-CoV-2 vaccinations.[4,14,26,27] Mycophenolate mofetil, methotrexate, rituximab, and TNF inhibitors had a negative effect on anti-SARS-CoV-2 antibody responses, especially in seronegative populations of patients.[4,26,27] By contrast, immunosuppression might be less relevant in seropositive patients, because we observed no detrimental effect on humoral response with these drugs, although we cannot draw any definitive conclusions because of the small sample of patients who were seronegative at day 28. The longer disease duration in our population of seronegative patients than in our seropositive patient population is probably not clinically important for immunogenicity, because age remained balanced between the groups.

Neutralising antibody activity before vaccination was higher in seropositive patients with RT-PCR-confirmed or serology-confirmed previous infection who were symptomatic than in those who were asymptomatic, in accordance with previous reports that neutralising antibody activity correlates positively with disease severity.[28] However, after the first dose of vaccine, both groups reached a similar peak without further increase after the second dose, suggesting that for seropositive patients, a single dose of vaccine results in a boost to the maximum level of response with CoronaVac, independent of the underlying immunosuppressive condition. However, other investigators have reported that asymptomatic or oligosymptomatic individuals who have been exposed to SARS-CoV-2 but are otherwise healthy had a different response after an mRNA-based vaccine (BNT162b2), with lower antibody responses after two doses than symptomatic individuals.[5]

In line with previous studies that included healthy individuals,[9,10] we found that seropositive patients with autoimmune rheumatic diseases had more vaccine-related adverse events than did seronegative patients, which could be related to exacerbated immunity after vaccination, although more data are needed to define the underlying mechanism.[8,19] Ebinger and colleagues[9] found that previously infected individuals had adverse post-vaccine symptoms more frequently than did individuals who had not been previously infected.

The main strength of our study was its prospective design, with all participants receiving vaccine within 2 days at one site, which enabled an adequate comparison of the kinetics of humoral response between study groups. Moreover, the inclusion of study groups balanced for sex and age, and similar groups of patients with autoimmune rheumatic diseases with regards to the diverse diagnoses allowed a more precise assessment of the specific effect of previous exposure to SARS-CoV-2 on the humoral response pattern in the different groups. SARS-CoV-2 vaccine responses might be affected by the presence of immune-mediated inflammatory diseases, age, and sex.[26] Treatment was also similar in the patient groups, which is relevant because glucocorticoids, immunosuppressives, and biological therapies have been reported to impair SARS-CoV-2 vaccine immunogenicity.[4,27] Additionally, few studies on pre-vaccination SARS-CoV-2-exposed individuals have focused on the detailed immunological analysis of neutralising antibodies;[6,7] the leading candidate for a surrogate marker of protection.[29] Notably, the ELISA kit we used to detect neutralising antibodies does not completely replace the gold standard live-virus neutralisation assay, but a comparison between the two tests revealed 98·2% sensitivity and 69·5% specificity.[30]

Our study limitations include the paucity of assessment of memory B-cell and T-cell responses,

which is relevant to assess the recall of antibody response.[6] Also, we have not assessed the effect of CoronaVac on disease activity, but previous large studies in patients with autoimmune rheumatic diseases reported that disease remains stable after SARS-CoV-2 vaccination.[31] The absence of mRNA vaccination as a comparator is another limitation.

In summary, we found that SARS-CoV-2-exposed patients with autoimmune rheumatic diseases have a robust response that plateaus between the first and second dose of CoronaVac, independent of disease or therapy. Our finding raises the possibility that the reduced immunogenicity observed in seronegative patients might not represent the optimum response potential after a first SARS-CoV-2 vaccination, and therefore emphasises the importance of at least a second dose of vaccine in these patients. Future studies are urgently needed to assess whether a third dose of vaccine would be of additional value regarding clinical protection against COVID-19.

Contributors

NEA, LVKK, SGP, ACM-R, EFNY, CGSS, TP, PRM, EGK, CAS, and EB conceived of and designed the study, participated in data collection and analysis, supervised clinical data management, wrote the manuscript, and revised the manuscript. NEA, LVKK, SGP, ACM-R, EFNY, CGSS, TP, CAS, EB, RF, SKS, PDS-B, DCOA, RMRP, LPCS, JMLV, and FW collected epidemiological and clinical data and assisted with the identification of SARS-CoV-2 infection and follow-up of patients. NEA, LVKK, and ACM-R verified the data and had access to raw data. NEA, LVKK, SGP, ACM-R, EFNY, CGSS, CAS, and EB had final responsibility for the decision to submit for publication. AMCS organised and supervised the vaccination protocol, ECS did the SARS-CoV-2 genotyping of positive RT-PCR samples. AJSD and LA supervised the processing of serum samples, SARS-CoV-2 specific antibody ELISAs and neutralisation assays, and SARS-CoV-2 RT-PCRs. All authors helped to edit the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Anonymised participant-level data will be made available on request directed to the corresponding author. Proposals will be reviewed by the Hospital das Clinicas 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 Clinicas da Universidade de São Paulo review board and the National Research Ethics Council and a Material Transfer Agreement in place.

Acknowledgments

We thank Antonio José Rodrigues Pereira, Solange Fusco, and Priscila Tagliaferro Rojo for the organisation of the infrastructure for blood sample collection and vaccination stations. We also thank the Registry Division, Security Division, IT Division, Pharmacy Division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, and all the volunteers participating in the study. This study was sponsored by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (number 2015/03756-4 to NEA, SGP, CAS, and EB; number 2019/17272-0 to LVKK; number 2017/14352-7 to TP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (305242/2019-9 to EB, 304984/2020-5 to CAS, 305556/2017-7 to RMRP, and 303379/2018-9 to SKS), and B3-Bolsa de Valores do Brasil. Instituto Butantan supplied the vaccine and had no other role in the trial.

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4.7 CoronaVac induz alta resposta imune em pacientes com doença hepática gordurosa metabólica

Um artigo publicado na plataforma de preprints SSRN da revista britânica The Lancet mostrou que a CoronaVac é segura e imunogênica para indivíduos com doença hepática gordurosa associada ao metabolismo (DHGM), sendo capaz de induzir produção de anticorpos IgG em 100% dos pacientes analisados.

Participaram do estudo 50 pessoas com DHGM e 50 indivíduos saudáveis para controle, que receberam o esquema vacinal completo de duas doses da CoronaVac. A média de idade foi de 42 anos no grupo DHGM e 40 anos no grupo controle.

Um mês após a segunda dose, anticorpos IgG específicos para proteína Spike foram detectados em 100% dos indivíduos de ambos os grupos. Seis meses depois da imunização, 94% dos pacientes DHGM e 98% dos controles mantiveram a produção de anticorpos IgG. Em relação aos anticorpos neutralizantes, 82% dos pacientes e 90% dos controles apresentaram soroconversão.

O imunizante foi bem tolerado pelas pessoas com DHGM e não teve impacto no status da doença. Além disso, não houve diferença significativa na incidência geral de reações adversas entre os dois grupos e todos os efeitos relatados foram leves.

De acordo com os autores, "nosso trabalho é o primeiro estudo prospectivo de uma vacina contra Covid-19 em pacientes com DHGM publicado até o momento. Os resultados sugerem que é seguro e eficaz administrar a CoronaVac em pacientes com DHGM, e que esta vacina não afeta o estado da doença. Portanto, os pacientes com DHGM devem ser incluídos na imunização contra a SARS-CoV-2 como uma população altamente vulnerável com maior risco de morbidade e mortalidade".

A DHGM é a doença hepática mais frequente no mundo, atingindo quase 25% da população. Está associada a distúrbios metabólicos e cardiovasculares, como obesidade, resistência à insulina, hipertensão arterial, dislipidemia e diabetes tipo 2.

Publicado em: 05/10/2021

Evaluation of Immune Response and Disease Flares in metabolic-associated fatty liver disease (MAFLD) Patients Following SARS-CoV-2 Vaccination: a prospective study

Qianru Zhu[1,2*], jin Gao[3*], Jiaping Gu[4], Lu Shen[4], Jing Liu[5], Yu Song[6], Xiying Gong[6], Yutong Chen[4], Jie Liao[6], Yining He[4], Siyi Zhang[4], Lei Sun[4], Li Shao[1,4#], Jie Li[7,8#], Junping Shi[5#]

* Contributed equally

[1]Department of Translational Medicine Platform, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China;

[2]State Key Laboratory of Quality Research in Chinese Medicines, Faculty of Chinese Medicine, Macau University of Science and Technology, Macau, P.R. China;

[3]Department of Clinical Laboratory, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China;

[4]Medical college of Hangzhou Normal University, Hangzhou, Zhejiang, China;

[5]Department of Infectious & Hepatology Diseases, Metabolic Disease Center, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China;

[6]The Fourth School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China;

[7]Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China;

[8]Institute of Viruses and Infectious Diseases, Nanjing University, Nanjing, Jiangsu, China.

Correspondence to:

Li Shao, Department of Translational Medicine Platform, The Affiliated Hospital of Hangzhou Normal University, Hangzhou, Zhejiang, China; Medical college of Hangzhou Normal University, Hangzhou, Zhejiang, China; Email: 20200006@hznu.edu.cn;

Jie Li, Institute of Viruses and Infectious Diseases, Nanjing University, Nanjing, Jiangsu, China; Department of Infectious Diseases, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China; Email: lijier@sina.com;

Junping Shi, Department of Hepatology & Infectious Disease, Metabolic Disease Center, The Affiliated Hospital of Hangzhou Normal University, Wenzhou Road, Hangzhou, Zhejiang, China; Email: 20131004@hznu.edu.cn

Word Count: 2394; Abstract: 300; Tables: 2; Figures: 4.

Abbreviations

MAFLD	Metabolic associated fatty liver disease
Nab	Neutralizing antibody
GMT	Geometric Mean Titers
BMI	Body mass index
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GGT	γ-glutamyl transpeptidase
Alb	Albumin
STB	Total bilirubin
ALP	Alkaline phosphatase
LDL-c	Low-density lipoprotein cholesterol
HDL-c	High-density lipoprotein cholesterol
TC	Total cholesterol
TG	Triglyceride
Glu	Glucose
HOMA-IR	homeostatic model assessment of insulin resistance
hs-CRP	high-sensitive C-reactive protein
UA	Urid acids
Cr	Creatine

Author Contributions

JP Shi conceptualized and supervised the study, QR Zhu, L Shao, J Li, and JP Shi designed the study, QR Zhu, J Gao, JP Gu, L Shen, J Liu, Y Song, XY Gong, YT Chen, J Liao, YN He, SY Zhang collected data, QR Zhu, L Shao, J Li drafted the manuscript, QR Zhu, L Shao, J Li interpreted data, all authors critically reviewed or revised the manuscript and approved the final version of the manuscript.

Declaration of Interests

JP Shi reports grants from Project of Key Medical Disciplines of Hangzhou for the Department of infectious & Hepatology. QR Zhu reports grants from the Health and Science and Technology Planning Project of Hangzhou municipal Health Commission, during the conduct of the study. All authors declare no competing interests.

Research in context

Evidence before this study

In patients with metabolic-associated fatty liver disease (MAFLD), existing retrospective data on the risk of adverse outcomes with SARS-CoV-2 infection have been reported. Although the development of SARS-CoV-2 vaccines has shown encouraging safety and efficacy data in many clinical trials, However, concerns have been raised recently about SARS-CoV-2 vaccine responses in patients with MAFLD, such as safety, immunogenicity, and disease flares.

Added value of this study

To our knowledge, this is the first prospective study of the safety, immunogenicity, and disease flares of SARS-CoV-2 vaccine in MAFLD populations. We found that SARS-CoV-2 vaccination does not promote disease progression of MAFLD and metabolic comorbidities, and MAFLD patients show a robust immune response after SARS-CoV-2 vaccination in the short term, but this response does not seem to be sustained in the long term. Furthermore, NAFLD fibrosis score was a negatively predictor of neutralizing maintenance.

Implications of all the available evidence

Although previous studies reported that metabolic disorders might be significant risk factors of hospitalization and severity in COVID-19 patients, and the effectiveness of vaccination for the MAFLD population is uncertain. Our study showed that a two-dose regimen of CoronaVac vaccination in MAFLD patients was safe and well tolerated. The neutralizing antibody responses appeared to be robust in MAFLD patients who completed vaccination, which conferred 82% protection against COVID-19, and SARS-CoV-2 vaccine did not affect disease flares in MAFLD patients. Therefore, MAFLD patients should be involved in immunization to against SARS-Cov-2. However, liver fibrosis/cirrhosis maybe affect the neutralizing antibody maintenance in MAFLD patients, then future studies should consider booster doses in those with undetectable and suboptimal antibody responses.

Summary（300/300 words）

Background The ongoing COVID-19 pandemic has led to the focused application of resources toward developing vaccines to prevent COVID-19. However, the efficacy and safety profiles of vaccines against SARS-CoV-2 in patients with metabolic associated fatty liver disease (MAFLD) are still unknown. We aimed to evaluate the safety, tolerability, seroreactivity, and disease flares after SARS-CoV-2 vaccination in MAFLD patients.

Methods For this prospective observational study, we recruited patients receiving two doses SARS-CoV-2 vaccine (CoronaVac). Neutralizing antibody to the SARS-CoV-2 spike receptor-binding domain and IgG to SARS-COV-2 spike-specific were evaluated on Day 0, Day 28, Day 57, and Day 180. All participants with available data were included in the safety and immunogenicity, and disease flares analyses.

Findings 50 MAFLD patients and 50 healthy controls receiving a 0-28 interval vaccination procedure were enrolled. The seroconversion rates of neutralizing antibodies were 16% in MAFLD group (Log_{10} Geometric Mean Titers (GMT): median 0·783, IQR: 0·719-0·971) and 32% in non-MAFLD group (0·884, IQR: 0·716-1·027) on day 28, and 82% in MAFLD group (1·206, IQR: 1·053-1·467), 90% of non-MAFLD group (1·360, IQR: 1·130-1·464) on day 57, respectively. However, the neutralizing antibody titer in two groups fell below the seropositivity cut-off value on day 180 (MAFLD group 0.928, IQR: 0·773-1·057 vs. non-MAFLD group 0·907, IQR: 0·810-1·009). There was no significant difference in the overall incidence of adverse reactions after two-dose vaccinations between two groups. Furthermore. disease flares were not found in MAFLD group after two-dose vaccinations. On

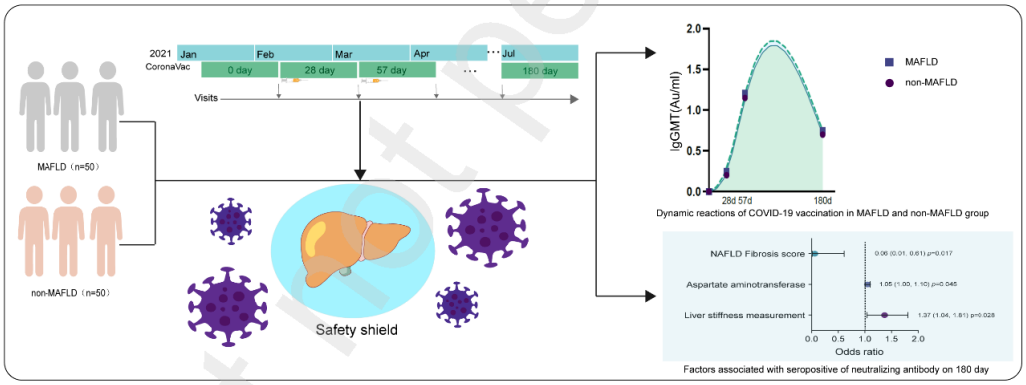
multivariable analysis, NAFLD fibrosis score was negatively associated with seropositive of neutralizing antibody on 180 days (OR 0·03, 95% CI 0·001-0·58, P = 0·022).

Interpretation Two-dose regimen of CoronaVac vaccination in MAFLD patients was safe and well tolerated. MAFLD patients showed a robust immune response after SARS-COV-2 vaccination, which conferred 82% protection against COVID-19 and vaccination does not affect MAFLD disease status.

Keywords COVID-19; SARS-COV-2 vaccination; MAFLD; safety; immunogenicity; disease flares

Funding Project of Key Medical Disciplines of Hangzhou, the Health and Science and Technology Planning Project of Hangzhou municipal Health Commission (No. A20210205).

Graphical abstract



Introduction

The persistent COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to high morbidity and mortality worldwide.[1] Metabolic-associated fatty liver disease (MAFLD), formerly named as non-alcoholic fatty liver disease (NAFLD), is the most common chronic liver disease, affecting about a quarter of the world's adult population,[2,3] which often concurrent with elements of metabolic syndromes, such as diabetes, obesity, or hyperlipidemia are

more susceptible to infection and also induce worse outcomes in COVID-19.[4,5] Early data provided evidence that metabolic syndrome was associated with chronic low-grade inflammation that compromised the immune system and caused microvascular endothelial dysfunction, which was particularly vulnerable to COVID-19 infection and disease progression.[6-8] Furthermore, COVID-19 infection is reported to associate with disease flares in MAFLD patients.[9]

Although encouraging safety and efficacy data of SARS-CoV-2 vaccines has shown in many clinical trials,[10-12] but these studies only included a small number of participants with pre-existing liver disease, such like liver transplantation.[13,14] Recently, Wang and colleagues demonstrated that COVID-19 vaccination is safe and effective in NAFLD patients, while this retrospective study did not set up the control group.[15] However, concerns have been raised recently about SARS-CoV-2 vaccine responses in MAFLD patients, such as safety, immunogenicity, and disease flares. Thus, our study aimed to examine the safety, efficacy, and changes of multiple metabolic indicators of SARS-CoV-2 vaccines in patients with MAFLD.

Methods

Study design and participants

We performed a prospective, observational cohort study that recruited adults (>18 years) receiving SARS-CoV-2 vaccination between 12 January 2021 to 4 February 2021 at the affiliated hospital of Hangzhou Normal University. All participants received two doses of an inactivated vaccine against SARS-CoV-2 (0·5 mL/dose, Sinovac life science, Beijing, China) with a 28-day interval. Hepatic steatosis was defined as a controlled attenuation parameter measurement of 248 dB/m or more.[16,17]

MAFLD diagnosed by hepatic steatosis plus any of the following three metabolic disorders according to the definition proposed by the international expert group[18]: 1) overweight/obesity (≥23 kg/m^2); 2) type-2-diabetes mellitus or 3) metabolic dysregulation. Metabolic dysregulation was defined as the presence of at least two of the following metabolic risk abnormalities: 1) Waist circumference ≥ 90/80 cm in men and women; 2) Blood Pressure ≥130/85 mmHg or use of antihypertensive medications; 3) Triglyceride (TG) ≥ 150 mg/dL or use of lipid-lowing medications; 4) HDL-cholesterol (HDL-c) < 40/50 mg/dL for male and female or use of lipid-lowing medications; 5) prediabetes (fasting glucose levels 100-125 mg/dL, 2h glucose levels 140-199 mg/dL or HbA1c 5·7%-6·4%; 6) homeostatic model assessment of insulin resistance (HOMA-IR) ≥2·5.[19] We also included immunized non-MAFLD participants without hepatic steatosis, diabetes, and were of normal weight from the same hospital. The study was approved by local Hospital Ethics Committee (2021(E2)-KS-049) and written informed consent was obtained from patients involved before enrolment when data were collected. This trial had been registered in Chinese ClinicalTrials.gov (ChiCTR2100042717).

Procedures

Blood samples were captured before vaccination (Day 0), 28 days after the first vaccine dose (Day 28), 28 days after the second dose vaccination (Day 57), and 180 days after the first vaccine dose (Day 180). Telephone consultations evaluated reactogenicity and safety of each patient within 28 days after injection. Adverse events were graded according to the following scale: grade 1 (mild; does not interfere with activity); grade 2 (moderate; interferes with activity), grade 3 (severe; prevents daily activity), and grade 4 (potentially life-threatening; emergency department visit or hospital admission).

[20] Seroreactivity and biochemical indicators were detected at each time point. Neutralizing antibodies (NAb) to the receptor-binding domain (RBD) of SARS-CoV-2 spike protein was detected by iFlash 2019-nCoV NAb assay (SHENZHEN YHLO BIOTECH CO., LTD, Shenzhen, China, Cat#C86109), which is a paramagnetic particle chemiluminescent immunoassay (CLIA) for the qualitative detection of SARS-CoV-2 NAb in human serum and plasma using the automated iFlash immunoassay system, and the cut-off value of 10·00 AU/mL for the antibody.[21] IgG to SARS-COV-2 spike-specific were detected by magnetic particle chemiluminescence immunoassay using SARS-CoV-2 IgG detection kit (Beijing Hotgen Biotech Co., Ltd.). The cut-off was set as 1·00 Au/ml according to the manufacturer's guidelines.

Statistical analysis

All participants with available data were included in the safety and immunogenicity analyses. Statistics were computed in IBM SPSS Statistics 26 (Armonk, NY: IBM Corp). The significance threshold for p values was less than 0·05 after correction for multiple comparisons. We used the Pearson χ^2 test or Fisher's exact test for the analysis of categorical outcomes. We calculated Geometric Mean Titers (GMT) and corresponding IQR of the log-transformed antibody titre then used the t-test method to compare the log-transformed antibody titre. Repeated measures ANCOVA, as implemented under the mixed model,[22] was applied with change from baseline as the dependent variable, group, time, and the group by time interaction as independent variables. The approximate normality of each outcome and the change score of the outcome was confirmed by examination. Age, sex, BMI, and hypertension were included as covariates to ensure statistical balance was not captured by randomization, and reduce error

variance. Logistic regression analysis was used to investigate the association of seroconversion of Nab at day 180 with various metabolic indicators.

Role of the funding source

The funder of this study participated in study design, data collection, data analysis, and data interpretation in collaboration with all investigators.

Results

Study design and participants

A total of 164 subjects were screened in the study, and divided into MAFLD group and non-MAFLD group after matching the age. Finally, 60 people were included in the MAFLD group and 60 people in the non-MAFLD group. However, 10 MAFLD patients and 10 non-MAFLD participants did not complete vaccination. Finally, 50 subjects with MAFLD and 50 non-MAFLD subjects were enrolled in our study, respectively (Figure 1). The clinical characteristics of study participants were summarized in Table 1. The mean age of the MAFLD group was 42·10 (9·87), and 39·88(10·50) in the non-MAFLD group. MAFLD patients were more likely to have higher BMI and waist circumference, lower HDL-c and higher level of TG, as well as liver enzyme (alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), high-sensitive C reactive protein (hs-CRP), and HOMA-IR, compared with non-MAFLD patients ($p<0·05$) (Table 1).

Safety

The overall incidence of adverse reactions was 19 (18%) of 100 participants within 28 days after the first dose vaccination, 9 (18%) in the MAFLD group, and 10 (20%) in the non-MAFLD group, with no significant difference between the two groups. All adverse reactions were mild and self-limiting. Reported adverse events were graded according to China National Medical Products Administration guidelines,[23] The most common symptom was injection-site pain, which was reported by5 (10%) participants in the MAFLD group, 5 (10%) in the non-MAFLD group, followed by fatigue (4%), dizziness (1%). Furthermore, there was still no significant difference in the overall incidence of adverse events between two groups within 28 days after vaccinations, which was similar to the results performed in the phase 2 trial of CoronaVac vaccine[24] (Figure 2, appendix p 2).

Immunogenicity

All individuals were assayed for anti-SARS-CoV-2 spike IgG responses and neutralizing antibodies to the RBD of SARS-CoV-2 Spike Protein. At baseline, none of the participants had any detectable neutralizing antibodies to live SARS-CoV-2. The seroconversion rates of neutralizing antibodies were 16% (8/50) in MAFLD group (Log_{10} GMT: median 0·783 [IQR: 0·719-0·971]) and 32% (16/50) in non-MAFLD group (0·884 [0·716-1·027]) on 28 days after the first dose vaccination (Day 28). Furthermore, seroconversion rates were 82% (41/50) in the MAFLD group (1·206 [1·053–1·467]) and 90% (45/50) in non-MAFLD group (1·360 [1·130-1·464] on 28 days after the second dose vaccination (Day 57). However, the neutralizing antibody titer of 19 (38%) MAFLD patients (0·928 [0·773-1·057] 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**

As shown in Table 2, NAFLD fibrosis score (NFS), liver stiffness measurement, AST, HDL-c, triglyceride, and GMT of neutralizing antibody on 28 days and 57 days were significantly associated with seropositive of neutralizing antibody on 180 days by univariate analyses. Then, on multivariable analysis, the most parsimonious model that optimized prediction only included NFS, which meant that the odds of seropositive of neutralizing antibody at 180 days were higher in those who with lower NFS (OR 0·03, 95% CI 0·001, 0·58) (Table 2).

Discussion

Previous studies reported that the incidence of COVID-19 was higher in MAFLD group than in non-MAFLD group. [25,26] In addition, metabolic disorders might also be significant risk factors of hospitalization and severity in COVID-19 patients. [27,28] Therefore, it's urgently needed to explore the SARS-CoV-2 vaccine responses in MALFD patients as those patients may be uniquely susceptible to COVID-19 infection and disease progression. To the best of our knowledge, this is the first prospective report of the safety and immunogenicity of SARS-CoV-2 vaccine in MAFLD populations. Our study indicated that there was no significant difference in the overall incidence of adverse reactions after two-dose vaccinations between two groups, and SARS-CoV-2 vaccine did not affect the biochemical indicators in MAFLD patients. Furthermore, we detected that NAFLD fibrosis score was inversely associated with seropositive of neutralizing antibody on 180 days.

Similar to the general population[29], side effects related to the SARS-CoV-2 vaccine in MAFLD patients were mild and self-limiting, and the most common symptom was injection-site pain, followed by fatigue, dizziness, and diarrhea. No serious adverse events were reported in MAFLD patients. Our

results indicated that a two-dose regimen of 3 ug of inactivated CoronaVac vaccine administered 28 days apart to MAFLD patients was safe and well tolerated. Furthermore, we did not find changes of biochemical indicators, especially ALT, AST, γ-GGT after vaccinations in MAFLD patients, which means that CoronaVac vaccination might not affect the disease status and also prove the safety of SARS-CoV-2 vaccine in special population.

Vaccine immunogenicity is broadly assumed to require neutralizing antibodies, although its protection role against COVID-19 remains incompletely defined. Our results showed that two-dose CoronaVac induced neutralizing antibody and spike-specific IgG in MAFLD patients still comparable, which was consistent with previous study,[24] Similar to the study performed by Wang et al.,[29] CoronaVac elicited a high immune response in our cohort in the short term, with 82% vaccine efficacy in MAFLD group at 28 days after two-dose vaccinations. However, the GMT of Nab declined to below the positive cutoff titer after 6 months of vaccination in our cohort, which was also consistent with the results of Pan et al.'s study using the same vaccine.[30] Pan et al.'s study also found that a third dose vaccination, given at an interval of 6-8 months after the second dose could lead to a significant rebound in antibody levels, which indicating that booster vaccination may be necessary.

On multivariable logistic regression analysis, few variables were associated with seroconversion rates of neutralizing antibodies after vaccination and liver steatosis, abnormal liver function, and elevated BMI were not associated with the poor antibodies responses, which provides encouraging evidence for MAFLD patients, who should be more actively involved in SARS-CoV-2 immunization. However, NFS was inversely correlated with the seropositive of neutralizing antibody at 6 months, which implies

306 that liver fibrosis/cirrhosis could be an indicator for neutralizing antibody maintenance in MAFLD
307 patients. In fact, previous study indicated that SARS-CoV-2 infection in cirrhosis patients was
308 associated with 2.43-times mortality hazard, and the presence of cirrhosis among chronic liver disease
309 patients infected with SARS-CoV-2 were associated with 3.39-times mortality hazard.[32] In a
310 prospective study from USA, among 79 cirrhotic patients receiving two dose of mRNA vaccines or
311 single dose of Johnson & Johnson vaccine, 15 had suboptimal antibody response and 3 had
312 undetectable antibody, and cirrhosis was indicated to be associated with poor antibody response.[31]
313 Lower immune response was anticipated since humoral immunity is critical for antibody response after
314 the vaccination, but patients with cirrhosis are considered immunocompromised and the response was
315 disappointingly low, while these findings and precise mechanism merit further research.
316 Nevertheless, we acknowledge the following limitations. First, the sample size of the study is small.
317 Besides, this study does not evaluate T cell responses and the production of memory cells between the
318 two groups and data on immune persistence needs further study. Furthermore, the study lacks a
319 comparison to convalescent samples, especially in the absence of a correlate of protection, but these
320 have been taken into account in our further research.
321
322 Despite these limitations, we believe our observations are very important and meaningful. Our study is
323 the first prospective study of COVID-19 vaccine in MAFLD patients to date. It is safe and effective to
324 receive the SARS-Cov-2 vaccine in MAFLD patients, which does not affect disease status. Therefore,
325 MAFLD patients should be involved in immunization to against SARS-Cov-2 as the highly vulnerable
326 patient population with higher morbidity and mortality risk. However, immune response does not seem

to be sustained in the long term is a major concern and liver fibrosis/cirrhosis maybe affect the neutralizing antibody maintenance in MAFLD patients, then a third dose could be necessary to boost immunity. However, future studies should consider booster doses in those with undetectable and suboptimal antibody responses.

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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, creatine. * p<0·05.

Table 1: Patient baseline characteristics, comorbidities by MAFLD stature

Characteristics	MAFLD group (N=50)	non-MAFLD group (N=50)	*p*
Age (years)	42·10 (9·87)	39·88 (10·50)	0·279
Sex (male/Female)	30/20	9/41	0·0001
Body mass index, BMI (kg/m^2)	21·04 (1·35)	26·84 (3·27)	**0·000**
Waist circumference (cm)	92·22 (10·29)	75·56 (6·37)	**0·000**
Controlled attenuation parameter, CAP (dB/m) [a]	300·72 (36·87)	201·32 (42·72)	**0·000**
Liver stiffness measurement, LSM (kPa)	5·66 (2·00)	4·21 (1·14)	**0·000**
Fibrosis-4 index, FIB4	-3·18 (0·03)	-3·17 (0·19)	0·714
NAFLD fibrosis score, NFS	0·91 (0·51)	0·84 (0·40)	0·459
Total bilirubin (μmol/L)	19·57 (7·66)	20·42 (6·63)	0·551
Albumin (g/L)	48·52 (2·73)	48·17 (2·18)	0·485
Alanine aminotransferase, ALT (U/L)	32·27 (24·64)	13·84 (8·35)	**0·000**
Aspartate aminotransferase, AST (U/L)	25·43 (11·51)	18·00 (4·09)	**0·000**
Alkaline phosphatase, ALP (U/L)	72·96 (18·98)	61·44 (15·50)	**0·001**
γ-glutamyl transpeptidase, γGGT (U/L)	34·78 (28·30)	18·10 (12·20)	**0·000**
LDL-cholesterol, LDL-c (mmol/L)	3·33 (0·78)	2·86 (0·65)	**0·000**
HDL-cholesterol, HDL-c (mmol/L)	1·17 (0·22)	1·50 (0·35)	**0·001**
Total cholesterol, TC (mmol/L)	5·16 (0·91)	4·72 (0·80)	**0·012**
Triglyceride, TG (mmol/L)	1·44 (0·74)	0·91 (0·37)	**0·000**
Glucose, Glu (mmol/L)	4·32 (1·64)	3·97 (0·87)	0·185
HOMA-IR	3·00 (2·9)	1·29 (0·64)	**0·000**
Creatinine (μmol/L)	64·44 (14·35)	54·76 (11·27)	**0·000**
Uric Acid, UA (μmol/L)	363·12 (101·43)	265·16 (60·60)	**0·000**
hs-CRP (mg/L)	2·05 (3·27)	0·55 (0·62)	**0·003**
Leukocyte count (10^9/L)	6·83 (1·54)	5·93 (1·22)	**0·002**
Platelets count (10^9/L)	253·22 (56·24)	245·34 (62·94)	0·511
Red blood cell count (10^9/L)	5·07 (0·51)	4·65 (0·46)	**0·000**
Lymphocytes (10^9/L)	2·33 (0·56)	1·87 (0·48)	**0·000**
hemoglobin (g/L)	150·62 (17·70)	137·64 (14·37)	**0·000**
Comorbidity, N **(%)**			
Hypertension	27 (54)	6 (12)	**0·000**
Diabetes	3 (6)	0 (0)	0·242

Results are expressed as mean (SD) / count (%), [a] represent the number of MAFLD patients diagnosed by CAP were 46, hs-CRP represents high-sensitivity C-reactive protein, HOMA-IR represents Homeostasis model assessment insulin resistance.

Hypertension was defined as systolic blood pressure ⩾130 or diastolic blood pressure⩾85 mmHg.

Table 2: Factors associated with seropositive of neutralizing antibody on 180 day

Characteristics	Univariable Analysis			Multivariable Analysis		
	B	OR (95% CI)	*p*	B	OR (95% CI)	*p*
NAFLD Fibrosis score, NFS	-2·75	0·06 (0·01, 0·61)	**0·017**	-3·71	0·03 (0·001, 0·58)	**0·022**
Liver stiffness measurement, LSM (kPa)	0·31	1·37 (1·04, 1·81)	**0·028**	0·30	1·34 (0·87, 2·08)	0·185
Aspartate aminotransferase, AST (U/L)	0·05	1·05(1·00, 1·10)	**0·045**	-0·01	0·99 (0·92, 1·06)	0·718
HDL-cholesterol (mmol/L)	-1·92	0·15 (0·03, 0·71)	**0·017**	-0·78	0·46(0·04, 5·06)	0·525
Triglyceride (mmol/L)	1·16	3·18 (1·55, 6·49)	**0·002**	0·86	2·37 (0·73, 7·72)	0·152
GMT of Nab at day 57	1·78	1·02 (1·00, 1·04)	**0·018**	1·12	0·08 (0·40, 23·48)	0·279
GMT of Nab at day 28	2·79	1·13 (1·04, 1·23)	**0·005**	2·45	11·59 (0·67, 201·75)	0·093

OR, odds ratio; CI, confidence interval; GMT, Geometric Mean Titers.

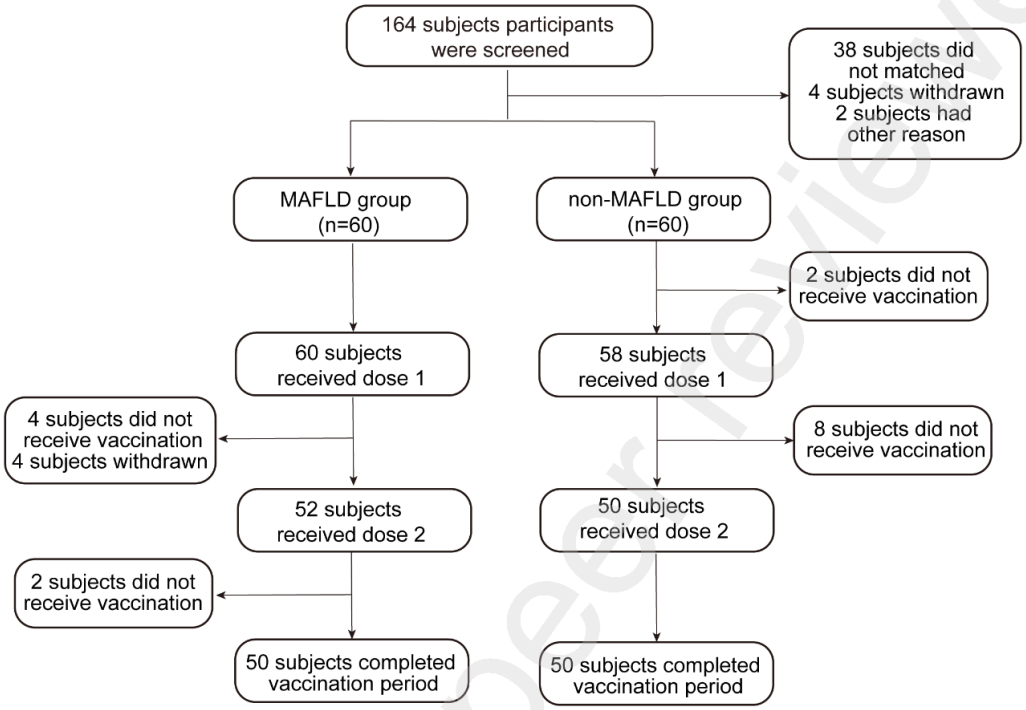


Figure 1: Screening and vaccine administration

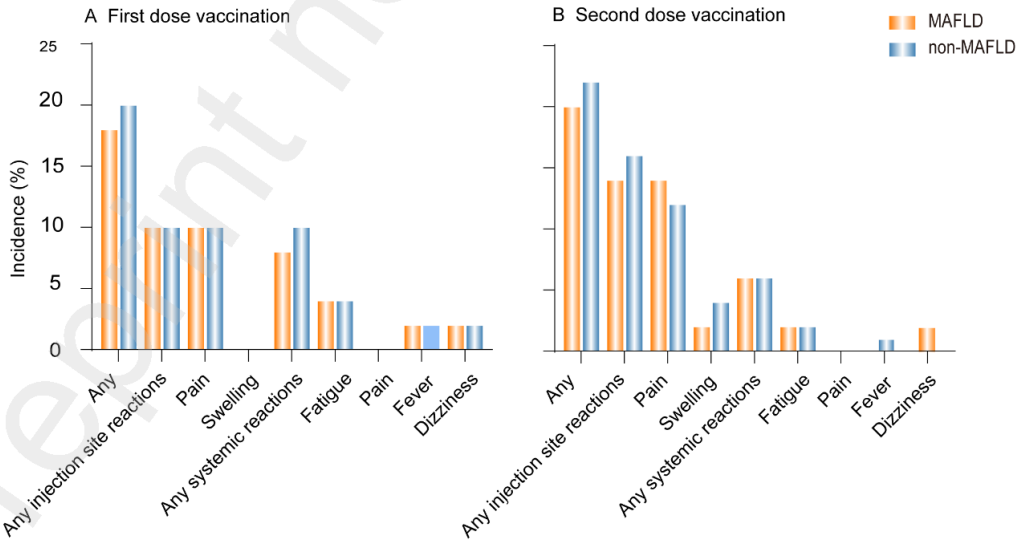


Figure 2: Adverse reactions of COVID-19 vaccination in MAFLD and non-MAFLD group

Incidence of adverse reactions reported within 28 days after the first dose vaccination (A) and the second dose vaccination (B) between the two groups.

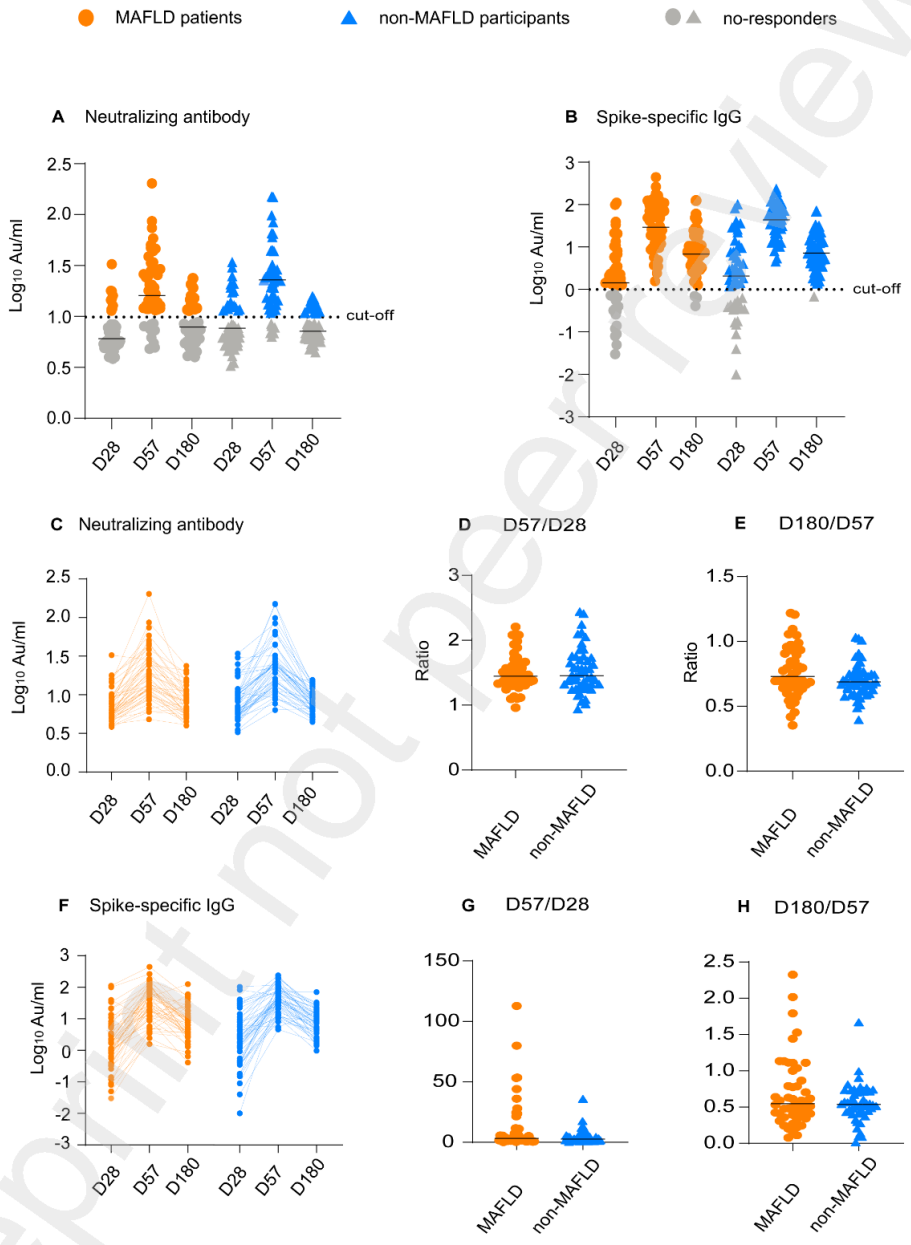


Figure 3: Serological response to COVID-19 vaccine

Antibody titres of neutralizing antibodies (A) and RBD-specific IgG (B) to live SARS-CoV-2 at different

timepoints after vaccination. The horizontal line represents the threshold of specific response. Short bars represent the mean values of titres. Sample comparisons tested by Mann-Whitney U and no significant differences. Line chart represents production and regression of neutralizing antibody and spike-specific IgG (C, F). Ratio of Day 57 to Day 28 represents the production of neutralizing antibody (D) and spike-specific IgG (G) between the two groups, Ratio of Day 180 to Day57 represents the regression of neutralizing antibody (E) and spike-specific IgG (H).

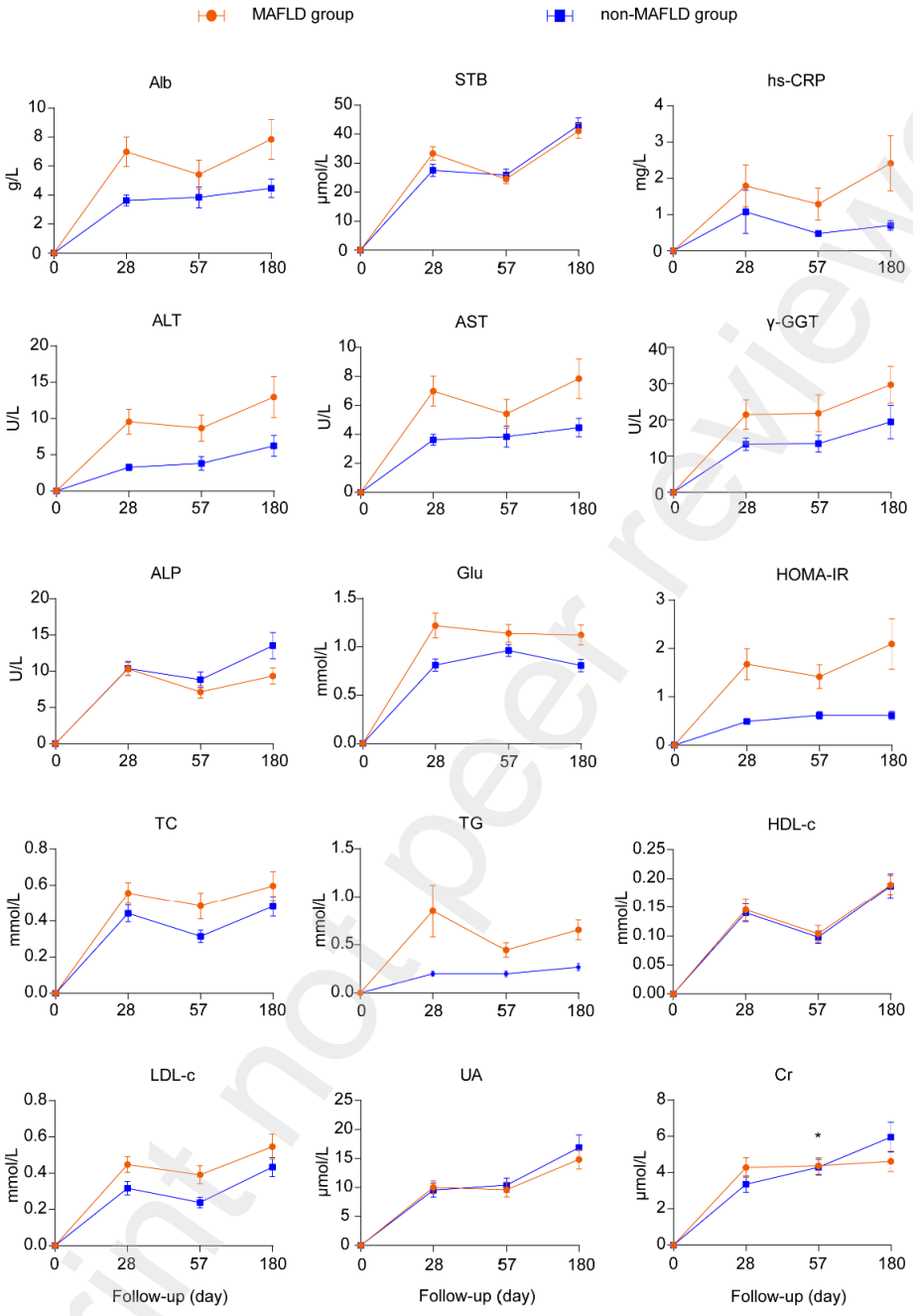


Figure 4: Dynamic absolute changes of biochemical indicators at different timepoints

Dynamic absolute changes of biochemical indicators at different timepoints were shown as mean (SE). Alb, Albumin; STB, total bilirubin; hs-CRP, high-sensitive C-reactive protein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; γ-GGT, γ-glutamyl transpeptidase; ALP, Alkaline

phosphatase; Glu, glucose; HOMA-IR, Homeostasis model assessment insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; UA, uric acids; Cr, creatine. * $p<0{\cdot}05$.

4.8 CoronaVac é segura e imunogênica para pacientes com miopatias autoimunes sistêmicas

Um estudo clínico de fase 4 conduzido pela Faculdade de Medicina da Universidade de São Paulo, publicado na revista científica Rheumatology, apresentou evidências de que a CoronaVac é segura e induz resposta imune em pacientes com miopatias autoimunes sistêmicas. Trata-se de um grupo heterogêneo de doenças sistêmicas raras que acometem principalmente os músculos estriados esqueléticos, podendo também atingir pulmões, coração e trato gastrintestinal.

Seis semanas após completarem o esquema vacinal de duas doses da CoronaVac, os 37 pacientes que participaram da pesquisa apresentaram uma atividade média de neutralização semelhante aos 79 indivíduos controles não imunocomprometidos (57,2% vs. 63%). Já a frequência de produção de anticorpos neutralizantes foi de 51,4% nos pacientes e de 77,2% nos controles.

Em relação à produção de anticorpos IgG, 64,9% dos pacientes apresentaram soroconversão, sendo que a titulação geométrica média de anticorpos IgG ficou em 7,9.
Os autores do estudo destacam que, apesar de apresentarem uma menor imunogenicidade em comparação com pessoas saudáveis, algo esperado em indivíduos imunossuprimidos, os pacientes desenvolveram uma boa resposta ao SARS-CoV-2. Além disso, não foi observado nenhum efeito adverso moderado ou grave, comprovando a segurança da CoronaVac nessa população. A frequência de reações adversas leves foi similar em ambos os grupos.

Durante o acompanhamento, seis indivíduos (três pacientes e três controles) tiveram Covid-19, sendo cinco entre a primeira e a segunda dose e apenas um após a segunda dose. Todos desenvolveram sintomas leves e sem necessidade de hospitalização.

Publicado em: 19/10/2021

RHEUMATOLOGY

Rheumatology 2021;00:1–11
https://doi.org/10.1093/rheumatology/keab773
Advance access publication 19 October 2021

Original article

Systemic autoimmune myopathies: a prospective phase 4 controlled trial of an inactivated virus vaccine against SARS-CoV-2

Samuel K. Shinjo[1], Fernando H. C. de Souza[1], Isabela B. P. Borges[1], Alexandre M. dos Santos[1], Renata Miossi[1], Rafael G. Misse[1], Ana C. Medeiros-Ribeiro[1], Carla G. S. Saad[1], Emily F. N. Yuki[1], Sandra G. Pasoto[1], Léonard V. K. Kupa[1], Carina Ceneviva[2], Júlia C. Seraphim[1], Tatiana N. Pedrosa[1], Margarete B. G. Vendramini[1], Clóvis A. Silva[3], Nádia E. Aikawa[1,3] and Eloisa Bonfá [1]

Abstract

Objectives. To evaluate immunogenicity and safety of an inactivated SARS-CoV-2 vaccine in systemic autoimmune myopathies (SAMs) and the possible influence of baseline disease parameters, comorbidities and therapy on immune response.

Methods. This prospective controlled study included 53 patients with SAMs and 106 non-immunocompromised control group (CTRL). All participants received two doses of the Sinovac-CoronaVac vaccine (28-day interval). Immunogenicity was assessed by anti-SARS-CoV-2 S1/S2 IgG seroconversion (SC), anti-S1/S2 IgG geometric mean titre (GMT), factor increase GMT (FI-GMT), neutralizing antibodies (NAb) positivity, and median neutralizing activity after each vaccine dose (D0 and D28) and six weeks after the second dose (D69). Participants with pre-vaccination positive IgG serology and/or NAb and those with RT-PCR confirmed COVID-19 during the protocol were excluded from immunogenicity analysis.

Results. Patients and CTRL had comparable sex ($P>0.99$) and age ($P=0.90$). Immunogenicity of 37 patients and 79 CTRL-naïve participants revealed at D69, a moderate but significantly lower SC (64.9% *vs* 91.1%, $P<0.001$), GMT [7.9 (95%CI 4.7–13.2) *vs* 24.7 (95%CI 30.0–30.5) UA/ml, $P<0.001$] and frequency of NAb (51.4% *vs* 77.2%, $P<0.001$) in SAMs compared with CTRL. Median neutralizing activity was comparable in both groups [57.2% (interquartile range (IQR) 43.4–83.4) *vs* 63.0% (IQR 40.3–80.7), $P=0.808$]. Immunosuppressives were less frequently used among NAb+ patients *vs* NAb- patients (73.7% *vs* 100%, $P=0.046$). Type of SAMs, disease status, other drugs or comorbidities did not influence immunogenicity. Vaccine-related adverse events were mild with similar frequencies in patients and CTRL ($P>0.05$).

Conclusion. Sinovac-CoronaVac is safe and has a moderate short-term immunogenicity in SAMs, but reduced compared with CTRL. We further identified that immunosuppression is associated with diminished NAb positivity.

Trial registration. COVID-19 CoronaVac in Patients With Autoimmune Rheumatic Diseases and HIV/AIDS (CoronavRheum), http://clinicaltrials.gov/ct2/show/NCT04754698

Key words: anti-SARS-CoV-2 vaccine, COVID-19, immunogenicity, myositis, neutralizing antibodies, safety

Rheumatology key messages

- Sinovac-CoronaVac is safe for patients with systemic autoimmune myopathies (SAMs).
- Anti-SARS-CoV-2 S1/S2 IgG seroconversion rates were of moderate effect.
- SAM patients have a moderate NAb response but it is reduced compared to the control group.

[1]Division of Rheumatology, [2]Central Laboratory Division and [3]Pediatric Rheumatology Unit, Childrens' Institute, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, SP, Brazil (BR)

Submitted 24 July 2021; accepted 9 October 2021

Correspondence to: Samuel Katsuyuki Shinjo, Av. Dr. Arnaldo, 455, 3° andar, sala 3184, Cerqueira César, CEP 01246-903, Sao Paulo, SP, Brazil. E-mail: samuel.shinjo@usp.br

Introduction

Since the first case in Wuhan, China, in December 2019, the novel coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to more than four million deaths and ~220 million confirmed cases worldwide up to August 2021 [1].

Several studies have identified risk factors associated with severe COVID-19, such as cardiovascular diseases and other comorbidities, male gender and age [2–4]. In addition, systemic autoimmune rheumatic diseases patients may have a worse COVID-19 associated prognosis [5, 6], due to the disease-associated immune dysregulation and immunosuppressive drugs.

Among these systemic autoimmune rheumatic diseases, idiopathic inflammatory myopathies or systemic autoimmune myopathies (SAMs) are a group of rare and heterogeneous diseases that affect primarily the striated skeletal muscles, including DM, PM, antisynthetase syndrome (ASSD), immune-mediated necrotizing myopathies (IMNM), inclusion body myositis, neoplasia-associated myositis and myositis-overlap syndromes [7–9]. Other tissues and systems may be also involved, such as skin, heart, joint, lung and gastrointestinal tract [7].

Gupta *et al.* [10] report challenges for SAMs patients in a large descriptive study during the COVID-19 pandemic, particularly health problems attributed to the pandemic, need to increase or facing of obstacles in the acquisition of medicines, hospitalization for disease-related complications, and reduction of physical exercises. More than a half of patients with SAMs had underlying cardiovascular risk factors and frequently required an increase in drug therapy due to worsening in health-related problems during the pandemic, resulting in a high risk for severe COVID-19 infection. Moreover, patients with SAMs are susceptible to general or opportunistic infections [11, 12]. The use of high doses of glucocorticoids and immunosuppressive drugs are potential risk factors associated with these complications [11]. Therefore, in the context of the COVID-19 pandemic, it becomes extremely important to establish strategic measures to protect these patients against SARS-CoV-2.

An extensive and intensive task force around the world has been combating and containing the SARS-CoV-2 through the development of COVID-19 vaccines. There are, however, few studies evaluating safety and immunogenicity after at least one vaccine dose or two shots of the messenger RNA (mRNA) (BioNTech/Pfizer, Moderna or BNT162b2) and Oxford/Astra- Zeneca/ChAdOx1 nCoV-19 anti-SARS-CoV-2 vaccines in systemic autoimmune rheumatic diseases populations, including <20 SAMs patients [13–19]. Our group has recently reported an overall adequate anti-SARS-CoV-2 IgG seroconversion rate (70.4%) with Sinovac-CoronaVac vaccine in 910 naïve adult autoimmune rheumatic diseases patients compared with 182 age and sex-matched subjects' frequencies showing a diminished frequency of COVID-19 incident cases after immunization [20]. However, none of these studies specifically assessed SAMs and its peculiar disease factors and treatment with an age- and sex-balanced population, in order to more accurately define vaccine response in this group of patients.

Therefore, the present study aimed to evaluate the safety and immunogenicity of Sinovac-CoronaVac vaccine in patients with SAMs compared with a control (CTRL) population, as well as to analyse the potential harmful effect of disease parameters, comorbidities and therapy on vaccine-induced antibody response.

Patients and method

Study design

This prospective phase 4 controlled study is within the protocol of a larger phase 4 trial (clinicaltrials.gov #NCT04754698) that assessed the immunogenicity and safety of the Sinovac-CoronaVac COVID-19 vaccine in a large sample of patients with systemic autoimmune rheumatic diseases [20]. The present study was conducted at a single tertiary centre in Sao Paulo (Brazil). The study had three in-person visits that occurred mostly on 9–10 February 2021 (D0—first vaccine dose), on 9–10 March 2021 (D28—second vaccine dose) and on 19 April 2021 (D69). For those unable to attend, we set a 15-day period for the recap.

The study was conducted according to the Declaration of Helsinki and local regulations and was approved by Comissão de Ética para Análise de Projetos de Pesquisa (CAPPesq) and Comissão Nacional de Ética em Pesquisa (CONEP) – the local and national ethical committees, respectively (CAAE: 42566621.0.0000.0068). Written informed consent was obtained from participants before enrolment.

Participants, inclusion and exclusion criteria

SAMs patients

Patients with SAMs from the Inflammatory Myopathy Outpatient Clinics were invited to participate in the study if they were 18 years or older, and if they fulfilled the EULAR/ACR2017 classification criteria for the inflammatory myopathies [8], and patients with ASSD fulfilled the criteria used by Behrens Pinto *et al.* (2020) [21]. All patients with ASSD had a positive anti-Jo-1 antibody.

Exclusion criteria

Exclusion criteria were history of anaphylactic response to vaccine components, acute febrile illness or symptoms compatible to COVID-19 at vaccination, Guillain–Barre syndrome, decompensated heart failure, demyelinating disease, previous vaccination with any SARS-CoV-2 vaccine, history of live virus vaccine up to four weeks before, history of inactivated virus vaccine up to two weeks before vaccination, history of having received blood products up to six months before vaccination,

cancer-associated myopathies, and inflammatory myopathies overlapping syndromes. Participants with pre-vaccination positive COVID-19 anti-S1/S2 IgG serology and/or SARS-CoV-2 cPass virus-neutralization antibodies (NAb) were excluded from immunogenicity analysis. Patients with RT-PCR confirmed COVID-19 infection after the first vaccine dose and during the protocol were excluded from the immunogenicity analysis.

Seventy SAMs patients were initially selected to participate after the review of the last 3-month medical records using an electronic database (Fig. 1). We preferentially selected patients with well-controlled disease to avoid hospitalizations or changes in therapy during the next three months of study. Selection of patients began within three weeks of the initial protocol, immediately after the emergency's approval of the vaccine in Brazil and invitations began after the ethics committee sanction of the trial. Among the invited patients, 17 patients were excluded due to refusal to participate ($n=3$), hospitalization ($n=1$), difficult coming to the hospital in the pre-established dates for vaccination ($n=5$), scheduled to receive rituximab within short period of vaccination ($n=3$) and disease activity ($n=5$). SAMs patients and CTRL+ groups were balanced for age (up to $\pm$ 5 years' difference) and sex, using an Excel program for random selection of individuals in each category, with a 1 SAM : 2 CTRL ratio. Fifty-three patients comprised the study group, and 106 individuals with no autoimmune rheumatic disease or other immunosuppressive condition and without immunosuppressive therapy composed the CTRL group, who were recruited among healthcare workers from our centre. None of them had received the previous anti-SARS-CoV-2 vaccine.

Demographic data, comorbidities, disease activity parameters and treatments

The patients were clinically assessed, and a standardized interview was performed by physicians with expertise in SAMs. The following data were collected: current age, ethnicity, sex, type of SAMs, disease duration, comorbidities (e.g. systemic arterial hypertension, diabetes mellitus, dyslipidaemia, obesity, myocardial infarction, interstitial lung disease and stroke), habits (smoking) and current therapy (e.g. glucocorticoids, immunosuppressive and immunobiological drugs).

The disease status at D0 (first vaccine dose) was assessed using the International Myositis Assessment and Clinical Studies Groups (IMACS) core set measures, which included application of questionnaires based on scores of the Manual Muscle Testing-8 (MMT-8), Myositis Disease Activity Assessment Visual Analogue Scales (MYOACT), HAQ, global assessment of the disease by the physician and by the patient using the Visual Analogue Scale (VAS) [22–24]. The serum levels of creatine phosphokinase (CPK, reference value: 26–192 U/l) were also tested only at the baseline of the protocol (D0).

Vaccination protocol

The vaccination protocol for patients with SAMs and CTRL consisted of a two-dose schedule of the COVID-19 vaccine. The first dose with blood collection was given mostly on 9–10 February 2021 (D0), the second dose with blood collection on 9–10 March 2021 (D28), and the last blood collection occurred on 19 April 2021 (D69). In case of incident COVID-19 between vaccine doses, the second dose was delayed four weeks after the beginning of symptoms. Ready-to-use syringes loaded with CoronaVac (Sinovac Life Sciences, Beijing, China, batch #20200412), that consists of 3 μg in 0.5 ml of β-propiolactone inactivated SARS-CoV-2 (derived from the CN02 strain of SARS-CoV-2 grown in African green monkey kidney cells – Vero 25 cells) with aluminum hydroxide as an adjuvant were administered intramuscularly in the deltoid area.

Immunogenicity evaluation

Primary immunogenicity evaluation included seroconversion rates of total anti-SARS-Cov-2 S1/S2 IgG and presence of NAb at D69. Secondarily, immunogenicity was assessed by anti-S1/S2 IgG seroconversion and presence of NAb at D28 (after vaccine first dose); geometric mean titres of anti-S1/S2 IgG and their factor-increase in GMT (FI-GMT) at D28 and D69; and median (interquartile range) neutralizing activity of NAb at D28 and D69. In order to assess these outcomes, blood samples (20 ml) from all participants were obtained at days D0 (baseline – immediately before first vaccine dose), D28 (immediately before the second dose) and D69 (six weeks after the second dose). Sera were stored in a -70°C freezer.

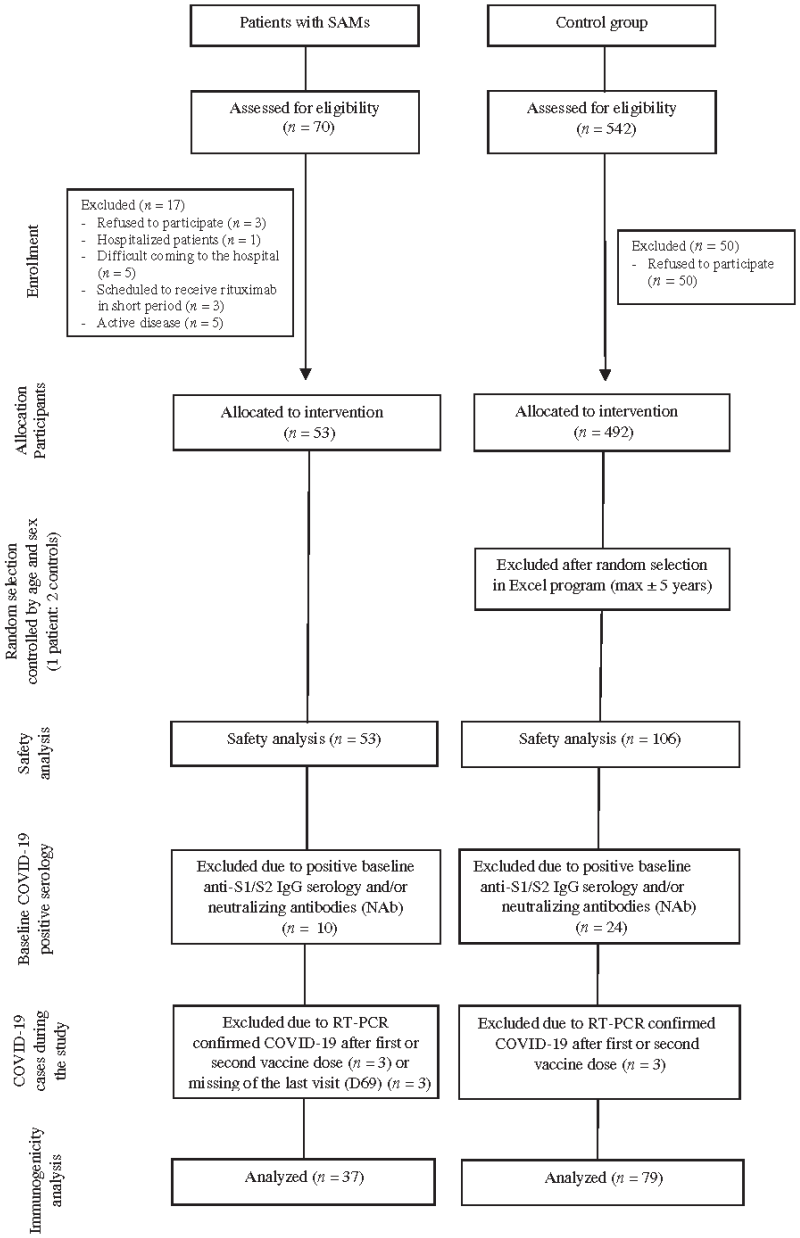
Anti-SARS-CoV-2 S1/S2 IgG antibodies

A chemiluminescent immunoassay was used to measure human IgG antibodies against the S1 and S2 proteins in the RBD (Indirect ELISA, LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin, Italy). Seroconversion rate (SC) was defined as positive serology (>15.0 UA/ml) post-vaccination, taking into consideration that only patients with pre-vaccination negative serology were included. Geometric mean titres (GMT) and 95% CI of these antibodies were also calculated at all time points, attributing the value of 1.9 UA/ml (half of the lower limit of quantification 3.8 UA/ml) to undetectable levels (<3.8 UA/ml). The factor increase in GMT (FI-GMT) is the ratio of the GMT after vaccination to the GMT before vaccination, showing the growth in titres. They are also presented and compared as geometric means and 95% CI.

NAb

The SARS-CoV-2 neutralizing antibodies analysis was performed according to manufacturer instructions using sVNT Kit (GenScript, Piscataway, NJ, USA). This analysis detects circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor-binding domain of the viral spike glycoprotein with the angiotensin-converting enzyme 2 cell surface receptor. The tests were performed on the ETI-MAX-

FIG. 1 Flow chart of the present study



Nab: neutralization antibodies; SAMS: systemic autoimmune myopathies.

3000 equipment (DiaSorin, Italy). The samples were classified as either 'positive' (inhibition ≥30%) or 'negative' (inhibition <30%), as suggested by the manufacturer [25]. The frequency of positive samples was calculated at all time points. Median [interquartile range (IQR) 25th–75th] of the percentage of neutralizing activity only for positive samples were calculated at all time points.

Vaccine adverse events and incident cases of COVID-19

Patients and CTRL were advised to report any adverse events of the vaccine and they received on D0 (first dose) and on D28 (second dose) a standardized diary for local and systemic manifestations. Vaccine adverse event severity was defined according to World Health Organization (WHO) definition [1]. Additionally, all

patients and CTRL were instructed to communicate any manifestation associated or not with COVID-19 through telephone, smartphone instant messaging, or email. Independent vaccine experts monitored the study regarding anything adverse for data safety.

RT-PCR for SARS-CoV-2 incident cases

Clinical samples for SARS-CoV-2 RT-PCR consisted of naso- and oropharyngeal swabs, collected at our central laboratory [26] or another laboratory if the patient was unable to come to our hospital.

Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate the distribution of each parameter. The results were presented as mean (S.D.), median (IQR 25th–75th) for continuous variables, whereas the categorical variables were presented as frequency (%). Continuous variables were compared by *t*-Student or Mann–Whitney test for intergroup comparisons when applicable, whereas categorical variables were compared using the χ^2 or Fisher's exact tests when applicable. Specifically, continuous data regarding anti-S1/S2 IgG serology titres are presented as geometric means (95% CI) and compared with the same tests, but in neperian (ln) logarithm-transformed data. Comparisons of ln-transformed IgG titres between SAMs and CTRL in the three time points (D0, D28 and D69) were performed using generalized estimating equations (EEG) with normal marginal distribution and gamma distribution, respectively and identity binding function assuming first-order autoregressive correlation matrix between moments. Results were followed by Bonferroni multiple comparisons to identify differences between groups and time points. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences, version 20.0 (IBM-SPSS for Windows. 20.0, Chicago, IL, USA).

Results

Participants

Fifty-three patients with SAMs (25 with ASSD, 24 with DM and 4 with IMNM) with median disease duration of 6.0 (4.5–9.0) years, and 106 CTRL were prospectively assessed. SAMs and CTRL had comparable current age ($P = 0.925$), female sex ($P > 0.999$) and ethnicity distribution ($P = 0.312$) (Table 1). The disease duration was 6.0 (4.5–9.0) months. Seven (13.2%) patients with SAMs and seven (6.9%) CTRL ($P = 0.166$) were unable to attend on the defined days; therefore, they had up to 15 days for the recap.

Comorbidities were balanced in SAMs and CTRL, except for a higher prevalence of systemic arterial hypertension, dyslipidaemia and obesity in patients with SAMs compared with CTRL (Table 1). Interstitial lung disease occurs only in patients with SAMs, whereas one stroke case occurred in CTRL. There were no cases of arterial or venous thrombosis, chronic kidney disease, pulmonary hypertension, hemorrhage, liver disease, cancer, tuberculosis and HIV in both groups.

All patients had stable or low disease activity, based on the IMACS core set scores at baseline (Table 1). Concerning current treatment, 15 (28.3%) patients were under prednisone with current median dose of 6.3 (5.0–13.8) mg/day and the cumulative dose of the six previous months was 1.6 (1.1–4.8) g. In addition, 44 (83.0%) patients were using immunosuppressive drugs, six (11.3%) patients were under rituximab and one (1.9%) tofacitinib (Table 1). None of the immunosuppressive drugs, including CYC, rituximab and mycophenolate mofetil were discontinued in patients with SAMs.

Vaccine immunogenicity

Samples

For this assessment, 16 patients with SAMs were excluded: 10 patients had pre-vaccination positive COVID-19 IgG serology or NAb positivity, three patients had RT-PCR confirmed COVID-19 after the first dose of vaccine until D69, two patients who did not attend the final visit, and one patient deceased (not related to COVID-19). In the CTRL group, 24 individuals were excluded from immunogenicity analysis for positive anti-S1/S2 IgG and/or NAb at D0 and another three for RT-PCR confirmed COVID-19 during the protocol.

Anti-SARS-CoV-2 IgG antibodies

Humoral response to Sinovac-CoronaVac is shown in Table 2. Analysis of SARS-CoV-2 S1/S2 IgG response revealed that six weeks after vaccine second dose, SC rates were moderate but lower than CTRL (64.9% *vs* 91.1%, respectively; $P < 0.001$). GMT and FI-GMT were also significantly lower in patients with SAMs compared with CTRL ($P < 0.001$ and $P < 0.001$, respectively) (Table 2).

NAb

After complete vaccination, NAb positivity was also moderate but reduced when compared with CTRL (51.4% *vs* 77.2%, $P < 0.01$), whereas the median NAb was comparable in both groups after the first [39.2 (38.4–52.5) *vs* 46.6 (36.9–73.3), $P = 0.573$] and second dose [57.2 (43.4–83.4) *vs* 63.0 (40.3–80.7), $P = 0.808$] (Table 3).

Factors associated with seroconversion and NAb positivity among patients with SAMs

Patients with NAb positivity used less often immunosuppressive drugs than those without NAb (73.7% *vs* 100%, $P = 0.046$). Likewise, the median of patient global activity (VAS) was lower in the former group [1.0 (0.0–3.0) *vs* 2.0 (2.0–3.0), $P = 0.029$] (Table 4), although both groups were characterized by mild value alterations.

Vaccine tolerance and safety

Sinovac-CoronaVac vaccine tolerance and safety analysis is shown in Table 5. No moderate/severe adverse events were observed. The frequency of mild symptoms

Table 1 Baseline characteristics of patients with systemic autoimmune myopathies and controls

	SAMs ($n = 53$)	CTRL ($n = 106$)	*P*-value
Demographics			
Current age (years)	50.7 (11.1)	50.5 (10.6)	0.925
Disease duration (years)	6.0 (4.5–9.0)	–	–
Female sex	40 (75.5)	80 (75.5)	>0.999
White ethnicity	28 (52.8)	47 (44.3)	0.312
Comorbidities and habits			
Systemic arterial hypertension	28 (52.8)	38 (35.8)	0.041
Diabetes mellitus	10 (18.9)	18 (17.0)	0.768
Dyslipidaemia	14 (26.4)	7 (6.6)	0.001
BMI $\geq$30 kg/m^2	26 (49.1)	27 (25.5)	0.003
Myocardial infarction	2 (3.8)	2 (1.9)	0.601
Interstitial lung disease	19 (35.8)	0	–
Stroke	0	1 (0.9)	–
Current smoking	2 (3.8)	11 (10.4)	0.222
Type of diseases			
DM	24 (45.3)	–	–
Antisynthetase syndrome	25 (47.2)	–	–
IMNM	4 (7.5)	–	–
Disease status			
HAQ (0.0–3.0)	0.0 (0.0–0.0)		
Patients' EVA (0–10)	1.0 (0.0–3.0)		
Physician's EVA (0–10)	0.0 (0.0–1.0)		
MMT-8 (0–80)	80 (80–80)		
MYOACT (0–60)	0.0 (0.0–0.0)		
Creatine phosphokinase (U/l)	110 (78–174)		
Current therapy			
Prednisone (current use)	15 (28.3)	–	–
Dose (mg/day)	6.3 (5.0–13.8)		
Cumulative dose[a] (g)	1.6 (1.1–4.8)		
Immunosuppressive drugs	44 (83.0)	–	–
Mycophenolate mofetil	19 (35.8)	–	–
MTX	11 (20.8)	–	–
AZA	8 (15.1)	–	–
LEF	6 (11.3)	–	–
Ciclosporin	3 (5.7)	–	–
CYC	2 (3.8)	–	–
Rituximab	6 (11.3)	–	–
Tofacitinib	1 (1.9)	–	–

Results are expressed in mean (S.D.), median (interquartile range 25th–75th), and *n* (%). CTRL: control group; HAQ: Healthy Assessment Questionnaire; IMNM: immune-mediated necrotizing myopathies; MMT: manual muscle testing; MYOACT: Myositis Disease Activity Assessment Visual Analogue Scales; SAMs: systemic autoimmune myopathies; VAS: Visual Analogue Scale. [a]Last six months.

was comparable in patients with SAMs and CTRL, except for significantly higher prevalence of headache in patients with SAMs at the first vaccine dose (26.4% *vs* 8.5%, $P = 0.002$). No differences were observed in the frequencies of myalgia or muscle weakness among groups.

COVID-19 incident cases

A total of six incident symptomatic cases of COVID-19 confirmed by RT-PCR were identified among SAMs ($n = 3$) and CTRL ($n = 3$) throughout the study period. Three CTRL individuals and two patients with SAMs had COVID-19 between the first and second dose, whereas one patient had COVID-19 three weeks after the second dose. All participants had mild symptoms and none required hospitalization.

Discussion

To our knowledge, this is the largest study demonstrating a short-term disease safety and moderate immunogenicity of anti-SARS-CoV-2 inactivated vaccine in patients with SAMs but reduced compared with an age and sex-balanced non-immunocompromised control group. We further identified that immunosuppressive therapy reduces antibody response.

Table 2 Seroconversion rates and anti-SARS-CoV-2 S1/S2 IgG GMT in naïve patients with myositis and control group

	Before vaccine First dose	After vaccine First dose (D28)			After vaccine Second dose (D69)		
	GMT	SC	GMT	FI-GMT	SC	GMT	FI-GMT
SAMs ($n=37$)	2.1 (1.9–2.3)	3 (8.1)	3.3 (2.5–4.3)[a]	1.5 (1.2–2.0)[a]	24 (64.9)[a]	16.6 (9.7–28.3)[a,b]	7.9 (4.7–13.2)[a]
CTRL ($n=79$)	2.4 (2.1–2.7)	27 (34.2)	9.6 (7.2–12.9)	4.1 (3.2–5.1)	72 (91.1)	58.5 (48.4–70.8)[c,d]	24.7 (20.0–30.5)
P-value (SAMs *vs* CTRL)	0.630	0.005	<0.001	<0.001	<0.001	<0.001	<0.001

Results are expressed in mean (95% CI) or frequency (%). CTRL: control group; FI-GMT: factor increase of geometric mean titres; GMT: geometric mean titres (AU/ml); SAMs: systemic autoimmune myopathies; SC: seroconversion. Frequencies of SC are presented as number (%), and they were compared using two-sided χ^2 test between SAMs and CTRL at D28 and D69. Anti-S1/S2 IgG were expressed as geometric means (CI95%). Titers were compared between SAM and CTRL and between time points (D0, D28 and D69) using generalized estimating equations (EEG) with normal marginal distribution and gamma distribution, respectively. Results were followed by Bonferroni multiple comparisons to identify differences between groups and time points. [a]$P<0.001$ for longitudinal comparison of GMT in SAMs at D69 *vs* baseline. [b]$P<0.001$ for longitudinal comparison of GMT in SAMs at D69 *vs* D28. [c]$P<0.001$ for longitudinal comparison of GMT in controls at D28 and D69 *vs* baseline. [d]$P<0.001$ for longitudinal comparison of GMT in controls at D69 *vs* D28.

Table 3 Neutralizing antibodies and neutralizing activity in naïve patients with myositis in comparison to control group

	After vaccine first dose		After vaccine second dose	
	Subjects with positive NAb	Neutralizing activity (%)	Subjects with positive NAb	Neutralizing activity (%)
SAMs ($n=37$)	5 (13.5)[a]	39.2 (38.4–52.5)	19 (51.4)[a]	57.2 (43.4–83.4)
CTRL ($n=79$)	26 (32.9)	46.6 (36.9–73.3)	61 (77.2)	63.0 (40.3–80.7)

Results are expressed in median (25th–75th) or frequency (%). CTRL: control group; NAb: neutralizing antibodies; SAMs: systemic autoimmune myopathies. [a]$P<0.01$ in comparison to controls.

One advantage of the present study was the prospective analysis with a representative sample of patients with well-defined SAMs taking into consideration that they are a group of patients with rare conditions and the strict exclusion criteria applied herein. Another strength of the present study was that patients had comparable age and sex of the CTRL, as immunogenicity can vary according to these parameters [27, 28]. We also excluded cancer-associated myopathies and other associated autoimmune conditions in order to have a more homogeneous population [29]. A limitation of the present study is the inclusion of patients solely from a tertiary care centre who may not represent the full spectrum of SAMs and could result in an overestimation of the disease or drug complications in the context of a more severe disease.

All individuals were followed with three scheduled face-to-face appointments, telephone calls and smartphone instant messaging, which allowed a precise monitoring of vaccine-induced adverse effects in all phases of the study. The exclusion of pre-vaccination seropositive participants and those with RT-PCR confirmed COVID-19 during the study period were also relevant, allowing a more accurate evaluation of this vaccine response. The strict schedule for blood sample collection and vaccination in two days aimed to guarantee that most patients with SAMs and CTRL would be vaccinated in the same timeframe during the pandemic, precluding the possible confounding nonlinear relationship between the elapsed time and immune response.

Currently, most studies on the immunogenicity and safety of the anti-SARS-CoV-2 vaccines in patients with systemic autoimmune rheumatic diseases evaluated distinct vaccines, mainly mRNA or vector-borne vaccines [13–19]. Regarding safety, all those studies related acceptable rates of adverse events [13–20], without apparent impact on disease activity. However, specifically for SAMs, the number of patients was small [14–19], and they were not evaluated with specific and validated instruments for SAMs. The current study adds data about the safety of the inactivated vaccine in well-controlled patients with SAMs, using specific and validated instruments at baseline [22–24]. Importantly,

Table 4 Baseline characteristics of patients regarding to seroconversion for anti-SARS-CoV-2 S1/S2 IgG, and neutralizing antibodies positivity

	Patients with SC ($n = 24$)	Patients without SC ($n = 13$)	*P*-value	Patients with Nab ($n = 19$)	Patients without Nab ($n = 18$)	*P*-value
Demographic data						
Current age (years)	50.0 (11.7)	55.0 (8.9)	0.187	48.8 (11.6)	54.9 (9.4)	0.090
Current age >60 years	3 (12.5)	2 (15.4)	>0.999	2 (10.5)	3 (16.7)	0.660
Female sex	16 (66.7)	12 (92.3)	0.119	13 (68.4)	15 (83.3)	0.447
White ethnicity	14 (58.3)	6 (46.2)	0.478	11 (57.9)	9 (50)	0.630
Diseases						
DM	11 (45.8)	6 (46.2)	>0.999	7 (36.8)	10 (55.6)	0.330
Antisynthetase syndrome	11 (45.8)	6 (46.2)	>0.999	10 (52.6)	7 (38.9)	0.515
IMNM	2 (8.4)	1 (7.6)	>0.999	2 (10.6)	1 (5.5)	>0.999
Disease parameters						
HAQ (0.0–3.0)	0.0 (0.0–1.2)	0.0 (0.0–0.0)	0.537	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.746
Patients' EVA (0–10)	1.0 (0.0–2.8)	3.0 (2.0–3.0)	0.058	1.0 (0.0–3.0)	2.0 (2.0–3.0)	**0.029**
Physician's EVA (0–10)	0.0 (0.0–0.0)	0.0 (0.0–3.0)	0.387	0.0 (0.0–0.0)	0.0 (0.0–3.0)	0.221
MMT-8 (0–80)	80 (80–80)	80 (79–80)	0.353	80 (80–80)	80 (80–80)	0.558
MYOACT (0–60)	0.0 (0.0–10.0)	0.0 (0.0–3.5)	0.479	0.0 (0.0–1.0)	0.0 (0.0–0.8)	0.940
Creatine phosphokinase (U/l)	121 (89–183)	99 (74–189)	0.460	124 (81–181)	111 (74–189)	0.663
Prednisone						
Current use	6 (25)	7 (53.8)	0.096	5 (26.3)	8 (44.4)	0.298
Dose (mg/day)	6.3 (2.5–20.0)	5 (2.5–30.0)	0.945	10.0 (7.3)	9.1 (8.9)	0.847
Dose >10 mg/day	2 (8.3)	3 (23.1)	0.321	2 (10.5)	3 (16.7)	0.660
Immunosuppressive drugs	19 (79.2)	13 (100)	0.140	14 (73.7)	18 (100)	**0.046**
Mycophenolate mofetil	7 (29.2)	8 (61.5)	0.056	6 (31.5)	9 (50)	0.254
MTX	7 (29.2)	1 (7.7)	0.216	5 (26.3)	3 (16.7)	0.693
AZA	4 (16.7)	2 (15.4)	1.000	3 (15.7)	3 (16.7)	>0.999
LEF	3 (12.5)	0 0	0.538	2 (10.5)	1 (5.6)	>0.999
Ciclosporin	0	2 (15.4)	–	0	2 (11.1)	–
CYC	1 (4.2)	1 (7.7)	1.000	1 (5.3)	1 (5.6)	1.000
Rituximab	3 (12.5)	3 (23.1)	0.643	2 (10.5)	4 (22.2)	0.405

Results are expressed in mean (s.d.), median (interquartile range 25th–75th) and frequency (%). Bold text indicates significance. IMNM: immune-mediated necrotizing myopathies; Nab: neutralization antibodies; SAMs: systemic autoimmune myopathies; SC: seroconversion.

vaccine safety was demonstrated by the absence of severe or moderate adverse events related to vaccination with only mild and self-limiting side effects.

We observed that patients with SAMs had a moderate immune response to this vaccine and within the standards established by Food and Drugs Administration (FDA) and European Medicine Agency for Emergency Use Authorization of pandemic vaccines [30, 31]. In addition, the WHO recently approved the Sinovac-CoronaVac COVID-19 vaccine for emergency use [32]. However, after complete vaccination, the immunogenicity was lower compared with CTRL, but with SC rates comparable to the 64% reported for the pandemic influenza A H1N1 inactivated vaccine in a study of 1,600 autoimmune rheumatic disease patients [33]. Our findings with Sinovac-CoronaVac vaccine confirm and extends Furer *et al.*'s study [19] which assessed serum IgG antibody levels against SARS-CoV-2 proteins after the second dose of BNT162b2 mRNA COVID-19 vaccine and showed significantly reduced vaccine-induced immunogenicity in a small SAMs population ($n = 19$). We further demonstrated that NAb rates, now recognized as one of the major predictors of SARS-CoV-2 immune protection [34] were also moderate but lower than CTRL.

In contrast, after the first dose there was a negligible vaccine response (SC and NAb positivity) reinforcing the importance of the second dose for these patients. However, among patients who develop NAb, NAb activity was comparable for both groups after the first and second dose.

Further analysis of possible interference of clinical and laboratory parameters, comorbidities and type of SAMs in vaccine immunogenicity revealed that solely immunosuppressive drugs hampered the NAb positivity. This finding is in line with the reported reduced vaccine response in patients under mycophenolate mofetil therapy [17, 19, 20], rituximab [17–20], MTX [19, 20] and abatacept [19, 20] after different kinds of vaccines and their schedules [13–20]. Accordingly, in the present study, >80% of patients were under immunosuppressive drugs, especially mycophenolate mofetil in one third of

Table 5 Adverse events of Sinovac-CoronaVac vaccination in patients with systemic autoimmune myopathies and control group

	After vaccine first dose			After vaccine second dose		
	SAMs ($n = 53$)	CTRL ($n = 106$)	*P*-value	SAMs ($n = 50$)	CTRL ($n = 106$)	*P*-value
No symptoms	27 (50.9)	66 (62.3)	0.172	27 (54.0)	63 (59.4)	0.431
Local reactions[a]	11 (20.8)	18 (17.0)	0.561	11 (22.0)	19 (17.9)	0.579
Pain	9 (17.0)	15 (14.2)	0.638	11 (22.0)	17 (16.0)	0.390
Erythema	0	1 (0.9)	—	3 (6.0)	3 (2.8)	0.390
Swelling	0	4 (3.8)	—	4 (8.0)	6 (5.7)	0.728
Bruise	0	4 (3.8)	—	1 (2.0)	2 (1.9)	>0.999
Pruritus	2 (3.8)	1 (0.9)	0.258	2 (4.0)	6 (5.7)	>0.999
Induration	2 (3.8)	1 (0.9)	0.258	2 (4.0)	4 (3.8)	>0.999
Systemic reactions	23 (43.4)	34 (32.1)	0.161	16 (32.0)	31 (29.3)	0.775
Fever	2 (3.8)	0	—	0	3 (2.8)	—
Malaise	5 (9.4)	3 (2.8)	0.118	3 (6.0)	9 (8.5)	0.752
Somnolence	8 (15.1)	11 (10.4)	0.387	6 (12.0)	12 (11.3)	0.931
Lack of appetite	2 (3.8)	3 (2.8)	>0.999	1 (2.0)	5 (4.7)	0.664
Nausea	1 (1.9)	1 (0.9)	>0.999	1 (2.0)	10 (9.4)	0.104
Vomiting	0	0	—	0	1 (0.9)	—
Diarrhea	2 (3.8)	7 (6.6)	0.719	1 (2.0)	6 (5.7)	0.428
Abdominal pain	2 (3.8)	4 (3.8)	>0.999	2 (4.0)	5 (4.7)	>0.999
Vertigo	5 (9.4)	5 (4.7)	0.248	2 (4.0)	6 (5.7)	>0.999
Tremor	0	0	—	0	0	—
Headache	14 (26.4)	9 (8.5)	**0.002**	8 (16.0)	19 (17.9)	0.731
Fatigue	6 (11.3)	8 (7.5)	0.429	5 (10.0)	15 (14.1)	0.445
Sweating	2 (3.8)	3 (2.8)	>0.999	3 (6.0)	1 (0.9)	0.100
Myalgia	5 (9.4)	5 (4.7)	0.248	5 (10.0)	9 (8.5)	0.783
Muscle weakness	3 (5.7)	2 (1.9)	0.334	4 (8.0)	7 (6.6)	0.748
Arthralgia	4 (7.5)	6 (5.7)	0.732	5 (10.0)	8 (7.5)	0.627
Back pain	5 (9.4)	6 (5.7)	0.377	1 (2.0)	9 (8.5)	0.168
Cough	4 (7.5)	7 (6.6)	>0.999	3 (6.0)	7 (6.6)	>0.999
Sneezing	2 (3.8)	6 (5.7)	0.720	1 (2.0)	11 (10.4)	0.104
Coryza	1 (1.9)	10 (9.4)	0.101	3 (6.0)	8 (7.5)	>0.999
Stuffy nose	0 0	3 (2.8)	0.551	2 (4.0)	6 (5.7)	>0.999
Sore throat	3 (5.7)	5 (4.7)	>0.999	1 (2.0)	7 (6.6)	0.438
Shortness of breath	0	2 (1.9)	—	1 (2.0)	3 (2.8)	>0.999
Conjunctivitis	0	0	—	0	1 (0.9)	—
Pruritus	1 (1.9)	3 (2.8)	>0.999	1 (2.0)	5 (4.7)	0.664
Skin rash	1 (1.9)	2 (1.9)	>0.999	1 (2.0)	2 (1.9)	>0.999

Results are presented in frequency (%). Bold text indicates significance. [a]At the injection site. CTRL: control group; SAMs: systemic autoimmune myopathies.

patients, but also, at lower frequencies, MTX and rituximab. Although we could not show any specific drug effect due to the limited sample size, probably pooled analysis of these drugs was responsible for the interference in NAb positivity. In contrast to Furer *et al.* [19], that found a deleterious effect of glucocorticoids even at low dose [6.7 (6.3) mg/day of prednisone], we failed to show such interference with a very similar dose, also probably due to sample size.

Our patients had stable or low disease activity, according to inclusion criteria and IMACS core set measures at baseline and precluded any interpretation regarding the effect of disease activity in vaccine response, in spite of an association between mild elevated VAS of patient global activity and reduced frequency of NAb positivity. Therefore, further studies of SARS-CoV-2 vaccines with a large population of SAMs, including analysis of effect of individual immunosuppressive drugs, disease activity and different subtypes of SAMs will be necessary.

Patients with systemic autoimmune rheumatic diseases, including SAMs, may be at a higher risk for COVID-19 infection. Preliminary ACR guidelines recommended that patients with rheumatic and musculoskeletal diseases should be promptly vaccinated for COVID-19 [35]. Recent reports have also suggested that immunosuppressive drugs should be suspended for patients after COVID-19 vaccinations, particularly for

those under mycophenolate mofetil, MTX, CYC and rituximab to improve immunogenicity [36, 37]. Although our patients were in low disease activity, we choose not to withdraw medications due to the risk of reactivation and lack of definitive findings about each drug suspension at this specific population. Moreover, the current recommendations were not available during the study design.

There are limitations in the present study. First, inclusion of patients with different SAMs subtypes and from only one tertiary care centre, who may not represent the full spectrum of SAMs and could result in an overestimation of the disease activity or drug complications in the context of a more severe disease. Second, the sample size was not calculated because we used a convenience sample. Third, the FI-GMT and GMT values were not assessed for individual immunosuppressive drugs because of the small representation of each medication.

In conclusion, our data demonstrated that Sinovac-CoronaVac inactivated vaccine is safe and has a moderate short-term immunogenicity in inactive or low disease activity SAMs patients, although inferior compared with the CTRL. We further confirmed that immunosuppressive drugs have a deleterious effect on vaccine-induced antibody production, affecting in particular NAb positivity rates. These findings support the recommendation of SARS-CoV-2 vaccination for SAMs patients.

Acknowledgements

We thank the contribution of the Central Laboratory Division, Registry Division, Security Division, IT Division, Superintendence, Pharmacy Division and Vaccination Center (CRIE) for their technical support. We also thank the volunteers for handling the biological material and those responsible for the medical follow-up of all participants.

Funding: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (#2015/03756–4 to N.E.A., S.G.P., C.A.S. and E.B., #2019/17272–0 to L.V.K.K., #2019/21211–6 to J.C.S.; #2019/11776-6 to S.K.S.); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #305242/2019–9 to E.B., #304984/2020–5 to C.A.S., #303379/2018–9 to S.K.S.), B3 - Bolsa de Valores do Brasil to E.B., and Faculdade de Medicina da USP—SP to S.K.S. Instituto Butantan supplied the study product and had no other role in the trial.

Disclosure statement: The authors have declared no conflicts of interest.

Data availability statement

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. Anonymised data are available on request from the corresponding author.

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5. É eficaz em idosos

5.1 Estudo chileno com mais de dez milhões de pessoas mostra que efetividade da CoronaVac é superior a 86%, inclusive entre idosos

Um artigo publicado no The New England Journal of Medicine nesta quinta (2) mostrou mais uma vez que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, é efetiva (ou seja, tem eficácia comprovada no "mundo real" e não somente em um estudo controlado de ensaios clínicos) contra casos de Covid-19 e variantes do SARS-CoV-2, inclusive entre as pessoas com mais de 60 anos.

A pesquisa foi realizada no Chile e mostrou que a proteção da CoronaVac foi de 65,9% contra infecções por Covid-19, de 87,5% contra hospitalizações, de 90,3% contra internações em Unidades de Terapia Intensiva (UTI) e de 86,3% contra mortes. Para o grupo totalmente vacinado acima de 60 anos, a efetividade da vacina foi de 66,6% para a proteção contra infecções, de 85,3% contra hospitalizações, de 89,2% contra internações na UTI e de 86,5% para a prevenção de morte relacionada à doença.

A análise foi feita entre fevereiro e maio com cerca de 10,2 milhões de pessoas. O estudo de coorte (estudo observacional que acompanha indivíduos ao longo de um período de tempo para determinar características e evolução do grupo) contou com participantes acima dos 16 anos cadastrados no Fundo Nacional de Saúde (FONASA), programa nacional de saúde chileno, que cobre cerca de 80% da população.

Os participantes foram divididos em três grupos: não vacinados, vacinados com apenas uma dose e totalmente vacinados. Os testes para detecção da Covid-19 foram exames RT-PCR (98,1%) e testes rápidos de antígeno (1,9%). Durante o período da análise, as UTIs no Chile operavam com 93,5% da sua capacidade.

O país andino tem as taxas mais elevadas de realização de testes para detecção da Covid-19 na América Latina e um sistema padronizado de informação pública para estatísticas vitais ao estudo. O Ministério da Saúde do país utilizou 13,98 milhões de doses da vacina CoronaVac desde o começo da campanha de vacinação, em fevereiro.

Outro estudo de efetividade da CoronaVac foi realizado pelo Butantan no município paulista de Serrana. O chamado Projeto S vacinou quase toda a população adulta do município (28 mil pessoas) entre fevereiro e abril de 2021 e concluiu que o imunizante causou uma redução de 80% no número de casos sintomáticos de Covid-19, de 86% nas internações e de 95% nos óbitos.

Publicado em: 03/09/2021

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 2, 2021 VOL. 385 NO. 10

Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile

Alejandro Jara, Ph.D., Eduardo A. Undurraga, Ph.D., Cecilia González, M.D., Fabio Paredes, M.Sc., Tomás Fontecilla, M.Sc., Gonzalo Jara, B.S.E., Alejandra Pizarro, M.D., Johanna Acevedo, M.S., Katherinne Leo, B.S.E., Francisco Leon, M.B.A., Carlos Sans, B.S.E., Paulina Leighton, B.S.E., Pamela Suárez, B.S.E., Heriberto García-Escorza, M.S., and Rafael Araos, M.D.

ABSTRACT

BACKGROUND

Mass vaccination campaigns to prevent coronavirus disease 2019 (Covid-19) are occurring in many countries; estimates of vaccine effectiveness are urgently needed to support decision making. A countrywide mass vaccination campaign with the use of an inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine (CoronaVac) was conducted in Chile starting on February 2, 2021.

METHODS

We used a prospective national cohort, including participants 16 years of age or older who were affiliated with the public national health care system, to assess the effectiveness of the inactivated SARS-CoV-2 vaccine with regard to preventing Covid-19 and related hospitalization, admission to the intensive care unit (ICU), and death. We estimated hazard ratios using the extension of the Cox proportional-hazards model, accounting for time-varying vaccination status. We estimated the change in the hazard ratio associated with partial immunization (≥14 days after receipt of the first dose and before receipt of the second dose) and full immunization (≥14 days after receipt of the second dose). Vaccine effectiveness was estimated with adjustment for individual demographic and clinical characteristics.

RESULTS

The study was conducted from February 2 through May 1, 2021, and the cohort included approximately 10.2 million persons. Among persons who were fully immunized, the adjusted vaccine effectiveness was 65.9% (95% confidence interval [CI], 65.2 to 66.6) for the prevention of Covid-19 and 87.5% (95% CI, 86.7 to 88.2) for the prevention of hospitalization, 90.3% (95% CI, 89.1 to 91.4) for the prevention of ICU admission, and 86.3% (95% CI, 84.5 to 87.9) for the prevention of Covid-19–related death.

CONCLUSIONS

Our results suggest that the inactivated SARS-CoV-2 vaccine effectively prevented Covid-19, including severe disease and death, a finding that is consistent with results of phase 2 trials of the vaccine. (Funded by Agencia Nacional de Investigación y Desarrollo and others.)

From the Ministry of Health (A.J., C.G., F.P., T.F., G.J., A.P., J.A., K.L., F.L., C.S., P.L., P.S., H.G.-E., R.A.), Facultad de Matemáticas (A.J.) and Escuela de Gobierno (E.A.U.), Pontificia Universidad Católica de Chile, Millennium Nucleus Center for the Discovery of Structures in Complex Data (A.J.), Millennium Initiative for Collaborative Research in Bacterial Resistance (E.A.U., R.A.), the Research Center for Integrated Disaster Risk Management (E.A.U.), Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo (R.A.), and the Advanced Center for Chronic Diseases (R.A.) — all in Santiago, Chile; and the CIFAR Azrieli Global Scholars Program, CIFAR, Toronto (E.A.U.). Address reprint requests to Dr. Araos at Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Clínica Alemana Universidad del Desarrollo, Av. Las Condes 12461, Las Condes 7590943, Chile, or at rafaelaraos@udd.cl.

Drs. Jara, Undurraga, and Araos contributed equally to this article.

This article was published on July 7, 2021, and updated on July 13, 2021, at NEJM.org.

N Engl J Med 2021;385:875-84.
DOI: 10.1056/NEJMoa2107715

THE CORONAVIRUS DISEASE 2019 (COVID-19) pandemic has imposed an enormous disease burden worldwide, with more than 159 million cases and approximately 3.3 million deaths reported as of May 10, 2021.[1] Covid-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and the severity ranges from mild symptoms to life-threatening disease.[2] Older age and underlying conditions substantially increase the case fatality rate.[3,4] Nonpharmaceutical interventions, such as social distancing, face masks, and contact tracing, have so far been the mainstay of health policy strategies to reduce viral spread and limit demands on health care.[5,6] New Covid-19 vaccines are beginning to change this situation. On December 2, 2020, the first vaccine tested in a large, randomized clinical trial was approved in the United Kingdom,[7,8] although some countries began vaccinations before clinical results were available. Several effective vaccines against Covid-19 have been developed and approved in record time,[8-12] and numerous new vaccines are in the final stages of clinical trials.[13]

Mass vaccination campaigns to prevent Covid-19 are now occurring in many countries.[14] Preliminary results of the effectiveness of other Covid-19 vaccines across different populations have been published, including studies at the national level in Israel[15] and Scotland[16] and studies involving essential frontline workers at specific locations in the United States.[17-19] Estimates of vaccine effectiveness in the prevention of Covid-19 are essential because they reflect real-world challenges, such as logistics, cold chains, vaccination schedules, and follow-up, and also involve more diverse populations than those selected in randomized clinical trials, such as older or immunocompromised persons or those with coexisting conditions. Despite being the standard for assessing vaccine efficacy, phase 3 clinical trials have some limitations, such as restrictive inclusion criteria and implementation under strict experimental conditions that may not resemble a mass vaccination rollout.[20] Thus, large observational studies to estimate the effectiveness of new vaccines in real-world settings are an essential complement to randomized, controlled trials.[21]

Existing vaccine-effectiveness estimates have focused on the BNT162b2 messenger RNA (mRNA) vaccine (Pfizer–BioNTech), the ChAdOx1 nCoV-19 vaccine (Oxford–AstraZeneca), and the mRNA-1273 vaccine (Moderna).[15-19] Several countries are conducting vaccination campaigns with the use of an inactivated SARS-CoV-2 vaccine (CoronaVac) amid a record surge of Covid-19 cases worldwide.[1,13] A total of 22 primarily low- and middle-income countries have approved the CoronaVac vaccine for emergency use. Despite its global importance, limited evidence is available on the efficacy or effectiveness of this vaccine.

Phase 1–2 trials of the CoronaVac vaccine[22] were carried out in China among participants 18 to 59 years of age[23] and in participants 60 years of age or older.[24] The findings suggested that the vaccine was safe and immunogenic in most patients 14 days after receipt of the second dose. Phase 3 clinical trials are taking place in Brazil, Chile, Indonesia, and Turkey (ClinicalTrials .gov numbers, NCT04456595, NCT04651790, NCT04508075, and NCT04582344, respectively). Efficacy results from these trials have not yet been published, but reported efficacy estimates from the manufacturers with regard to mild Covid-19 have varied substantially among the sites: 50.7% (95% confidence interval [CI], 35.6 to 62.2) in Brazil, 65.3% in Indonesia, and 83.5% (95% CI, 65.4 to 92.1) in Turkey.[25-28] In addition, preliminary estimates from an observational study involving vaccinated health care workers (from a preprint server) suggested that at least one dose of the CoronaVac vaccine was 49.6% (95% CI, 11.3 to 71.4) effective against Covid-19 in Manaus, Brazil, a location where the P.1 (or gamma) variant, which is considered to be a variant of concern by the Centers for Disease Control and Prevention,[29] is predominant (occurred in approximately 75% of the test results).[30] No estimates of the effectiveness of the CoronaVac vaccine with regard to preventing Covid-19 in the general population or in persons who have received full vaccination are publicly available.

On February 2, 2021, Chile began a mass vaccination campaign with the CoronaVac vaccine (Section S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).[31] The Public Health Institute of Chile approved the CoronaVac vaccine for emergency use on January 20, 2021; the vaccine is to be administered in a two-dose schedule, with doses separated by 28 days. The vaccination campaign prioritized older adults, beginning at 90 years of age or older; frontline health care workers; and persons with underlying conditions. The government relied on the existing health care infrastructure to roll the vaccines out to the eligible

population where they lived. Vaccination rollout was organized by means of a publicly available national schedule that assigned specific dates to eligible groups. Eligible persons needed to show up at the nearest vaccination site with their identification; they did not need to make an appointment (Figs. S3 and S4). A national immunization registry keeps track of the vaccination schedules. As of May 10, 2021, the Ministry of Health has administered 13.98 million doses of the CoronaVac vaccine (7.62 million first doses and 6.36 million second doses).[32] Vaccine introduction and scale-up of the campaign occurred during a period with the highest incidence rates of Covid-19 since the beginning of the pandemic in Chile.

We used a rich administrative observational data set to provide estimates of the effectiveness of the CoronaVac vaccine in preventing Covid-19 and related hospitalization, admission to the intensive care unit (ICU), and death in the Chilean population. We estimated the effectiveness of the administration of one vaccine dose and of two doses (the complete schedule), with adjustment for relevant demographic and clinical confounders of the association between vaccination and Covid-19 outcomes. We conducted robustness checks to test whether vaccine effectiveness would be affected by differences in health care access between the vaccinated and unvaccinated groups, and we provide vaccine-effectiveness estimates among persons 16 to 59 years of age and among those 60 years of age or older.

METHODS

STUDY POPULATION AND DESIGN

We used a prospective observational cohort at the national level. The study cohort included participants 16 years of age or older who were affiliated with Fondo Nacional de Salud (FONASA), the national public health insurance program, which includes approximately 80% of the Chilean population. A detailed description of the vaccination campaign is provided in the Supplementary Appendix. Eligibility criteria included an age of 16 years or more, affiliation with FONASA, and receipt of at least one dose of the CoronaVac vaccine between February 2 and May 1, 2021, or no receipt of any Covid-19 vaccination. We excluded participants with a probable or confirmed SARS-CoV-2 infection, as assessed by reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay or antigen testing, on or before February 2, 2021, and persons who had received at least one dose of the BNT162b2 vaccine. We did not focus on the effectiveness of the BNT162b2 vaccine because these estimates have been provided elsewhere.[15,17] We focused on the results regarding the CoronaVac vaccine because they are the mainstay of the vaccination strategy in Chile. However, we provide estimates of the effectiveness of the BNT162b2 vaccine in the Supplementary Appendix as a validation of the procedures used here.

All persons 16 years of age or older are eligible to receive the vaccine, according to the national vaccination schedule. We classified participants into three groups: those who were not vaccinated, those who were partially immunized (≥14 days after receipt of the first vaccine dose and before receipt of the second dose), and those who were fully immunized (≥14 days after receipt of the second dose).

The study team was entirely responsible for the design of the study and for the collection and analysis of the data. The authors vouch for the accuracy and completeness of the data. The first, second, and last authors wrote the first draft of the manuscript.

OUTCOMES AND COVARIATES

We estimated vaccine effectiveness using four primary outcomes: laboratory-confirmed Covid-19, hospitalization for Covid-19, admission to the ICU for Covid-19, and Covid-19–related death. For all the outcomes, we considered the time from the beginning of follow-up (February 2, 2021) to the onset of symptoms as the end point. Vaccine-effectiveness estimates regarding Covid-19 cases included the more severe outcomes. All suspected cases of Covid-19 in Chile are notified to health authorities by means of an online platform and are confirmed by laboratory testing. In our study, cases of Covid-19 and related deaths were those in persons with laboratory-confirmed infection, which corresponds to code U07.1 in the *International Classification of Diseases, 10th Revision*.

We controlled for several patient characteristics that could confound the association between vaccination and outcomes, including age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19. These conditions included chronic kidney disease, diabetes, cardiovascular disease, stroke, chronic obstructive pulmonary disease, hematologic dis-

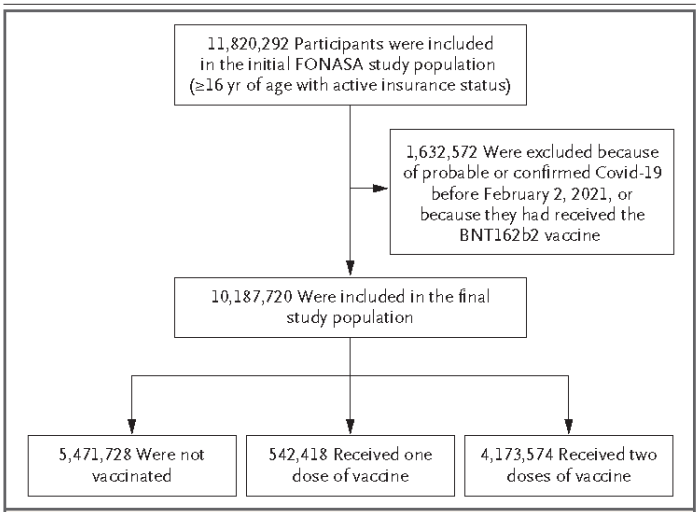

Figure 1. Study Participants and Cohort Eligibility.

Participants were at least 16 years of age, were affiliated with Fondo Nacional de Salud (FONASA; the national public health care system in Chile), and either had received at least one dose of the CoronaVac vaccine between February 2 and May 1, 2021, or had not received any vaccination. We excluded persons who had probable or confirmed coronavirus disease 2019 (Covid-19) according to reverse-transcriptase–polymerase-chain-reaction assay for severe acute respiratory syndrome coronavirus 2 and all persons who had been immunized with the BNT162b2 vaccine.

ease, autoimmune disease, human immunodeficiency virus infection, and Alzheimer's disease and other dementias.[4,33-35]

STATISTICAL ANALYSIS

Our analysis was broadly based on the analytic methods of Thompson et al.[17] for estimating vaccine effectiveness in the United States. We determined vaccine effectiveness by estimating the hazard ratio between the vaccinated and unvaccinated groups. On the basis of the observed information regarding the time to symptom onset from February 2, 2021, we estimated hazard ratios using the extension of the Cox proportional-hazards model, which allowed us to account for a time-varying vaccination status of the persons in the study. We evaluated the robustness of the model assumptions by fitting a stratified version of the extended Cox proportional-hazards model using the available predictors. Inference was based on a partial likelihood approach (Section S2).[17] We estimated the change in the hazard associated with partial immunization and full immunization, and both time-to-event analyses were performed separately. Because the immunity status induced by the CoronaVac vaccine is unknown during the 13 days between vaccine administration and partial or full immunization, those periods were excluded from the at-risk person-time in our analyses.[17]

We estimated the vaccine effectiveness as 1 minus the corresponding hazard ratio, obtained from a model including the previously described covariates, which was expressed as a percentage. We also provide the results with adjustment for the effect of sex and age only. To evaluate whether our effectiveness results were affected by potentially different access to health care between vaccinated persons and unvaccinated persons and according to the age distribution, we performed subgroup analyses involving the subgroup of persons with access to RT-PCR or antigen testing for SARS-CoV-2 and subgroups of persons 60 years of age or older and persons 16 to 59 years of age. Statistical analyses were conducted with the use of the survival package of R software, version 4.0.5.[36,37]

RESULTS

STUDY POPULATION AND VACCINATION ROLLOUT

Figure 1 shows the flow diagram of the study cohort. Of the 11,820,292 persons 16 years of age or older who were affiliated with FONASA, 10,187,720 were eligible for inclusion in the study. Table 1 shows the descriptive statistics for the approximately 10.2 million participants included in the study cohort. There were significant differences according to geographic region, sex, age, income group, nationality, and presence of underlying medical conditions, both in the incidence of Covid-19 and according to vaccination status (unvaccinated, vaccinated with only one dose, or vaccinated with two doses). Laboratory confirmation of infection was by RT-PCR assay in 98.1% of the cases and by antigen testing in 1.9%. Figure 2A shows the rapid rollout of the vaccination campaign, which started on February 2, 2021. Details of the vaccination campaign are provided in Section S1 and Figures S5 through S8. Figure 2B shows the crude cumulative incidence of Covid-19 during the study period among persons who had received one or two doses of vaccine or were unvaccinated.

VACCINE EFFECTIVENESS

There were approximately 615 million person-days in the unvaccinated group, 70 million person-days in the partially immunized group, and 92 million

Table 1. Characteristics of the Study Cohort, Overall and Those with Laboratory-Confirmed Covid-19, According to Vaccination Status.*

Characteristic	Cohort Participants		Persons with Covid-19		P Value	Unvaccinated Persons		Persons Vaccinated with One Dose		Persons Vaccinated with Two Doses		P Value
	no.	%	*no.*	%		*no.*	%	*no.*	%	*no.*	%	
Total	10,187,720	100	248,645	2.4	—	5,471,728	53.7	542,418	5.3	4,173,574	41.0	—
Sex												<0.001
Female	5,469,202	54.0	135,311	2.5	<0.001	2,775,436	50.8	272,044	5.0	2,421,722	44.3	
Male	4,718,518	46.0	113,334	2.4		2,696,292	57.1	270,374	5.7	1,751,852	37.1	
Age group												<0.001
16–19 yr	708,676	7.0	14,871	2.1	<0.001	670,451	94.6	8,192	1.2	30,033	4.2	
20–29 yr	2,017,676	20.0	59,645	3.0		1,655,595	82.1	55,854	2.8	306,227	15.2	
30–39 yr	1,867,491	18.0	54,480	2.9		1,446,544	77.5	59,166	3.1	361,781	19.4	
40–49 yr	1,423,770	14.0	39,993	2.8		851,622	59.8	165,487	11.6	406,661	28.6	
50–59 yr	1,457,564	14.0	37,539	2.6		434,694	29.8	184,268	12.6	838,602	57.5	
60–69 yr	1,365,940	13.0	23,669	1.7		221,738	16.2	41,693	3.1	1,102,509	80.7	
70–79 yr	870,082	8.5	11,778	1.4		111,592	12.8	16,412	1.9	742,078	85.3	
≥80 yr	476,521	4.7	6,670	1.4		79,492	16.7	11,346	2.4	385,683	80.9	
No. of coexisting conditions†												<0.001
0	6,880,426	68.0	168,401	2.4	0.04	4,447,684	64.6	394,030	5.7	2,038,712	29.6	
≥1	3,307,294	32.0	80,244	2.4		1,024,044	31.0	148,388	4.5	2,134,862	64.6	<0.001
Nationality												
Chilean	9,497,058	93.2	233,572	2.5	<0.001	4,913,208	51.7	513,604	5.4	4,070,246	42.9	
Non-Chilean	690,662	6.8	15,073	2.2		558,520	80.9	28,814	4.2	103,328		

* The study cohort included eligible persons who were affiliated with Fondo Nacional de Salud, the national public health insurance program, which collects, manages, and distributes funds for the public health care system in Chile. The model also included individual-level income and location (16 regions). Additional details are provided in Table S1. Covid-19 denotes coronavirus disease 2019.

† Coexisting conditions included chronic kidney disease, diabetes, cardiovascular disease (hypertension or myocardial infarction), stroke, chronic obstructive pulmonary disease, hematologic disease (lymphoma, leukemia, or myeloma), autoimmune disease (rheumatoid arthritis, juvenile idiopathic arthritis, or systemic lupus erythematosus), human immunodeficiency virus infection, and Alzheimer's disease and other dementias.

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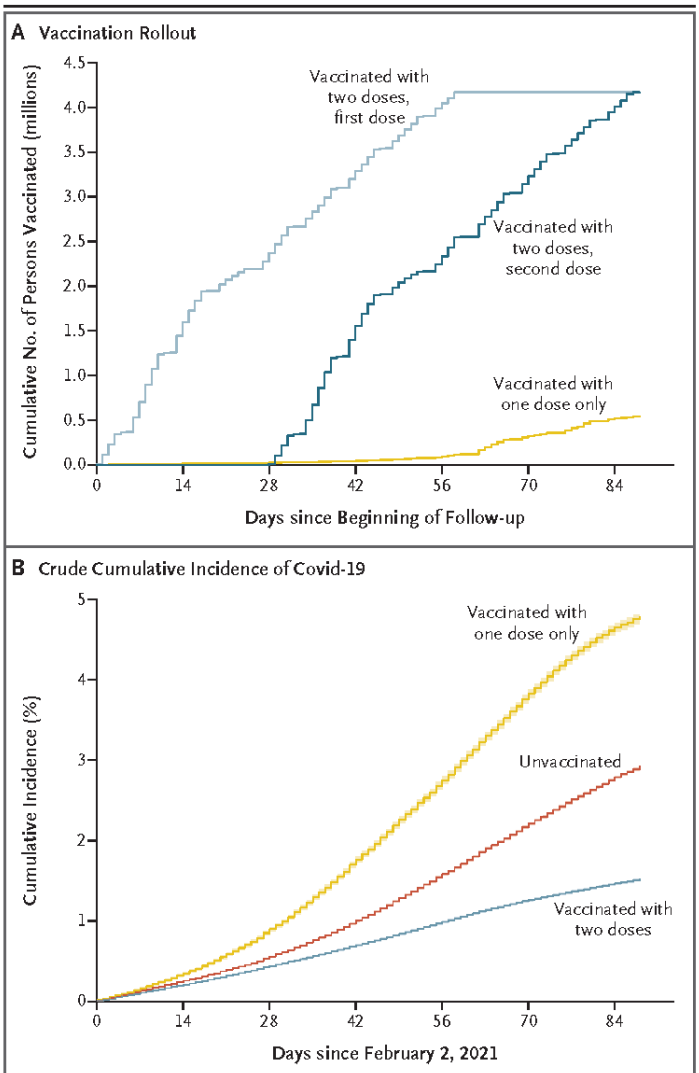

Figure 2. Vaccination Rollout and Crude Cumulative Incidence of Covid-19 in the Study Cohort.

Panel A shows the pace and coverage of the vaccination program among persons who received both doses of vaccine (first and second doses shown separately) or only one dose during the study period (February 2 through May 1, 2021). Panel B shows the crude cumulative incidence of Covid-19 during the study period among unvaccinated persons, among persons who had received only one dose of vaccine, and among persons who had received both doses of vaccine. The relatively high cumulative incidence of Covid-19 in the one-dose group should be interpreted with caution. As shown in Panel A, this group initiated vaccination approximately 40 days after the beginning of the vaccination campaign on February 2, 2021. Therefore, the incidence curve includes all cases that occurred from before vaccination up to 13 days after receipt of the first dose. Shading on the lines indicates 95% confidence intervals.

person-days in the fully immunized group during the study period (Table 2). We documented 218,784 cases of Covid-19, as well as 22,866 hospitalizations, 7873 ICU admissions, and 4042 deaths.

We estimated that the vaccine effectiveness among partially immunized persons (14 to 28 days after receipt of the first dose) was 15.5% (95% CI, 14.2 to 16.8) for the prevention of Covid-19 and 37.4% (95% CI, 34.9 to 39.9) for the prevention of hospitalization, 44.7% (95% CI, 40.8 to 48.3) for the prevention of admission to the ICU, and 45.7% (95% CI, 40.9 to 50.2) for the prevention of Covid-19–related death. In the fully immunized group, the estimated adjusted vaccine effectiveness was 65.9% (95% CI, 65.2 to 66.6) for the prevention of Covid-19 and 87.5% (95% CI, 86.7 to 88.2) for the prevention of hospitalization, 90.3% (95% CI, 89.1 to 91.4) for the prevention of ICU admission, and 86.3% (95% CI, 84.5 to 87.9) for the prevention of Covid-19–related death (Table 2). The vaccine-effectiveness estimates in the stratified model were consistent with these results.

We estimated that the adjusted vaccine effectiveness in the subgroup of fully immunized persons 60 years of age or older was 66.6% (95% CI, 65.4 to 67.8) for the prevention of Covid-19 and 85.3% (95% CI, 84.3 to 86.3) for the prevention of hospitalization, 89.2% (95% CI, 87.6 to 90.6) for the prevention of ICU admission, and 86.5% (95% CI, 84.6 to 88.1) for the prevention of Covid-19–related death (Table 3). Vaccine-effectiveness estimates among persons 16 to 59 years of age are provided in Table S3.

To address a potential concern that the observed vaccine effectiveness may have been driven by health care access, we conducted an analysis in the subgroup of persons who had undergone testing with an RT-PCR assay (98.1%) or antigen test (1.9%) during the analysis period. The results, conditional on whether testing was performed, showed larger effects for vaccination than when we included the complete cohort. Among fully immunized persons in this subgroup, the adjusted vaccine effectiveness was 72.9% (95% CI, 72.3 to 73.4) for the prevention of Covid-19 and 89.2% (95% CI, 88.5 to 89.8) for the prevention of hospitalization, 91.6% (95% CI, 90.5 to 92.5) for the prevention of ICU admission, and 87.8% (95% CI, 86.2 to 89.2) for the prevention of Covid-19–related death (Table S4).

DISCUSSION

We provide estimates of the effectiveness of administration of the CoronaVac vaccine in a countrywide mass vaccination campaign for the prevention of laboratory-confirmed Covid-19 and related hospitalization, admission to the ICU, and

Table 2. Effectiveness of CoronaVac Vaccine in Preventing Covid-19 Outcomes in Overall Study Cohort, According to Immunization Status.*

Outcome and Immunization Status	Study Cohort	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
	No. of Person-Days	No. of Persons	Incidence Rate *no. of events/ 1000 person-days*	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates† *percent*	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	Persons with Covid-19		Vaccine Effectiveness (95% CI)		
	No. of Person-Days	No. of Persons	Incidence Rate	Analysis Adjusted for Sex and Age	Analysis Adjusted for All Covariates*	Stratified Analysis†
			no. of events/ 1000 person-days		*percent*	
Covid-19						
Unvaccinated	75,707,905	15,597	0.2060	—	—	—
Partially immunized	35,675,604	8,333	0.2336	3.9 (0.9–6.8)	9.7 (6.9–12.4)	12.7 (9.8–15.5)
Fully immunized	66,563,272	7,510	0.1128	63.4 (62.0–64.6)	66.6 (65.4–67.8)	67.2 (66.0–68.4)
Hospitalization						
Unvaccinated	76,047,640	5,304	0.0697	—	—	—
Partially immunized	35,961,593	2,168	0.0603	29.2 (25.1–33.1)	35.0 (31.3–38.6)	38.6 (34.8–42.2)
Fully immunized	66,986,859	1,344	0.0201	83.4 (82.2–84.5)	85.3 (84.3–86.3)	85.4 (84.3–86.4)
Admission to ICU						
Unvaccinated	76,194,648	1,811	0.0238	—	—	—
Partially immunized	36,062,081	672	0.0186	38.2 (31.9–44.0)	44.5 (38.7–49.7)	47.0 (41.2–52.2)
Fully immunized	67,051,769	331	0.0049	87.5 (85.7–89.0)	89.2 (87.6–90.6)	89.3 (87.8–90.7)
Confirmed death						
Unvaccinated	76,169,386	1,999	0.0262	—	—	—
Partially immunized	36,053,806	768	0.0213	39.7 (33.8–45.1)	45.8 (40.4–50.7)	46.1 (40.5–51.2)
Fully immunized	67,045,620	402	0.0060	84.4 (82.3–86.2)	86.5 (84.6–88.1)	86.8 (85.0–88.4)

* The analysis was adjusted for age, sex, region of residence, income, nationality, and whether the patient had underlying conditions that have been associated with severe Covid-19.

† A stratified version of the extended Cox proportional-hazards model was fit to test the robustness of the estimates to model assumptions, with stratification according to sex, age, coexisting conditions, nationality, and income.

potential of this vaccine to save lives and substantially reduce demands on the health care system.

Our study has at least three main strengths. First, we used a rich administrative health care data set, combining data from an integrated vaccination system for the total population and from the Ministry of Health FONASA, which covers approximately 80% of the Chilean population. These data include information on laboratory tests, hospitalization, mortality, onset of symptoms, and clinical history in order to identify risk factors for severe disease. Information on region of residence also allowed us to control for differences in incidence across the country. We adjusted for income and nationality, which correlate with socioeconomic status in Chile and are thus considered to be social determinants of health. The large population sample allowed us to estimate vaccine effectiveness both for one dose and for the complete two-dose vaccination schedule. It also allowed for a subgroup analysis involving adults 60 years of age or older, a subgroup that is at higher risk for severe disease[3] and that is underrepresented in clinical trials. Second, data were collected during a rapid vaccination campaign with high uptake and during a period with one of the highest community transmission rates of the pandemic, which allowed for a relatively short follow-up period and for estimation of the prevention of at least four essential outcomes: Covid-19 cases and related hospitalization, ICU admission, and death. Finally, Chile has the highest testing rates for Covid-19 in Latin America, universal health care access, and a standardized, public reporting system for vital statistics, which limited the number of undetected or unascertained cases and deaths.[14]

Our study has several limitations. First, as an observational study, it is subject to confounding. To account for known confounders, we adjusted the analyses for relevant variables that could affect vaccine effectiveness, such as age, sex, underlying medical conditions, region of residence, and nationality. The risk of misclassification bias that would be due to the time-dependent performance of the SARS-CoV-2 RT-PCR assay is relatively low, because the median time from symptom onset to testing in Chile is approximately 4 days (98.1% of the tests were RT-PCR assays). In this 4-day period, the sensitivity and specificity of the molecular diagnosis of Covid-19 are high.[38] However, there may be a risk of selection bias. Systematic differences between the vaccinated and unvaccinated groups, such as health-seeking behavior or risk aversion, may affect the probability of exposure to the vaccine and the risk of Covid-19 and related outcomes.[39,40] However, we cannot be sure about the direction of the effect. Persons may be hesitant to get the vaccine for various reasons, including fear of side effects, lack of trust in the government or pharmaceutical companies, or an opinion that they do not need it, and they may be more or less risk-averse. Vaccinated persons may compensate by increasing their risky behavior (Peltzman effect).[40] We addressed potential differences in health care access by restricting the analysis to persons who had undergone diagnostic testing, and we found results that were consistent with those of our main analysis.

Second, owing to the relatively short follow-up in this study, late outcomes may not have yet developed in persons who were infected near the end of the study, because the time from symptom onset to hospitalization or death can vary substantially.[3,15] Therefore, effectiveness estimates regarding severe disease and death, in particular, should be interpreted with caution. Third, during the study period, ICUs in Chile were operating at 93.5% of their capacity on average (65.7% of the patients had Covid-19).[32] If fewer persons were hospitalized than would be under regular ICU operation, our effectiveness estimates for protection against ICU admission might be biased downward, and our effectiveness estimates for protection against death might be biased upward (e.g., if patients received care at a level lower than would usually be received during regular health system operation).

Fourth, although the national genomic surveillance for SARS-CoV-2 in Chile has reported the circulation of at least two viral lineages considered to be variants of concern, P.1 and B.1.1.7 (or the gamma and alpha variants, respectively),[41] we lack representative data to estimate their effect on vaccine effectiveness (Table S2). Results from a test-negative design study of the effectiveness of the CoronaVac vaccine in health care workers in Manaus, Brazil, where the gamma variant is now predominant, showed that the efficacy of at least one dose of the vaccine against Covid-19 was 49.6% (95% CI, 11.3 to 71.4).[30] Although the vaccine-effectiveness estimates in Brazil are not directly comparable with our estimates owing to differences in the target population, the vaccination schedule (a window of 14 to 28 days between doses is recommended in Brazil[42]), and immunization status, they highlight the importance of continued vaccine-effectiveness monitoring.

Overall, our study results suggest that the CoronaVac vaccine was highly effective in protecting against severe disease and death, findings that are consistent with the results of phase 2 trials[23,24] and with preliminary efficacy data.[27,28]

The research protocol was approved by the Comité Ético Científico Clínica Alemana Universidad del Desarrollo. The study was considered exempt from informed consent; no human health risks were identified. Research analysts are employees of the Chilean Ministry of Health; our use of data follows Chilean law 19.628 on private data protection.

Presented in part at the World Health Organization (WHO) Strategic Advisory Group of Experts on Immunization (SAGE) meeting on coronavirus disease 2019 (Covid-19) vaccines, April 29, 2021, and the WHO Vaccines Prioritization Working Group, April 21, 2021.

Supported by grants from the Agencia Nacional de Investigación y Desarrollo (ANID) Millennium Science Initiative Program, Millennium Nucleus Center for the Discovery of Structures in Complex Data (MIDAS) (NCN17_059, to Dr. Jara), and Millennium Initiative for Collaborative Research in Bacterial Resistance (MICROB-R) (NCN17_081, to Drs. Undurraga and Araos); the Advanced Center for Chronic Diseases, ANID Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias (FONDAP) (15130011, to Dr. Araos); and the Research Center for Integrated Disaster Risk Management ANID FONDAP (15110017, to Dr. Undurraga).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

Owing to data privacy regulations, the individual-level data in this study cannot be shared (Law N19.628). Aggregate data on vaccination and incidence are publicly available at https://github.com/MinCiencia/Datos-COVID19/.

We thank the attendees of the WHO meeting and working group for suggestions and comments; Drs. Ira Longini (University of Florida), Daniel Cohen (Tel Aviv University), Richard Peto (University of Oxford), Elizabeth Miller (Public Health England), and Myron M. Levine (University of Maryland); Drs. Catterina Ferreccio (Pontificia Universidad Católica de Chile), Ximena Aguilera (Universidad del Desarrollo), María T. Valenzuela (Universidad de Los Andes), Pablo Vial (Universidad del Desarrollo), Gonzalo Valdivia (Pontificia Universidad Católica de Chile), Fernando Otaiza (Ministerio de Salud), and Álvaro Erazo (Pontificia Universidad Católica de Chile), from the Chilean Covid-19 Advisory Group, for advice on the study design and interpretation of results; and Drs. José R. Zubizarreta (Harvard University) and Erika M.C. D'Agata (Brown University) for thoughtful suggestions.

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5.2 Estudo com 60 milhões de brasileiros mostra efetividade da CoronaVac acima de 70% contra hospitalizações e mortes, inclusive entre idosos

Uma pesquisa realizada com 60,5 milhões de brasileiros vacinados entre janeiro e junho de 2021 mostrou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, tem uma efetividade superior a 70% para evitar casos graves, internações em Unidades de Terapia Intensiva (UTIs) e mortes causadas por Covid-19, inclusive entre idosos. O estudo, que analisou a CoronaVac e a vacina da Astrazeneca/Fiocruz, é o maior já realizado no Brasil sobre a efetividade da vacinação contra o SARS-CoV-2.

Do total de pessoas avaliadas que haviam completado o esquema vacinal com CoronaVac (ou seja, tomado as duas doses), 72,6% apresentaram menor risco de hospitalização, 74,2% menor risco de admissão em UTI e 74% menor risco de morte. Em relação às pessoas entre 60 e 89 anos, a efetividade da vacina foi ainda melhor: 84,2% contra hospitalizações, 80,8% contra internações em UTI e 76,5% contra mortes.

O estudo foi realizado por pesquisadores das universidades federais da Bahia e de Ouro Preto, da Universidade de Brasília, da Universidade Estadual do Rio de Janeiro, da London School of Hygiene & Tropical Medicine e da Fundação Oswaldo Cruz Fiocruz. As conclusões foram publicadas no artigo "The effectiveness of Vaxzevria and CoronaVac vaccines: A nationwide longitudinal retrospective study of 61 million Brazilians (VigiVac-COVID19)", na plataforma de preprints MedRxiv.

Dos 60,5 milhões de brasileiros analisados no estudo, 21,9 milhões (36,2%) foram imunizados com a CoronaVac, e 38,6 milhões (63,8%) com a vacina da AstraZeneca/Fiocruz. Ao todo, 26,8 milhões de pessoas (44,4% do total) tinham 60 anos ou mais.

Para determinar a efetividade das vacinas em evitar casos graves de Covid-19, os pesquisadores confrontaram as informações de populações vacinadas com os dados nacionais do Sistema de Vigilância Epidemiológica da Gripe (SIVEP-Gripe), que reúne os casos notificados de hospitaliza-

ções e mortes causadas por vírus respiratórios, como é o caso do SARS-CoV-2.

O levantamento é extremamente importante não apenas pelo número elevado de indivíduos analisados, como por se tratar do primeiro levantamento feito nacionalmente para aferir a efetividade vacinal – que não é a mesma coisa que eficácia. Enquanto a investigação da eficácia é feita em condições ideais e controladas, por vezes em laboratório, a análise da efetividade vacinal é baseada em dados do mundo real, onde a vacina é colocada à prova diante de um conjunto diverso de pessoas, em diferentes condições.

Outro estudo de efetividade feito em relação à CoronaVac foi o Projeto S, realizado pelo Butantan em Serrana, interior de São Paulo. Por meio dele, a população do município (quase 28 mil adultos) foi vacinada entre fevereiro e abril de 2021. A pesquisa concluiu que o imunizante causou uma redução de 80% no número de casos sintomáticos de Covid-19, de 86% nas internações e de 95% nos óbitos. Além disso, mostrou que com uma cobertura vacinal de aproximadamente 75% da população adulta a pandemia pode ser controlada.

A eficácia da CoronaVac foi comprovada no Brasil por meio de um estudo clínico de fase 3 com 13 mil voluntários, todos profissionais da saúde, população altamente exposta à Covid-19. Os resultados finais demonstraram que a eficácia geral do imunizante pode chegar a 62,3% quando o intervalo entre a primeira e a segunda dose é de 21 a 28 dias. Os dados foram divulgados na plataforma de preprints SSRN, ligada à revista The Lancet, e estão em processo de revisão por pares.

Publicado em: 03/09/2021

The effectiveness of Vaxzevria and CoronaVac vaccines: A nationwide longitudinal retrospective study of 61 million Brazilians (VigiVac-COVID19).

Short Title: **Effectiveness of Vaxzevria and CoronaVac vaccines in Brazil**

Thiago Cerqueira-Silva [1,2], Vinicius de Araújo Oliveira, M.D. [,1,2,3], Julia Pescarini, Ph.D. [3,4], Juracy Bertoldo Júnior [2,3], Tales Mota Machado [5], Renzo Flores Ortiz, Ph.D. [3], Gerson Penna, M.D., Ph.D. [6], Maria Yury Ichihara, M.D., Ph.D. [3], Jacson Venâncio de Barros [7], Viviane S. Boaventura, M.D., Ph.D. [1,2], Mauricio L. Barreto, M.D., Ph.D. [2,3], Guilherme Loureiro Werneck, M.D., Ph.D. [8], Manoel Barral-Netto, M.D., Ph.D. [1,2,3]

Affiliations:

1.LIB and LEITV Laboratories, Instituto Gonçalo Moniz, Fiocruz, Salvador, Bahia, Brazil;

2. Universidade Federal da Bahia (UFBA), Salvador, Bahia, Brazil;

3. Center for Data Integration and Knowledge for Health (Cidacs / IGM / Fiocruz), Salvador, Bahia, Brazil;

4. London School of Hygiene and Tropical Medicine, London, United Kingdom

5. Universidade Federal de Ouro Preto, Ouro Preto, Brazil;

6. Núcleo de Medicina Tropical, Universidade de Brasília. Escola Fiocruz de Governo, Fiocruz Brasilia. Brasilia, DF, Brazil;

7. Department of Health Informatics, Ministry of Health, Brasília, Brazil

8. Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

Corresponding author:

Manoel Barral-Netto, MD

manoel.barral@fiocruz.br

Abstract

Background

High rates of virus transmission and the presence of variants of concern can affect vaccine effectiveness (VE). Both conditions occur in low-income countries, which primarily use viral vector or inactivated virus vaccine technologies. However, few VE analyses have been conducted in such countries, and most lack the power to evaluate effectiveness in subgroups, such as the elderly.

Methods

The present retrospective cohort study evaluated the effectiveness of Vaxzevria and CoronaVac vaccines for COVID-19-related infection in 60,577,870 Brazilian vaccinees from January 18 to June 30, 2021.

Study outcomes included documented infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Covid-19–related hospitalization, ICU admission and death. We estimated VE for each outcome as one minus the hazard ratio using Cox regression adjusted for age, sex, Brazilian deprivation index, and month/region of dose administration.

Results

Vaccination with Vaxzevria or CoronaVac was found to be effective against SARS-CoV-2 infection and highly effective against hospitalization, ICU admission and death in individuals up to 79 years. From 80-89 years of age, 91.2 (95CI: 89.1-92.9) VE against death was seen in Vaxzevria-vaccinated individuals versus 67.3 (95CI: 63.6-70.6) for Coronvac. Above 90 years, 70.5 (95CI: 51.4-82.1) protection was conferred to Vaxzevria-vaccinated individuals versus 35.4 (95CI: 23.8-45.1) in Coronavac-vaccinated individuals

Conclusions

Both vaccines demonstrated overall effectiveness against severe COVID-19 up to 80 years of age. Our results suggest that individuals aged 90 years or older may benefit from an expedited third booster dose. Ongoing evaluations, including any additional vaccines authorized, are crucial to monitoring long-term vaccine effectiveness.

Background

Several COVID-19 vaccines have proved efficacious, and many of them are being extensively used around the world.[1–4] While high-income countries preferentially administer mRNA-based vaccines, lower- and middle-income countries have employed vaccines based on viral vectors or inactivated virus technologies. A timely evaluation of the effectiveness of the currently available vaccines across different regions is essential for a comprehensive understanding of vaccine impact, considering significant variations in vaccination schedules, virus transmission and the emergence of viral variants, in addition to social and cultural standards and local health system conditions.

Brazil is one of the countries most affected by the pandemic, with high rates of transmission. The Brazilian COVID-19 vaccination program initially relied on the vaccines Vaxzevria/Fiocruz (previously Oxford-AstraZeneca or ChAdOx-1), approved in 181 countries, and Sinovac's CoronaVac/Butantan, approved in 39 countries.[5] The recommended interdose interval in Brazil for Vaxzevria is 12 weeks versus 2-4 weeks for CoronaVac. The period between doses of Vaxzevria has varied in several countries.[7] However, CoronaVac has been applied at distinct intervals,[1,8] making direct comparisons difficult. Additionally, several early publications on vaccine effectiveness (VE) evaluated only the initial dose or were limited to analyzing effectiveness against symptomatic infection[9,10] and hospitalization[10,11], i.e., ICU admission and death were not addressed.

Nationwide evaluations of the effectiveness of COVID-19 vaccines in Brazil offers advantages, as this country's large population is distributed throughout several regions with considerable differences in socio-economic aspects and access to medical facilities. Nonetheless, data collection systems are identical throughout the entire country, offering a comprehensive source of data to perform a countrywide VE evaluations. The COVID-19 vaccination campaign was initiated nationwide on January 18, 2021. By June, a large number

of vaccinees had received either Vaxzevria/Fiocruz or CoronaVac/Butantan vaccines, allowing for a detailed evaluation of the effectiveness of both vaccines while considering several outcomes and stratified age ranges, making it possible to examine in detail specific age effects previously not investigated.

A significant issue regarding the VE of vaccines against COVID-19 is the degree of circulation of distinct SARS-CoV-2 variants of concern (VOC) in different regions. During the course of the present study, the Gamma variant was the most frequent in all regions of Brazil.[12] Importantly, the literature contains few reports on the VE of Vaxzevria and Coronavac against the Gamma variant.[1,10,13]

The present study aimed to evaluate the effectiveness of Vaxzevria and Coronavac vaccines in 60,577,870 Brazilian vaccinee with respect to several different outcomes: COVID-19 related infection, hospitalization, ICU admission and death, between January 18 and June 30, 2021.

Methods

Study design and datasets

We conducted a retrospective cohort using individual-level information on demographic, clinical characteristics, and SARS-COV-2 laboratory tests from the Brazilian federal health registries. The Brazilian Ministry of Health Department of Informatics (DATASUS) provided unidentified datasets of the COVID-19 Vaccination Campaign dataset (SI-PNI), the Acute Respiratory Infection Suspected Cases dataset (e-SUS Notifica), and the National Epidemiological Surveillance System registry for Severe Acute Respiratory Infection/Illness (SIVEP-Gripe). A key-coded individual identification number present in the three datasets was used for a deterministic linkage and then removed from the resulting linked dataset used

in our analyses. No personally identifiable data was accessed at any stage. Codebooks, scripts and public dataset version will be available at https://vigivac.fiocruz.br

SI-PNI is a data warehouse run by DATASUS with all the vaccine doses administered by health services in Brazil. From SI-PNI, we extracted information on the COVID-19 vaccine received either Sinovac CoronaVac or Vaxzevria (under the names AstraZeneca/Fiocruz or Covishield/ChAdOx1-S), and the dates of the first and second doses. Overall and age-specific Brazilian population estimates for 2021 corrected the all-cause deaths reported in 2020 overall and age were retrieved from the Brazilian Institute of Geography and Statistics.[14] Open version of the SI-PNI dataset is available at opendatasus-SI PNI.

The e-SUS Notifica is a national online health surveillance information system where acute respiratory infections cases and COVID-19 suspected or confirmed cases are registered. and has been used as a data source for epidemiological research.[15] Open version of e-SUS Notifica is available at opendatasus-eSUS Notifica.

SIVEP-Gripe is the national system used to register SARI-related hospitalizations and deaths caused by influenza or other respiratory viruses. The system is a registry for new respiratory infections since the H1N1 pandemic in 2009 and widely used as a source for epidemiological studies.[16–18] All COVID-19 related SARI hospitalizations and deaths (independent of hospitalization) are registered in the system. Open version of the 2021 SIVEP-Gripe dataset is available at opendatasus-SIVEP

From both SIVEP-Gripe and eSUS-Notifica, we extracted information on the date of symptom onset, RT-PCR, and antigen test results for SARS-CoV-2, and from SIVEP-Gripe, we got data of hospitalization, admission to ICU, and hospitalization outcome (discharge or death).

Study population

We included all individuals who received the COVID-19 vaccine first dose between January 18th, 2021, and June 30th, 2021. The study individuals were followed retrospectively to assess infection, hospitalization, admission to ICU, and death with a laboratory-confirmed diagnosis of SARS-CoV-2 up to June 30th, 2021.

We excluded individuals (i) vaccinated with vaccines besides Vaxzevria or CoronaVac, (ii) with inconsistent vaccine records (i.e., individuals who received the second dose without the first dose, received doses from different vaccines or interval between doses less than 14 days), (iii) with confirmed COVID-19 before the date of vaccine administration, and (iv) with missing data for essential covariates (i.e., sex or age).

Exposure and outcomes

We defined vaccination status for each vaccine based on the time elapsed since the administration of a vaccine dose:

1. ≤13 days after the first dose (the reference period)
2. ≥14 days after the first dose and without the second dose (partially vaccinated)
3. ≥14 days after the second dose (fully vaccinated)

We defined the period up to 13 days after the first dose as the reference period for VE estimation based on results of a Phase III randomized controlled trial[8] and three test-negative studies.[11,19,20] The time-lapsed between the date of the first dose and the development of an effective immune response is used to detect bias in test-negative case-control studies to estimate vaccine effectiveness, the theoretical frame for such use has been discussed by Hitchings et al.[21] We also analyzed vaccine effectiveness for 1 to 13 days after the second dose, with the results presented in supplementary table S1).

Laboratory confirmation of COVID-19 with a positive RT-PCR or antigen test result) was required for inclusion in the analyses. The outcomes analyzed were infection, hospitalization, admission to an intensive care unit (ICU), and death by COVID-19. We considered the time

between day one of the first or second vaccination up to the symptom's onset for each outcome. Individuals whose symptoms started on the same day of the first vaccination dose were assigned one day of follow-up time. Death was considered at any time regardless of prior hospitalization. ICU admission was considered at any time point between the admission and the discharge or death dates.

Statistical analyses

In the primary analysis, we used a Cox regression model to estimate the hazard ratio (HR) of COVID-19 infection, hospitalization, ICU admission, and death for partially and fully vaccinated individuals. The model was adjusted for age, sex, region of residence, socioeconomic status, and month of the 1st dose. We used the Brazilian Deprivation Index (*Índice Brasileiro de Privação*-IBP), a municipality-level measure of material deprivation, as an indicator of socioeconomic status.[21] We estimated vaccine effectiveness (VE) as 1-HR, obtained from a model including all covariates, and reported as a percentage. We also reported the crude VE for each outcome. In addition, we performed a stratified analysis by age groups (<60, 60–69, 70–79, 80–89, ≥90 years) to investigate whether VE was modified by age.

To assess the robustness of our findings, we repeated the principal analysis defining as the reference period the time elapsed up to 10 days after the date of the first dose, as it is expected that the vaccines' protection increases with time. Additionally, we examined the VE for hospitalization, ICU admission and death using clinical suspected cases besides laboratory confirmed ones.

Analyses were performed using the R statistical software (R Core Team) and its H2O package.[23,24] Descriptive statistics were presented as frequencies and percentages. We used

the 95% confidence intervals (CI) of the estimated measures of association for interpreting the findings.

RESULTS

From January 18 to June 30, 2021, 61,783,842 individuals received at least one dose of one of the two COVID-19 vaccines analyzed in this study, and 60,577,870 (98.1%) met the eligibility criteria and were included in the analysis (Figure 1). The majority (63.8%, n=38,664,633 individuals) received at least one dose of Vaxzevria and the remaining (36.2%, n=21,933,237 individuals) received at least one dose of CoronaVac. The majority of our cohort comprised women (56.1%) and individuals aged 60 years or older (44.4%). Compared to individuals that received CoronaVac, individuals that received Vaxzevria were younger (29.3% vs. 70.9% of individuals aged 60 years or older), and a lower proportion had completed the full vaccine schedule (10.6% vs. 82.7%). Vaccination with CoronaVac occurred mainly from January to April 2021, while Vaxzevria was administered predominantly after March 2021 (Figure 2). Among those who received the second dose, the median time between the first and second doses was 85 days (IQR 83–90) for Vaxzevria and 27 days (IQR 21–28) for CoronaVac. Individuals who received at least one dose of Vaxzevria or CoronaVac were mostly women (54.6% vs. 58.7% respectively) and from the southeast region of the country (44.1% vs. 46.3%, respectively) (Table 1).

Table 2 shows the COVID-19 VE analysis results, including number of events and incidence rate per 1000 person-days and supplementary table S1 shows the crude and adjusted VE analysis . We observed that individuals with full vaccination schedule (i.e., ≥ 14 days after the second dose) with Vaxzevria had a 70.0% (95% CI 68.6 to 71.3) lower risk of infection, 86.8% (95% CI 85.2 to 88.2) lower risk of hospitalization, 88.1% (95% CI 85.4 to 90.3) lower risk of ICU admission, and 90.2% (95% CI 88.3 to 91.8) lower risk of death. Partial vaccination (i.e., ≥14 days after the first dose up to the second dose) with Vaxzeria was

associated with a 32.7% lower risk of infection (95% CI 31.9 to 33.5) and at least 50% lower risk of hospitalization (51.7%; 95% CI 50.4 to 52.9), ICU admission (53.6%; 95% CI 51.4 to 55.6), and death (49.3%; 95% CI 47.0 to 51.5). Complete vaccination with CoronaVac was associated with a 54.2 (95% CI 53.4-55.0) lower risk of infection, 72.6% (95% CI 71.6 to 73.6) lower risk of hospitalization, 74.2% (95% CI 72.6 to 75.7) lower risk of ICU admission, and 74.0% (95% CI 72.6 to 75.3) lower risk of death. Partial vaccination with CoronaVac was associated with less than 50% of reduction in the risk of infection (16.2%; 95% CI 15.1 to 17.4), hospitalization (26.5%; 95% CI 24.6 to 28.4), ICU admission (28.1%; 95% CI 24.9 to 31.1), and death (29.4%; 95% CI 26.7 to 32.0).

When stratifying the analysis by age, complete vaccination with Vaxzevria or CoronaVac presented a similar VE within all age groups, with the exception among individuals aged 90 years or older (Table S2, Figure 3).

In the analysis using the reference period of up to 10 days after the first dose, we found VE point and interval estimates similar to those found in the primary analysis for both Vaxzeria and Coronavac vaccines (Table S3). The results using all clinical suspected and laboratory confirmed cases for the outcomes of hospitalization, ICU admission and death were qualitatively equal to those found in primary analysis (Table S4).

DISCUSSION

Here we present nationwide results on the effectiveness of vaccination with CoronaVac/Butantan and Vaxzevria/Fiocruz after the first six months of the vaccination campaign in Brazil. Analyzing data from almost 61 million individuals vaccinated with at least one dose, our results demonstrate strong evidence of 70.0% and 54.2% protection against infection after full vaccination with Vaxzevria and CoronaVac, respectively. Vaxzevria offered approximately 90% effectiveness against hospitalization, ICU admission

and death, while CoronaVac provided approximately 75% protection following full vaccination.

Our findings regarding the Coronovac/Butantan vaccine are compatible with a previous Brazilian efficacy study[24], but lower than the 83.5% protection reported by a Turkish efficacy trial.[8] The effectiveness determined by a cohort study in Chile was also higher than our findings for infection (66.5% vs. 54.2%) as well as hospitalization (87.5% vs. 72.6%). Differences between the study in Chile and the present analyses of Brazilian vaccinees may be partially explained by the higher frequency of younger individuals in the Chile study (51.2% vs. 29.1% of individuals younger than 60 years old). During the vaccination campaign, Brazil experienced health system collapse in several states, which may have influenced death rates, especially between February and May, likely affecting CoronaVac estimates more markedly due to its greater availability of this vaccine in the early stages of the vaccination program. Another reason for these differences could be the increased circulation of the Gamma lineage detected in these countries, which has been estimated at 28.6% in Chile and 69.6% in Brazil during both study periods.[1,12] In plasma samples obtained from individuals fully vaccinated with CoronaVac, a reduced capacity to neutralize the Gamma variant was observed.[1] Furthermore, 9.9% of the Brazilian population was fully vaccinated from January to May 2021, compared to almost 35.4% of Chile's population. This may have contributed to lower viral transmission in Chile compared to Brazil.[1]

For Vaxzevria, our findings of 70.0% effectiveness against infection exceeded the levels of 66.7% effectiveness reported in a combined analysis of four clinical trials conducted in the UK, South Africa, and Brazil.[7] Effectiveness against hospitalization was consistent with the 80% and 89% protection observed in studies in Scotland[3] and England,[11] respectively. Additionally, our findings support the high level of protection offered by Vaxzevria despite the abundant circulation of the Gamma variant in Brazil during the period studied. Few

studies have reported on the VE of Vaxzevria in populations infected by VOCs.[1,9,10,13,20] Studies analyzing effectiveness against VOCs have mainly focused on protection against symptomatic infection or hospitalization.[9,10,13] Taken together, the findings reported herein combined with data in the literature confirm a consistently high rate of protection against moderate to severe COVID-19 in real-world studies, despite abundant circulation of VOCs.

Protection was shown to vary according to age group. The VE of CoronaVac/Butantan was close to 80% against death in individuals aged up to 79 years of age. However, a reduction in effectiveness was observed after 80 years of age, with only 35.4% protection against death seen in individuals over 90. In contrast, the Vaxzevria/Fiocruz vaccine achieved close to 90% protection against death in individuals aged less than 90 years, while a VE of 70.5% was found in those older than 90 years of age. It is reasonable to attribute the observed reduction in effectiveness to immunosenescence, which is commonly associated with a higher frequency of comorbidities, and may imply higher death rates. In the context of limited vaccine availability, the precise identification of age limits at which point immune protection becomes impaired can provide valuable evidence to inform public health measures. Considering the current scenario in Brazil, our findings demonstrate the eventual need for a vaccine booster dose in individuals aged 80 years or older who received CoronaVac, as well as for individuals over 90 years immunized with Vaxzevria.

The differences evidenced in effectiveness between Vaxzevria and CoronaVac may be related to the distinct technologies used be each of these two products, as well as how they influence immunogenicity. Both vaccines analyzed herein activate immunological mechanisms and trigger a neutralizing antibody response against viral particles. However, CoronaVac, a whole-cell inactivated vaccine, elicits a less potent cellular response than Vaxzevria, an adenoviral-vectored vaccine.[25] Additionally, Vaxzevria was shown to induce a higher peak neutralizing antibody response than CoronaVac.[27] Thus, the intrinsic

characteristics of each formulation may serve to explain differences observed in both clinical trials and vaccine effectivity studies.[1,26,28]

A relevant strength of our study is its large sample size, due to the use of the complete dataset covering the Brazilian COVID-19 vaccination campaign from January to June 2021. This large sample allowed us to identify the age limits in which immune protection becomes impaired, especially with regard to CoronaVac. Sensitivity analyses further confirmed the robustness of our findings. However, our study is also subject to some limitations. First, as VE was estimated using observational data, our analysis is subject to data availability and, therefore, to potential confounders. Although our analyses were not controled for comorbidities, crude and adjusted VE estimates were similar. In addition, comorbidities have been identified as the causal pathway between age and COVID-19 severity. Therefore, by controlling for age, we are also indirectly controlling for comorbidities.[29] Second, in contrast to many VE studies, the reference period used herein for comparison purposes was 1-13 days after vaccination. Although using early post-vaccination as a reference may underestimate VE, previous studies have used a similar approach and obtained VE results similar to those found in clinical trials.[30,31] The early post-vaccination period can also be used as a bias indicator related to differences in SARS-CoV-2 infection risk. Additionally, the effectiveness results of the present report are similar, in the pertinent age ranges, to reports on both vaccines using distinct approaches.[1,19,20] Finally, we also performed sensitivity analysis, which demonstrated similar results when a 0-10 day reference period was applied.

Using the data available in Brazil, we estimated overall VE for each vaccine evaluated as well as by age group. Vaxzevria/Fiocruz and CoronaVac/Butantan were both shown to be highly protective against severe COVID-19 in the population aged up to 80 years, yet due to decreased VE an early booster dose may be considered for those over 80 years of age who received CoronaVac, and especially for individuals aged over 90 years regardless of which of

these two vaccines were administered. Despite high population adherence, the vaccination campaign is evolving unevenly throughout Brazil, and continuous monitoring of VE in the current context may provide sound evidence to inform public health measures.

ETHICAL CONSIDERATIONS

The Brazilian National Commission in Research Ethics approved the research protocol (CONEP approval number 4.921.308). The study was considered exempt from informed consent; no human health risks were identified. All work presented here used unidentified secondary data in accordance with the Brazilian Personal Data Protection General Law (LGPD). Data was manipulated in a secure computing environment, ensuring protection against data leakage and records reidentification.

DECLARATION OF INTERESTS

VO, VB, MB, and MB-N are employees from Fiocruz, a federal public institution, which manufactures Vaxzevria in Brazil, through a full technology transfer agreement with AstraZeneca. Fiocruz allocates all its manufactured products to the Ministry of Health for the public health service (SUS) use. All other authors report no potential competing interest.

FUNDING

This study was partially supported by a donation from the "Fazer o bem faz bem" program.

DATA SHARING

We used third-party data, provided by the Brazilian Ministry of Health. Any request for access to the data shall be directed to DATASUS - Ministry of Health Brazil:

https://datasus.saude.gov.br/

ACKNOWLEDGMENTS

The authors thank DATASUS for its excellent work in providing unidentified databases. GW, MB, and MB-N are research fellows from CNPq, the Brazilian National Research Council. The authors would also like to thank Andris K. Walter for English language revision and manuscript copyediting assistance.

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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	**Persons with two doses N=4,107,650**	**Total N=38,664,633**	**Persons with only one dose N=3,794,753**	**Persons with two doses N=18,138,484**	**Total N=21,933,237**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Sex (Female)	18,603,771 (53.8)	2,509,503 (61.1)	21,113,274 (54.6)	2,136,515 (56.3)	10,739,832 (59.2)	12,876,347(58.7)
Age group						
<20	279,896 (0.8)	18,880 (0.5)	298,776 (0.8)	36,246 (1.0)	57,185 (0.3)	93,431 (0.4)
20-29	2,369,858 (6.9)	284,973 (6.9)	2,654,831 (6.9)	294,281 (7.8)	832,301 (4.6)	1,126,582 (5.1)

30-39	3,935,033 (11.4)	427,267 (10.4)	4,362,300 (11.3)	351,089 (9.3)	1,204,701 (6.6)	1,555,790 (7.1)
40-49	7,143,476 (20.7)	386,696 (9.4)	7,530,172 (19.5)	988,384 (26.0)	1,091,683 (6.0)	2,080,067 (9.5)
50-59	12,198,475 (35.3)	280,890 (6.8)	12,479,365 (32.3)	671,336 (17.7)	863,722 (4.8)	1,535,058 (7.0)
60-69	7,899,957 (22.9)	751,488 (18.3)	8,651,445 (22.4)	631,203 (16.6)	5,211,550 (28.7)	5,842,753 (26.6)
70-79	401,161 (1.2)	591,043 (14.4)	992,204 (2.6)	611,335 (16.1)	6,701,411 (36.9)	7,312,746 (33.3)
80-89	284,210 (0.8)	1,234,312 (30.0)	1,518,522 (3.9)	163,675 (4.3)	1,712,040 (9.4)	1,875,715 (8.6)
≥90	44,917 (0.1)	132,101 (3.2)	177,018 (0.5)	47,204 (1.2)	463,891 (2.6)	511,095 (2.3)
Region of residence						
Central West	2,568,166 (7.4)	342,173 (8.3)	2,910,339 (7.5)	246,240 (6.5)	1,359,139 (7.5)	1,605,379 (7.3)

Northeast	825,655 (2.4)	1,074,931 (26.2)	1,900,586 (4.9)	769,299 (20.3)	4,412,161 (24.3)	5,181,460 (23.6)
North	2,453,059 (7.1)	507,337 (12.4)	2,960,396 (7.7)	242,527 (6.4)	1,165,657 (6.4)	1,408,184 (6.4)
Southeast	15,479,240 (44.8)	1,582,019 (38.5)	17,061,259 (44.1)	2,083,624 (54.9)	8,077,669 (44.5)	10,161,293 (46.3)
South	5,621,171 (16.3)	575,822 (14.0)	6,196,993 (16.0)	427,859 (11.3)	3,021,915 (16.7)	3,449,774 (15.7)
Missing	178,789 (0.5)	25,368 (0.6)	204,157 (0.5)	25,204 (0.7)	101,943 (0.6)	127,147 (0.6)
Brazilian Municipal Deprivation Index						
1	7,140,436 (20.7)	776,055 (18.9)	7,916,491 (20.5)	788,353 (20.8)	3,973,481 (21.9)	4,761,834 (21.7)

2	6,616,814 (19.1)	712,784 (17.4)	7,329,598 (19.0)	994,456 (26.2)	3,456,814 (19.1)	4,451,270 (20.3)
3	7,071,108 (20.5)	833,540 (20.3)	7,904,648 (20.4)	729,322 (19.2)	3,751,664 (20.7)	4,480,986 (20.4)
4	6,925,602 (20.0)	853,682 (20.8)	7,779,284 (20.1)	595,008 (15.7)	3,580,458 (19.7)	4,175,466 (19.0)
5	6,624,234 (19.2)	906,221 (22.1)	7,530,455 (19.5)	662,410 (17.5)	3,274,124 (18.1)	3,936,534 (17.9)
Missing	178,789 (0.5)	25,368 (0.6)	204,157 (0.5)	25,204 (0.7)	101,943 (0.6)	127,147 (0.6)

The study participants were included if they received first dose of CoronaVac of Vaxzevria between January 18 and June 30, 2021. The Brazilian Municipal Deprivation Index works as proxy for socioeconomic status.

Table 2. Vaccine effectiveness of Vaxzevria and CoronaVac in Brazil for COVID-19 infection, hospitalization, ICU admission, and death.

	Vaxzevria/Fiocruz				CoronaVac/Butantan			
	Person-days	Events	Incidence per 1000 person-days	VE % (95% CI)*	Person-days	Events	Incidence per 1000 person-days	VE % (95% CI)*
Infection								
Reference period	474,317,595	76,780	0,1619	Ref	272,340,929	47,523	0,1745	Ref
Partially vaccinated	1,183,986,976	119,195	0.1007	32.7 (31.9-33.5)	431,038,009	55,495	0.1287	16.2 (15.1-17.4)
Fully	98,266,804	6,271	0.0638	70.0 (68.6-	1,184,435,889	108,998	0.0920	54.2 (53.4-55.0)

vaccinated				71.3)				
Hospitalization								
Reference period	474,679,253	18,420	0.0389	Ref	272,540,206	15,080	0.0553	Ref
Partially vaccinated	1,189,453,888	20,998	0.0177	51.7 (50.4-52.9)	434047110	14,484	0.0334	26.5 (24.6-28.4)
Fully vaccinated	99,464,137	574	0.0058	86.8 (85.2-88.2)	1192845239	20,299	0.0170	72.6 (71.6-73.6)
ICU admission								
Reference period	474,760,394	6,272	0.0132	Ref	272,599,778	5,643	0.0207	Ref
Partially	1,190,575,743	7,129	0.0060	53.6 (51.4-	435,127,028	5,291	0.0122	28.1 (24.9-31.1)

vaccinated				55.6)				
Fully vaccinated	99,558,609	184	0.0018	88.1 (85.4-90.3)	1,194,037,275	6,971	0.0058	74.2 (72.6-75.7)
Death								
Reference period	474,761,099	6,255	0.0131	Ref	272,587,083	7,529	0.0276	Ref
Partially vaccinated	1,190,384,840	8,518	0.0072	49.3 (47.0-51.5)	434,742,763	6,988	0.0161	29.4 (26.7-32.0)
Fully vaccinated	99,567,659	249	0.0025	90.2 (88.3-91.8)	1,193,883,495	9,600	0.0080	74.0 (72.6-75.3)

Reference period: ≤13 days after the first dose; Partially vaccinated: ≥14 days after the first dose and without the second dose; Fully vaccinated: ≥14 days after the second dose. ICU denotes intensive care unit.

* Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level.

Table S1. Crude and adjusted Vaccine effectiveness of Vaxzevria and CoronaVac in Brazil for COVID-19 infection, hospitalization, ICU admission and death.

	Vaxzevria/Fiocruz		CoronaVac/Butantan	
	CRUDE VE % (95% CI)	ADJUSTED VE % (95% CI)*	CRUDE VE % (95% CI)	ADJUSTED VE % (95% CI)*
Infection				
Reference period	—	—	—	—
Partially vaccinated	27.4 (26.5-28.2)	34.0 (33.2-34.7)	14.1 (12.9-15.3)	16.4 (15.2-17.5)
Fully vaccinated until 13 days	49.0 (47.3-50.6)	56.9 (55.3-58.5)	38.2 (37.2-39.1)	40.3 (39.4-41.2)
Fully vaccinated	63.2 (61.7-64.7)	70.0 (68.6-71.3)	52.5 (51.7-53.3)	54.2 (53.4-55.0)
Hospitalization				
Reference period	—	—	—	—
Partially vaccinated	45.3 (43.8-46.7)	52.2 (50.9-53.4)	24.1 (22.1-26.0)	26.6 (24.6-28.4)
Fully vaccinated until 13 days	53.8 (50.5-56.9)	69.6 (67.2-71.8)	55.0 (53.6-56.4)	57.3 (56.0-58.6)
Fully vaccinated	79.0 (76.5-81.2)	86.8 (85.2-88.2)	71.0 (70.0-72.0)	72.6 (71.6-73.6)
ICU admission				
Reference period	—	—	—	—
Partially vaccinated	46.5 (44.0-48.9)	54.0 (51.8-56.0)	25.3 (22.1-28.4)	28.1 (24.9-31.1)

Fully vaccinated until 13 days	51.5 (45.6-56.8)	69.2 (65.0-72.8)	55.8 (53.5-57.9)	58.1 (55.9-60.1)
Fully vaccinated	80.2 (76.0-83.7)	88.1 (85.4-90.3)	72.6 (70.9-74.2)	74.2 (72.6-75.7)
Death				
Reference period	—	—	—	—
Partially vaccinated	39.7 (37.0-42.3)	49.3 (47.0-51.5)	26.9 (24.2-29.6)	29.4 (26.7-32.0)
Fully vaccinated until 13 days	31.9 (24.9-38.3)	72.1 (69.1-74.9)	56.2 (54.3-58.1)	58.7 (56.9-60.4)
Fully vaccinated	74.8 (70.0-78.8)	90.2 (88.3-91.8)	72.1 (70.7-73.5)	74.0 (72.6-75.3)

* Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level.

Table S2. Vaccine effectiveness of Vaxzevria and CoronaVac in Brazil by age groups for COVID-19 infection, hospitalization, ICU admission and death.

	Vaxzevria/Fiocruz					CoronaVac/Butantan				
	<60	**60-69**	**70-79**	**80-89**	**≥90**	**<60**	**60-69**	**70-79**	**80-89**	**≥90**
Infection										
Partially vaccinated	38.8 (37.9-39.7)	23.1 (21.3-24.9)	25.9 (20.3-31.1)	28.2 (24.5-31.7)	-43.0 (-71.2 to -19.5)	13.8 (11.6-16.0)	15.4 (13.0-17.8)	25.0 (23.1-26.9)	1.5 (-3.0 to 5.9)	-19.3 (-30.5 to -9.2)
Fully vaccinated until 13 days	54.4 (51.9-56.8)	72.2 (68.2-75.8)	60.9 (56.4-65.0)	57.9 (55.1-60.5)	21.5 (1.4-37.6)	31.1 (29.2-32.9)	38.1 (36.1-40.0)	52.5 (51.2-53.8)	37.1 (33.9-40.1)	9.1 (0.3-17.2)
Fully vaccinated	62.5 (60.2-64.7)	78.5 (73.3-82.6)	79.2 (75.7-82.2)	78.3 (76.4-80.1)	46.9 (30.9-59.3)	44.6 (43.0-46.2)	55.9 (54.3-57.4)	61.9 (60.7-63.1)	57.1 (54.7-59.5)	31.7 (24.4-38.2)
Hospitalization										
Partially vaccinated	64.1 (62.6-65.5)	44.9 (42.4-47.4)	32.9 (25.2-39.8)	32.9 (28.0-37.4)	-31.1 (-66.1 to -3.4)	33.7 (27.1-39.7)	29.5 (25.8-33.0)	32.5 (29.9-35.1)	8.2 (2.1-13.8)	-16.2 (-31.2 to -2.9)

Fully vaccinated until 13 days	83.8 (77.7-88.2)	83.3 (77.3-87.8)	71.9 (66.4-76.5)	66.6 (63.3-69.7)	34.9 (11.1-52.4)	67.1 (62.8-70.8)	60.2 (57.6-62.6)	62.2 (60.4-63.9)	42.7 (38.6-46.6)	12.4 (0.6-22.8)
Fully vaccinated	94.2 (89.8-96.6)	91.7 (84.3-95.6)	88.4 (84.6-91.2)	86.9 (84.9-88.7)	54.9 (35.4-68.5)	84.2 (81.3-86.7)	78.2 (76.3-79.8)	74.0 (72.6-75.4)	63.0 (59.9-66.0)	32.7 (22.8-41.3)
ICU admission										
Partially vaccinated	65.1 (62.5-67.6)	48.9 (44.8-52.7)	37.4 (25.1-47.7)	33.9 (25.6-41.3)	-35.4 (-110.9 to 13.1)	32.1 (19.4-42.8)	29.0 (23.1-34.5)	33.1 (28.8-37.1)	18.1 (8.6-26.6)	-27.8 (-59.6 to -2.3)
Fully vaccinated until 13 days	83.2 (70.2-90.6)	82.4 (71.2-89.3)	69.3 (59.5-76.7)	68.0 (62.3-72.8)	5.8 (-60.4 to 44.7)	69.1 (61.1 - 75.4)	61.7 (57.7-65.4)	60.9 (57.9-63.6)	46.4 (39.5-52.5)	11.3 (-12.3 to 29.9)
Fully vaccinated	95.5 (85.8-98.6)	93.2 (78.7-97.9)	87.4 (80.5-91.9)	89.3 (86.0-91.8)	39.7 (-11.7-67.5)	80.8 (74.5-85.6)	78.7 (75.8-81.3)	75.7 (73.5-77.8)	65.1 (59.9-69.7)	37.2 (18.4-51.6)
Death										
Partially vaccinated	64.8 (61.8-67.6)	45.4 (41.0-49.4)	37.1 (26.9-45.8)	38.1 (32.2-43.4)	-40.6 (-84.5 to -7.1)	41.7 (26.4-53.9)	35.7 (30.3-40.7)	38.2 (34.7-41.5)	10.1 (2.7-1.07)	-22.1 (-40.7 to -5.9)

Fully vaccinated until 13 days	80.7 (57.6-91.2)	88.5 (78.9-93.7)	77.2 (70.5-82.4)	71.3 (67.4-74.7)	45.2 (19.4-62.8)	66.1 (54.9-74.5)	64.1 (60.3-67.4)	65.5 (63.2-67.6)	46.9 (41.9-51.5)	10 (-4.4 to 22.4)
Fully vaccinated	93.3 (72.1-98.4)	89.6 (71.8-96.2)	92.5 (88.1-95.3)	91.2 (89.1-92.9)	70.5 (51.4-82.1)	76.5 (66.9-83.3)	78.7 (76.6-80.0)	78.3 (76.6-80.0)	67.3 (63.6-70.6)	35.4 (23.8-45.1)

*Obtained through Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level

Table S3. Robustness analysis with different time windows as reference period

	Vaxzevria/Fiocruz VE % (95% CI)	CoronaVac/Butantan VE % (95% CI)
Reference Period:	0-10 days	0-10 days
Infection		
Partially vaccinated	33.2 (32.3-34.0)	16.5 (15.2-17.8)
Fully vaccinated until 13 days	55.5 (53.7-57.3)	38.0 (36.9-39.0)
Fully vaccinated	69.8 (68.2-71.3)	54.6 (53.7-55.5)
Hospitalization		
Partially vaccinated	51.3 (49.9-52.7)	25.5 (23.4-27.6)
Fully vaccinated until 13 days	67.6 (64.8-70.1)	55.4 (53.8-56.8)
Fully vaccinated	86.0 (84.1-87.6)	72.5 (71.4-73.6)
ICU admission		
Partially vaccinated	53.7 (51.3-56.0)	27.8 (24.3-31.1)
Fully vaccinated until 13 days	67.2 (62.4-71.3)	56.7 (54.2-59.0)
Fully vaccinated	87.4 (84.3-89.9)	74.1 (72.3-75.8)
Death		
Partially vaccinated	48.2 (45.6-50.6)	28.8 (25.8-31.6)
Fully vaccinated until 13 days	70.4 (66.8-73.7)	57.9 (55.8-59.9)
Fully vaccinated	89.2 (86.9-91.1)	73.7 (72.1-75.2)

Table S4: Percentage of events with laboratory confirmation and VE using all cases (laboratory and clinical suspected)

	Vaxzevria/Fiocruz				Coronavac/Butantan			
	Events-Laboratory Confirmed	Events-Confirmed or Clinical Suspected	% Confirmed	VE* (95% CI)	**Events-Laboratory Confirmed**	Events-Confirmed or Clinical Suspected	% Confirmed	VE* (95% CI)
Hospitalization								
Reference period	18,420	23,368	78.8	Ref	15,080	19,672	76.6	Ref
Partially vaccinated	20,998	27,946	75.1	50.7 (49.6-51.9)	14,484	19,182	75.5	25.5 (23.8-27.2)
Fully vaccinated	574	845	67.9	85.8 (84.3-87.1)	20,299	26,836	75.6	71.5 (70.6-72.4)
ICU admission								
Reference period	6,272	7,693	81.5	Ref	5,643	7,176	78.6	Ref
Partially vaccinated	7,129	9,164	77.8	52.4 (50.5-54.3)	5,291	6,875	77.0	26.9 (24.1-29.6)
Fully vaccinated	184	262	70.2	87.5 (85.1-89.5)	6,971	9,015	77.3%	73.2 (71.8-74.6)

Death								
Reference period	6,255	7,749	80.7	Ref	7,529	9,608	78.4	Ref
Partially vaccinated	8,518	11,091	76.8	47.8 (45.7-49.8)	6,988	9,043	77.3	28.7 (26.3-31.0)
Fully vaccinated	249	359	69.4	89.5 (87.8-91.0)	9,600	12,262	78.2	73.4 (72.2-74.6)

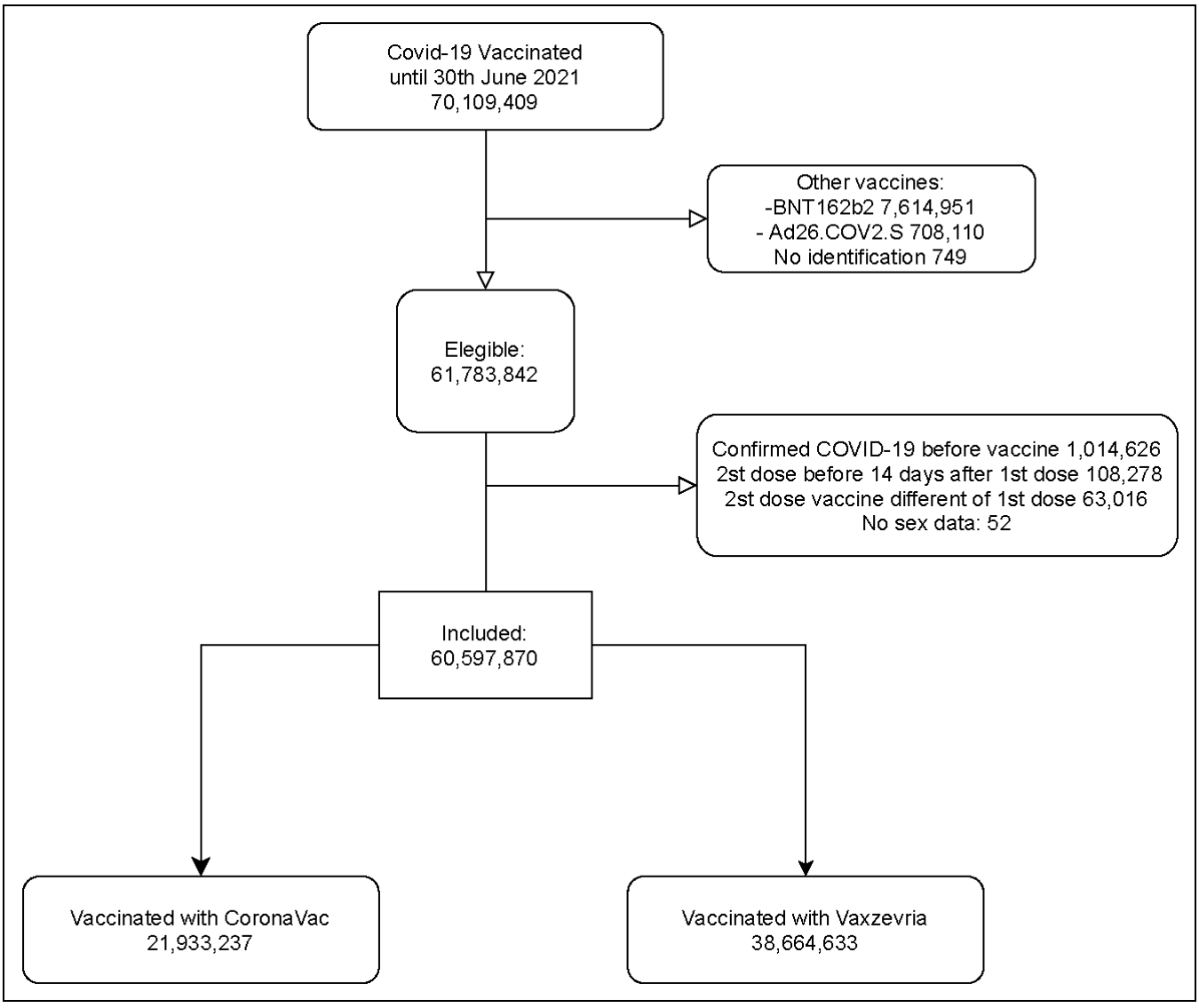
*Obtained through Cox regression model adjusted for age, sex, region of residence, month of administration of first dose and municipal deprivation level

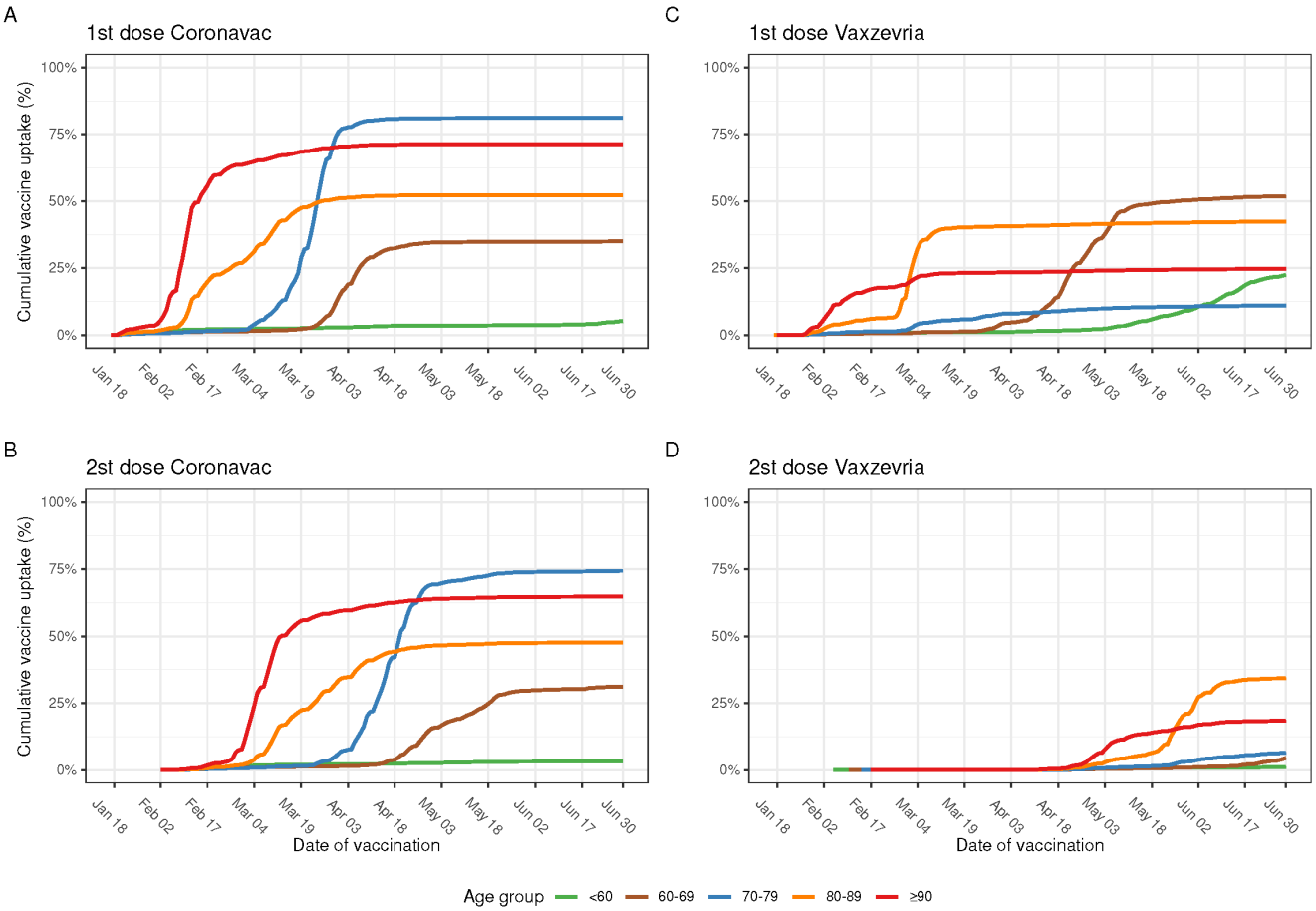
Figures legends

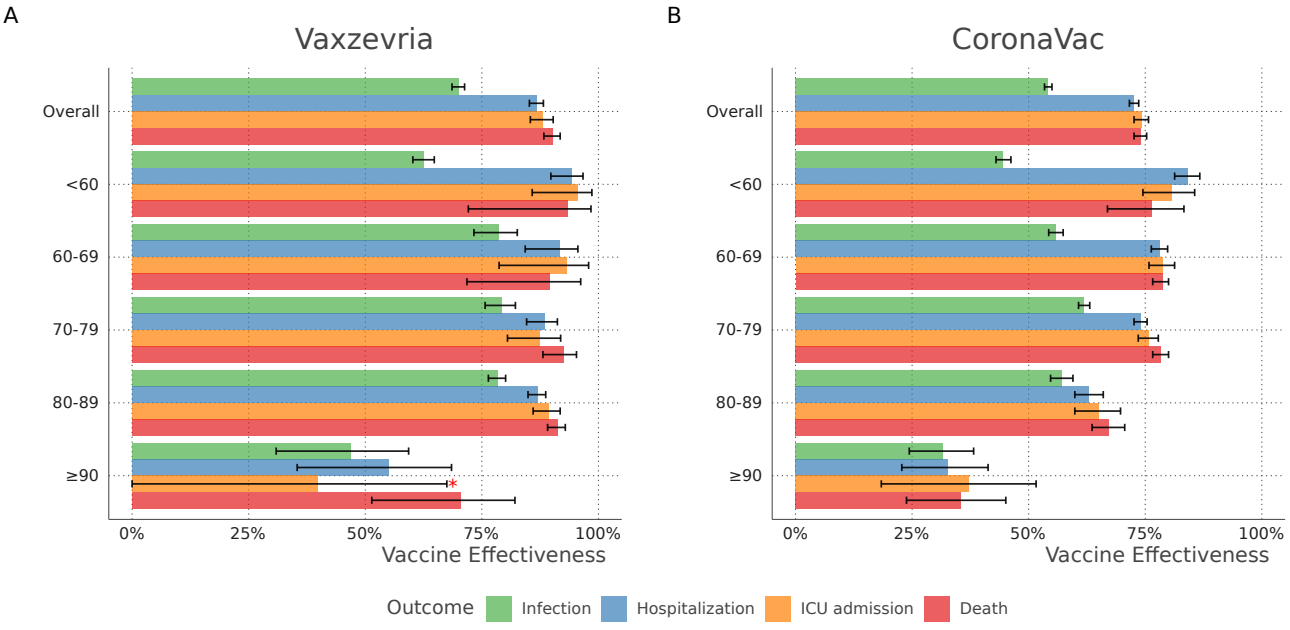
Figure 1. Flowchart of the selection of the study individuals vaccinated between 18th January and 30 June 2021. Eligible participants received at least one dose of CoronaVac or Vaxzevria vaccine between January 18 and June 30, 2021. We excluded persons with confirmed COVID-19 diagnosis in 2021 before the first dose and all persons with different vaccines from CoronaVac or Vaxzevria

Figure 2. Coverage of first and second dose of CoronaVac and Vaxzevria in Brazil during the study period. The panels A, B, C and D shown the rate and coverage of the vaccination program regarding CoronaVac and Vaxzevria, A and C regarding first dose between January 18 and June 30 and panels B and D the second dose until 30 June 2021.

Figure 3. Vaccine effectiveness of Vaxzevria and CoronaVac in Brazil by age group. VE (1-Hazard Ratio) was obtained through Cox regression adjusted for age, sex, region of residence, the month of administration of first dose, and municipal deprivation level (IBP). *The point estimate and confidence interval for ICU admission in ≥90 y.o. are 39.7 (95%CI -11.7 to 67.5%), the large confidence interval is reflect of the small sample size and number of events in this group, 35 in the reference period and 33 in the fully vaccinated.


Covid-19 Vaccinated
until 30th June 2021
70,109,409
Other vaccines:
-BNT162b2 7,614,951
- Ad26.COV2.S 708,110
No identification 749
Elegible:
61,783,842
Confirmed COVID-19 before vaccine 1,014,626
2st dose before 14 days after 1st dose 108,278
2st dose vaccine different of 1st dose 63,016
No sex data: 52
Included:
60,597,870
Vaccinated with CoronaVac
21,933,237
Vaccinated with Vaxzevria
38,664,633


A
1st dose Coronavac
C
1st dose Vaxzevria
B
2st dose Coronavac
D
2st dose Vaxzevria
Cumulative vaccine uptake (%)
100%
75%
50%
25%
0%
Jan 18
Feb 02
Feb 17
Mar 04
Mar 19
Apr 03
Apr 18
May 03
May 18
Jun 02
Jun 17
Jun 30
Date of vaccination
Age group
<60
60-69
70-79
80-89
≥90


A
Vaxzevria
B
CoronaVac
Overall
<60
60-69
70-79
80-89
≥90
*
0%
25%
50%
75%
100%
Vaccine Effectiveness
Outcome
Infection
Hospitalization
ICU admission
Death

5.3 Estudo atesta a eficácia da CoronaVac contra a variante gama (P.1) entre idosos

Nesta quarta (21), foi publicado um novo estudo que atesta a eficácia da Coronavac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, na prevenção da variante gama (P.1, amazônica) do vírus SARS-CoV-2 em idosos com mais de 70 anos. O artigo Effectiveness of the CoronaVac vaccine in the elderly population during a P.1 variant-associated epidemic of Covid-19 in Brazil já está disponível na plataforma de preprints MedRxiv, onde são disponibilizadas pesquisas ligadas à saúde que estão em processo de revisão.

A eficácia da vacina contra hospitalizações 14 dias após a aplicação da segunda dose foi de 59%, e contra mortes, de 71,4%. O indicador variou com o aumento de idade: entre os indivíduos com idade de 70 a 74 anos, a eficácia foi de 61,8% contra a doença sintomática, de 80,1% contra hospitalizações e de 86% contra mortes.

"Em resumo, ficou evidenciado que um esquema de duas doses de CoronaVac foi eficaz na prevenção de casos sintomáticos de Covid-19 e na prevenção de desfechos clínicos mais graves entre idosos frente à variante gama", afirmam os autores no artigo.

O trabalho foi realizado por pesquisadores ligados à Secretaria de Saúde do Estado de São Paulo, à Organização Pan-Americana de Saúde, à Universidade de São Paulo e às universidades norte-americanas da Flórida e de Yale, entre outras instituições. Foram investigados 43.774 adultos com 70 anos ou mais, residentes no estado de São Paulo, todos sintomáticos para Covid-19.

O objetivo da pesquisa era estimar a eficácia da CoronaVac contra a Covid-19 sintomática na população idosa do estado de São Paulo durante a ampla circulação da variante gama. De acordo com o boletim epidemiológico da Rede de Alerta das Variantes do SARS-CoV-2, iniciativa liderada pelo Instituto Butantan e que realiza o sequenciamento genômico de resultados diagnósticos positivos para Covid-19, essa cepa é dominante no estado de São Paulo, concentrando mais de 90% dos casos.

Os autores concluem que, embora outras pesquisas ainda devam contribuir para reafirmar a eficácia do CoronaVac contra a variante gama, os resultados fornecem evidências que suportam o uso da vacina no Brasil e nos demais países da América do Sul que enfrentam a disseminação da variante gama do SARS-CoV-2.

Publicado em: 23/07/2021

Effectiveness of the CoronaVac vaccine in the elderly population during a Gamma variant-associated epidemic of COVID-19 in Brazil: A test-negative case-control study

Otavio T. Ranzani[1,2]*, Matt D.T. Hitchings[3,4]*, Murilo Dorion[5], Tatiana Lang D'Agostini[6], Regiane Cardoso de Paula[6], Olivia Ferreira Pereira de Paula[6], Edlaine Faria de Moura Villela[6], Mario Sergio Scaramuzzini Torres[7], Silvano Barbosa de Oliveira[8,9], Wade Schulz[10], Maria Almiron[8], Rodrigo Said[8], Roberto Dias de Oliveira[11], Patricia Vieira da Silva[12], Wildo Navegantes de Araújo[8,9,13], Jean Carlo Gorinchteyn[14], Jason R. Andrews†[15], Derek A.T. Cummings†[3,4], Albert I. Ko†[5,16], Julio Croda†[5,12,17]

1-Barcelona Institute for Global Health, ISGlobal, Barcelona, Spain
2-Pulmonary Division, Heart Institute (InCor), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil
3-Department of Biology, University of Florida, Gainesville, FL, USA
4-Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA
5-Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA
6-Disease Control Coordination of the São Paulo State Department of Health, São Paulo, Brazil
7-Municipal Health Secretary of Manaus, Brazil, AM, Brazil
8-Pan American Health Organization, Brasília, DF, Brazil
9-Universidade de Brasília, Brasília, DF, Brazil
10-Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, USA
11-State University of Mato Grosso do Sul - UEMS, Dourados, MS, Brazil
12-Universidade Federal de Mato Grosso do Sul - UFMS, Campo Grande, MS, Brazil
13-National Institute for Science and Technology for Health Technology Assessment, Porto Alegre, RS, Brazil
14-Health Secretariat of the State of São Paulo, São Paulo, Brazil
15-Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, USA
16-Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil
17-Fiocruz Mato Grosso do Sul, Fundação Oswaldo Cruz, Campo Grande, MS, Brazil

*Authors contributed equally
†Authors contributed equally

Correspondence to: Prof Julio Croda, Universidade Federal de Mato Grosso do Sul and Fundação Oswaldo Cruz, julio.croda@fiocruz.br

Keywords
COVID-19; CoronaVac; inactivated whole-virus vaccine, Gamma variant; test-negative study; case-control study; Brazil

Word count: 3,840

ABSTRACT

Objective To estimate the effectiveness of the inactivated whole-virus vaccine, CoronaVac, against symptomatic COVID-19 in the elderly population of São Paulo State, Brazil during widespread circulation of the Gamma variant.

Design Test negative case-control study.

Setting Health-care facilities in São Paulo State, Brazil.

Participants 43,774 adults aged 70 years or older who were residents of São Paulo State and underwent SARS-CoV-2 RT-PCR testing from January 17 to April 29, 2021. 26,433 cases with symptomatic COVID-19 and 17,622 symptomatic, test negative controls were selected into 7,950 matched pairs, according to age, sex, self-reported race, municipality of residence, prior COVID-19 status and date of RT-PCR testing.

Intervention Vaccination with a two-dose regimen of CoronaVac.

Main outcome measures RT-PCR confirmed symptomatic COVID-19 and COVID-19 associated hospitalizations and deaths.

Results Adjusted vaccine effectiveness against symptomatic COVID-19 was 18.2% (95% CI, 0.0 to 33.2) in the period 0-13 days after the second dose and 41.6% (95% CI, 26.9 to 53.3) in the period ≥14 days after the second dose. Adjusted vaccine effectiveness against hospitalisations was 59.0% (95% CI, 44.2 to 69.8) and against deaths was 71.4% (95% CI, 53.7 to 82.3) in the period ≥14 days after the second dose. Vaccine effectiveness ≥14 days after the second dose declined with increasing age for the three outcomes, and among individuals aged 70-74 years it was 61.8% (95% CI, 34.8 to 77.7) against symptomatic disease, 80.1% (95% CI, 55.7 to 91.0) against hospitalisations and 86.0% (95% CI, 50.4 to 96.1) against deaths.

Conclusions Vaccination with CoronaVac was associated with a reduction in symptomatic COVID-19, hospitalisations and deaths in adults aged 70 years or older in a setting with extensive Gamma variant transmission. However, significant protection was not observed until completion of the two-dose regimen, and vaccine effectiveness declined with increasing age amongst this elderly population.

Summary boxes

What is already known on this topic

- Randomised controlled trials (RCT) have yielded varying estimates (51 to 84%) for the effectiveness of the inactivated whole-virus vaccine, CoronaVac, against symptomatic COVID-19.
- Current evidence is limited on whether CoronaVac is effective against severe disease or death caused by the SARS-CoV-2 variant of concern, Gamma, or in the setting of extensive Gamma variant circulation.
- More evidence is needed for the real-world effectiveness of CoronaVac and other inactivated vaccines among elderly individuals, a population that was underrepresented in RCTs of these vaccines.

What this study adds

- A two-dose regimen of CoronaVac provides significant protection against symptomatic COVID-19, hospitalisations and deaths among adults ≥70 years of age in the setting of widespread Gamma variant transmission.
- Significant protection did not occur until ≥14 days after administration of the second dose of CoronaVac.
- The effectiveness of CoronaVac declines with increasing age in the elderly population.

Introduction

The coronavirus disease (COVID-19) pandemic has caused 3.9 million deaths worldwide as of early July 2021,[1] and has imparted disproportionately high mortality and morbidity on the elderly.[2] A key question is whether the authorised COVID-19 vaccines are effective in the elderly, who may have impaired immune responses[3,4] and are underrepresented in randomised controlled trials (RCTs).[5–7] mRNA and adenovirus vector-based vaccines have been shown to be effective against COVID-19 in elderly individuals,[8,9] but evidence is limited for the effectiveness of inactivated vaccines in these populations.[7,10–12]

CoronaVac, an inactivated whole-virus vaccine, has been approved by 32 countries and jurisdictions,[10] and has been implemented as part of mass vaccination campaigns in low-income and middle-income countries, many of which are experiencing COVID-19 epidemics due to the emergence of SARS-CoV-2 variants of concern (VOC). RCTs of a two-dose CoronaVac regimen in healthcare workers and the general population have yielded varying estimates (51 to 84%) of vaccine efficacy against symptomatic COVID-19.[5,7,10] The World Health Organisation (WHO) Emergency Use Listing (EUL) procedure approved CoronaVac in early June 2021, but identified an evidence gap for the effectiveness of this vaccine in adults aged 60 and above.[11] The WHO EUL cited an observational study in Chile,[10,12] which found that the adjusted effectiveness of CoronaVac, starting 14 days after the second dose, was 66.6% among adults aged 60 years and older. During the study period, the variant of concern (VOC) Gamma was detected in 28.6% of SARS-CoV-2 genomes.[12] Furthermore, evidence from RCTs or observational studies have not

addressed whether CoronaVac provides significant protection after administration of the first vaccine dose or in the setting of widespread VOC transmission.[5,10,11]

Brazil has experienced one of the world's highest COVID-19 burdens during the pandemic with more than 18 million cases and 526,000 deaths as of early July 2021.[1,13] VOCs, and in particular the Gamma variant, have played an important role in the recent epidemic wave in Brazil which began in early 2021.[14–16] The Gamma variant, which was first detected in Manaus, has increased transmissibility,[16] has accrued mutations associated with decreased *in vitro* seroneutralisation,[17–19] and at present, accounts for the majority of SARS-CoV-2 isolates genotyped in Brazil from 1 January 2021.[14,20] In the setting of a large Gamma variant-associated epidemic in São Paulo, the most populous state in Brazil, we conducted a matched, test-negative,[21] case-control study to evaluate the real-world effectiveness of CoronaVac against symptomatic COVID-19 and severe clinical outcomes in the elderly population.

Methods

Study setting

The State of São Paulo (23°3'S, 46°4'W) has 645 municipalities and 46 million inhabitants, among which 3.23 million are ≥70 years of age.[22] The state experienced three successive COVID-19 epidemic waves during which 2,997,282 cases (cumulative incidence rate: 6,475 per 100,000 population) and 100,649 deaths (cumulative mortality: 217 per 100,000 population) have been reported as of 9 May 2021 (Figure 1A, Supplementary Figure 1).[23] The State Secretary of Health of Sao Paulo (SES-SP) initiated a COVID-19 vaccination campaign for the

general population on 17 January 2021 according to an age-based prioritisation strategy (Figure 1, B-D) and is administering a two-dose regimen of CoronaVac, separated by a two to four week interval, and a two-dose regimen of ChAdOx1, separated by a 12 week interval.[24] As of 29 April 2021, 8.63 million doses (5.16 first and 3.47 second million doses) have been administered of CoronaVac and 2.06 million doses (1.987 first and 0.07 second million doses) of ChAdOx1.

Study design

We conducted a matched test-negative case-control study to estimate the effectiveness of CoronaVac in reducing the odds of symptomatic RT-PCR-confirmed COVID-19 in adults ≥70 years of age from São Paulo State during the period from 17 January 2021, the start of COVID-19 vaccination, to 29 April 2021. Test-negative design studies have provided estimates of vaccine effectiveness in concordance with those obtained from RCTs[25,26] and have been used extensively to evaluate vaccines against respiratory infections,[27] including COVID-19.[8,21] We chose the test-negative design because of the feasibility of accessing information on individuals who received SARS-CoV-2 testing from São Paulo State surveillance systems and the opportunity to control for potential biases, such as healthcare-seeking behaviour and access to testing.[21] The study population was adults ≥70 years of age who had a residential address in São Paulo State, underwent SARS-CoV-2 RT-PCR testing during the study period, and had complete and consistent information between data sources on age, sex, residence, and vaccination and testing status and dates. We matched symptomatic test-negative controls to COVID-19 cases by date of testing to address potential sources of bias that may vary during the course of an

epidemic, as well as by participant characteristics of age, gender, self-reported race, municipality of residence, and prior COVID-19 status.

The study design and statistical analysis plan were specified in advance of extracting information from data sources and are described in a publicly available protocol (https://github.com/juliocroda/VebraCOVID-19) and the Supplement. In the protocol, we pre-specified power thresholds for conducting analyses on the effectiveness of CoronaVac and ChAdOx1. These thresholds were achieved for CoronaVac but not for ChAdOx1 because of lower rates of ChAdOx1 administration in the population. We therefore restricted the evaluation of vaccine effectiveness to CoronaVac. The study was approved by the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021).

Data Sources

We obtained individual-level information on demographic characteristics, comorbidities, SARS-CoV-2 testing, and COVID-19 vaccination during the study period by extracting information on 6 May 2021 from the SES-SP laboratory testing registry (GAL), the national surveillance databases for COVID-19-like illnesses (e-SUS) and severe acute respiratory illness (SIVEP-Gripe), and the SES-SP vaccination registry (Vacina Já). Notification of suspected COVID-19 cases and SARS-CoV-2 testing results is compulsory in Brazil. The information technology bureau of the São Paulo State Government (PRODESP) linked individual-level records from the four databases using CPF numbers (Brazilian citizens' unique identifier code) and provided anonymised datasets. We

retrieved information on SARS-CoV-2 variants from genotyped isolates deposited in the GISAID database.[20]

Selection of cases and matched controls

Cases were selected from the study population who had symptomatic COVID-19, defined as an individual who had a COVID-19-like illness; had a positive SARS-CoV-2 RT-PCR test result from a respiratory sample which was collected within 10 days after the onset of symptoms; and did not have a positive RT-PCR test in the preceding 90-day period. Controls were selected from the study population who had a COVID-19-like illness; had a negative SARS-CoV-2 RT-PCR test result from a respiratory sample that was collected within 10 days after the onset of symptoms;[21] and did not have a positive RT-PCR test in the prior 90 days during the study period or in the subsequent 14 days. Cases and controls were excluded if they received the ChAdOx1 vaccine before sample collection for RT-PCR testing. COVID-19-like illness was defined as the presence of one or more reported COVID-19 related symptoms.[28]

We matched one test-negative control to each case according to RT-PCR sample collection date (±3 days); age category (5-year age bands, e.g, 70-74, 75-79 years); municipality of residence; self-reported race (defined as brown, black, yellow, white, or indigenous);[29] and previous symptomatic events that were reported to the surveillance systems[28] between February 1, 2020 and January 16, 2021, as a proxy for previous COVID-19 infection. Matching factors were chosen from variables that were associated with vaccination coverage or timing, and with SARS-

CoV-2 infection risk or healthcare access (see protocol in Supplement).[21] Upon identification of each case, a single control was randomly chosen from the set of all eligible matching controls.

Statistical analysis

We estimated the effectiveness of CoronaVac against symptomatic COVID-19 during the periods 0-13 and ≥14 days after the second vaccine dose and ≥14 days after a single vaccine dose. Furthermore, we estimated the effectiveness of a single dose during the period 0-13 days after the first dose, when the vaccine has no or limited effectiveness.[5,30,31] An association during this period may serve as an indicator of unmeasured confounding in the effectiveness estimate.[32] The reference group for vaccination status was individuals who had not received a first vaccine dose before the date of sample collection.

We used conditional logistic regression to estimate the odds ratio (OR) of vaccination among cases and controls. 1-OR provided an estimate of vaccine effectiveness under the assumptions of a test-negative design.[33] We included age and COVID-19-associated comorbidities (cardiovascular, renal, neurological, haematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression) as covariates in the model. We evaluated nonlinearity for age using restricted cubic splines and chose the parsimonious model comparing nested models with a likelihood ratio test. Furthermore, we conducted a *post hoc* sensitivity analysis that incorporated the calendar date of RT-PCR sample collection in the model to evaluate potential residual confounding that may not be addressed by the matching criteria

We estimated the vaccine effectiveness against acute respiratory illness (ARI) associated hospitalizations and deaths in a *post hoc* analysis. In separate analyses, we selected matched pairs in which the case had the secondary outcome of interest.[34,35] We fit the same conditional logistic regression model as for the primary outcome.

We conducted a pre-specified analysis of vaccine effectiveness among age sub-groups for the primary and secondary outcomes, but could not perform analyses stratified by previous COVID-19 documented infection because of small numbers. Additional *post hoc* analyses were performed of vaccine effectiveness for the primary outcome for subgroups stratified by sex, number of chronic comorbidities (none vs. at least one), the two most frequent chronic comorbidities (cardiovascular disease and diabetes), and region of residence ("Grande São Paulo" health region vs. others). Interaction terms were incorporated into the model to evaluate the association of each subgroup of interest with vaccine effectiveness ≥14 days after the second dose.

Power calculation

Our protocol specified that we would conduct proposed analyses after achieving ≥80% power to identify a vaccine effectiveness of 40% against symptomatic COVID-19 for the comparison of ≥14 days after the second dose of CoronaVac and not receiving a vaccine dose. The power was simulated fitting conditional logistic regressions on 1,000 simulated datasets. After extracting the surveillance databases on May 6, 2021 and generating matched case-control pairs, we determined that the power of the study was 99.9% and proceeded to conduct the pre-specified

analyses. We did not perform an analysis for ChAdOx1 since the simulated power was 31% to identify a vaccine effectiveness of 40% for the comparison of ≥28 days after the first dose of ChAdOx1 and not receiving a vaccine dose. All analyses were done in R, version 4.0.2.

Results

COVID-19 epidemic and vaccination campaign in São Paulo State

São Paulo State experienced three COVID-19 epidemic waves during which peak incidence occurred in July 2020 for the first wave (Supplementary Figure 1), January 2021 for the second wave and March 2021 for the third wave (Figure 1A). The second wave was preceded in November 2020 by an increase in the prevalence of the Zeta variant among genotyped isolates from São Paulo State deposited into the GISAID database (Figure 1E). The third wave was preceded in January 2021 by an increase in the prevalence of the Gamma variant among genotyped isolates. The Gamma variant replaced other SARS-CoV-2 variants[20] and accounted for 79% (3,834/4,887) of the genotyped isolates that were reported in GISAID during the study period and 86% (3,584/4,192) of genotyped isolates that were reported between 1 March to 29 April 2021 when the majority of discordant case-control pairs were identified (Supplementary Figure 2). The vaccination campaign, initiated on January 17, 2021, achieved an estimated coverage of roughly 85% for the first (2.82 million) and 65% for second (2.10 million) CoronaVac doses among adults ≥70 years of age by April 29, 2021 (Figure 1B-D). After initiation of the vaccination campaign and during the third epidemic wave, COVID-19 incidence increased and peaked in late March in all age groups except for adults ≥90 years of age (Figure 1A).

Study population

Among 43,774 individuals eligible for study inclusion (Figure 2), 15,852 (36.2%) who provided 15,900 RT-PCR test results were selected into 7,950 matched case and control pairs. There were 38 individuals that contributed two times as controls and 10 individuals one time as control and one time as case. Table 1 shows the characteristics of eligible individuals with positive and negative RT-PCR tests and selected cases and matched controls. A higher proportion of cases had reported comorbidities than controls. Supplementary Table 1 shows the distribution of matched pairs according to the vaccination status of cases and controls at the time of RT-PCR testing. The majority of discordant pairs, based on vaccination status, were selected after 14 March 2021 (Supplementary Figure 3). Cases and controls who completed the two dose vaccine regimen had similar inter-dose intervals (mean 29 vs. 25 days). Likewise, cases and controls who were vaccinated had similar distributions for the intervals between administration of vaccine doses and RT-PCR testing (Table 1 and Supplementary Figure 3). The characteristics of the matched case and control pairs which were selected for the analysis of secondary outcomes of hospitalisation (n=8,078) and death (n=4,104) are shown in Supplementary Tables 2 and 3.

Vaccine effectiveness

The adjusted effectiveness of the two-dose CoronaVac schedule against symptomatic COVID-19 was 18.2% (95% CI 0.0 to 33.2) in the period 0-13 days and 41.6% (95% CI 26.9 to 53.3) in the period ≥14 days after administration of the second dose (Table 2). We did not identify a significant reduction or increase in the odds of COVID-19 in the time periods following a single vaccine dose, including the period 0-13 days which serves as a potential bias-indicator.

Increasing number of comorbidities was significantly associated with increased odds of COVID-19. In a sensitivity analysis including calendar date of testing as a covariate, vaccine effectiveness was 19.3% (95% CI 1.3 to 34) in the period 0-13 day and 42.3% (95% CI 27.7 to 53.9) in the period ≥14 days after administration of the second dose.

In the period starting 14 days after the second dose, the adjusted effectiveness of the two-dose schedule was 59.0% (95% CI 44.2 to 69.8) against hospitalisation and 71.4% (95% CI 53.7 to 82.3) against deaths (Table 2). In general, statistically significant protection was not observed until after the second dose, and the vaccine effectiveness in the "bias-indicator" period 0-13 days after the first dose was low.

Vaccine effectiveness against symptomatic COVID-19 in the period ≥14 days after the second dose declined with increasing age and was 61.8% (95% CI 34.8 to 77.7) among individuals 70-74 years old, 48.9% (95% CI 23.3 to 66.0) among 75-79 years old, and 28.0% (95% CI 0.6 to 47.9) among individuals ≥80 years of age ($p_{interaction}$ = 0.05)(Figure 3). The same pattern was observed for hospitalisations ($p_{interaction}$ = 0.04) and deaths ($p_{interaction}$ = 0.19), yielding effectiveness of 80.1% (95% CI 55.7 to 91.0) for hospitalisations and 86.0% (95% CI 34.8 to 77.7) for deaths among the 70-74 years age group (Figure 3 and Supplementary Table 4).

Vaccine effectiveness against symptomatic COVID-19 disease did not differ among sub-groups defined by sex, presence of comorbidities, reported cardiovascular disease, or regions of residence. However, individuals with reported diabetes had lower protection than those

without reported diabetes (VE 26.9% vs. 45.6% , $p_{interaction}$ = 0.12) during the period starting 14 days after the 2nd dose (Supplementary Table 5 and Supplementary Figure 4).

Discussion

This test-negative case-control study found that a two-dose schedule of CoronaVac had a real-world effectiveness of 41.6% (95% CI 26.9 to 53.3) against symptomatic COVID-19, 59.0% (95% CI 44.2 to 69.8) against COVID associated hospitalisations, and 71.4% (95% CI 53.7 to 82.3%) against COVID-19 associated deaths among those ≥70 years during a Gamma variant-associated epidemic in Brazil. Furthermore, we have addressed several evidence gaps for the use of this vaccine: 1) vaccination with CoronaVac demonstrated an effectiveness against COVID-19, including associated severe outcomes, in the setting of widespread Gamma transmission which was similar to that found in the Brazilian RCT conducted prior to the emergence of Gamma,[5] 2) the vaccine did not confer significant protection until 14 days after completion of the two dose regimen; and 3) vaccine effectiveness declined with increasing age among adults ≥70 years of age.

Research in context

A key evidence gap, as raised in the WHO EUL for Coronavac,[11] has been the effectiveness of this vaccine in the elderly population, since this age group was not represented in the Brazilian and Turkish RCTs.[5,7,10,11] We found that CoronaVac had an effectiveness in the elderly population that was similar to that observed in RCTs of younger populations and similar to estimates of vaccine effectiveness in adults ≥60 years of age from a retrospective cohort study

in Chile.[10,12] However, we observed a significant decline in vaccine effectiveness against symptomatic COVID-19 with increasing age from 61.8% (95% CI 34.8 to 77.7) in adults 70-74 year olds to 28.0% (95% CI 0.6 to 47.9) in adults ≥80 years of age. These findings parallel real-world evidence for the BNT162b2 mRNA vaccine, which found reduced effectiveness in residents of long-term care facilities in Denmark,[36] skilled nursing facilities in the USA,[37] and the general population with ≥70 years in Finland[38] and ≥80 years of age in Israel.[39] As well as a slower immune response and lower peak of neutralising antibodies than younger populations, elderly individuals seem to have faster decay of antibodies titers.[4] Together, these findings suggest that effective COVID-19 vaccination of the very elderly (≥80 years) population may require specific vaccines or vaccination schemes.

Vaccine effectiveness was greater against severe outcomes than against symptomatic COVID-19 in all age subgroups among the elderly. This finding, consistent with RCTs and observational studies for multiple COVID-19 vaccines and across settings,[5,6,9,10,12] suggests that vaccination will reduce morbidity and mortality even if effectiveness at preventing infections is reduced among the elderly. The direct comparison of the effectiveness against hospitalisation with other vaccines and between countries is not straightforward, because hospitalisation is dependent on admission triage policies that change according to age and hospital bed availability. Therefore, a patient above 80 years with symptomatic COVID-19 has higher likelihood of being admitted compared to younger patients even if not severe, and this likelihood varies between public and private facilities and whether the health system is overwhelmed.[13] Thus, we cannot generalise our findings for protection against hospitalisations without considering this context. We

evaluated vaccine effectiveness at the individual level, not accounting for the indirect effect and the total effect from the vaccination campaign. A preliminary aggregated analysis using weekly times series of COVID-19 deaths in Brazil found a relative decrease in mortality among those ≥70 years compared with all ages after the vaccination with CoronaVac and ChAdOx1,[40] suggesting a discernible impact of vaccination on mortality at the population level. Additional investigation is required to address the duration of protection conferred by Coronavac.[7,19,21]

The absence of demonstrable effectiveness of CoronaVac until completion of the two dose regimen has profound implications for its use in an epidemic response. In contrast to COVID-19 vaccines that confer protection after the first dose,[9,41] we did not detect significant effectiveness for CoronaVac until ≥14 days after the second dose (more than six weeks after the first dose).[19] Our findings suggest that in countries where CoronaVac supplies are constrained and are experiencing high SARS-CoV-2 transmission, vaccination should prioritise completion of the two-dose regimen among the highest risk populations and avoid expanding to broader segments for which provisions for a second dose have not been secured.

Our study did not directly address the question whether vaccination with CoronaVac was effective against Gamma-variant-associated COVID-19 since we have no data on whether the analysed cases were due to Gamma variant. However, 90% (1,790/1,999) of the discordant pairs in this matched case-control study were selected during the period 1 March to 29 April 2021, when Gamma accounted for 85% of the genotyped isolates during surveillance in São Paulo state. A test-negative study in Canada evaluated ≥70 years individuals and estimated an

adjusted vaccine effectiveness of single-dose mRNA vaccines of 61% (95% CI 45-72) against the VOC Gamma compared to 72% (95% CI 58-81) for non-VOC.[42] Although further studies are required to determine the effectiveness of CoronaVac against Gamma and additional VOCs, our findings provide supportive evidence for the use of CoronaVac in countries in South America which are experiencing epidemics due to extensive spread of Gamma[20] and are administering mass vaccination with CoronaVac as part of the epidemic response.

Strengths and limitations of this study

This study has several strengths which include the large sample size and geospatial coverage, comprising the state of São Paulo with 46 million inhabitants distributed across 645 municipalities. We implemented a pre-specified publicly-available protocol, which is in accordance with the recent WHO guideline for COVID-19 vaccine effectiveness evaluation.[21] Using a test-negative design, we have addressed biases that affect observational vaccine effectiveness studies, such as health-seeking behaviour and access. Additionally, after matching and adjustment, the "bias-indicator" association between recent vaccination with a single dose 0-13 days before sample collection was close to null, suggesting that vaccinated and unvaccinated individuals did not differ in their underlying risk of testing positive for SARS-CoV-2.[8,32,43]

Our study had limitations. We could not assess the influence of a previous SARS-CoV-2 infection on vaccine effectiveness since passive surveillance identified few individuals with a positive RT-PCR or rapid antigen test before the study period. Prior to the start of the vaccination campaign, the estimated seroprevalence of COVID-19 in inhabitants who were ≥60 years of age

in the capital of São Paulo State was 19.9% (95% CI, 14.9-29.9) in January 2021.[44] Our estimates of vaccine effectiveness may therefore be subject to downward bias as unvaccinated individuals were at lower risk of reinfection. We attempted to exclude false-negative RT-PCR tests by excluding as controls patients with a subsequent positive test within 14 days after the initial testing and including only tests performed 10 days of symptom onset.[21] In addition, we restricted our study population to elderly individuals because they were a priority group for vaccination and received the large majority of CoronaVac doses during the initial stages of the campaign in Brazil; as a result, a direct comparison of the effectiveness of CoronaVac between older and younger populations was not possible. Our analyses were also limited by the lack of more refined covariates, such as frailty and chronic illness status, which could influence vaccine effectiveness in the very elderly and would not be addressed by age and reported comorbidities *per se*. Finally, although we matched for calendar time of SARS-CoV-2 testing (±3 days),[21] we cannot exclude the possibility of time-varying changes in behaviour or testing practices among participants that were not addressed by our matching criteria and may introduce bias. However, estimates of vaccine effectiveness remained similar in the sensitivity analysis that adjusted for calendar date of RT-PCR sample collection.

In summary, we found that a two-dose schedule of CoronaVac was effective in preventing symptomatic COVID-19 and more severe clinical outcomes among elderly individuals and in a setting with extensive Gamma variant transmission. However, the delayed onset of vaccine-mediated protection underscores the need to prioritise vaccine supplies and maximise the number of individuals who complete the two-dose schedule, when CoronaVac is used as part of a mass vaccination campaign that is implemented in response to a COVID-19 epidemic.

Author contributions

All authors conceived the study. OTR, MDTH and MD completed analyses with guidance from JRA, DATC, AIK, and JC. MSST, OFPP, OTR and MDTH curated and validated the data. OTR and MDTH wrote the first draft of the manuscript. TLD, RCP, OFPP, EFMV, MA, RS, JCG, WNA provided supervision. All authors contributed to, and approved, the final manuscript. JC is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Declaration of interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethics approval

The study was approved by the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021).

Data sharing

Deidentified databases as well as the R codes will be deposited in the repository https://github.com/juliocroda/VebraCOVID-19

Public and Patient Involvement

Members of the public or patients were not involved in setting the research question or the outcome measures, nor were they involved in developing plans for the design of the study. No patients were asked to advise on interpretation or writing up of results.

Transparency statement

The lead author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained.

Dissemination declaration

Results will be disseminated to the public in Manaus and across Brazil. It is not possible to disseminate results to individuals who were selected into the study due to anonymisation of the data.

Acknowledgements

We are grateful for the Pan American Health Organization's support and the São Paulo State in making the databases available for analysis. JC is supported by the Oswaldo Cruz Foundation (Edital Covid-19 – resposta rápida: 48111668950485). OTR is funded by a Sara Borrell fellowship (CD19/00110) from the Instituto de Salud Carlos III. OTR acknowledges support from the Spanish Ministry of Science and Innovation through the Centro de Excelencia Severo Ochoa 2019-2023 Program and from the Generalitat de Catalunya through the CERCA Program.

Role of the funding source

All funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The Health Secretary of State of São Paulo and PRODESP reviewed the data and findings of the study, but the academic authors retained editorial control. OTR, MDTH, MSST, and JC had full access to de-identified data in the study and OTR and MDTH verified the data, and all authors approved the final version of the manuscript for publication.

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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.

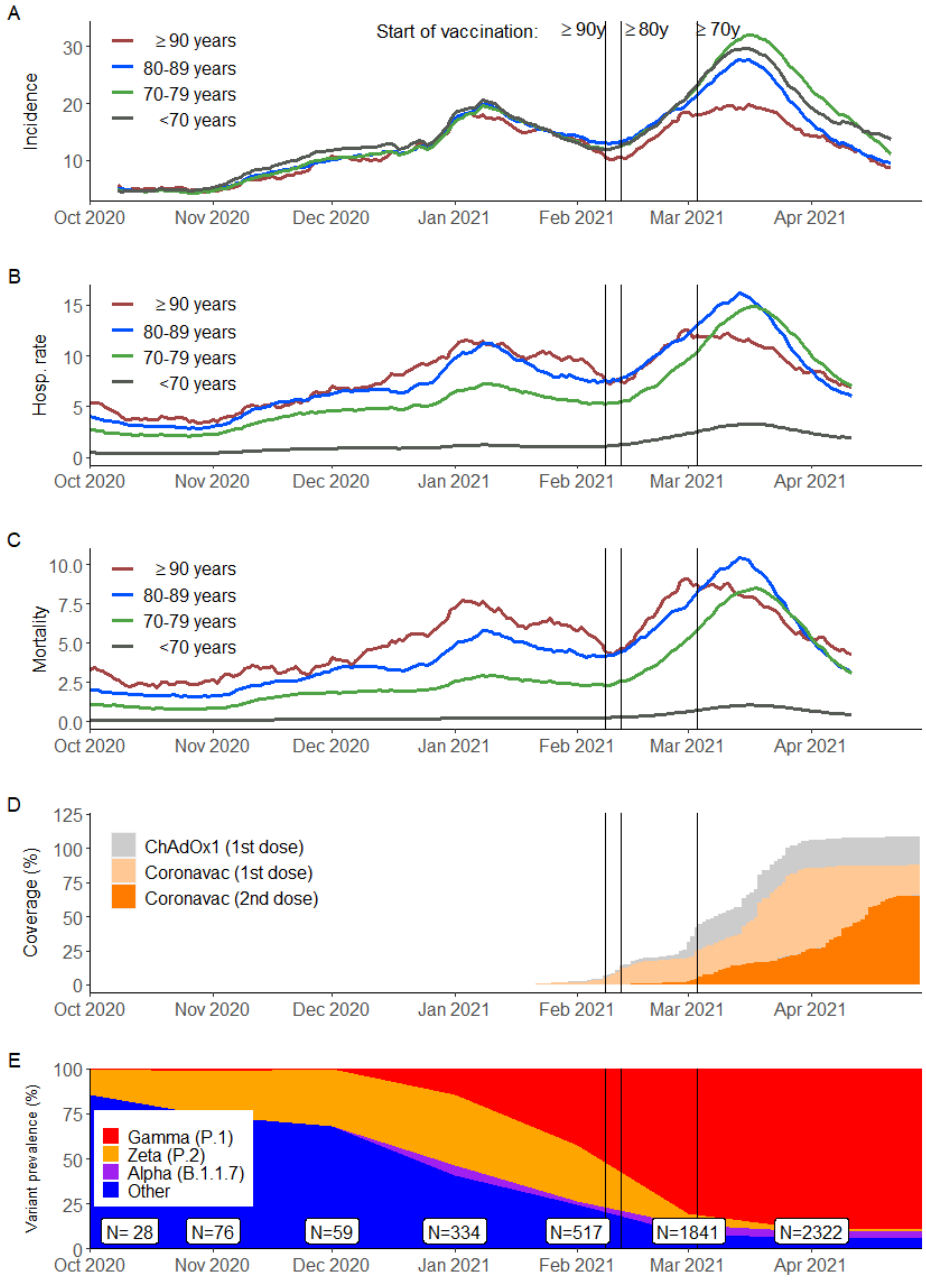

Figure 2. Flowchart of the identification of the study population from surveillance databases and selection of matched cases and controls.

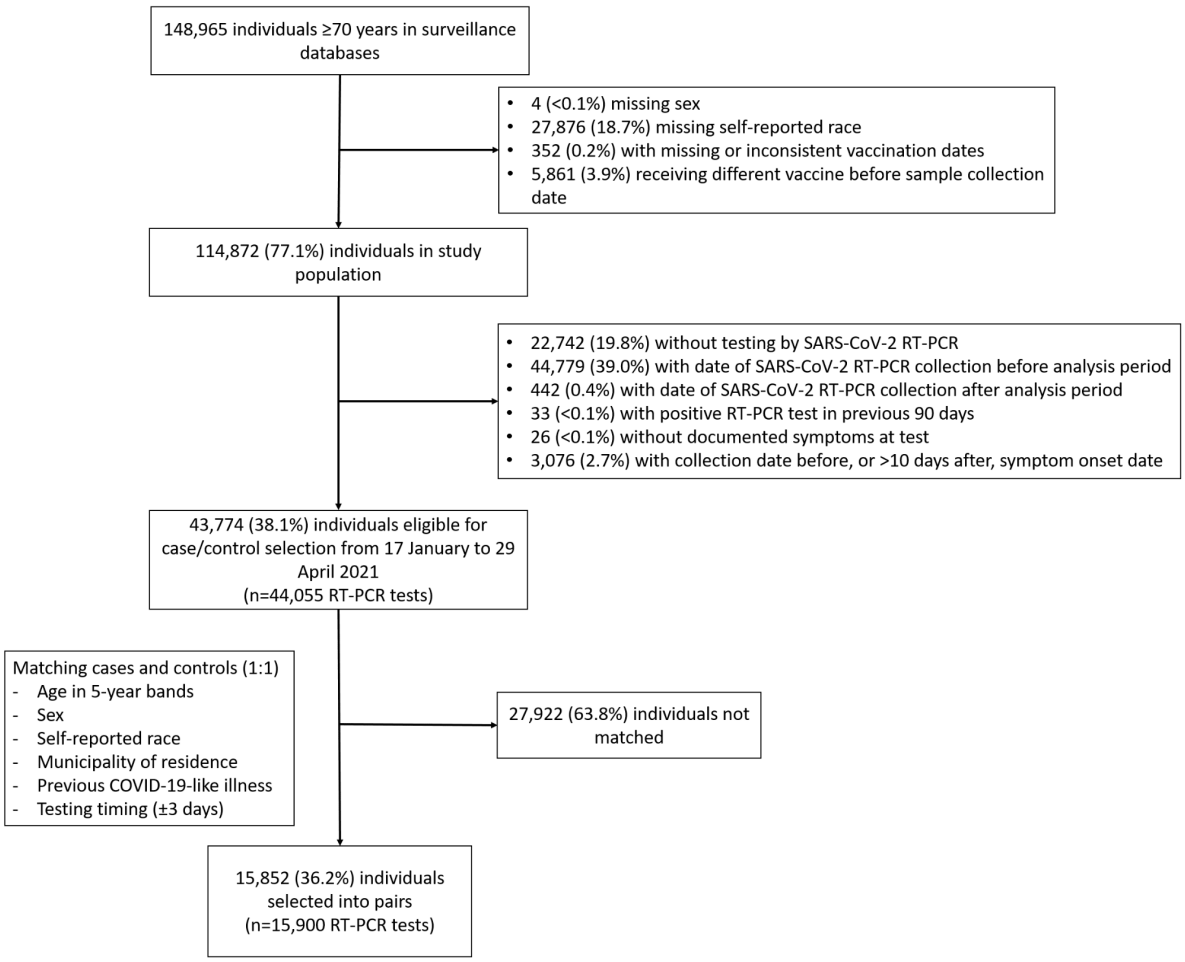

Figure 3. Adjusted vaccine effectiveness during the period ≥14 days after the second CoronaVac dose for subgroups of adults ≥70 years of age. Estimates of vaccine effectiveness were obtained from a conditional logistic regression model that included covariates of age and the number of comorbidities and incorporated an interaction term between the category of interest and the period ≥14 days after the second CoronaVac dose.

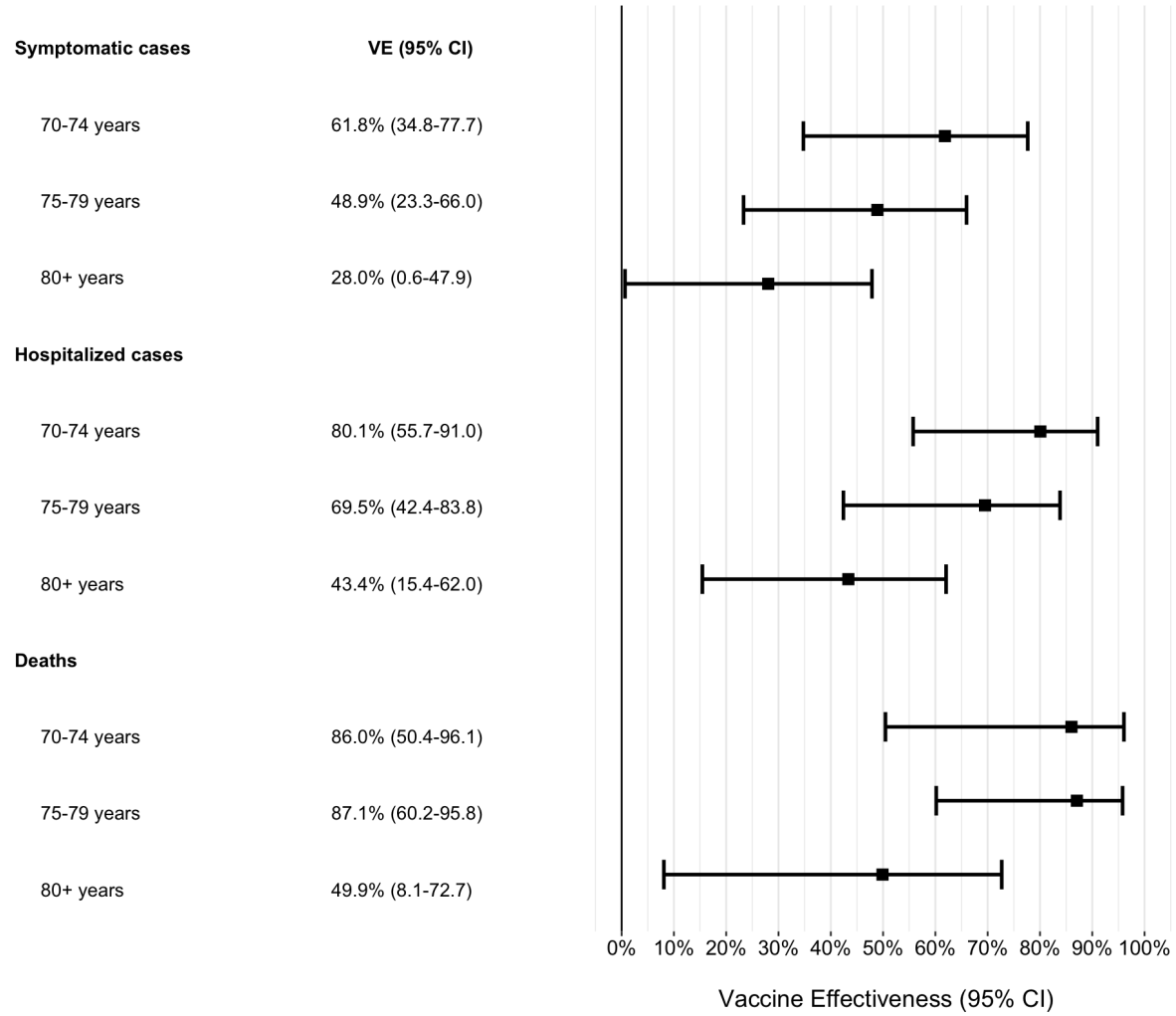

Table 1. Characteristics of adults ≥70 years of age who were eligible for matching and selected into case-test negative pairs.

	Eligible cases and controls		Matched pairs	
Characteristics*	**Test-negative (n=17,622)^**	**Test-positive (n=26,433)^**	**Controls (n=7,950)^**	**Cases (n=7,950)^**
Demographics				
Age, mean (SD), years	77.53 (6.8)	76.71 (6.2)	76.15 (5.8)	76.15 (5.8)
Age categories, n (%)				
70-79 years	12,123 (68.8)	19,673 (74.4)	6,150 (77.4)	6,150 (77.4)
80-89 years	4,301 (24.4)	5,437 (20.6)	1,510 (19.0)	1,510 (19.0)
≥90 years	1,198 (6.8)	1,323 (5.0)	290 (3.6)	290 (3.6)
Male sex, n (%)	7,689 (43.6)	12,431 (47.0)	3,276 (41.2)	3,276 (41.2)
Self-reported race[†], n (%)'				
White/Branca	13,415 (76.1)	19,796 (74.9)	6,420 (80.8)	6,420 (80.8)
Brown/Pardo	3,192 (18.1)	4,983 (18.9)	1,301 (16.4)	1,301 (16.4)
Black/Preta	785 (4.5)	1,258 (4.8)	191 (2.4)	191 (2.4)
Yellow/ Amarela	226 (1.3)	390 (1.5)	38 (0.5)	38 (0.5)
Indigenous/Indigena	4 (0.0)	6 (0.0)	-	-
Residence in "Grande São Paulo" Health Region, n (%)	12,381 (70.3)	16,538 (62.6)	4,259 (53.6)	4,259 (53.6)
Comorbidities				
Reported number[‡], n (%)				
None	10,027 (56.9)	12,668 (47.9)	4,510 (56.7)	3,564 (44.8)
One or two	6,984 (39.6)	12,548 (47.5)	3,151 (39.6)	3,994 (50.2)
Three or more	611 (3.5)	1,217 (4.6)	289 (3.6)	392 (4.9)
Cardiovascular disease , n (%)	5,293 (30.0)	10,079 (38.1)	2,375 (29.9)	3,252 (40.9)
Diabetes, n (%)	3,233 (18.3)	6,533 (24.7)	1,314 (19.0)	2,092 (26.3)
Prior SARS-CoV-2 exposure[**]				

Previous symptomatic events notified to the surveillance systems**, n (%)	685 (3.9)	354 (1.3)	35 (0.4)	35 (0.4)
Positive SARS-CoV-2 test result ††, n (%)	66 (0.4)	13 (0.0)	1 (0.0)	4 (0.1)
Interval between symptoms onset and RT-PCR testing, median (p25-p75), days	3 [2-5]	4 [2-6]	3 [1-5]	4 [2-6]
ARI associated hospitalisations, n (%)	4,524/17,484 (25.9)	12,987/26,221 (49.5)	2,065/7,889 (26.2)	4,039/7,883 (51.2)
ARI associated deaths, n (%)	912/16,710 (5.5%)	7,054/24,508 (28.8%)	729/7,557 (9.6%)	2,052/7,359 (27.9%)
Interval between symptoms onset and hospitalization, median (p25-p75), days	3 [2-6]	7 [4-10]	3 [2-6]	7 [4-10]
Interval between symptoms onset and deaths, median (p25-p75), days	8 [4-13]	14 [9-21]	8 [4-15]	15 [10-22]
Vaccination status				
Not vaccinated, n (%)	11,986 (68.0)	17,233 (65.2)	5,485 (69.0)	5,561 (69.9)
Single dose, within 0-13 days, n (%)	1,446 (8.2)	2,976 (11.3)	747 (9.4)	762 (9.6)
Single dose, ≥14 days, n (%)	1,797 (10.2)	3,312 (12.5)	843 (10.6)	851 (10.7)
Two doses, within 0-13 days, n (%)	1,041 (5.9)	1,533 (5.8)	437 (5.5)	421 (5.3)
Two doses, ≥14 days, n (%)	1,352 (7.7)	1,379 (5.2)	438 (5.5)	355 (4.5)
Interval between first and second dose, mean (SD), days	25 (6)	30 (12)	25 (6)	29 (11)
Interval between first dose and RT-PCR testing, mean (SD), days	28 (19)	23 (16)	24 (17)	23 (16)
Interval between second dose and RT-PCR testing, mean (SD), days	20 (15)	17 (14)	18 (15)	17 (14)

*Continuous variables are displayed as mean (SD); categorical variables are displayed as n (%).
^These numbers refer to RT-PCR tests and represent 43,774 individuals for the eligible cases and controls and 15,852 individuals in the matched cases and controls.
†Race/skin colour as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas).[27]
‡Comorbidities included: cardiovascular, renal, neurological, haematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression.
**Prior to the start of the study on 17 January, 2021 and after systematic surveillance was implemented on 1 February, 2020.
** Reported illness with COVID-19 associated symptoms in the eSUS and SIVEP-Gripe databases.
†† Defined as a positive SARS-CoV-2 RT-PCR or antigen detection test result.

Table 2: Effectiveness of CoronaVac against symptomatic COVID-19, hospitalisations and deaths in adults ≥70 years of age.

	Unadjusted Analysis			Adjusted Analysis^		
Symptomatic COVID-19 (n=15,900)	**OR (95% CI)**	**VE (95% CI)**	**p-value**	**OR (95% CI)**	**VE (95% CI)**	**p-value**
Single dose, within 0-13 days vs. unvaccinated*	0.97 (0.85-1.12)	2.7% (-11.7-15.3)	0.70	0.98 (0.85-1.12)	2.5% (-12.2-15.3)	0.72
Single dose, ≥14 days vs. unvaccinated*	0.91 (0.78-1.05)	9.5% (-5.3-22.3)	0.20	0.90 (0.77-1.04)	10.5% (-4.4-23.3)	0.16
Two doses, within 0-13 days vs. unvaccinated*	0.81 (0.66-0.98)	19.5% (1.9-34.0)	0.03	0.82 (0.67-1.00)	18.2% (0.0-33.2)	0.05
Two doses, ≥14 days vs. unvaccinated*	0.60 (0.48-0.74)	40.5% (25.8-52.3)	<0.001	0.58 (0.47-0.73)	41.6% (26.9-53.3)	<0.001
COVID-19 associated hospitalisations (n=8,078)						
Single dose, within 0-13 days vs. unvaccinated*	0.89 (0.74-1.07)	11.3% (-7.0-26.4)	0.21	0.84 (0.68-1.02)	16.4% (-2.2-31.6)	0.08
Single dose, ≥14 days vs. unvaccinated*	0.85 (0.70-1.04)	14.6% (-4.2-30.0)	0.12	0.83 (0.66-1.01)	18.5% (-1.0-34.2)	0.06
Two doses, within 0-13 days vs. unvaccinated*	0.62 (0.47-0.81)	38.1% (18.8-52.8)	0.001	0.59 (0.44-0.79)	40.9% (20.7-55.9)	<0.001
Two doses, ≥14 days vs. unvaccinated*	0.47 (0.36-0.63)	52.7% (37.2-64.4)	<0.001	0.41 (0.30-0.56)	59% (44.2-69.8)	<0.001
COVID-19 associated deaths (n=4,104)						
Single dose, within 0-13 days vs. unvaccinated*	0.92 (0.72-1.18)	8.2% (-17.7-28.4)	0.50	0.93 (0.71-1.21)	7.4% (-21.3-29.2)	0.58
Single dose, ≥14 days vs. unvaccinated*	0.76 (0.57-1.00)	24.5% (0.0-43.0)	0.05	0.68 (0.50-0.93)	31.6% (7.1-49.7)	0.02
Two doses, within 0-13 days vs. unvaccinated*	0.40 (0.27-0.59)	60.4% (40.6-73.5)	<0.001	0.36 (0.23-0.55)	64.4% (44.6-77.1)	<0.001
Two doses, ≥14 days vs. unvaccinated*	0.34 (0.22-0.52)	66.2% (47.8-78.1)	<0.001	0.29 (0.18-0.46)	71.4% (53.7-82.3)	<0.001

ARI - acute respiratory illness

*At date of index sample collection for cases and controls.

^ Models adjusted by age (linear term for symptomatic and hospitalisation, restricted cubic spline for deaths) and number of comorbidities (None, One or Two, Three or more)

Supplementary appendix

Supplement to: Effectiveness of the CoronaVac vaccine in the elderly population during a Gamma variant-associated epidemic of COVID-19 in Brazil: A test-negative case-control study

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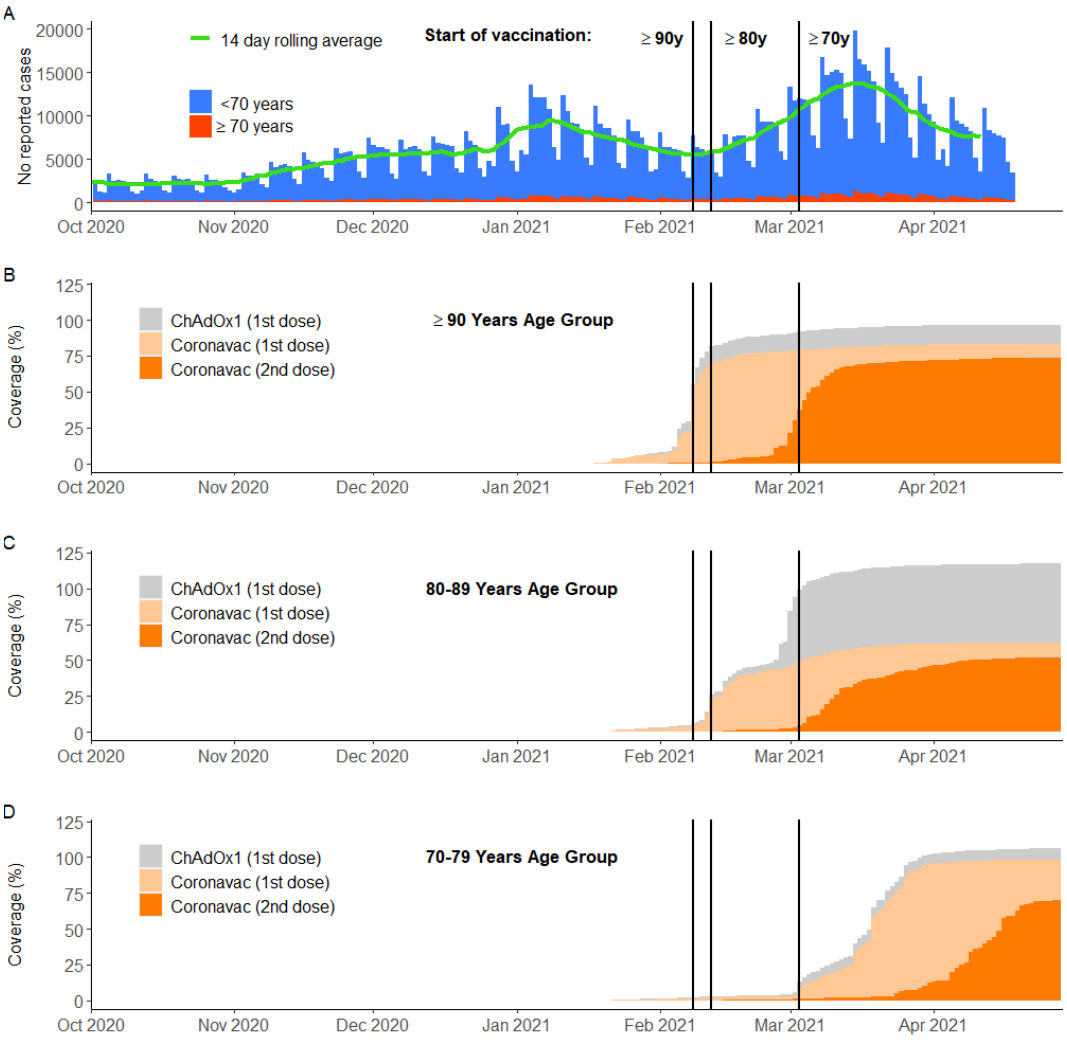
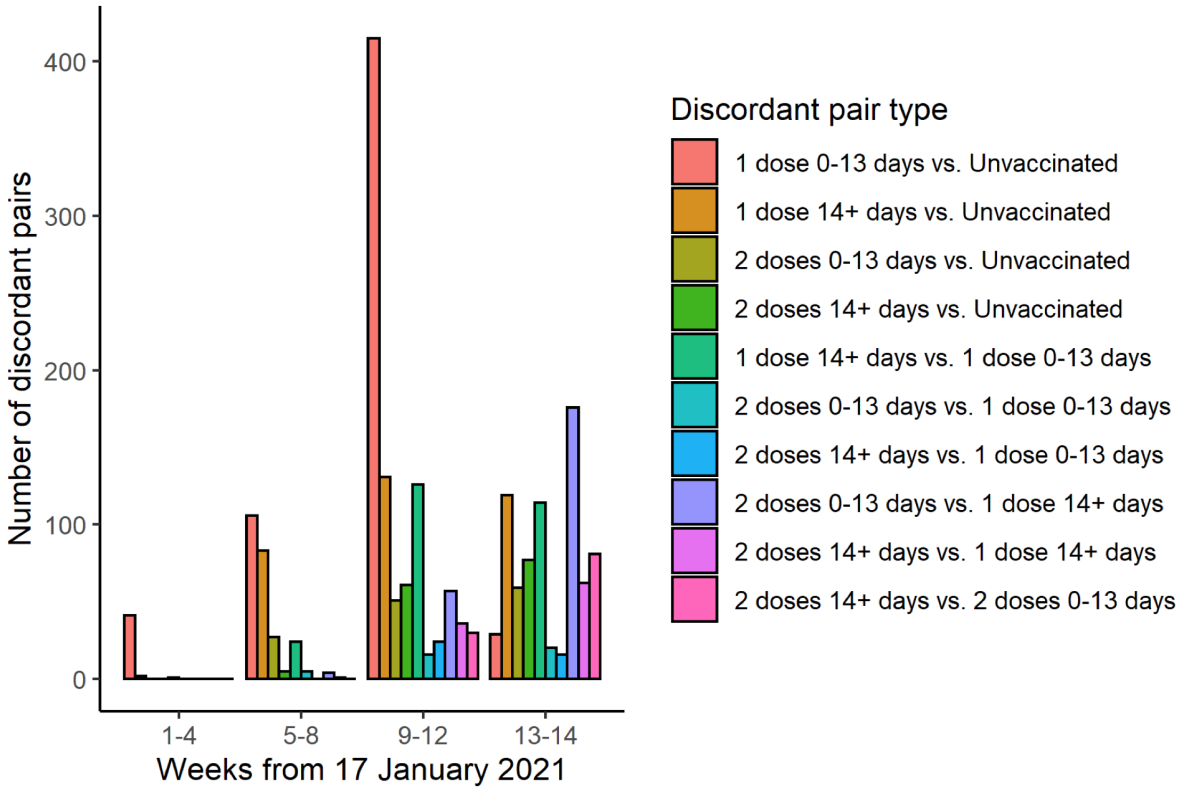
Supplementary Figure 1. Daily cases and vaccine coverage by age.

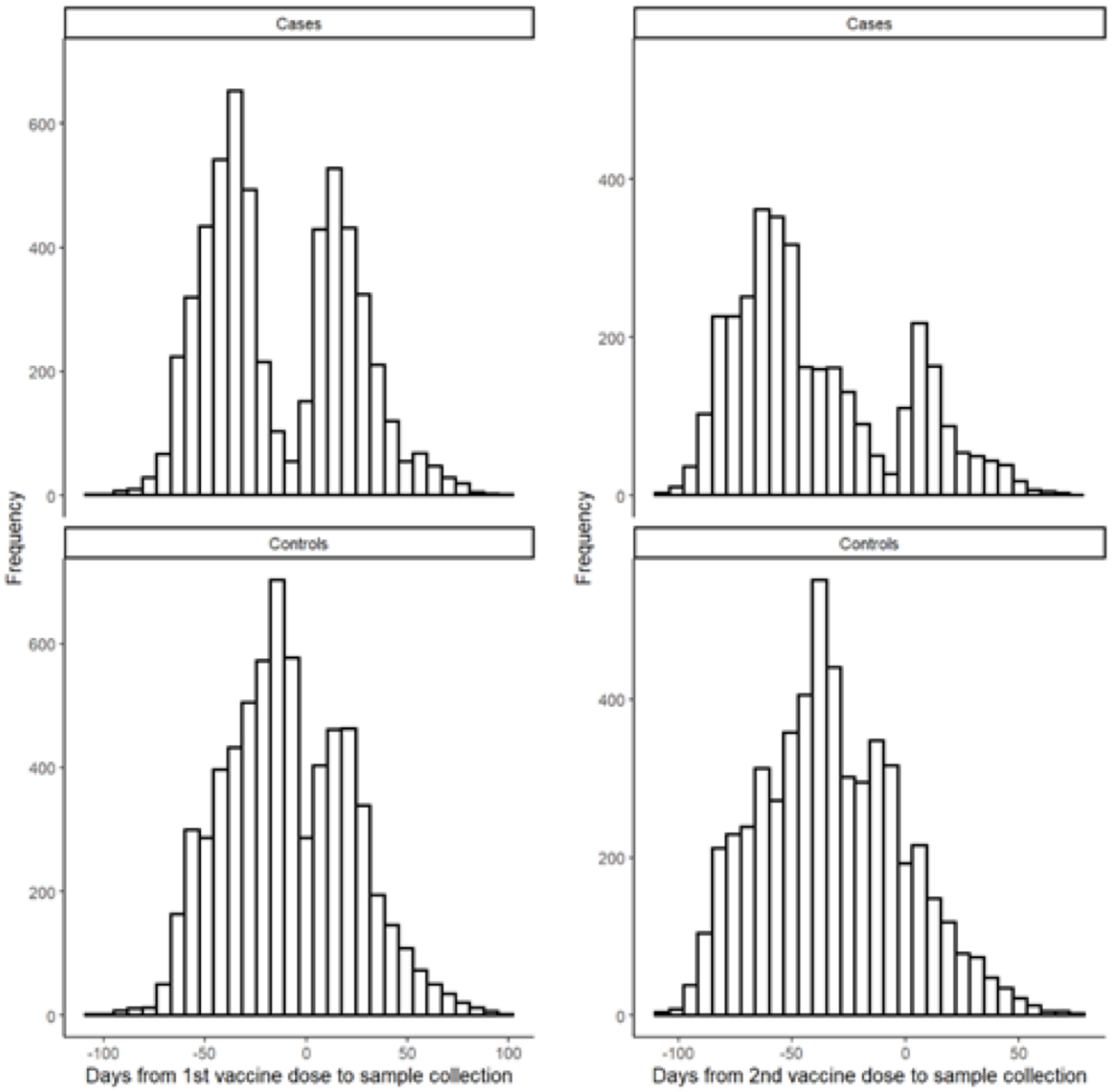
Panel A shows the daily cases of reported COVID-19 from Mar 15, 2020 to Apr 29, 2021 in São Paulo State, Brazil, with the green line representing the 14-day rolling average of counts. Panels B, C and D show the cumulative vaccination coverage for age groups >90y, 80y-89y, and 70y-79y, respectively. Population 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.

	Eligible cases and controls		Matched pairs	
Characteristics*	**Test-negative (n=17,622)^**	**Test-positive (n=26,433)^**	**Controls (n=4,039)^**	**Cases (n=4,039)^**
Demographics				
Age, mean (SD), years	77.53 (6.78)	76.71 (6.19)	77.22 (6.41)	77.25 (6.38)
Age categories, n (%)				
70-79 years	12,123 (68.8)	19,673 (74.4)	2847 (70.5)	2847 (70.5)
80-89 years	4,301 (24.4)	5,437 (20.6)	965 (23.9)	965 (23.9)
≥90 years	1,198 (6.8)	1,323 (5.0)	227 (5.6)	227 (5.6)
Male sex, n (%)	7,689 (43.6)	12,431 (47.0)	1771 (43.8)	1771 (43.8)
Self-reported race[†], n (%)'				
White/Branca	13,415 (76.1)	19,796 (74.9)	3251 (80.5)	3251 (80.5)
Brown/Pardo	3,192 (18.1)	4,983 (18.9)	644 (15.9)	644 (15.9)
Black/Preta	785 (4.5)	1,258 (4.8)	115 (2.8)	115 (2.8)
Yellow/ Amarela	226 (1.3)	390 (1.5)	29 (0.7)	29 (0.7)
Indigenous/Indigena	4 (0.0)	6 (0.0)	-	-
Residence in "Grande São Paulo" Health Region, n (%)	12,381 (70.3)	16,538 (62.6)	1783 (44.1)	1783 (44.1)
Comorbidities				
Reported number[‡], n (%)				
None	10,027 (56.9)	12,668 (47.9)	2213 (54.8)	1127 (27.9)
One or two	6,984 (39.6)	12,548 (47.5)	1661 (41.1)	2566 (63.5)
Three or more	611 (3.5)	1,217 (4.6)	165 (4.1)	346 (8.6)
Cardiovascular disease , n (%)	5,293 (30.0)	10,079 (38.1)	1241 (30.7)	2201 (54.5)
Diabetes, n (%)	3,233 (18.3)	6,533 (24.7)	793 (19.6)	1439 (35.6)
Prior SARS-CoV-2 exposure[**]				

Previous symptomatic events notified to the surveillance systems**, n (%)	685 (3.9)	354 (1.3)	13 (0.3)	13 (0.3)
Positive SARS-CoV-2 test result [††], n (%)	66 (0.4)	13 (0.0)	0 (0.0)	2 (0.0)
Interval between symptoms onset and RT-PCR testing, median (p25-p75), days	3 [2-5]	4 [2-6]	3 [1-5]	4 [2-6]
ARI associated hospitalisations, n (%)	4,524/17,484 (25.9)	12,987/26,221 (49.5)	1,252/4,009 (31.2)	4,039/4,039 (100)
ARI associated deaths, n (%)	912/16,710 (5.5%)	7,054/24,508 (28.8%)	446/3,795 (11.8)	1,939/3,470 (55.9)
Interval between symptoms onset and hospitalization, median (p25-p75), days	3 [2-6]	7 [4-10]	3 [2-6]	7 [4-10]
Interval between symptoms onset and deaths, median (p25-p75), days	8 [4-13]	14 [9-21]	8 [4-15]	15 [10-23]
Vaccination status				
Not vaccinated, n (%)	11,986 (68.0)	17,233 (65.2)	2656 (65.8)	2746 (68.0)
Single dose, within 0-13 days, n (%)	1,446 (8.2)	2,976 (11.3)	413 (10.2)	408 (10.1)
Single dose, ≥14 days, n (%)	1,797 (10.2)	3,312 (12.5)	445 (11.0)	463 (11.5)
Two doses, within 0-13 days, n (%)	1,041 (5.9)	1,533 (5.8)	230 (5.7)	196 (4.9)
Two doses, ≥14 days, n (%)	1,352 (7.7)	1,379 (5.2)	295 (7.3)	226 (5.6)
Interval between first and second dose, mean (SD), days	25 (6)	30 (12)	25 (6)	29 (12)
Interval between first dose and RT-PCR testing, mean (SD), days	28 (19)	23 (16)	25 (19)	24 (18)
Interval between second dose and RT-PCR testing, mean (SD), days	20 (15)	17 (14)	20 (16)	20 (16)

*Continuous variables are displayed as mean (SD); categorical variables are displayed as n (%).

^These numbers refer to RT-PCR tests and represent 43,774 individuals for the eligible cases and controls and 8,059 individuals in the matched cases and controls.

[†]Race/skin colour as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas).

[‡]Comorbidities included: cardiovascular, renal, neurological, haematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression.

[**]Prior to the start of the study on 17 January, 2021 and after systematic surveillance was implemented on 1 February, 2020.

** Reported illness with COVID-19 associated symptoms in the eSUS and SIVEP-Gripe databases.

[††] Defined as a positive SARS-CoV-2 RT-PCR or antigen detection test result

Supplementary Table 3. Characteristics of adults ≥70 years of age who were eligible for matching and selected into case-test negative pairs for the death analysis.

	Eligible cases and controls		Matched pairs	
Characteristics*	**Test-negative (n=17,622)^**	**Test-positive (n=26,433)^**	**Controls (n=2,052)^**	**Cases (n=2,052)^**
Demographics				
Age, mean (SD), years	77.53 (6.78)	76.71 (6.19)	77.69 (6.57)	77.76 (6.53)
Age categories, n (%)				
70-79 years	12,123 (68.8)	19,673 (74.4)	1396 (68.0)	1396 (68.0)
80-89 years	4,301 (24.4)	5,437 (20.6)	523 (25.5)	523 (25.5)
≥90 years	1,198 (6.8)	1,323 (5.0)	133 (6.5)	133 (6.5)
Male sex, n (%)	7,689 (43.6)	12,431 (47.0)	962 (46.9)	962 (46.9)
Self-reported race[†], n (%)'				
White/Branca	13,415 (76.1)	19,796 (74.9)	1654 (80.6)	1654 (80.6)
Brown/Pardo	3,192 (18.1)	4,983 (18.9)	320 (15.6)	320 (15.6)
Black/Preta	785 (4.5)	1,258 (4.8)	61 (3.0)	61 (3.0)
Yellow/ Amarela	226 (1.3)	390 (1.5)	17 (0.8)	17 (0.8)
Indigenous/Indigena	4 (0.0)	6 (0.0)	-	-
Residence in "Grande São Paulo" Health Region, n (%)	12,381 (70.3)	16,538 (62.6)	982 (47.9)	982 (47.9)
Comorbidities				
Reported number[‡], n (%)				
None	10,027 (56.9)	12,668 (47.9)	1105 (53.8)	535 (26.1)
One or two	6,984 (39.6)	12,548 (47.5)	868 (42.3)	1304 (63.5)
Three or more	611 (3.5)	1,217 (4.6)	79 (3.8)	213 (10.4)
Cardiovascular disease , n (%)	5,293 (30.0)	10,079 (38.1)	633 (30.8)	1142 (55.7)
Diabetes, n (%)	3,233 (18.3)	6,533 (24.7)	396 (19.3)	754 (36.7)
Prior SARS-CoV-2 exposure[**]				

Previous symptomatic events notified to the surveillance systems**, n (%)	685 (3.9)	354 (1.3)	7 (0.3)	7 (0.3)
Positive SARS-CoV-2 test result ††, n (%)	66 (0.4)	13 (0.0)	0 (0.0)	1 (0.0)
Interval between symptoms onset and RT-PCR testing, median (p25-p75), days	3 [2-5]	4 [2-6]	3 [1-5]	4 [2-6]
ARI associated hospitalisations, n (%)	4,524/17,484 (25.9)	12,987/26,221 (49.5)	645/2,035 (31.7)	1,939/2,025 (95.8)
ARI associated deaths, n (%)	912/16,710 (5.5%)	7,054/24,508 (28.8%)	255/1,940 (13.1)	2,052/2,052 (100)
Interval between symptoms onset and hospitalization, median (p25-p75), days	3 [2-6]	7 [4-10]	3 [2-6]	6 [4-10]
Interval between symptoms onset and deaths, median (p25-p75), days	8 [4-13]	14 [9-21]	8 [4-12]	15 [10-22]
Vaccination status				
Not vaccinated, n (%)	11,986 (68.0)	17,233 (65.2)	1362 (66.4)	1425 (69.4)
Single dose, within 0-13 days, n (%)	1,446 (8.2)	2,976 (11.3)	218 (10.6)	225 (11.0)
Single dose, ≥14 days, n (%)	1,797 (10.2)	3,312 (12.5)	226 (11.0)	236 (11.5)
Two doses, within 0-13 days, n (%)	1,041 (5.9)	1,533 (5.8)	117 (5.7)	79 (3.8)
Two doses, ≥14 days, n (%)	1,352 (7.7)	1,379 (5.2)	129 (6.3)	87 (4.2)
Interval between first and second dose, mean (SD), days	25 (6)	30 (12)	25 (6)	24 (5)
Interval between first dose and RT-PCR testing, mean (SD), days	28 (19)	23 (16)	24 (18)	22 (17)
Interval between second dose and RT-PCR testing, mean (SD), days	20 (15)	17 (14)	19 (16)	20 (15)

*Continuous variables are displayed as mean (SD); categorical variables are displayed as n (%).
^These numbers refer to RT-PCR tests and represent 43,774 individuals for the eligible cases and controls and 4,099 individuals in the matched cases and controls.
†Race/skin colour as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas).
‡Comorbidities included: cardiovascular, renal, neurological, haematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression.
**Prior to the start of the study on 17 January, 2021 and after systematic surveillance was implemented on 1 February, 2020.
** Reported illness with COVID-19 associated symptoms in the eSUS and SIVEP-Gripe databases.
†† Defined as a positive SARS-CoV-2 RT-PCR or antigen detection test result.

Supplementary Table 4. Adjusted vaccine effectiveness during the period ≥14 days after the second CoronaVac dose for subgroups of adults ≥70 years of age.

Estimates of vaccine effectiveness were obtained from a conditional logistic regression model that included covariates of age and the number of comorbidities and incorporated an interaction term between the category of interest and the period ≥14 days after the second CoronaVac dose.

Outcome	OR (95% CI)	VE (95% CI)	p-value for interaction
Symptomatic cases (n=15,900)			
70-74 (n=8,178)	0.38 (0.22-0.65)	61.8% (34.8-77.7)	0.05
75-79 (n=4,122)	0.51 (0.34-0.77)	48.9% (23.3-66.0)	
80+ (n=3,600)	0.72 (0.52-0.99)	28.0% (0.60-47.9)	
Hospitalisations (n=8,078)			
70-74 (n=3,596)	0.20 (0.09-0.44)	80.1% (55.7-91.0)	0.04
75-79 (n=2,098)	0.31 (0.16-0.58)	69.5% (42.4-83.8)	
80+ (n=2,384)	0.57 (0.38-0.85)	43.4% (15.4-62.0)	
Deaths (n=4,104)			
70-74 (n=1,652)	0.14 (0.04-0.50)	86.0% (50.4-96.1)	0.19
75-79 (n=1,140)	0.13 (0.04-0.40)	87.1% (60.2-95.8)	
80+ (n=1,312)	0.50 (0.27-0.92)	49.9% (8.1-72.7)	

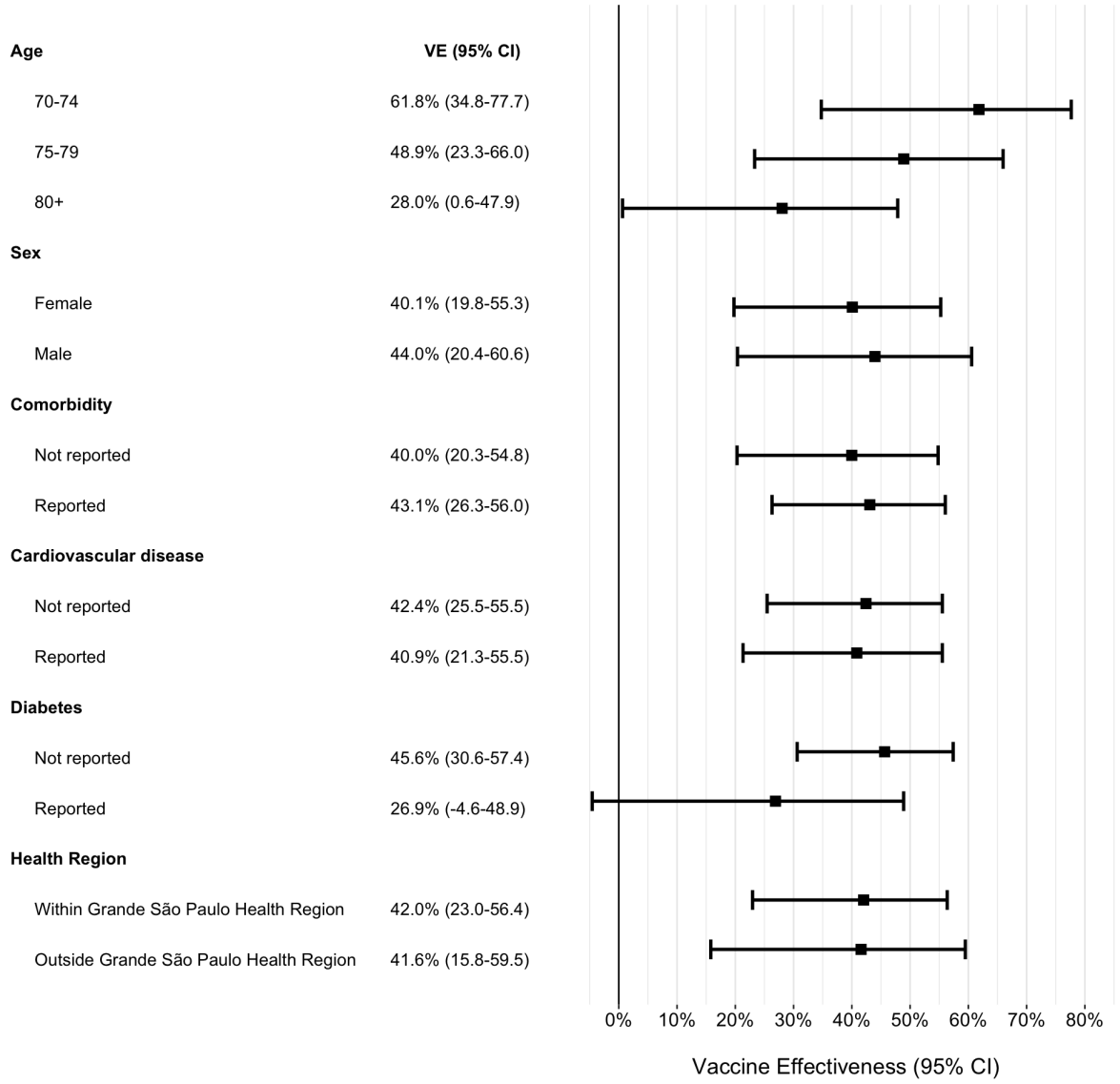
Supplementary Table 5. Estimated effectiveness of CoronaVac ≥14 days after the second dose, in subgroups of adults ≥70 years of age.

All models are adjusted by age (continuous) and number of comorbidities, and include an interaction term between the subgroup of interest and vaccinations with 2 doses, ≥14 days after second vaccine dose.

Subgroup	Adjusted OR (95% CI)	Adjusted VE (95% CI)	p-value for interaction
Age			
70-74 (n=8,178)	0.38 (0.22-0.65)	61.8% (34.8-77.7)	0.05
75-79 (n=4,122)	0.51 (0.34-0.77)	48.9% (23.3-66.0)	
80+ (n=3,600)	0.72 (0.52-0.99)	28.0% (0.60-47.9)	
Sex			
Females (n=9,348)	0.60 (0.45-0.80)	40.1% (19.8-55.3)	0.85
Males (n=6,552)	0.56 (0.39-0.80)	44.0% (20.4-60.6)	
Comorbidities			
No reported (n=8,074)	0.60 (0.45-0.80)	40.0% (20.3-54.8)	0.81
Reported (n=7,826)	0.57 (0.44-0.74)	43.1% (26.3-56.0)	
Cardiovascular disease			
No reported (n=10,273)	0.58 (0.45-0.75)	42.4% (25.5-55.5)	0.86
Reported (n=5,627)	0.59 (0.45-0.79)	40.9% (21.3-55.5)	
Diabetes			
No reported (n=12,294)	0.54 (0.43-0.69)	45.6% (30.6-57.4)	0.12
Reported (n=5,627)	0.73 (0.51-1.05)	26.9% (-4.6-48.9)	
Health regional area			
“Grande São Paulo” (n=7,382)	0.58 (0.44-0.77)	42% (23.0-56.4)	0.66
Not “Grande São Paulo” (n=8,518)	0.58 (0.41-0.84)	41.6% (15.8-59.5)	

Supplementary Figure 4. Adjusted vaccine effectiveness during the period ≥14 days after the second CoronaVac dose for subgroups of adults ≥70 years of age.

Estimates of vaccine effectiveness were obtained from a conditional logistic regression model that included covariates of age (continuous) and the number of comorbidities and incorporated an interaction term between the category of interest and the period ≥14 days after the second CoronaVac dose.



Protocol for the Teste-Negative Case-Control Study in São Paulo State

Version 01.3 / April 30th 2021

Released in https://github.com/juliocroda/VebraCOVID-19/

PROTOCOL

Evaluation of Vaccine Effectiveness in Brazil against COVID-19 (VEBRA-COVID) Sub-Study: A Test-Negative Case-Control Study on the Effectiveness of COVID-19 Vaccines amongst the General Population of São Paulo State in Brazil

Version: 01.3 / April 30th 2021

Table 1. Protocol Revisions

Changes in Version 1.3	Justification
Addition of ChAdOx1 exposure times	We added the time windows following the first and second doses of ChAdOx1 to be 0-13 days, 14-27 days and ≥28 days
Revised expected vaccine effectiveness	In the VEBRA-COVID analysis of the elderly (≥70 years of age) in São Paulo, we aimed to answer the research question of whether vaccines had a real-world effectiveness of public health value rather than whether they had a real-world effectiveness that was consistent with efficacy estimates from RCTs. Thus, we powered the study for a real world effectiveness above a lower threshold of 40%, below which the value of the vaccination would require reconsideration.
Change of matching criteria from CEP (5 digits) to Municipality and self-reported race	We based this decision on three main reasons: 1 – A great proportion of municipalities in São Paulo State has a unique CEP (zipcode), so everyone in that municipality has the same CEP. For these municipalities, we would lose within municipality socioeconomic information 2 – We observed a larger proportion of invalid CEPs mainly in the e-SUS database compared with the SIVEP-Gripe database, which may introduce potential bias since SIVEP-Gripe has a higher proportion of severe COVID-19 cases 3 – A significant number of unique CEPs were inconsistently placed in more than one municipality.
Addition of outcomes for the cohort analysis of test-positive cases	We added ICU admission and respiratory support, occurring within 21 days of initial SARS-CoV-2 test positivity. We also changed hospitalization from occurring within 14 days to within 21 days of initial SARS-CoV-2 test positivity.

I. Background

Since the emergence of severe acute respiratory virus coronavirus 2 (SARS-CoV-2), Brazil has experienced one of the world's highest incidence and mortality rates in the world, with over 13 million reported infections as of the middle of April 2021.[1–3] São Paulo, the most populous state in Brazil (~ 46 million inhabitants), is the state with highest number of cases and deaths: 2,827,833 cases and 92,548 deaths as by April 24th 2021.[4] Variants of Concern (VOC) also had a key role on the recent several surges in Brazil and São Paulo State. The P.1 VOC, which was first detected in Manaus on Jan 12, 2021, [5–7] and now consists the majority of new infections, being dominant in several states in Brazil. P1. has accrued mutations associated with decreased neutralization,[8,9] and has since spread throughout Brazil, synchronizing the epidemic in country in a scenario of relaxed non-pharmacological interventions.

The rapid development of novel vaccines against COVID-19 allowed countries to start vaccine distribution programs within a year of the identification of the novel virus. Among the first vaccines to be developed was Sinovac's CoronaVac vaccine.[10–12] Phase III trials were conducted in Turkey, Chile, Singapore and Brazil. The Brazilian trial was conducted among a study population of healthcare professionals, and reported that the effectiveness of CoronaVac after 14 days following completion of a two dose schedule was 50.7% (95% CI 36.0-62.0) for all symptomatic cases of COVID-19, 83.7% (95% CI 58.0-93.7) for cases requiring medical attention, and 100% (95% CI 56.4-100) for hospitalized, severe, and fatal cases.[12] CoronaVac was approved for emergency use on 17 January in Brazil, and used to vaccinate healthcare workers and the general population. AstraZeneca-Oxford's ChAdOx1 vaccine[13,14] was approved on the same day and was administered beginning on 23 January 2021. In Brazil, ChAdOx1 schedule is for 12 weeks between first and second dose.

As vaccine programs continue, there has been much interest in estimation of vaccine effectiveness through observational studies, and specifically in settings where VOC are circulating. Such studies have advantages over clinical trials, including increased size and follow-up time, and reduced cost. However, as vaccinated and unvaccinated individuals are likely different in their SARS-CoV-2 risk and healthcare access, these studies must address bias through design and analysis. Several studies have demonstrated the effectiveness of COVID-19 vaccines against infection caused by the B.1.1.7 variant.[15] However, large-scale real-world investigations on vaccine effectiveness have not been conducted in regions where the P.1 variant is prevalent.

We propose a test-negative case-control study[16,17] of the general population from the São Paulo State to evaluate the effectiveness of COVID-19 vaccines in preventing symptomatic disease in a setting of widespread P.1 VOC transmission.[6] The study will initially evaluate the effectiveness of COVID-19 vaccines, CoronaVac and ChAdOx1 amongst the population with age ≥70 years, since the vaccination campaign prioritized this age group in its first months. We will expand the study population as additional age groups become eligible for vaccination. Furthermore, we expect that additional vaccines will be approved and will evaluate their effectiveness. We will therefore continue to amend the protocol and its objectives accordingly to address these new questions.

II. Objectives

To estimate the effectiveness of COVID-19 vaccines against symptomatic SARS-CoV-2 infection amongst the general population from the São Paulo State. Our initial analyses will focus on estimating vaccine effectiveness in the age group of ≥70 years.

III. Methods

1. Study Design: We will conduct a retrospective matched case-control study, enrolling cases who test positive for SARS-CoV-2 and controls who test negative for SARS-CoV-2 amongst the general population (Section 3) as of the day that the COVID-19 vaccination campaign was initiated at the study sites. The study will evaluate vaccine effectiveness on the primary outcome of symptomatic SARS-CoV-2 infection. We will identify cases and matched controls by extracting information from health surveillance records and ascertain the type and data of vaccination by reviewing the state COVID-19 vaccination registry. In this design, one minus the odds ratio (1-OR) of vaccination comparing cases and controls estimates the direct effect of vaccination on the disease outcome. In a separate

analysis, we will assess the association between vaccination and hospitalization and/or death among individuals who have tested positive for SARS-CoV-2.

2. IRB and Ethics Statement: The protocol has been submitted to the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021). The work of investigators at the University of Florida, Yale University, Stanford University, and Barcelona Institute for Global Health was conducted to inform the public health response and was therefore covered under Public Health Response Authorization under the US Common Rule.

Study Details

Study Site: The State of São Paulo (23°3′S, 46°4'W) is the most populous state in Brazil: an estimated population of 46,289,333 in 2020. São Paulo State has 645 municipalities and its capital, São Paulo city, has 12 million inhabitants. São Paulo State reported 2,827,833 COVID-19 cases (cumulative incidence rate: 6,109 per 100,000 population) and 92,548 deaths (cumulative mortality: 200 per 100,000 population), by 24/04/2021. The State Secretary of Health of Sao Paulo (SES-SP) initiated its COVID-19 vaccination campaign on 17 January 2021 and is administering two vaccines, CoronaVac and ChAdOx1. As of 24 April 2021, 10.7 million doses (6.9 million first doses and 3.8 million second doses) have been administered in the State.

Data Sources and Integration: We will identify eligible cases and controls from the State of São Paulo who test positive and negative, respectively, from the *state laboratory testing registry* of public health laboratory network; 2) Determine vaccination status from *state vaccination registries*; and 3) Extract information from *national healthcare and surveillance databases* that will be used to define outcomes, match controls to cases, determine vaccination status, serve as covariates for post-stratification and provide a source for cross-validation of information from databases. Registries are not available which enables constructing a cohort of people eligible for vaccination in the general population. Data sources for this study will include:

- State laboratory testing registry (**GAL**) of the network of public health laboratories
- State COVID-19 vaccination registry (**Vacina Já**)
- National surveillance database of severe acute respiratory illnesses (**SIVEP-Gripe**) created by Ministry of Health Brazil in 2009
- National surveillance system of suspected cases of COVID-19 (**e-SUS**) from mild to moderate "influenza like illness", created by the Ministry of Health Brazil in 2020

The databases will be integrated by the São Paulo State Government – PRODESP - using CPF numbers (Brazilian citizens' unique identifier code) and send to the VEBRA-COVID group anonymized. The database will be updated on a bi-weekly basis.

Study Population

Inclusion criteria:
- Has a residential address in the State of São Paulo,
- Eligible to receive a COVID-19 vaccine based on age,
- With complete information, which is consistent between databases, on age, sex, and residential address
- With consistent vaccination status and dates for those who were vaccinated.

Exclusion criteria:
- Does not have a residential address in the State of São Paulo,
- Not eligible to receive a COVID-19 vaccine based on age,
- With missing or inconsistent information on age, sex, or city of residence
- With existing but inconsistent vaccination status or dates.

Case definition and eligibility: We will use information from integrated GAL/SIVEP-Gripe/e-SUS databases to identify cases that are defined as eligible members of the study population (as defined above, Study Population) who:

- Had a sample with a positive SARS-CoV-2 RT-PCR, which was collected between January 17, 2021 and 7 days prior to database extraction of information
- Did not have a positive RT-PCR test in the 90 day period preceding the index positive RT-PCR result
- Have complete and consistent data on SARS-CoV-2 RT-PCR test results

Control definition and eligibility: We will use integrated GAL/SIVEP-Gripe/e-SUS databases to identify eligible controls. Controls are defined as eligible members of the study population who:

- Had a sample with a negative SARS-CoV-2 RT-PCR result, which was collected after January 17, 2021,
- Did not have a positive RT-PCR test in the 90 day period preceding the index positive RT-PCR result
- Did not have a subsequent positive RT-PCR test in the 7-day period following the index positive RT-PCR result
- Have complete and consistent data on SARS-CoV-2 PCR test result

When studying each vaccine, individuals that received another vaccine are eligible for selection as a case and/or control until the day they receive their vaccine, i.e. we will consider test positive and test negative cases for RT-PCR collected before the day of receipt of the other vaccine.

Matching: Test-negative controls will be matched 1:1 to the cases. We chose the matching factors to balance the ability to reduce bias and to enroll sufficient case-control pairs. Matching factors will include variables that are anticipated to be causes of the likelihood of receiving the vaccine, risk of infection and likelihood of receiving PCR testing for SARS-CoV-2 (see Figures 1-5):

- Age, categorized as 5-years age bands (e.g., 70-74, 75-79 years),
- Sex,
- Municipality,
- Self-reported race,
- Window of ±3 days between collection of RT-PCR positive respiratory sample for cases and collection of RT-PCR negative respiratory sample for controls. If the date of respiratory sample collection is missing, the date of notification of testing result will be used.

We will use the standard algorithms to conduct matching which include: 1) setting a seed, 2) locking the database, 4) creating a unique identifier for matching after random ordering, 5) implementing exact matching based on matching variables, sampling controls at random if more than one available per case within strata.

An individual who fulfils the control definition and eligibility and later has a sample tested that fulfils the case definition and eligibility can be included in the study as both a case and a control. An individual who fulfils the control definition for multiple different sample collection dates can be included in the study as a control for each collection date, up to a maximum of three times.

Exposure definition:
CoronaVac vaccination:

- Received the first vaccine dose, and not having received a second dose, in the following time periods relative to sample collection for their PCR test:
  - 0-13 days
  - ≥14 days
- Received the second dose in the following time periods relative to sample collection for their PCR test:
  - 0-13 days
  - ≥14 days

ChAdOx1vaccination:

- Received the first vaccine dose, and not having received a second dose, in the following time periods relative to sample collection for their PCR test:

  - 0-13 days
  - 14-27 days
  - ≥28 days

- Received the second dose in the following time periods relative to sample collection for their PCR test:
  - 0-13 days
  - ≥14 days

Statistical Analyses: We will evaluate the effectiveness of CoronaVac and ChAdOx1for the following SARS-CoV-2 infection outcomes:

- Primary: Symptomatic COVID-19, defined as one or more reported COVID-19 related symptom with onset within 0-10 days before the date of their positive RT-PCR test

- Secondary:
  - COVID-19 associated hospitalization within 21 days of the symptom onset
  - COVID-19 associated ICU admission within 21 days of the symptom onset
  - COVID-19 associated respiratory support
  - COVID-19 associated death within 28 days of symptom onset

We will evaluate vaccine effectiveness for the primary outcome according to the test-negative design. Table 1 shows a list of all planned analyses in the test-negative design. The test-negative design may introduce bias when evaluating outcomes of hospitalizations and deaths during an epidemic. We will therefore perform time to event/logistic regression analysis of test positive cases to evaluate the association of vaccination status and the risk for hospitalization, ICU admission, COVID-19 respiratory support, and death after infection.

Our initial analyses will focus on estimating vaccine effectiveness in the population with age ≥70 years of age who were the initial priority group of the COVID-19 vaccination campaign.

Case-control analysis: Analyses of the primary outcome will be restricted to case and control pairs who are matched based on the presence of a COVID-19 related symptom before or at the time of testing.

We will use conditional logistic regression to estimate the odds ratio (OR) of vaccination among cases and controls, accounting for the matched design, where 1-OR provides an estimate of vaccine effectiveness under the standard assumptions of a test-negative design. For the CoronaVac analysis, the reference group will be individuals who have not received a first dose of CoronaVac by the date of respiratory sample collection. For the ChAdOx1 analysis, the reference group will be individuals who have not received a first dose of ChAdOx1by the date of respiratory sample collection. Date of notification of the testing result will be used if the date of respiratory sample collection is missing. To evaluate potential biases and the timing of vaccine effectiveness after administration, we will evaluate the windows of vaccination status corresponding: A) 0-13 days and ≥14 days after the 1st dose and 0-13 days and ≥14 days after the 2nd dose of CoronaVac; and B) 0-13 days, 14-27 days and ≥28 after the 1st dose and0-13 days and ≥14 days after the 2nd dose of ChAdOx1.

We will include the following covariates in the adjusted model, which we hypothesize are predictive of vaccination, the risk of SARS-CoV-2 infection and COVID-19 severity and healthcare access and utilization:

- Age as continuous variable
- Comorbidities (None, 1-2, ≥3 comorbidities)
- Evidence of prior SARS-CoV-2 infection (defined as positive PCR test, antigen test or rapid antibody test)

Although data on comorbidities is available through e-SUS and SIVEP-Gripe, this data may have different degrees of missingness between databases and between cases and control groups. Adjusting for comorbidities using complete case data will likely introduce bias. We will explore the feasibility of multiple imputation of comorbidity in a sensitivity analysis. Additional sensitivity analyses will evaluate potential effect modification of the vaccine effectiveness by history of a positive RT-PCR, antigen or serological test result prior to the vaccination campaign and age subgroups.

Survival/logistic regression analysis of hospitalization, ICU, respiratory support and death: We will perform additional analyses for hospitalization and death amongst individuals who test positive and estimate the hazards according to vaccination status at the date of positive test, adjusting for covariates described in the case-control analyses. Sensitivity analyses will be conducted to evaluate the association of influence of a positive RT-PCR, antigen or serological test result prior to the vaccination campaign.

Sample size calculations and timing of analyses: The power of a matched case-control study depends on the assumed odds ratio and the number of discordant pairs (i.e. pairs in which the case is exposed and the control is unexposed, or vice versa), which is a function of the assumed odds ratio and the expected prevalence of exposure among controls. Moreover, the estimate of the odds ratio for one level of a categorical variable compared to baseline is determined by the distribution of all discordant pairs. As vaccine coverage and incidence are changing over time, the latter in ways we cannot predict, and there is no power formula for this analysis, we will simulate power and enroll individuals until we have reached a target power, which we can assess without analyzing the data. In particular, after determining the number of discordant case-control pairs for each combination of exposure categories, we will randomly assign one of each pair to each relevant exposure type according to a Bernoulli distribution, with the probability determined by the assumed odds ratio comparing the two categories. We will run an unadjusted conditional logistic regression on the simulated dataset to determine the p-value, and estimate the power as the proportion of N=1,000 simulations that return $p<0.05$. Code to perform the power calculation can be found at https://github.com/mhitchings/VEBRA_COVID-19.

Timing of final analyses: We will perform an analysis of the primary outcome upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥14 days after the second dose for the CoronaVac. For the ChAdOx1, we will perform an analysis of effectiveness of at least one dose upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥28 days after the first dose. In addition, we will perform an analysis of effectiveness of two doses upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥14 days after the second dose. We chose a vaccine effectiveness of 40% to address the question of whether vaccination with CoronaVac and ChAdOx achieved a threshold of real-world effectiveness, below which the public health value of vaccination may need to be reconsidered.

Privacy: Only SES-SP, São Paulo State data management had access to the identified dataset to linkage the datasets by name, date of birth, mother's name and CPF. After the linkage, the CPF was encrypted and the de-identified dataset was sent to the team for analysis.

Working group: Matt Hitchings, Otavio T. Ranzani, Julio Croda, Albert I. Ko, Derek Adam Cummings, Wildo Navegantes de Araujo, Jason R. Andrews, Roberto Dias de Oliveira, Patricia Vieira da Silva, Mario Sergio Sacaramuzzini Torres, Wade Schulz, Tatiana Lang D Agostini, Edlaine Faria de Moura Villela, Regiane A. Cardoso de Paulo, Olivia Ferreira Pereira de Paula, Jean Carlo Gorinchteyn

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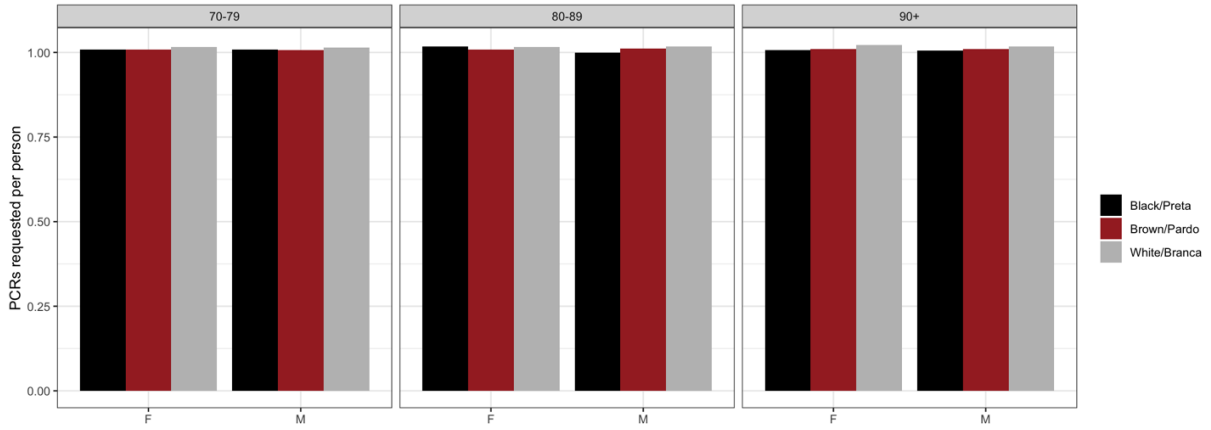


Figure 1: PCR testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

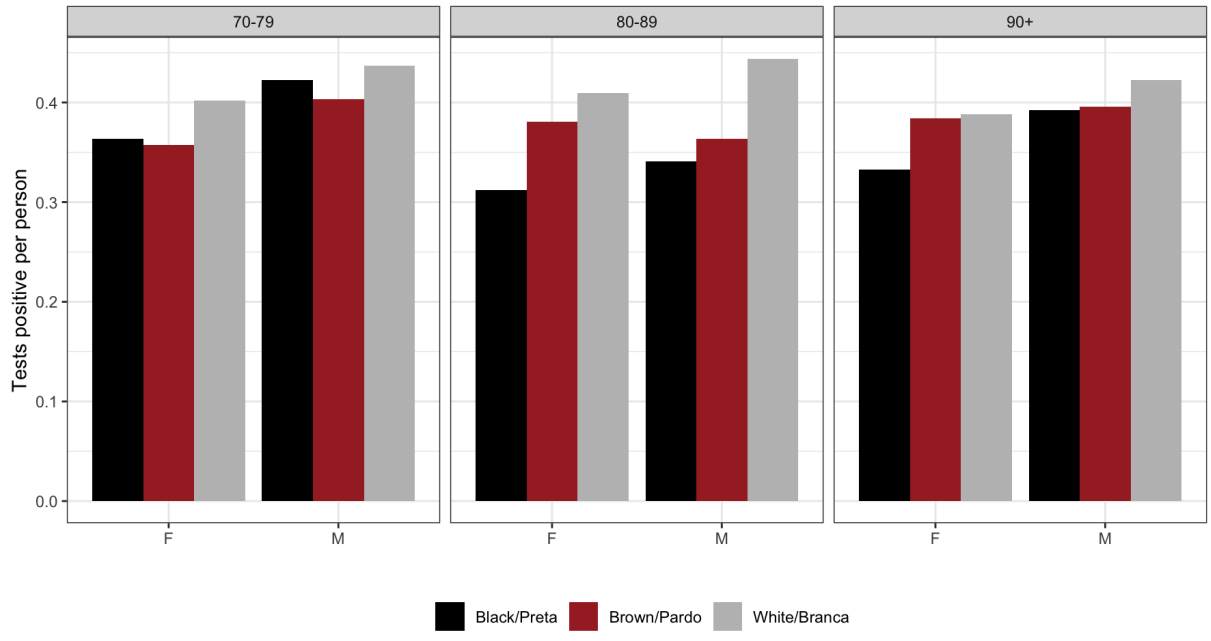


Figure 2: PCR positive testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

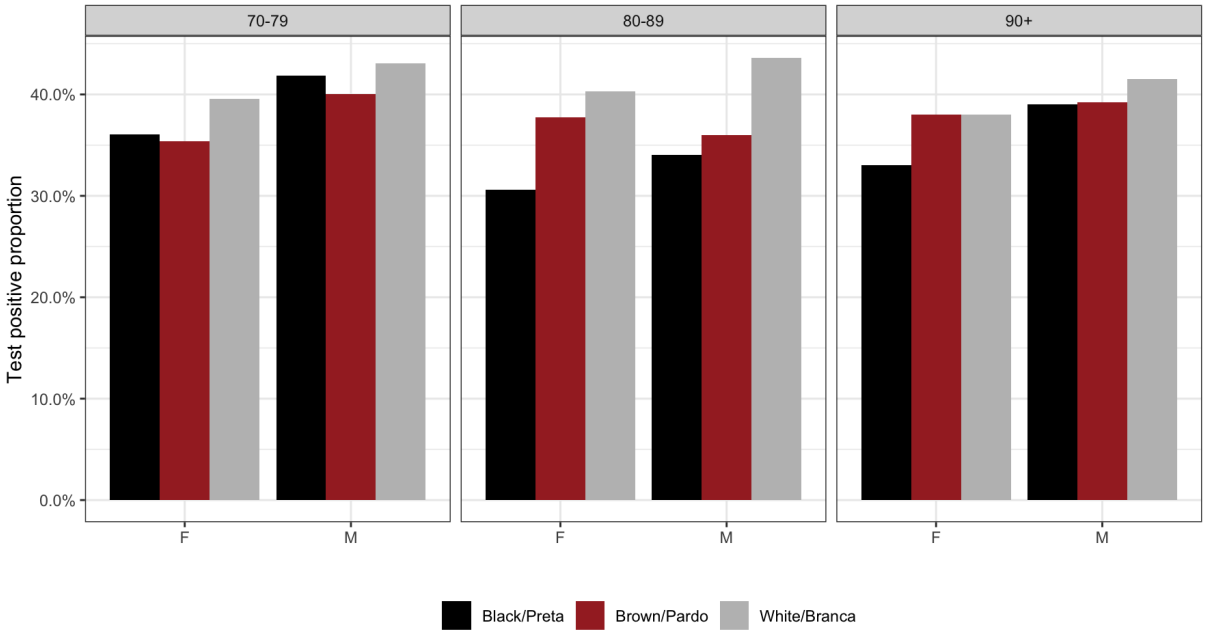


Figure 3: PCR positive proportion by age, sex and self-reported race (from data extracted on April 07, 2021)

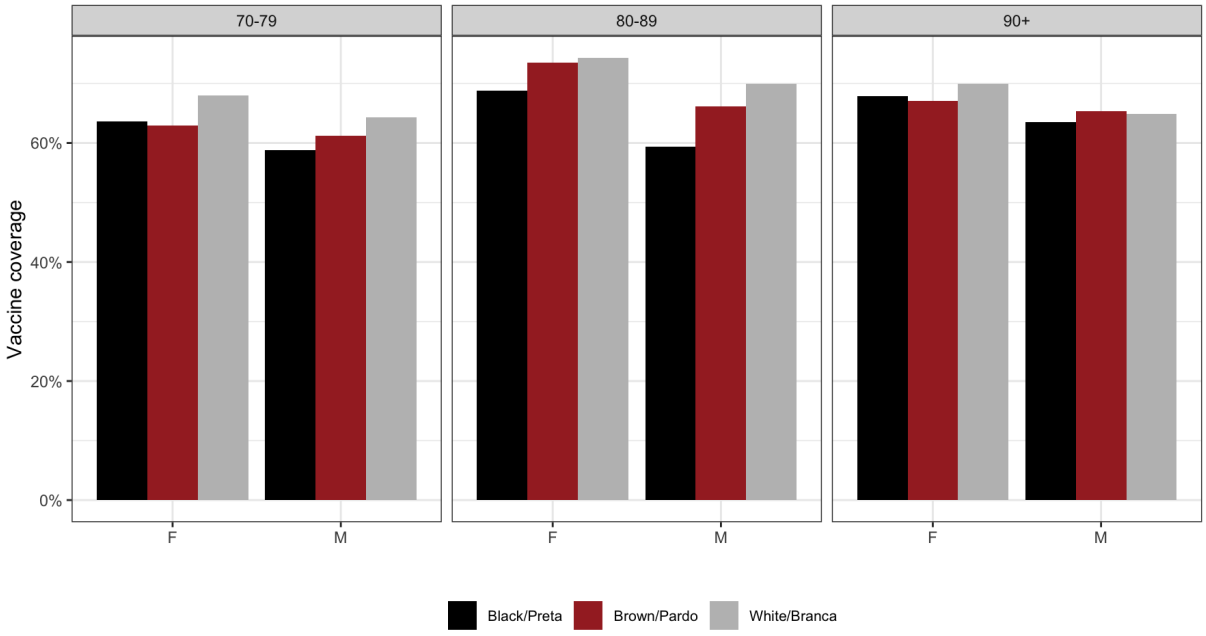
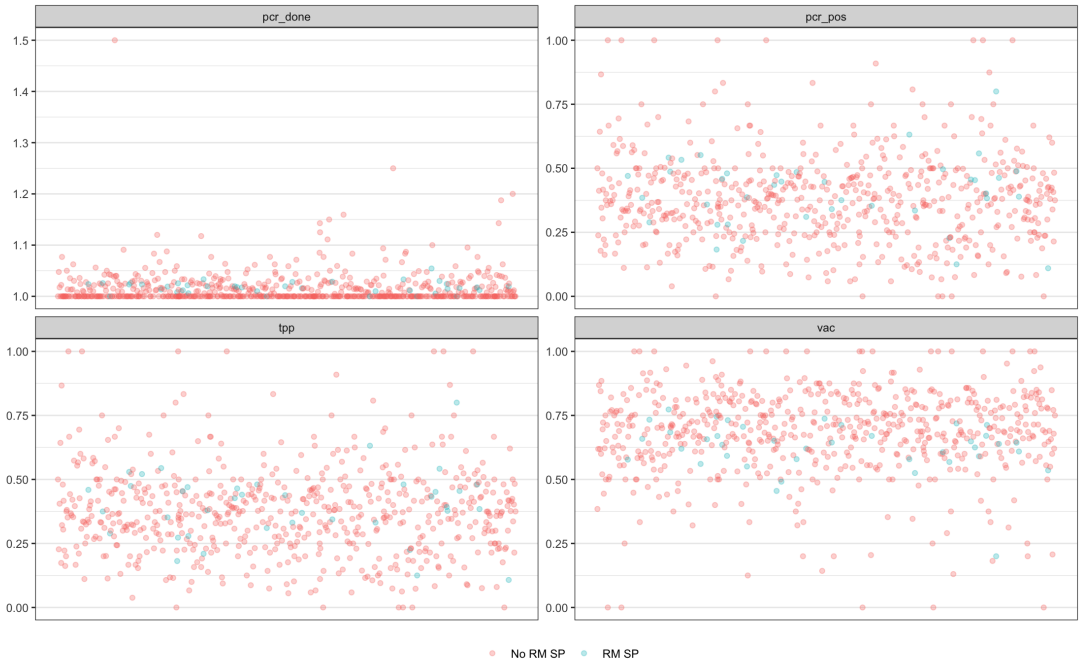


Figure 4: Vaccine coverage by age, sex and self-reported race (from data extracted on April 07, 2021)

Panel A. Indicators by Municipality



Panel B. Indicators by Municipality and Race

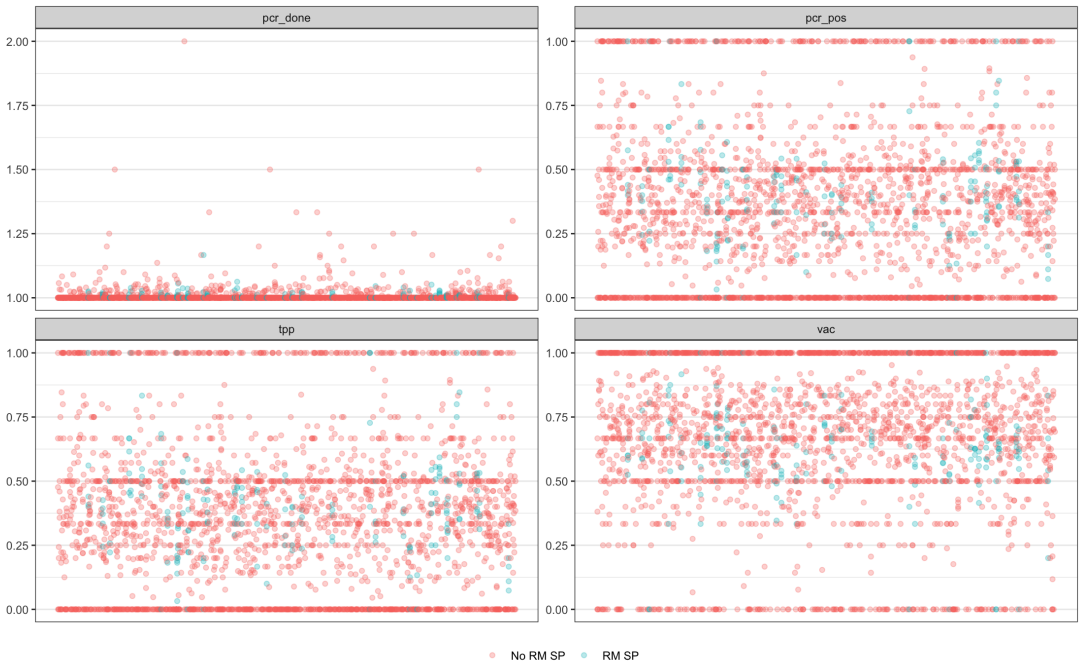
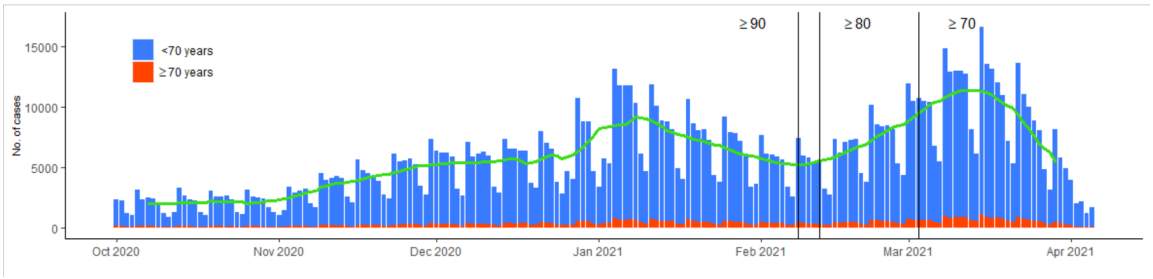


Figure 5: PCR testing rate (pcr_done), PCR positive testing rate (pcr_pos), positivity proportion (tpp) and vaccine coverage (vac) by each municipality (A) and municipality and race (B). RM SP denotes metropolitan area of São Paulo city (from data extracted on April 07, 2021)

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.

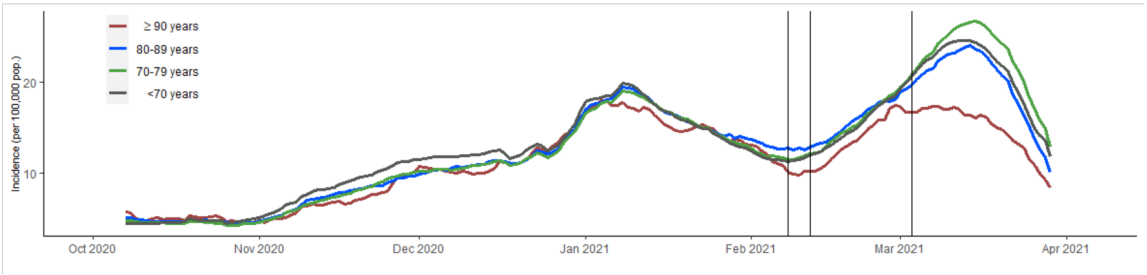


Table 1: Table of planned analyses

Analysis	Exposure	Outcome
CoronaVac		
Primary outcome, primary exposure	Two-dose regimen of CoronaVac in the period starting 14 days after administration of the 2nd dose	Positive test for SARS-CoV-2, with at least one COVID-19 symptom reported 0-10 days before sample collection date
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of CoronaVac in the period 0-13 days after administration of the 2nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of CoronaVac, in the period starting 14 days after administration of the 1st dose	
Primary outcome, bias indicator	One-dose regimen of CoronaVac, in the period 0-13 days after administration of the 1st dose	
ChAdOx1		
Primary outcome, primary exposure	One-dose regimen of ChAdOx1 in the period starting 28 days after administration of the 1st dose	Positive test for SARS-CoV-2, with at least one COVID-19 symptom reported 0-10 days before sample collection date
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1 in the period ≥14 days after administration of the 2nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1 in the period 0-13 days after administration of the 1st dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1, in the period starting 14-27 days after administration of the 1st dose	
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1, in the period starting 0-13 days after administration of the 2nd dose	
Primary outcome, bias indicator	One-dose regimen of ChAdOx1, in the period 0-13 days after administration of the 1st dose	

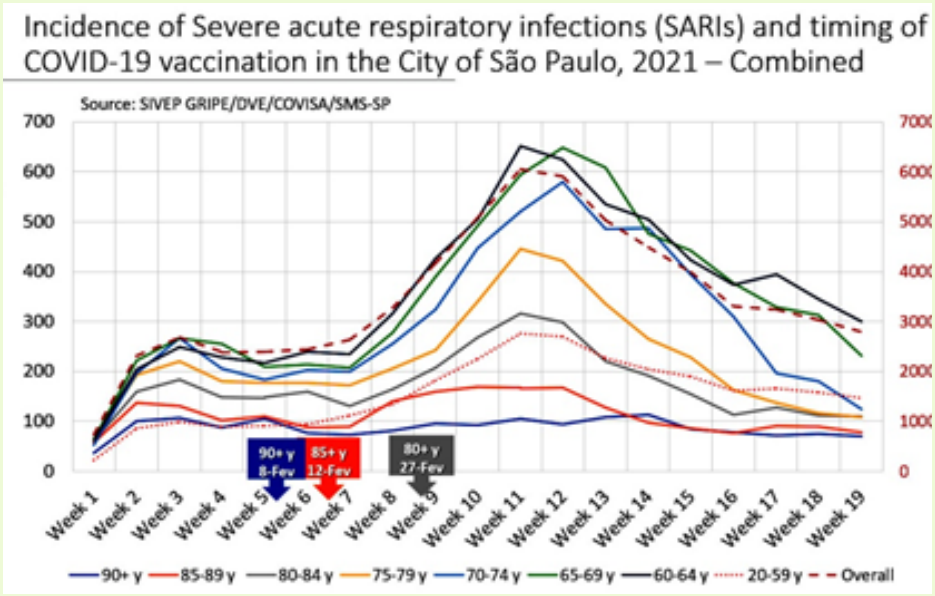
5.4 CoronaVac está associada à queda da mortalidade de idosos por Covid-19, demonstram estudos

Estudos realizados por pesquisadores do Brasil, dos Estados Unidos e da Espanha demonstraram que a aplicação da CoronaVac, vacina do Butantan contra a Covid-19, levou à queda na internação e nos óbitos por SARS-CoV-2 de pacientes idosos, inclusive em contextos onde predomina a variante P.1 do novo coronavírus (cepa amazônica).

Segundo o artigo "Estimativa do impacto inicial da imunização contra Covid-19 em mortes entre idosos no Brasil", a escalada da vacinação entre idosos no país está associada a uma queda considerável na mortalidade desse público na comparação com pessoas mais jovens.

Na relação entre janeiro-fevereiro (quando poucos idosos haviam tomado a segunda dose) e abril, a queda no número de mortes na população acima dos 80 anos foi de 25% para 13%.

Entre a primeira semana epidemiológica e o dia 22 de abril de 2021, 171.517 mortes foram atribuídas ao Covid-19 no Sistema de Informações sobre Mortalidade do Ministério da Saúde. O gráfico a seguir mostra que há uma clara aceleração nas mortes e partir da semana 9 (início de março), quando a variante P.1 começa a predominar no Brasil.



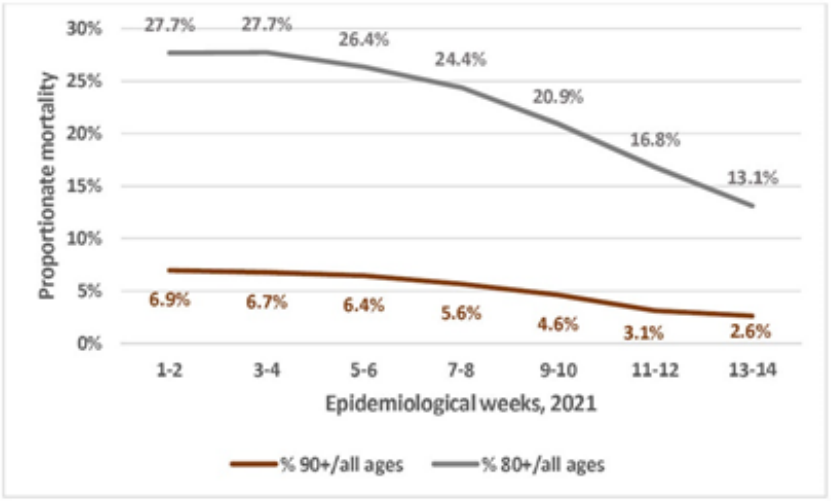
Já entre as semanas epidemiológicas 13 e 14 (em abril, quando cerca de 10 milhões de pessoas haviam recebido a segunda dose), começa a haver uma desaceleração no número de mortes, especialmente em pessoas acima de 70 anos. No gráfico fica evidente que não houve aumento no número de casos positivos no grupo acima de 90 anos, o que demonstra que a vacina se tornou efetiva em conter, neste grupo etário, a força de infecção do vírus.

Além disso, o estudo "Efetividade da vacina CoronaVac na população idosa durante a epidemia de Covid-19 associada à variante P.1 no Brasil", realizado entre janeiro e abril com 15 mil casos de pessoas acima dos 70 anos do estado de São Paulo, mostrou que a efetividade da vacina em um contexto onde predomina a variante P.1 aumenta com o tempo e não tem variação significativa em relação à eficácia geral da vacina, sendo de 49,4% 21 dias após a segunda dose. Ela é maior, porém, nos idosos mais jovens: no público entre 70 e 74 anos, a eficácia é de 61,8%.

Dados de efetividade de estudos feitos com o uso da vacina de forma rotineira podem variar e, portanto, devem ser interpretados com cautela. Sem contar que as pesquisas variam do ponto de vista metodológico e analisam momentos epidemiológicos distintos.

É necessário ressaltar que a previsão de eficácia dos estudos está baseada na relação entre os números da vacinação e os números de casos confirmados e mortes por Covid-19. As pesquisas não se baseiam em indicadores de internação clínica. O objetivo primordial da CoronaVac é reduzir o número de óbitos e internações hospitalares, diminuindo o impacto da doença sobre a perda de vidas e o sistema de saúde.

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.



Estudos realizados no Brasil e em outros países têm demonstrado que a CoronaVac é eficaz contra as novas variantes, comprovadamente a P.1 e a P.2, e que protege todos os grupos etários, inclusive os idosos, contra a mortalidade por Covid-19. Mas é importante salientar que nenhuma vacina impede que uma pessoa seja infectada pelo coronavírus.

Outro ponto relevante é que qualquer vacina gera uma resposta imune menor em pessoas mais idosas. Isso não quer dizer que elas estejam menos protegidas contra a doença, mas sim, que o organismo responde menos a um antígeno novo – uma característica que não se relaciona à efetividade da vacina em si, mas aos processos naturais do sistema imunológico.

Todos os fabricantes de vacinas, inclusive o Butantan e a Sinovac, estão avaliando a atualização da vacina. Mas nada indica, neste momento, a necessidade de uma terceira dose, e essa possibilidade ainda não foi estudada pela ciência.

Por isso, continua sendo primordial que todas as pessoas, de todas as idades, tomem a segunda dose da vacina. Os imunizantes sendo aplicados atualmente no Brasil contra a Covid-19 exigem a aplicação de duas doses para alcançarem sua eficácia máxima (com intervalos de tempo que variam de fabricante para fabricante). Pesquisas comprovam que a vacina não é suficientemente eficaz com a aplicação de somente uma dose.

Publicado em: 25/05/2021

EClinicalMedicine 38 (2021) 101036

Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: https://www.journals.elsevier.com/eclinicalmedicine

Research paper

Estimating the early impact of vaccination against COVID-19 on deaths among elderly people in Brazil: Analyses of routinely-collected data on vaccine coverage and mortality

Cesar G. Victora[a,b,*], Marcia C. Castro[b], Susie Gurzenda[b], Arnaldo C. Medeiros[c], Giovanny V.A. França[c], Aluisio J.D. Barros[a]

[a] International Center for Equity in Health, Federal University of Pelotas, Pelotas, RS, Brazil
[b] Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, MA, United States
[c] Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasilia, DF, Brazil

ARTICLE INFO

Article History:
Received 12 May 2021
Revised 29 June 2021
Accepted 1 July 2021
Available online 16 July 2021

Keywords:
COVID-19
Brazil
Immunization
Mortality

ABSTRACT

Background: Vaccination against COVID-19 in Brazil started in January 2021, with health workers and the elderly as the priority groups. We assessed whether there was an impact of vaccinations on the mortality of elderly individuals in a context of wide transmission of the SARS-CoV-2 gamma (P.1) variant.
Methods: By May 15, 2021, 238,414 COVID-19 deaths had been reported to the Brazilian Mortality Information System. Denominators for mortality rates were calculated by correcting population estimates for all-cause deaths reported in 2020. Proportionate mortality at ages 70–79 and 80+ years relative to deaths at all ages were calculated for deaths due to COVID-19 and to other causes, as were COVID-19 mortality rate ratios relative to individuals aged 0–69 years. Vaccine coverage data were obtained from the Ministry of Health. All results were tabulated by epidemiological weeks 1–19, 2021.
Findings: The proportion of all COVID-19 deaths at ages 80+ years was over 25% in weeks 1–6 and declined rapidly to 12.4% in week 19, whereas proportionate COVID-19 mortality for individuals aged 70–79 years started to decline by week 15. Trends in proportionate mortality due to other causes remained stable. Mortality rates were over 13 times higher in the 80+ years age group compared to that of 0–69 year olds up to week 6, and declined to 5.0 times in week 19. Vaccination coverage (first dose) of 90% was reached by week 9 for individuals aged 80+ years and by week 13 for those aged 70–79 years. Coronavac accounted for 65.4% and AstraZeneca for 29.8% of all doses administered in weeks 1–4, compared to 36.5% and 53.3% in weeks 15–19, respectively.
Interpretation: Rapid scaling up of vaccination coverage among elderly Brazilians was associated with important declines in relative mortality compared to younger individuals, in a setting where the gamma variant predominates. Had mortality rates among the elderly remained proportionate to what was observed up to week 6, an estimated additional 43,802 COVID-related deaths would have been expected up to week 19.
Funding: CGV and AJDB are funded by the Todos pela Saúde (São Paulo, Brazil) initiative.

Introduction

In early 2021, Brazil became the global epicenter of the COVID-19 pandemic [1] with an average of over 2000 daily deaths in recent months [2]. The gamma or P.1 variant, initially identified in Manaus in late 2020 [3] has rapidly spread throughout the country [4]. Although genomic analyses are infrequent, in April and May 2021 the new variant accounted for three out of every four samples subjected to viral sequencing [5].

Vaccination against COVID-19 was started in late January 2021, with two types of vaccines being offered: Coronavac (Sinovac, China) and AZD1222 (Oxford-AstraZeneca, UK). Vaccination has been initially targeted at four priority groups: health workers, the elderly (starting with those aged 85 years or more, and gradually vaccinating younger age groups), indigenous populations, and institutionalized individuals. By May 28, 41,478,005 Brazilians had received the first dose, and 19,604,603 the second dose [6].

Vaccination campaigns have been associated with reductions in hospital admissions and mortality among targeted population

* Corresponding author at: International Center for Equity in Health, Federal University of Pelotas, Rua Marechal Deodoro 1160, Pelotas, RS, 96020-220, Brazil
E-mail address: cvictora@equidade.org (C.G. Victora).

https://doi.org/10.1016/j.eclinm.2021.101036

Research in context

Evidence before this study

Brazil has been one of the world's hotspots for COVID-19 in 2021, largely due to the rapid spread of the SARS-CoV-2 gamma variant. Vaccination of the elderly population started in mid-January with the Coronavac (Sinovac, China) and Oxford/AstraZeneca (UK) vaccines. Although the efficacy of both vaccines has been established in phase-3 trials against the original variant of SARS-CoV-2, little is known about their protection against the gamma variant.

Added value of this study

By May 27, 2021, approximately 95% of Brazilians aged 80+ years had received the first vaccine dose. We analyzed data from the Ministry of Health database of over 450,000 COVID-19 deaths since the beginning of the pandemic, including 238,414 deaths in 2021.

Up to mid-February 2021, the deaths of individuals aged 80+ years due to COVID-19 remained almost constant at 25–30% of all reported COVID-19 deaths at any age. Starting in mid-February, proportionate mortality in the elderly started to fall steadily to under 13% in the first half of May. Similar trends were observed for individuals aged 70–79 years, after a time lag that was consistent with the later increase in vaccination coverage in this age group.

Trends in mortality due to other causes were stable, indicating a specific impact on COVID-19 deaths.

Implications of all the available evidence

Confirming early reports from cohorts of vaccinated health workers, our nationwide findings suggest that vaccination against SARS-CoV-2 in Brazil, which largely relied on the Coronavac vaccine in the first trimester of 2021, was associated with an important decline in relative mortality among the elderly compared to younger individuals, in a setting where the gamma variant accounted for three quarters of samples with information on sequencing cases in April-May 2021.

groups, in several of the early starting countries [7-9]. Yet, there is limited evidence on the efficacy of the two vaccines being delivered in Brazil against the gamma variant that currently accounts for the majority of cases in the country. Two observational studies among health care workers in Manaus [10] and São Paulo [11] suggested that the Coronavac provided partial protection against symptomatic illness in settings where gamma accounted for 75% and 47% of all infections, respectively, at the time of the study. Yet, there is growing concern that high SARS-CoV-2 incidence rates such as those observed in Brazil in early 2021 will lead to the appearance of new variants of concern as well as increase in the risk of vaccine escape [12].

To evaluate the real-life effectiveness of the vaccination campaign in Brazil, we analyzed time trends in mortality due to COVID-19 using a database of over 450,000 registered COVID-19 deaths since the beginning of the pandemic. We hypothesized that mortality would fall more rapidly among the elderly, who were the initial target group of the vaccination campaign, than among younger Brazilians.

Methods

Data sources

Data on COVID-19 deaths were obtained from the Ministry of Health Mortality Information System [13] including deaths reported until May 27, 2021. Coverage of the death registration system has been estimated at over 95% by 2010 [14]. As of 2016, the Global Burden of Disease project assigned four out of five stars for the system's coverage and quality of cause of death ascertainment [15], and by 2019 5.6% of all deaths were coded as due to ill-defined causes (França GA, unpublished data). We analyzed deaths for which the underlying cause was coded as B34.2, which included codes U07.1 (COVID-19, virus identified) and U07.2 (COVID-19, virus not identified) [16]. For 84% of 2021 deaths, presence of the virus was confirmed in a laboratory (preliminary results based on investigation of 163,637 deaths).

Data on COVID-19 vaccination coverage were obtained from a dataset made available by the Brazilian Ministry of Health [6]. The data are updated daily and consist of an individual level dataset including personal information and information on the vaccination (type and dose) along with whether it is the first or second dose received and the priority group for the person vaccinated. Data through May 15, 2021 were included in this analysis.

Population estimates

Population estimates for July 1, 2020 by age and sex were obtained from the Brazilian Institute for Geography and Statistics (IBGE) [17]. Due to the excess mortality observed in 2020 and the higher COVID-19 mortality among the elderly [18], the population numbers from IBGE for 2020 are overestimated, particularly at older ages. Since vaccination started in Brazil in early January 2021, it is imperative to obtain an adjusted estimated population that more closely reflects the Brazilian population by the end of 2020. We considered the total deaths that were reported in 2020 (for all causes, as reported in the Mortality Information System), and the expected deaths as implied in the IBGE estimates. We excluded the additional number of deaths from the published 2020 estimates and used that adjusted population as the denominator in our analyses. All adjustments were made by age and sex. All calculations were done in R (R Core Team, 2020).

Data analyses

Mortality results were analyzed in two ways. First, we calculated proportionate mortality by dividing the number of COVID-19 deaths at ages 70–79 and 80+ years by the total number of COVID-19 deaths at all ages. Our main analyses described mortality by epidemiological week in 2021, which are supported by analyses by month of death during 2020. To investigate whether age-specific trends in proportionate mortality were specific to COVID-19 deaths, we also investigated trends due to other causes of death. Second, we calculated COVID-19 age-specific mortality rates by dividing the numbers of weekly deaths from the Mortality Information System by the estimated population by age group, as described above. Mortality rates at ages 70–79 and 80+ years were then divided by rates for the age range 0–9 years in the same week, resulting in mortality rate ratios.

Formal statistical tests were not performed as all results are based on the full country population, rather than samples. Analyses were carried out using Stata version 16 (StataCorp, College Station, TX, USA).

Ethics approval

All analyses were based on anonymized databases that are available at the Brazilian Ministry of Health website [6].

Role of funding source

The funders did not play any role in the preparation of the manuscript, nor on the decision to publish.

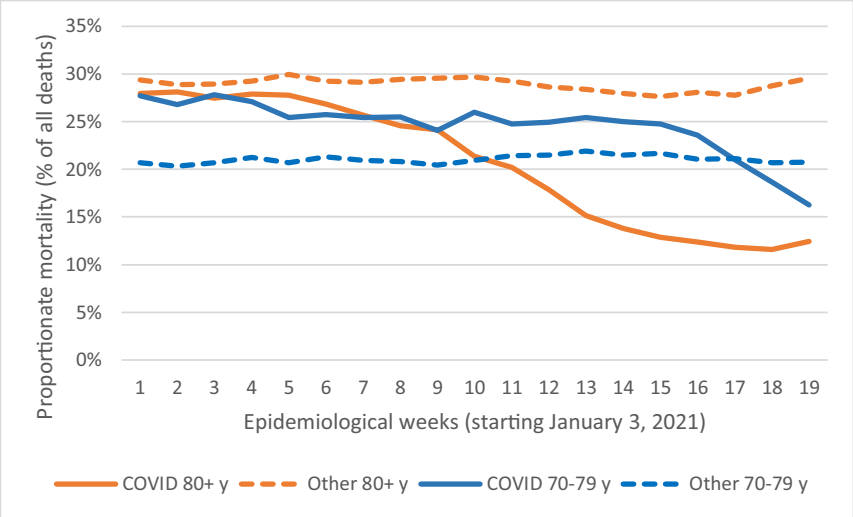


Fig. 1. Proportionate mortality of individuals aged 70–79 and 80 or more years due to COVID-19 and all other causes, relative to deaths due to the same causes at all ages by epidemiological weeks, Brazil, 2021.

Results

From the beginning of the first epidemiological week in 2021 (January 3) to May 15, 238,414 deaths in the Mortality Information System were assigned to COVID-19 and 447,817 to other causes. Supplementary Table 1 shows the absolute number of COVID-19 deaths for epidemiological weeks 1–19 of 2021 (January 3 to May 15). There was rapid acceleration in deaths from week 9 (early March) when the gamma variant became the dominant strain. Results for weeks 17–19 (April 25 to May 15) are likely affected by registration delay but remain useful for comparing age-specific proportionate mortality and mortality rate ratios. Table 1 does not include deaths occurring after epidemiological week 19 (May 16 or later) as these are more markedly affected by delay than earlier deaths.

Fig. 1 shows that proportionate COVID-19 mortality of individuals aged 80+ years fell rapidly from week 6 onwards, whereas proportionate mortality due to non-COVID causes remained relatively stable at just under 30%. Up to May 27, an additional 7,733 deaths had been reported for epidemiological weeks 20 and 21, of which 13.1% were among individuals aged 80+, a finding that is consistent with the levels achieved by week 15. Fig. 1 also shows that proportionate mortality for individuals aged 70–79 years remained at around 25% up to week 15, when it started to decline sharply. For the same age group, proportionate mortality due to other causes remained stable at just over 20% of deaths at any age.

Supplementary Fig. 1 shows that proportionate mortality at ages 80+ years fell in all regions of the country. The trend was less marked in the North region (where the Amazon is located) than in the rest of the country. Supplementary Fig. 2 expands the time series by showing proportionate mortality based on 453,244 COVID-19 deaths that occurred since the beginning of the pandemic in the country. From May 2020 (when the monthly number of deaths exceeded 15,000) to January 2021, proportionate mortality at ages 80+ remained between 25% and 30%, with a sharp reduction starting in mid-February 2021. Proportionate mortality at ages 70–79 years remained above 20% until March 2021, with a substantial decline in April–May. Also showing data for 2020 and 2021, Supplementary Fig. 3 demonstrates that the decline in proportionate mortality was observed for men and women, although proportionate mortality for women aged 80+

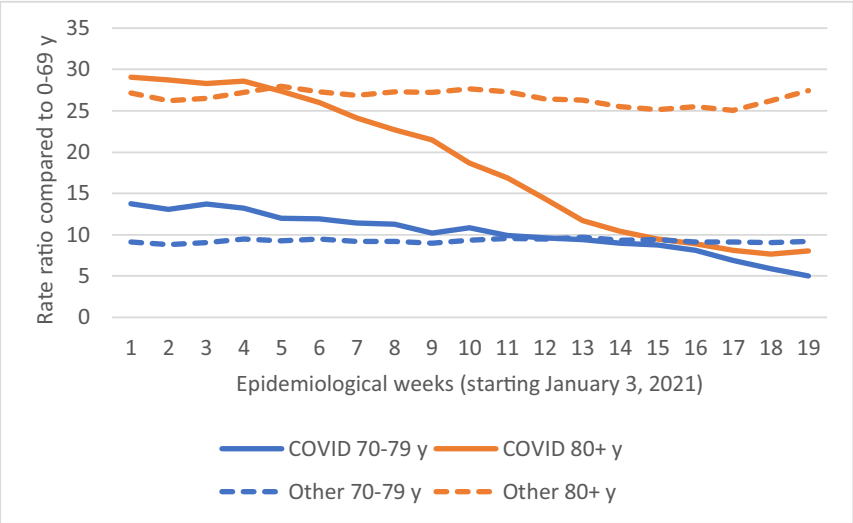


Fig. 2. Mortality rate ratios: mortality rates at ages 70–79 and 80+ years divided by mortality rate at ages 0–69 years by epidemiological weeks, Brazil 2021.

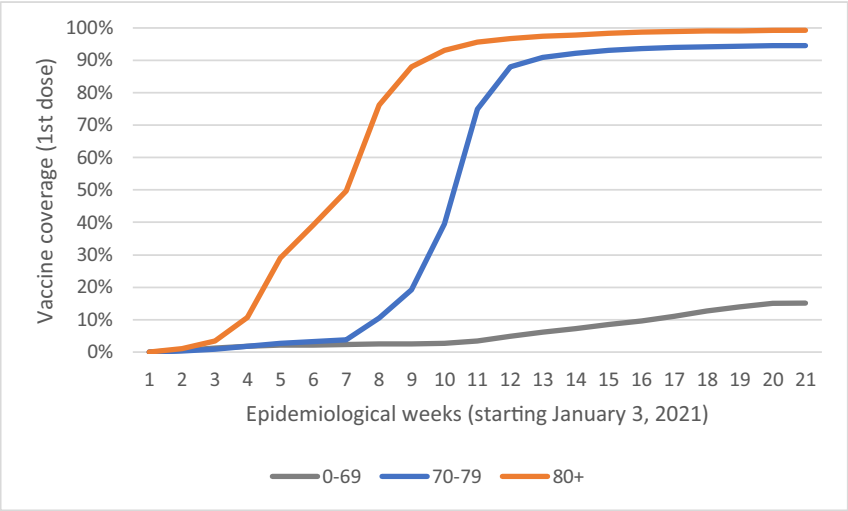


Fig. 3. Covid-19 vaccination coverage (first dose) by age group by epidemiological week, Brazil, 2021.

years tended to be higher than for men, likely due to higher life expectancy of women resulting in fewer deaths in those aged under 80 years.

Fig. 2 shows time trends in mortality rate ratios using the age group 0–69 years as the reference. The mortality rate ratio for persons aged 80+ years fell from over 27 in January and early February to 8 in week 19. The decline in the rate ratio for ages 70–79 was more gradual, from 13.8 in week 1 to 5.0 in week 19. Mortality rate ratios for non-COVID causes remained stable over time.

Fig. 3 shows vaccine coverage for individuals aged 70–79 and 80+ years over time. The increase in coverage was consistent with prioritization of older population groups, with 50% coverage reached for individuals aged 80+ years in the first half of February and over 80% by the second half, stabilizing at around 95% in March. For 70–79-year-olds, 50% coverage was reached by week 11 and 90% coverage by week 19. Coverage among younger age groups was largely restricted to priority groups including health workers, indigenous peoples and people living in institutions. In weeks 1–4, Coronavac accounted for 65.4% of all doses given and AstraZeneca for 29.8% whereas the corresponding percentages for weeks 15–19 were 36.5% and 53.3%. Pfizer/BioNTech (Germany) and Serum Institute (India) accounted for the remaining doses in the recent period.

The downturn in proportionate mortality due to COVID-19 started at about the sixth week of 2021. Had the number of deaths among individuals aged 80+ years continued to increase at the same rate as deaths among people aged 0–69 years, one would expect 70,015 such deaths during the 13-week period from mid-Feb to mid-May. Yet, 32,624 deaths were reported, or 37,401 fewer than expected under the scenario of similar trends as for the 0–69 years age group. A similar calculation was performed for deaths among 70–79-year-olds, among whom proportionate mortality started to decline around week 15. Compared to 13,838 deaths in weeks 15–19, 20,238 would be expected if mortality behaved similarly to that observed for 0–69-year-olds. Adding the two estimates, 43,802 deaths may have been avoided by the decline in mortality among the elderly.

Discussion

We found evidence that, although dissemination of the gamma variant led to increases in reported COVID-19 death at all ages, the proportion of deaths among the elderly started to fall rapidly from the second half of February 2021. This proportion had been stable at around 25–30% since the beginning of the epidemic in early 2020 but is now below 13% in May 2021.

Estimates of proportionate mortality must be interpreted with caution. We now describe how we handled potential caveats in these analyses.

First, the absolute number of deaths in the elderly may be reduced due to smaller number of persons at risk, resulting from high mortality in 2020 due to COVID-19 and other causes. In an estimated population of approximately 815 thousand Brazilians aged 90+ years in 2020, there were approximately 144 thousand deaths in the calendar year, of which about 10% were reported as being caused by COVID-19. To address this potential caveat, our calculations of mortality rates for 2021 were based on population estimates at the beginning of the year from which all-cause deaths had already been deducted.

Second, proportionate mortality may be spuriously reduced among the elderly if the gamma variant of concern disproportionally affected younger individuals, either in terms of infection rates or of infection-fatality rates. The EPICOVID-19 study has been monitoring prevalence of antibodies against SARS-CoV-2 through household surveys in nine large cities in the state of Rio Grande do Sul since April 2020. In early February 2021, antibody prevalence levels were 9.6%, 11.3%, 10.0% and 8.3% for unvaccinated individuals aged 10–19, 20–39, 40–59, and 60+ years, respectively (AJD Barros, personal communication). The state has been strongly affected by the recent pandemic wave, yet there is no evidence of important age patterns in antibody prevalence.

Thirdly, our results based on ratios of mortality rates closely mirror the findings from the proportionate mortality analyses, showing that the rate ratio for individuals aged 80+ relative to those aged 0–69 years fell from 13.3 in January to 8.0 in April.

Lastly, our analyses of deaths due to causes other than COVID-19 showed that proportionate mortality and mortality rate ratios for the elderly remained stable over time, thus supporting the specificity of an impact on COVID-19 deaths.

Another potential limitation of our analyses is the underreporting of deaths and delays in reporting. Delays are particularly relevant for estimating mortality rates for recent periods, as only deaths that reached the system by May 27 were included. However, proportionate mortality by age groups would only be affected if delays varied systematically with age, which is unlikely. As discussed in the Introduction, the overall coverage of mortality statistics has been very high in Brazil for many years, and ill-defined causes represent 5.6% of all deaths. The mortality database for the present analyses includes

approximately 30% more deaths than the SIVEP-Gripe database on hospital admissions and mortality that has been employed in previous analyses of COVID-19 deaths in Brazil [18-20].

However, there is evidence that the excess mortality during 2020 relative to earlier years was not fully explained by deaths due to COVID-19. It is likely that some of such deaths were reported as having been due to other causes or to ill-defined conditions, but it is also possible that increases in non-COVID-19 deaths were because health services were under stress due to the large COVID-19 case load. Unless reporting patterns varied by age or calendar time, this limitation is unlikely to affect the present results particularly in light of the present finding that age patterns in deaths assigned to non-COVID causes remained stable.

The decline in mortality was observed for both sexes. Proportionate mortality at older ages was higher among women than for men, which is compatible with higher case-fatality of younger male adults, possibly related to comorbidities, given that existing serological surveys do not suggest differences in infection prevalence by sex [21,22]. The reductions in proportionate mortality were very similar across four of the five regions of the country. A decline was also observed in the fifth region (Northern Brazil including the Amazon), but proportionate mortality was lower at the beginning of the year than in the rest of the country, and the decline started later than in the rest of the country. The North region has been badly hit by the first and second waves of the pandemic, and high prevalence, high case-fatality, and the limited availability of health services in this region [23] may have led to a larger number of deaths among young adults. Even before the pandemic, life expectancy at birth in the North region was the shortest in the country at 72.9 years, compared to 73.9, 78.3, 78.6 and 75.8 in the Northeast, Southeast, South and Center-West, respectively [17].

The most likely explanation for the observed reductions in proportionate mortality and in rate ratios for the elderly is the rapid increase in vaccination coverage in these age groups, as has been described for other parts of the world [7-9]. The increase in vaccine coverage preceded the decline in mortality, and the decline at ages 80+ years preceded the decline at ages 70−79 years, which is in accordance with the vaccination calendar.

Our results are original in the sense that none of existing population-based mortality studies were carried out in a setting where the gamma variant is predominant. Recent observational studies in vaccinated health workers in Manaus and São Paulo [10,11] had already suggested that Coronavac provided some degree of protection against symptomatic illness in settings where gamma was prevalent. Coronavac accounted for most vaccinations in the 80+ years age group, who were immunized in January and February, with AstraZeneca vaccine accounting for the majority of recent doses. Individuals who received the latter are so far protected by a single dose given that the second dose is provided 12 weeks after the first, whereas the second dose of Coronavac has already been administered to a very high proportion of individuals aged 80+ years [24] as doses are given four weeks apart. The health worker study in São Paulo suggested that the number of cases started to drop after the first Coronavac dose, which is compatible with our findings [11]. This is supported by the results of a recent mass vaccination trial with Coronavac in the town of Serrana (population 27,000) carried out by Instituto Butantan. Following high coverage with Coronavac in early 2021, reductions of 86% in admissions and 95% in deaths were observed in the town by the end of May [25].

We attempted to provide an approximate estimate of lives saved among elderly Brazilians in the eight-week period since vaccination was accelerated throughout the country. The figure of over 40 thousand deaths averted is likely an underestimate, because it does not take into account lives saved among other priority groups for vaccination, such as health workers and indigenous populations. Also, by using the mortality in ages 0−69 years to predict expected deaths among those aged 70+ years, we are not accounting for lives saved by the vaccine among younger age groups – e.g., 60−69-year-olds - for whom coverage also increased, albeit at a slower rate.

Although it is not possible to make strong causal arguments on the basis of the data available for our analyses, our findings are consistent with the results of efficacy trials for both vaccines, with two observational studies in high-risk groups of health workers, [10,11] and with a population-based test-negative study of individuals aged 70+ years in São Paulo State, all of which suggested that vaccination was effective under real-life conditions [26]. Although it is not possible to rule out publication bias, our literature search did not identify any studies showing lack of effectiveness of the Brazilian vaccination campaign, and one would expect that studies showing lack of effectiveness of widely used vaccines would be as likely to be published as those reporting a positive impact. Regarding generalizability, our findings are consistent with the growing evidence of vaccination impact on cases, hospital admissions and deaths in other countries as reported in the lay press [27].

The main contribution brought by the present analyses is to provide large-scale supporting evidence for effectiveness of vaccination in a setting with wide circulation of the gamma variant. Because compliance with non-pharmaceutical interventions such as social distancing and mask use is limited in most of the country, rapid scaling up of vaccination remains as the most promising approach for controlling the pandemic in a country where over 500,000 lives have already been lost to COVID-19 by July 2021.

Funding

CGV and AJDB are funded by the Todos pela Saúde (São Paulo, Brazil) initiative.

Declaration of Interest

The authors declare no competing interest.

Contributors

CGV and MCC conceptualized the manuscript, and CGV wrote the first draft. GVAF and AM extracted the database. AJDB and SG analyzed the data. All authors revised the manuscript and collaborated to produce a revised draft. AJDB and GVAF verified the underlying data. All authors approved the final version.

Data sharing

All data are publicly available on the Brazilian Ministry of Health website [6].

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.101036.

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6. Protege crianças e adolescentes

6.1 CoronaVac é segura e gera forte resposta imune em crianças e adolescentes, confirma estudo

A CoronaVac, vacina contra a Covid-19 desenvolvida pela biofarmacêutica chinesa Sinovac Biotech e produzida no Brasil pelo Butantan, é segura para a população de três a 17 anos de idade e pode induzir uma forte produção de anticorpos no grupo pediátrico. As conclusões foram obtidas nos estudos clínicos de fases 1 e 2 conduzidos pela Sinovac com a aplicação da CoronaVac em crianças e adolescentes. Os resultados foram publicados no periódico científico The Lancet Infectious Diseases.

Este é o primeiro estudo do mundo a avaliar o uso de uma vacina contra a Covid-19 em uma população a partir dos três anos de idade. "Crianças e adolescentes com Covid-19 geralmente têm infecções leves ou assintomáticas em comparação aos adultos. Apesar disso, um pequeno número ainda pode estar em risco de doença grave e essa população ainda pode transmitir o vírus a outras pessoas. Portanto, é vital testar a segurança e a eficácia das vacinas contra a Covid-19 em grupos de idades mais jovens", disse o gerente geral da Sinovac, Gao Qiang, em comunicado publicado no site da farmacêutica.

O estudo randomizado, controlado e duplo-cego avaliou 550 crianças (71 na fase 1 e 479 na fase 2) de três a 17 anos para medir a segurança, a tolerabilidade e a imunogenicidade da aplicação de duas doses da Coronavac com um intervalo de 28 dias entre elas.

Um grupo tomou a vacina enquanto o outro recebeu placebo com hidróxido de alumínio, adjuvante não nocivo ao organismo presente na fórmula do imunizante. As análises apontaram que a vacina foi capaz de gerar anticorpos em 96% dos voluntários 28 dias após a segunda dose. Na fase 1, nenhum dos participantes tinha anticorpos neutralizantes contra o SARS-CoV-2 e, 28 dias após a vacinação, 100% deles apresentaram anticorpos.

Na fase 2, alguns voluntários receberam duas aplicações com dosagens menores (1,5µg) e outros receberam dosagens maiores (3µg). Enquanto no primeiro grupo 95% dos participantes apresentaram anticorpos no sangue, este número foi de 100% no segundo grupo. Por isso, os pesquisadores optaram por seguir apenas com a dosagem mais alta no ensaio clínico de fase 3, que ainda está em andamento.

As reações adversas foram de leves a moderadas, sendo dor no local da aplicação e febre as mais comuns, com desaparecimento dos sintomas em até 48 horas. 27% dos participantes relataram efeitos colaterais. Houve apenas um caso de evento adverso grave, não associado à vacina - uma criança teve pneumonia após receber placebo.

Publicado em: 08/07/2021

Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial

Bihua Han, Yufei Song*, Changgui Li*, Wanqi Yang, Qingxia Ma, Zhiwei Jiang, Minjie Li, Xiaojuan Lian, Wenbin Jiao, Lei Wang, Qun Shu, Zhiwei Wu, Yuliang Zhao, Qi Li, Qiang Gao*

Summary

Background A vaccine against SARS-CoV-2 for children and adolescents will play an important role in curbing the COVID-19 pandemic. Here we aimed to assess the safety, tolerability, and immunogenicity of a candidate COVID-19 vaccine, CoronaVac, containing inactivated SARS-CoV-2, in children and adolescents aged 3–17 years.

Methods We did a double-blind, randomised, controlled, phase 1/2 clinical trial of CoronaVac in healthy children and adolescents aged 3–17 years old at Hebei Provincial Center for Disease Control and Prevention in Zanhuang (Hebei, China). Individuals with SARS-CoV-2 exposure or infection history were excluded. Vaccine (in 0·5 mL aluminum hydroxide adjuvant) or aluminum hydroxide only (alum only, control) was given by intramuscular injection in two doses (day 0 and day 28). We did a phase 1 trial in 72 participants with an age de-escalation in three groups and dose-escalation in two blocks (1·5 μg or 3·0 μg per injection). Within each block, participants were randomly assigned (3:1) by means of block randomisation to receive CoronaVac or alum only. In phase 2, participants were randomly assigned (2:2:1) by means of block randomisation to receive either CoronaVac at 1·5 μg or 3·0 μg per dose, or alum only. All participants, investigators, and laboratory staff were masked to group allocation. The primary safety endpoint was adverse reactions within 28 days after each injection in all participants who received at least one dose. The primary immunogenicity endpoint assessed in the per-protocol population was seroconversion rate of neutralising antibody to live SARS-CoV-2 at 28 days after the second injection. This study is ongoing and is registered with ClinicalTrials.gov, NCT04551547.

Findings Between Oct 31, 2020, and Dec 2, 2020, 72 participants were enrolled in phase 1, and between Dec 12, 2020, and Dec 30, 2020, 480 participants were enrolled in phase 2. 550 participants received at least one dose of vaccine or alum only (n=71 for phase 1 and n=479 for phase 2; safety population). In the combined safety profile of phase 1 and phase 2, any adverse reactions within 28 days after injection occurred in 56 (26%) of 219 participants in the 1·5 μg group, 63 (29%) of 217 in the 3·0 μg group, and 27 (24%) of 114 in the alum-only group, without significant difference (p=0·55). Most adverse reactions were mild and moderate in severity. Injection site pain was the most frequently reported event (73 [13%] of 550 participants), occurring in 36 (16%) of 219 participants in the 1·5 μg group, 35 (16%) of 217 in the 3·0 μg group, and two (2%) in the alum-only group. As of June 12, 2021, only one serious adverse event of pneumonia has been reported in the alum-only group, which was considered unrelated to vaccination. In phase 1, seroconversion of neutralising antibody after the second dose was observed in 27 of 27 participants (100·0% [95% CI 87·2–100·0]) in the 1·5 μg group and 26 of 26 participants (100·0% [86·8-100·0]) in the 3·0 μg group, with the geometric mean titres of 55·0 (95% CI 38·9–77·9) and 117·4 (87·8–157·0). In phase 2, seroconversion was seen in 180 of 186 participants (96·8% [93·1–98·8]) in the 1·5 μg group and 180 of 180 participants (100·0% [98·0–100·0]) in the 3·0 μg group, with the geometric mean titres of 86·4 (73·9–101·0) and 142·2 (124·7–162·1). There were no detectable antibody responses in the alum-only groups.

Interpretation CoronaVac was well tolerated and safe and induced humoral responses in children and adolescents aged 3–17 years. Neutralising antibody titres induced by the 3·0 μg dose were higher than those of the 1·5 μg dose. The results support the use of 3·0 μg dose with a two-immunisation schedule for further studies in children and adolescents.

Funding The Chinese National Key Research and Development Program and the Beijing Science and Technology Program.

Lancet Infect Dis 2021; 21: 1645–53

Published Online June 28, 2021 https://doi.org/10.1016/S1473-3099(21)00319-4

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*Contributed equally to the manuscript

Hebei Provincial Center for Disease Control and Prevention, Shijiazhuang, Hebei Province, China (B Han MSc, M Li MSc, Z Wu MSc, Y Zhao MSc, Qi Li PhD); **Sinovac Biotech, Beijing, China** (Y Song BSc, W Yang MSc, L Wang MSc); **National Institutes for Food and Drug Control, Beijing, China** (C Li PhD); **Zanhuang County Center for Disease Control and Prevention, Zanhuang, Hebei Province, China** (Q Ma BSc, W Jiao BSc); **Beijing Key Tech Statistics Technology, Beijing, China** (Z Jiang PhD, Q Shu MSc); **Sinovac Life Sciences, Beijing, China** (X Lian MBA, Q Gao MSc)

Correspondence to:
Dr Qiang Gao, Sinovac Life Sciences, Beijing, China
gaoq@sinovac.com

or

Dr Qi Li, Hebei Provincial Center for Disease Control and Prevention, Shijiazhuang, Hebei Province, China
liqicdc226@163.com

Introduction

The ongoing COVID-19 pandemic, caused by SARS-CoV-2, has led to more than 174·5 million infections and more than 3·8 million deaths worldwide as of June 11, 2021.[1] Children and adolescents infected with SARS-CoV-2 are mainly mild or asymptomatic compared with adults, but a

Research in context

Evidence before this study

We searched PubMed on Apr 29, 2021, for published research articles, with no language or date restrictions, using the search terms of "SARS-CoV-2", "COVID-19", "vaccine", and "clinical trial". We identified several clinical trials of COVID-19 vaccines across different platforms, including mRNA, viral vector, protein subunit, and inactivated virus. The results from phase 1–3 studies have confirmed that different vaccines were safe, effective, and induced humoral antibody responses in adults. As of April 19, 2020, more than ten COVID-19 candidate vaccines have been rolled out in many countries for general population use. Although vaccine companies have started to assess the safety and efficacy of COVID-19 vaccines in populations of 6 months to 17 years of age, there are currently no authorised vaccines for use among children and adolescents under the age of 16. We previously assessed CoronaVac, an inactivated vaccine developed by Sinovac Life Sciences, in adults aged 18–59 years and those aged 60 years and older, and showed that it was safe and well tolerated. Seroconversion rates ranged from 92% to 100% after two doses of CoronaVac (3·0 μg and 6·0 μg) with two immunisation schedules (on days 0 and 14, or on days 0 and 28) in adults aged 18–59 years. Seroconversion rates were higher than 98% after two doses of CoronaVac (3 μg and 6 μg) with the 0–28 days schedule in patients aged 60 years and older.

Added value of this study

This is, we believe, the first report of an inactivated SARS-CoV-2 vaccine, CoronaVac, tested in children and adolescents aged 3–17 years. CoronaVac was found to be well tolerated and safe in this population. The seroconversion rates of neutralising antibody with both doses (1·5 μg and 3·0 μg) were over 96% after two-dose vaccination and the neutralising antibody titres induced by the 3·0 μg dose were higher than those induced by the 1.5 μg dose. Taken together, the 3·0 μg dose of CoronaVac induced higher immune responses compared with 1·5 μg dose.

Implications of all the available evidence

While a small number of children and adolescents with SARS-CoV-2 infection might be at risk for severe COVID-19 and complicated illnesses, they usually have mild or asymptomatic symptoms compared with adults. Nevertheless, children and adolescents can be important transmitters of SARS-CoV-2 in communities. Therefore, testing the effectiveness of COVID-19 vaccines in this population is important. CoronaVac was well tolerated and immunogenic in healthy children and adolescents aged 3–17 years in this trial, which supports the use of CoronaVac for further studies in this population.

relatively small number of children and adolescents might be at risk for severe COVID-19, especially those with underlying health comorbidities.[2–5] Studies have also found that the SARS-CoV-2 infection can lead to a serious complication called multisystem inflammatory syndrome in children, which includes myocardial dysfunction, shock, and respiratory failure requiring intensive care.[3,6,7] Furthermore, children and adolescents can be important transmitters of SARS-CoV-2 in communities.[8,9] Therefore, testing the effectiveness of COVID-19 vaccines in this population is important. As of June 11, 2021, a total of 287 candidate vaccines are in clinical or preclinical development.[10] The results from phase 3 trials of multiple vaccines across three platforms, including mRNA, viral vector, and inactivated virus, have confirmed that the vaccines are effective in preventing SARS-CoV-2 infection in adults,[11,12] and more than ten vaccines have been rolled out in many countries for general population use. No COVID-19 vaccines are authorised for use among children under the age of 12 years, but vaccine companies have been started to assess the safety and efficacy of various vaccine platforms among the population aged 6 months to 17 years.[13,14] The mRNA vaccine developed by Pfizer has shown 100% efficacy and robust antibody responses in adolescents aged 12–15 years.[15]

Purified inactivated viruses have traditionally been used for vaccine development. CoronaVac is an inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences (Beijing, China), which provided partial or complete protection in macaques following SARS-CoV-2 challenge, without observable antibody-dependent enhancement of infection.[16] The analyses from phase 1–3 trials have shown that CoronaVac was effective, immunogenic, and safe in adults aged 18 years and older.[12,17–19] Furthermore, another 11 inactivated COVID-19 candidate vaccines are in clinical evaluation, and several studies have also shown that the inactivated vaccines can induce neutralising antibody responses and have good safety profiles.[20–24]

The phase 1/2 trial of CoronaVac in children and adolescents was launched in October, 2020 to assess the safety, tolerability, and immunogenicity. Here we report the results of CoronaVac among healthy participants aged 3–17 years old.

Method

Study design and participants

We have done two phase 1/2 clinical trials of CoronaVac in participants aged 18–59 years and aged 60 years and older.[17,18] The preliminary immunogenicity and safety results supported the expansion of the trial to children and adolescents. We subsequently did a single-centre, randomised, double-blind, controlled, phase 1/2 trial to evaluate the safety, tolerability, and immunogenicity of CoronaVac in children and adolescents aged 3–17 years. On the basis of the results of previous trials and considering the low weight of this population, two different doses—1·5 μg and 3·0 μg—were adopted in this study.

This trial was run at Hebei Provincial Center for Disease Control and Prevention in Zanhuang (Hebei, China).

The phase 1 trial was an age de-escalation and dose-escalation study of 72 participants. Participants in each age group (3–5 years, 6–11 years, and 12–17 years) were recruited in order from the low-dose stage (block 1) to the high-dose stage (block 2). In block 1, participants were randomly assigned to receive either 1·5 μg vaccine or aluminum hydroxide adjuvant only (alum only, control) and participants in block 2 were randomly assigned to receive either 3·0 μg vaccine or alum only. In phase 1, 7 days of follow-up for safety were required before entering the next stage. The phase 2 trial was initiated only after all the participants in phase 1 had finished and passed a 7-days safety observation period after the first dose, as confirmed by the data monitoring committee. The required safety criteria were: no-life threatening vaccine-related adverse events (adverse reactions), no more than 15% of vaccinated participants reporting severe adverse reactions, and no other safety concerns in the opinion of the data monitoring committee. A total of 480 participants were recruited in phase 2, including 120 aged 3–5 years, 180 aged 6–11 years, and 180 aged 12–17 years.

Eligible participants were healthy children and adolescents aged 3–17 years. The key exclusion criteria included high-risk epidemiology history within 14 days before enrolment (eg, travel or residence history in communities with case reports, or contact history with someone infected with SARS-CoV-2), history of severe acute respiratory syndrome or SARS-CoV-2 infection (as reported by participants), axillary temperature of more than 37·0°, and history of allergy to any vaccine component. A complete list of exclusion criteria is listed in the protocol, which is available online.

Parents provided written informed consents, and participants 8–17 years of age also provided written assents before enrolment. The clinical trial protocol and informed consent form were approved by the Ethics Committee of Hebei CDC (IRB2020-005). The study was done in accordance with the requirements of Good Clinical Practice of China and the International Conference on Harmonisation.

Randomisation and masking

In phase 1, participants of block 1 and block 2 were randomly assigned (3:1) to either vaccine or alum only, and in phase 2, participants were randomly assigned (2:2:1) to either 1·5 μg, 3·0 μg of vaccine, or alum only. The randomisation codes for the phase 1 and phase 2 were generated by the randomisation statistician by means of block randomisation using SAS software (version 9.4). The randomisation code was assigned to each participant in sequence in the order of enrolment, and then the participants received the study vaccine labelled with the same code. The vaccine and alum only were completely identical in appearance, and all participants, investigators, and laboratory staff were masked to group allocation.

Procedures

CoronaVac is an inactivated vaccine candidate against SARS-CoV-2 infection. To prepare the vaccine, SARS-CoV-2 (CN02 strain) was propagated in African green monkey kidney cells (WHO Vero 10-87 Cells). At the end of the incubation period, the virus was harvested, inactivated with β-propiolactone, concentrated, purified, and finally adsorbed onto aluminum hydroxide. The aluminium hydroxide complex was then diluted in sodium chloride, phosphate-buffered saline, and water, before being sterilised and filtered for injection. The control was aluminum hydroxide adjuvant (alum only) with no virus. Both the vaccine and alum only were prepared in the Good Manufacturing Practice-accredited facility of Sinovac Life Science that was periodically inspected by the National Medical Products Administration committee for compliance. The production process of the vaccine in this trial was a highly automated bioreactor (ReadyToProcess WAVE 25, GE, Umea, Sweden), which was consistent with the production process of vaccine used in the phase 2 trial of adults aged 18–59 years and in the phase 1/2 trial of older adults aged at least 60 years.[17,18] Vaccine doses of 1·5 μg, or 3·0 μg in 0·5 mL of aluminium hydroxide diluent per dose and alum only in ready-to-use syringes were administered intramuscularly to participants on day 0 and day 28.

Participants were observed in the study site for at least 30 min after vaccination. For the first 7 days after each dose, parents or guardians of participants were required to record any injection-site adverse events (eg, pain, swelling, erythema), or systemic adverse events (eg, allergic reaction, cough, fever) on the diary cards. From day 8 to day 28 after each dose, safety data were collected by spontaneous report from the participants combined with the regular visit (which occurred on day 3, day 8 and day 28 after each dose in phase 1, and on day 8 and day 28 in phase 2). Solicited adverse events were recorded for 7 days after each dose and unsolicited adverse events for 28 days. The serious adverse events are recorded throughout the study and follow-up will continue until 12 months after the second dose. The reported adverse events were graded according to the China National Medical Products Administration guidelines.[25] The causal relationship between adverse events and vaccination was established by the investigators.

In the phase 1 trial, blood and urine samples were taken on day 3 after each dose and tested to investigate any abnormal changes of the haematology, biochemistry, and urine routine indexes. Blood samples were collected on day 0, 28, and 56 from participants in phase 1, and on day 0 and 56 in phase 2 to evaluate the neutralising antibody titres. The neutralising antibody titres to

For more on **exclusion criteria** see http://www.hebeicdc.cn/kygz/25011.jhtml

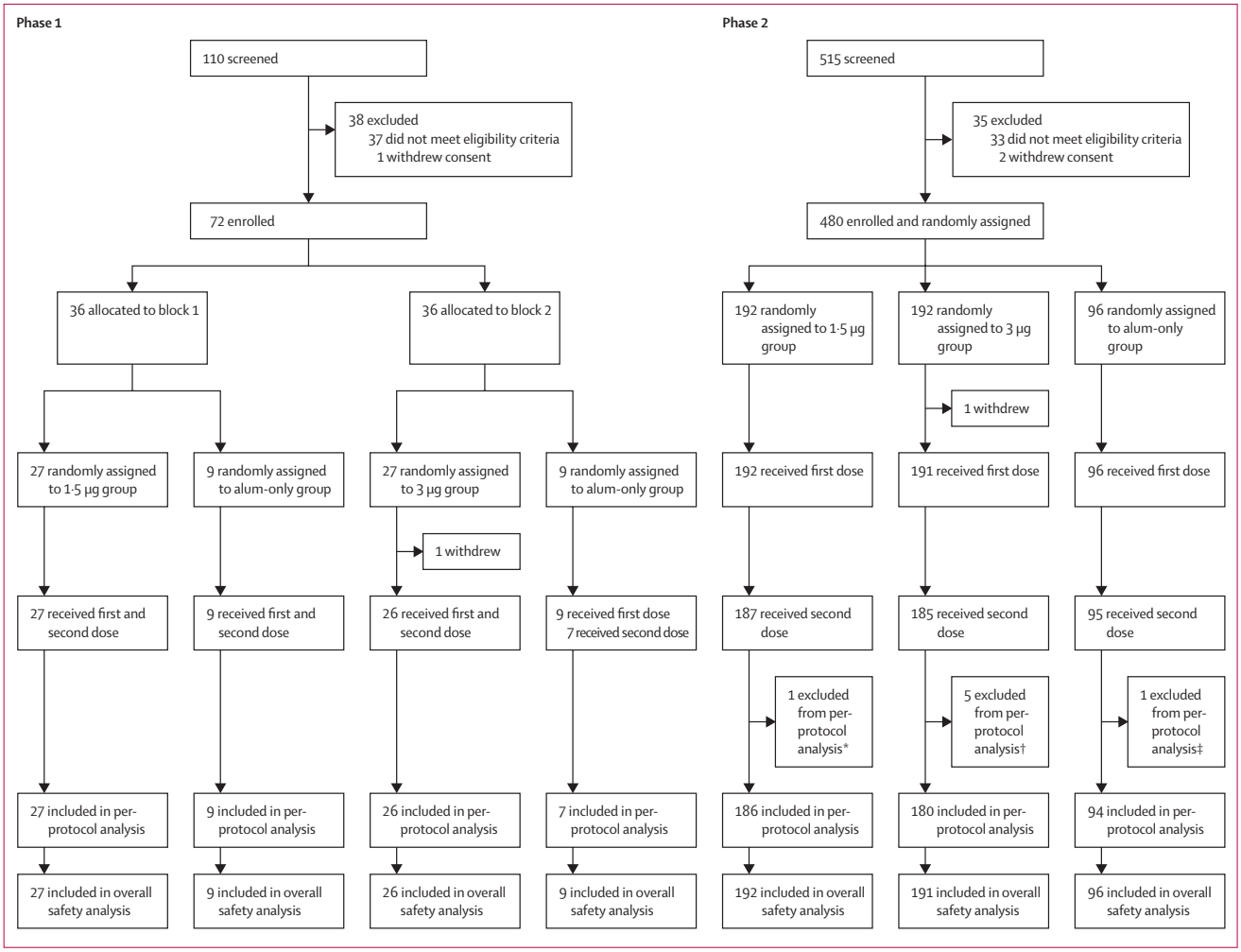

Figure 1: **Trial profile**
*One participant in the 1·5 μg group was excluded from the per-protocol analysis because he received tetanus immunoglobulin at day 14 after the second dose. †One participant in the 3 μg group was excluded from the per-protocol analysis because blood collection after vaccination was outside of the specified time window, and four did not have a blood sample taken 28 days after the second dose. ‡One participant in the alum only group was excluded from the per-protocol analysis because he did not have a blood sample taken 28 days after the second dose.

See Online for appendix

live SARS-CoV-2 (virus strain: SARS-CoV-2/human/CHN/CN1/2020, genebank number MT407649.1) was quantified by means of the microcytopathogenic effect assay.[26] Serum samples were inactivated at 56° for 30 min and serially diluted with cell culture medium in two-fold steps. The diluted serum samples were incubated with equal volume (50 μL) of the live SARS-CoV-2 virus suspension, with a 50% cell culture infective dose of 100 for 2 h at 37·0°. Vero cells ($1·0–2·0\times10^5$ cells per mL) were then added to the serum–virus suspensions in microplates in duplicate and incubated at 36·5° for 5 days. Cytopathic effects were recorded under microscopes and the neutralising antibody titre was calculated by the dilution number of 50% protective condition. Detection was done by the National Institute for Food and Drug Control. Further information on the method has been provided in the appendix (p 1).

Outcomes

The primary safety endpoint was any vaccine-related adverse events (adverse reactions) within 28 days after the administration of each dose of the study vaccine or alum only. Secondary safety endpoints were serious adverse events and any abnormal changes in laboratory measurements at day 3 after each dose. Laboratory index tests were prespecified only in the phase 1 trial. The primary immunogenic endpoint was the seroconversion rate of neutralising antibodies to live SARS-CoV-2 at day 28 after the second dose. Secondary immunogenic endpoints were geometric mean titre (GMT) of neutralising antibodies to

live SARS-CoV-2, as well as seropositive rates and geometric mean increase. Seroconversion was defined as a change from seronegative at baseline to seropositive or a four-fold titre increase if the participant was seropositive at baseline. The positive cutoff of the titre for neutralising antibodies to live SARS-CoV-2 was 1/8.

Statistical analysis

We assessed the safety endpoints in the safety population, which included all participants who had received at least one dose of vaccine or alum only. We assessed the immunogenicity endpoints in the per-protocol population, which included all participants who had randomly received two doses of vaccine or alum only, had antibody results available, and did not violate the trial protocol.

We did not determine the sample sizes on the basis of a statistical power calculation, but followed the requirements of the China National Medical Products Administration and Chinese Technical Guidelines for Clinical Trials of Vaccines—ie, recruitment of at least 20–30 participants in phase 1 and 300 participants in phase 2 trial.

We used the Pearson χ^2 test or Fisher's exact test for the analysis of categorical outcomes. We calculated the 95% CIs for all categorical outcomes using the Clopper-Pearson method. We calculated GMTs and corresponding 95% CIs on the basis of the standard normal distribution of the log-transformation antibody titre. We used the ANOVA method to compare the log-transformed antibody titres. When the comparison among all groups showed significant difference, we then did pairwise comparisons. Hypothesis testing was two-sided and we considered a p value of less than 0·05 to be significant.

An independent data monitoring committee consisting of one independent statistician, one clinician, and one epidemiologist was established before commencement of the study. Safety data were assessed and reviewed by the committee to ensure further proceeding of the study. We used SAS (version 9.4) for all analyses. This trial is registered with ClinicalTrials.gov, NCT04551547.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Employees of Sinovac Life Sciences and Sinovac Biotech, listed as the authors, contributed to the study design, data interpretation, clinical trial monitoring, writing or revising the manuscript.

Results

Between Oct 31, 2020, and Dec 2, 2020, 110 individuals were screened and 72 were enrolled in phase 1. Between Dec 12 and Dec 30, 2020, 515 individuals were screened and 480 were enrolled in phase 2. 550 (>99%) of

	Phase 1			Phase 2		
	1·5 μg group (n=27)	3 μg group (n=26)	Aluminium hydroxide only group (n=18)	1·5 μg group (n=192)	3·0 μg group (n=191)	Aluminium hydroxide only group (n=96)
Age, years	8·4 (4·2)	8·2 (4·0)	8·3 (4·0)	9·3 (3·9)	9·2 (3·8)	9·1 (4·0)
3–5	9 (33%)	9 (35%)	6 (33%)	48 (25%)	47 (25%)	24 (25%)
6–11	9 (33%)	9 (35%)	6 (33%)	72 (38%)	72 (38%)	36 (38%)
12–17	9 (33%)	8 (31%)	6 (33%)	72 (38%)	72 (38%)	36 (38%)
Sex						
Male	10 (37%)	12 (46%)	8 (44%)	105 (55%)	108 (57%)	54 (56%)
Female	17 (63%)	14 (54%)	10 (56%)	87 (45%)	83 (43%)	42 (44%)
Han ethnicity	27 (100%)	26 (100%)	18 (100%)	192 (100%)	191 (100%)	96 (100%)
Height, m	1·3 (0·2)	1·3 (0·3)	1·3 (0·3)	1·4 (0·2)	1·4 (0·2)	1·4 (0·2)
Weight, kg	34·3 (15·7)	35·0 (14·9)	34·9 (17·7)	40·4 (19·0)	37·9 (16·9)	39·2 (18·9)

Data are mean (SD) or n (%).

Table 1: **Baseline characteristics**

	1·5 μg group (n=219)	3·0 μg group (n=217)	Aluminium hydroxide only group (n=114)	Total (n=550)	p value*
Solicited adverse reactions within 0–7 days					
Any	51 (23%)	59 (27%)	22 (19%)	132 (24%)	0·28
Grade 1	39 (18%)	51 (24%)	15 (13%)	105 (19%)	0·065
Grade 2	16 (7%)	19 (9%)	9 (8%)	44 (8%)	0·82
Grade 3	2 (1%)	0	0	2 (<1%)	0·36
Injection site adverse reactions					
Pain	36 (16%)	35 (16%)	2 (2%)	73 (13%)	<0·0001
Grade 1	34 (16%)	35 (16%)	2 (2%)	71 (13%)	<0·0001
Grade 2	2 (1%)	0	0	2 (<1%)	0·36
Swelling	3 (1%)	6 (3%)	1 (1%)	10 (2%)	0·50
Grade 1	0	4 (2%)	0	4 (1%)	0·053
Grade 2	3 (1%)	3 (1%)	1 (1%)	7 (1%)	1·0
Induration	0	2 (1%)	0	2 (<1%)	0·20
Grade 1	0	2 (1%)	0	2 (<1%)	0·20
Erythema	0	1 (<1%)	0	1 (<1%)	0·60
Grade 1	0	1 (<1%)	0	1 (<1%)	0·60
Pruritus	3 (1%)	2 (1%)	0	5 (1 %)	0·64
Grade 1	3 (1%)	2 (1%)	0	5 (1%)	0·64
Systematic adverse reactions					
Fever	9 (4%)	11 (5%)	5 (4%)	25 (5%)	0·93
Grade 1	3 (1%)	2 (1%)	2 (2%)	7 (1%)	0·89
Grade 2	4 (2%)	10 (5%)	3 (3%)	17 (3%)	0·22
Grade 3	2 (1%)	0	0	2 (<1%)	0·36
Cough	5 (2%)	8 (4%)	5 (4%)	18 (3%)	0·47
Grade 1	1 (<1%)	4 (2%)	3 (3%)	8 (1%)	0·19
Grade 2	4 (2%)	4 (2%)	2 (2%)	10 (2%)	1·0
Headache	6 (3%)	4 (2%)	3 (3%)	13 (2%)	0·82
Grade 1	3 (1%)	3 (1%)	1 (1%)	7 (1%)	1·0
Grade 2	4 (2%)	1 (<1%)	2 (2%)	7 (1%)	0·39
Anorexia	3 (1%)	4 (2%)	2 (2%)	9 (2%)	0·92
Grade 1	1 (<1%)	3 (1%)	2 (2%)	6 (1%)	0·52
Grade 2	3 (1%)	1 (<1%)	0	4 (1%)	0·54

(Table 2 continues on next page)

	1·5 µg group (n=219)	3·0 µg group (n=217)	Aluminium hydroxide only group (n=114)	Total (n=550)	p value*
(Continued from previous page)					
Diarrhoea	2 (1%)	2 (1%)	4 (4%)	8 (1%)	0·16
Grade 1	2 (1%)	2 (1%)	4 (4%)	8 (1%)	0·16
Nausea	3 (1%)	2 (1%)	2 (2%)	7 (1%)	0·89
Grade 1	3 (1%)	2 (1%)	2 (2%)	7 (1%)	0·89
Mucocutaneous eruption	2 (1%)	2 (1%)	1 (1%)	5 (1%)	1·0
Grade 1	1 (<1%)	1 (<1%)	0	2 (<1%)	1·0
Grade 2	1 (<1%)	1 (<1%)	1 (1%)	3 (1%)	1·0
Vomiting	3 (1%)	1 (<1%)	1 (1%)	5 (1%)	0·85
Grade 1	3 (1%)	1 (<1%)	1 (1%)	5 (1%)	0·85
Muscle pain	4 (2%)	0	0	4 (1%)	0·078
Grade 1	2 (1%)	0	0	2 (<1%)	0·36
Grade 2	2 (1%)	0	0	2 (<1%)	0·36
Fatigue	1 (<1%)	1 (<1%)	1 (1%)	3 (1%)	1·0
Grade 1	1 (<1%)	1 (<1%)	1 (1%)	3 (1%)	1·0
Grade 2	1 (<1%)	0	0	1 (<1%)	1·0
Hypersensitivity	0	0	1 (1%)	1 (<1%)	0·21
Grade 1	0	0	1 (1%)	1 (<1%)	0·21
Unsolicited adverse reactions within 0–28 days					
Any	11 (5%)	15 (7%)	9 (8%)	35 (6%)	0·52
Grade 1	2 (1%)	3 (1%)	3 (3%)	8 (1%)	0·43
Grade 2	10 (5%)	12 (6%)	7 (6%)	29 (5%)	0·75
Overall adverse reactions within 0–28 days					
Any	56 (26%)	63 (29%)	27 (24%)	146 (27%)	0·55
Grade 1	40 (18%)	52 (24%)	18 (16%)	110 (20%)	0·16
Grade 2	22 (10%)	24 (11%)	15 (13%)	61 (11%)	0·67
Grade 3	2 (1%)	0	0	2 (<1%)	0·36

Data are n (%), representing the total number of participants who had adverse reactions (ie, adverse events related to vaccination). Results are broken down by dose and age group in the appendix (pp 2–10). *For differences across all groups.

Table 2: **Adverse reactions reported within 28 days after the first and the second dose of vaccine or alum only in phase 1 and phase 2**

552 enrolled participants received the first dose of vaccine or alum only (71 in phase 1 and 479 in phase 2) and were included in the safety population (figure 1). 69 (96%) participants in phase 1 received the second dose and all were eligible for the immunogenic evaluation at day 28 after the second dose (per-protocol population; figure 1). In phase 2, 467 (97%) participants received the second dose and 460 (96%) were included in the per-protocol population (figure 1). Seven participants were excluded because one received tetanus immunoglobulin at day 14 after the second dose, five did not have a blood sample taken at 28 days after the second dose, and one took a blood sample outside of the specified time window. The demographic characteristics of the participants were similar in terms of sex, mean age, height, weight, and ethnicity among groups. The mean age of study participants was 8·3 years (SD 4·0) in phase 1, including 24 (34%) of 71 participants aged 3–5 years, 24 (34%) aged 6–11 years, and 23 (32%) aged 12–17 years. The mean age of study participants was 9·2 years (3·9) in phase 2, including 119 (25%) of 479 participants aged 3–5 years, 180 (38%) aged 6–11 years, and 180 (38%) aged 12–17 years (table 1).

The safety data of the phase 1 and phase 2 trial were combined for analysis because the same batches of the vaccine and alum only and the same safety observation method were used. 146 (27%) of 550 participants reported at least one adverse reaction within 28 days of either vaccination, and the proportions of participants with any adverse reactions were similar across groups. Most adverse reactions were mild (grade 1) and moderate (grade 2) in severity. Only two (<1%) of 550 had grade 3 adverse reactions. Most adverse reactions occurred within 7 days after vaccination and participants recovered within 48 h. The most common reactions were injection site pain (73 [13%] participants) and fever (25 [5%]). Except for a higher prevalence of injection site pain in two vaccine groups than that in alum-only group, there

	1·5 µg group		3·0 µg group		Aluminium hydroxide only group		p value	
	Rate	% (95%) CI	Rate	% (95%) CI	Rate	% (95%) CI	Three groups	1·5-µg *vs* 3·0-µg group
Phase 1								
Total	27/27	100·0% (87·2–100·0)	26/26	100·0% (86·8–100·0)	0/16	0·0% (0·0–20·6)	<0·0001	1·0
3–5 years	9/9	100·0% (66·4–100·0)	9/9	100·0% (66·4–100·0)	0/5	0·0% (0·0–52·2)	<0·0001	1·0
6–11 years	9/9	100·0% (66·4–100·0)	9/9	100·0% (66·4–100·0)	0/6	0·0% (0·0–45·9)	<0·0001	1·0
12–17 years	9/9	100·0% (66·4–100·0)	8/8	100·0% (63·1–100·0)	0/5	0·0% (0·0–52·2)	<0·0001	1·0
Phase 2								
Total	180/186	96·8% (93·1–98·8)	180/180	100·0% (98·0–100·0)	0/94	0·0% (0·0–3·9)	<0·0001	0·030
3–5 years	46/46	100·0% (92·3–100·0)	45/45	100·0% (92·1–100·0)	0/24	0·0% (0·0–14·2)	<0·0001	1·0
6–11 years	68/69	98·6% (92·2–100·0)	68/68	100·0% (94·7–100·0)	0/35	0·0% (0·0–10·0)	<0·0001	1·0
12–17 years	66/71	93·0% (84·3–97·7)	67/67	100·0% (94·6–100·0)	0/35	0·0% (0·0–10·0)	<0·0001	0·059

Data are n/N (% [95% CI]).

Table 3: **Seroconversion rates of neutralising antibody responses to live SARS-CoV-2 28 days after the second dose**

were no significant differences in the prevalence of other solicited or unsolicited reactions among the three groups (table 2). In an exploratory analysis by age, the prevalence of adverse reactions was highest in participants aged 12–17 years (72 [35%] of 203 participants) followed by 3–5 years (37 [26%] of 143 participants) and 6–11 years (37 [18%] of 204 participants; appendix pp 8–10). As of June 12, 2021, only one participant in the alum-only group has reported one serious adverse event (pneumonia; appendix p 15), which was considered to be unrelated to vaccination. Additionally, only two (3%) of 71 participants at day 3 after the first dose and two (3%) of 69 participants after the second dose in phase 1 had a significant increase of laboratory indicator (appendix p 11).

In phase 1, none of the participants had any detectable neutralising antibody response against live SARS-CoV-2 at baseline (appendix p 12). The seroconversion rates at day 28 after the second dose were 27 (100%) of 27 participants in the 1·5 µg group (GMT 55·0 [95% CI 38·9–77·9]) and 26 (100%) of 26 in the 3·0 µg group (117·4 [87·8–157·0]). The GMT of the 3·0 µg group was significantly higher than that of the 1·5 µg group (p=0·0012; table 3, figure 2, appendix p 12). Testing for neutralising antibodies in all alum-only recipients was negative after vaccination (appendix p 12). In an exploratory analysis by age, seroconversion rates at day 28 after the second dose of 1·5 µg or 3·0 µg vaccine were all 100% in participants aged 3–5 years, 6–11 years, and 12–17 years, with the GMTs ranging from 45·9 to 212·6 (figure 2, appendix p 14).

In phase 2, none of the participants had any detectable neutralising antibody response at baseline (appendix p 13). After the second dose of vaccination, the seroconversion rates were 180 (95% CI 96·8% [93·1–98·8]) of 186 participants in the 1·5 µg group (GMT 86·4 [73·9–101·0]) and 180 (100·0% [98·0–100·0]) of 180 participants in the 3·0 µg group (142·2 [124·7–162·1]). The seroconversion rate and GMT of the 3·0 µg group were higher than those of the 1·5 µg group (p=0·030 and p<0·0001; table 3, figure 2, appendix p 13). Neutralising antibodies in all alum-only recipients were negative after vaccination (appendix p 13). In an exploratory analysis by age, the seroconversion rates at day 28 after the second dose were higher than 93% in the 1·5 µg and 3·0 µg groups for participants aged 3–5 years, 6–11 years, and 12–17 years, with the GMTs ranging from 78·3 to 146·0 (figure 2, appendix p 14).

Discussion

To our knowledge, this is the first report of immunogenicity and safety of COVID-19 candidate vaccine among children as low as 3 years old. We found that two

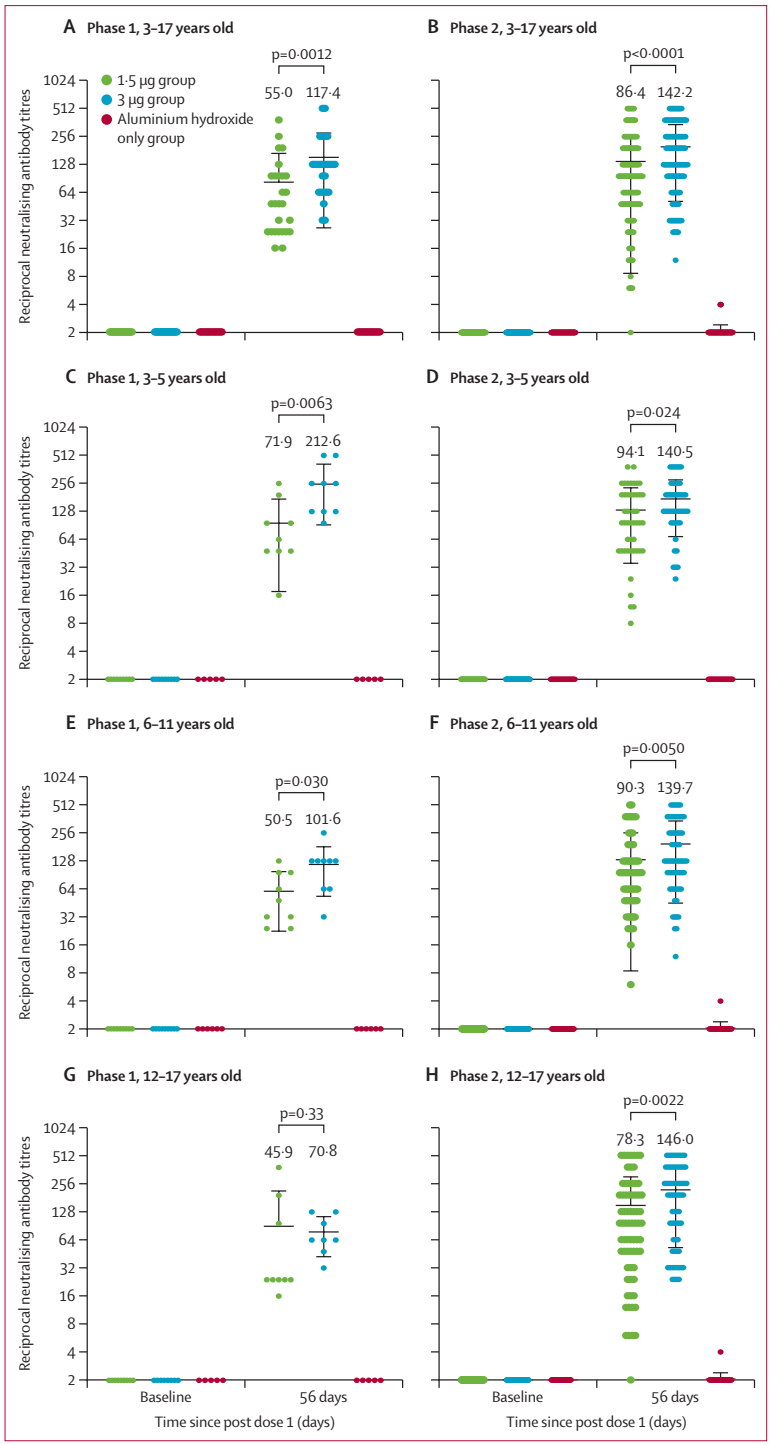

Figure 2: **Antibody titres of neutralising antibodies to live SARS-CoV-2 induced after two doses of CoronaVac or aluminium hydroxide diluent only in phase 1 and phase 2 trials**

GMT=geometric mean titre.The error bars indicate the 95% CI of the GMT and the spots indicate the individual antibody titres, with the number above the spots showing the GMT estimate. Only p values between 1·5 µg and 3·0 µg groups after the second vaccination are shown in the figure. All p values for all data are in the appendix (pp 12–13)

doses of the CoronaVac were safe and well tolerated at doses of 1·5 μg and 3·0 μg among children and adolescents aged 3–17 years old. The prevalence of adverse reactions in different dose groups was similar, indicating that there was no dose-related concern on safety. Most reactions were mild to moderate in severity and transient. Injection-site pain was the most reported symptom. The results were similar to our study of adults and elderly.[17,18] Furthermore, the higher grade 1 injection site pain reported by adolescents aged 12–17 years was the main reason for the higher prevalence of adverse reactions in this population compared with children aged 3–5 years and 6–11 years. None of the serious adverse events reported during the trial was related to vaccination.

CoronaVac was immunogenic in children and adolescents aged 3–17 years. The seroconversion rates of neutralising antibody in children and adolescents with both doses were over 96% after the two-dose vaccination. The GMTs of 142·2 in the 3·0 μg groups were higher than that of 86·4 in the 1·5 μg group in phase 2; however, even the GMT of 86·4 induced better immunogenicity compared with adults aged 18–59 years (44·1) and those aged 60 years and older (42·2) who received a 3·0 μg dose of vaccine with the same immunisation schedule.[17,18] Age plays an important role in antibody response to vaccine.[27] Decreasing responses to vaccination with increasing age have been shown in other vaccines, such as hepatitis B vaccine, seasonal influenza, pneumococcal disease, tetanus, pertussis, and diphtheria.[27,28] The results implied that a lower dose of vaccine could induce higher immune response in children and adolescents.

In an exploratory analysis stratified by age, we did not observe significant differences in neutralising antibody responses between age groups (3–5 years, 6–11 years, and 12–17 years) after the second vaccination (appendix p 14). GMTs in phase 1 decreased with age in recipients of the same vaccine, whereas they were similar in phase 2. Small sample size might account for the change trends of GMT in phase 1. In each age group, there were significant differences in GMTs between the 1·5 μg and 3·0 μg groups after the second dose, except in the group aged 12–17 years old in phase 1. Taken together, the 3·0 μg dose of CoronaVac induced higher immune responses in all age groups compared with the 1·5 μg dose.

Evidence from various studies supports the important role of T-cell responses to SARS-CoV-2 infection,[29] and such responses have been found with use of different vaccine platforms, including mRNA, viral vectors, and recombinant proteins.[30] In this study, T cell responses were not assessed, which was a limitation of the study design. However, a study in Chile found a significant induction of a T-cell response characterised by the secretion of interferon-gamma following vaccination of CoronaVac in a population aged 18 years and older,[19], which was different from the lower response observed in our phase 1 trial among adults aged 18–59 years.[17] Another inactivated SARS-CoV-2 vaccine, BBV152, has also been reported to induced a Th1-biased response.[21,24] Future studies are needed to assess the responses of type 1 and type 2 T-helper cells by inactivated vaccines.

This study has some further limitations. First, the sample size of this study is relatively small per age group and all study populations were of Han ethnicity. Further studies will be done in different regions and multiethnic populations to collect more data to provide scientific evidence for immune strategy. Second, at the time of the report, long-term immunogenicity and safety could not be available, although the participants will be followed up for at least 1 year. Finally, the calculated p values cannot support any powerful statistical conclusions in this study, which are only for reference and should be interpreted with caution.

In conclusion, CoronaVac was well tolerated and safe, and induced humoral responses in children and adolescents aged 3–17 years. Among the two doses evaluated, the neutralising antibody titres induced by a 3·0 μg dose were higher than those of the 1·5 μg dose. The results support the use of 3·0 μg dose with a two-immunisation schedule for further studies in children and adolescents.

Contributors

QL, QG, YZ, BH, and YS designed the trial and study protocol. BH, WY, and ML contributed to the literature search. All authors had access to data, and YS and QL verified the data. BH and WY wrote the first draft manuscript. QG, QL, YS, ML, XL, and YZ contributed to the data interpretation and revision of the manuscript. ZJ and QS contributed to data analysis. LW monitored the trial. QM and WJ were responsible for the site work including the recruitment, follow-up, and data collection, and ZW was the site coordinator. CL were responsible for the laboratory analysis. All the authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

QG and XL are employees of Sinovac Life Sciences. YS, WY, and LW are employees of Sinovac Biotech. All other authors declare no competing interests.

Data sharing

The individual participant-level data that underlie the results reported in this Article will be shared after de-identification (text, tables, figures, and appendices). This clinical trial is ongoing, and all the individual participant data will not be available until the immune persistence evaluation is completed. The data will be available immediately after publication and finalisation of the completed clinical study report for at least 6 months. Supporting clinical documents including the study protocol and statistical analysis plan and the informed consent form will be available immediately following publication of this Article for at least 1 year. Information on how to access the supporting clinical documents is available online. Researchers who provide a scientifically sound proposal will be allowed to access to the de-identified individual participant data. Proposals should be sent to the corresponding author. These proposals will be reviewed and approved by the sponsor, investigators, and collaborators on the basis of scientific merit. To gain access, data requestors will need to sign a data access agreement.

Acknowledgments

This study was funded by the National Key Research and Development Program and Beijing Science and Technology Program.

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7. Dose de reforço multiplica anticorpos

7.1 Dose de reforço da CoronaVac eleva proteção contra Covid-19 para 80%, de acordo com governo chileno

O Ministério da Saúde do Chile anunciou que a aplicação de uma dose de reforço da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19 aumenta a eficácia do imunizante para 80,2%, e eleva a proteção contra hospitalizações de 84% para 88%. A pesquisa, divulgada no início do mês, analisou o desempenho das três vacinas disponíveis no país (CoronaVac, Pfeizer e Astrazeneca) na prevenção de casos e internações pela doença com base na campanha nacional de vacinação contra o SARS-CoV-2.

A principal conclusão é que o uso da dose de reforço da CoronaVac traz resultados muito semelhantes aos das demais vacinas, aumentando de forma considerável os níveis de eficácia contra a Covid-19 sintomática. Em relação à proteção contra casos em geral, a vacina da Pfizer-BioNTech aumentou o indicador de 56% para 90%, e a da AstraZeneca, de 56% para 93%. Já contra hospitalizações, a Pfizer-BioNTech causou um aumento de 84% para 87% na proteção, e a AstraZeneca, de 84% para 96,3%.

O estudo incluiu 4.785.749 pessoas imunizadas com o esquema completo de duas doses da CoronaVac, das quais 2.017.878 receberam a dose de reforço a partir de 11 de agosto. Destes, 1.506.154 tomaram a dose de reforço da AstraZeneca, 371.592 receberam a dose de reforço da Pfizer e 140.132, da CoronaVac. Todos os participantes são maiores de 16 anos, sem histórico de infecção por SARS-CoV-2.

De acordo com o infectologista e assessor do Ministério da Saúde do Chile Rafael Araos, o estudo revela que a decisão de aplicar a dose adicional para prevenir a Covid-19 foi acertada. "As três vacinas que usamos como reforço em pessoas que foram vacinadas com a CoronaVac têm um efeito superpoderoso", afirmou o médico. "Os resultados são robustos e sugerem que o efeito da dose de reforço, com qualquer vacina, é altamente eficaz na prevenção da Covid-19 e hospitalizações."

Segundo dados oficiais, cerca de 15,2 milhões de chilenos (89% da população) já estão totalmente imunizados contra o SARS-CoV-2, e mais de 3,6 milhões de pessoas receberam a dose de reforço.

Publicado em: 29/10/2021

Ministerio de Salud

Gobierno de Chile

Immunization Campaign against SARS-CoV-2

Early estimates of the effectiveness of booster shots in Chile

Grupo para estudio de vacunas SARS-CoV-2 MINSAL (vCovid MINSAL)

October 2021

BACKGROUND

1ª ENTREGA DE RESULTADOS ESTUDIO DOSIS DE REFUERZO

- Evidence suggest that neutralizing antibodies against SARS-CoV-2 induced by vaccines wane over time, which may decrease their effect against Covid-19 and it consequences.
- The longitudinal effectiveness assessments performed by the Chile Ministry of Health showed a sharp discrease in the effectiveness to prevent Covid-19, specifically within the group immunized with inactivated vaccines early on.
- International studies have shown that the combination of vaccines is safe and effectively increase levels of SARS-CoV-2 neutralizing antibodies.

2

DESIGN AND METHODS

- We analized a cohort of people that are affiliated with the National Health Fund (FONASA):
  - **Aged 16 years or older**
  - **No history of SARS-CoV-2 infection** (confirmed of probable Covid-19).
  - **That have already received CoronaVac** as a primary immunization.
- The effectiveness was estimated for each vaccine booster and focuses on preventing Covid-19 or Covid-19 related hospitalization. Outcomes were compared to the unvaccinated population.

3

DESIGN AND METHODS

- The effectivenes was estimated 14 days after receiving the booster shot with any of the available vaccines.
- The comparison groups consisted of people that received the booster dose or not. All the people contributed (person-days) to the non vaccinated group before starting their vaccination schedule.
- The results are independent from age, sex, place of residence, presence of comorbilities, nationality and income level.

4

RESULTS | CHARACTERISTICS OF THE COHORT

1ª ENTREGA DE RESULTADOS ESTUDIO DOSIS DE REFUERZO

- 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

1ª ENTREGA DE RESULTADOS ESTUDIO DOSIS DE REFUERZO

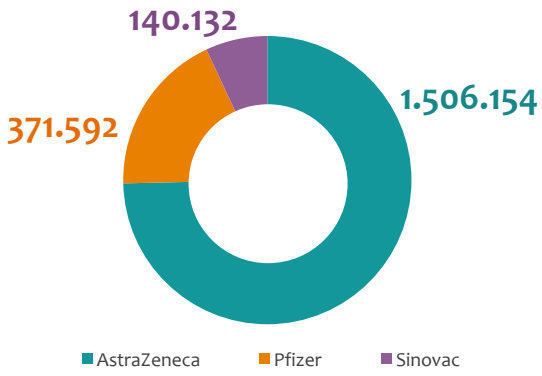
		Covid-19		Unvaccinated	Vaccinated			
					1 dose	2 doses	3 doses	
Characteristic	N (%)	N (row %)	p-value	N (row %)	N (row %)	N (%)	N (%)	p-value
Total	11,201,635 (100.0)	500,145 (4.5)	-	1,318,288 (11.7687)	719,263 (6.4211)	7,146,206 (63.7961)	2,017,878 (18.0141)	-
Region								
Arica	144,726 (1.3)	6,695 (4.6)	< 0.0001	21,489 (14.85)	10,608 (7.33)	92,597 (63.98)	20,032 (13.84)	< 0.0001
Tarapacá	200,869 (1.8)	8,828 (4.4)		32,257 (16.06)	12,694 (6.32)	131,619 (65.52)	24,299 (12.1)	
Antofagasta	329,632 (2.9)	10,659 (3.2)		43,639 (13.24)	24,239 (7.353)	218,761 (66.37)	42,993 (13.04)	
Atacama	191,906 (1.7)	5,991 (3.1)		23,938 (12.47)	12,995 (6.772)	127,012 (66.18)	27,961 (14.57)	
Coquimbo	531,115 (4.7)	17,518 (3.3)		59,364 (11.18)	34,141 (6.428)	356,775 (67.17)	80,835 (15.22)	
Valparaíso	1,212,562 (11)	44,364 (3.7)		150,740 (12.43)	71,947 (5.933)	747,759 (61.67)	242,116 (19.97)	
Metropolitana	4,098,579 (37)	184,233 (4.5)		505,690 (12.34)	267,377 (6.524)	2,530,109 (61.73)	795,403 (19.41)	
L.G.B. O'Higgins	629,292 (5.6)	24,266 (3.9)		60,130 (9.555)	33,564 (5.334)	428,027 (68.02)	107,571 (17.09)	
Maule	762,796 (6.8)	38,424 (5)		73,288 (9.608)	45,159 (5.92)	508,071 (66.61)	136,278 (17.87)	
Ñuble	348,527 (3.1)	14,062 (4)		32,392 (9.294)	16,905 (4.85)	236,007 (67.72)	63,223 (18.14)	
Biobío	1,054,437 (9.4)	54,087 (5.1)		101,632 (9.639)	61,591 (5.841)	686,255 (65.08)	204,959 (19.44)	
Araucanía	683,250 (6.1)	41,357 (6.1)		86,887 (12.72)	45,239 (6.621)	439,443 (64.32)	111,681 (16.35)	
Los Ríos	273,268 (2.4)	17,420 (6.4)		32,246 (11.8)	17,606 (6.443)	179,344 (65.63)	44,072 (16.13)	
Los Lagos	584,765 (5.2)	25,950 (4.4)		76,188 (13.03)	47,534 (8.129)	372,198 (63.65)	88,845 (15.19)	
Aysén	61,227 (0.55)	2,199 (3.6)		7,143 (11.67)	6,980 (11.4)	38,864 (63.48)	8,240 (13.46)	
Magallanes	94,684 (0.85)	4,092 (4.3)		11,265 (11.9)	10,684 (11.28)	53,365 (56.36)	19,370 (20.46)	

RESULTS

1ª ENTREGA DE RESULTADOS ESTUDIO DOSIS DE REFUERZO

4.785.749 people immunized with CoronaVac were included.

2.017.878 received one booster shot.



7

RESULTS

1ª ENTREGA DE RESULTADOS ESTUDIO DOSIS DE REFUERZO

INCREASED EFFECTIVINESS AGAINST COVID-19

14 DAYS AFTER THE BOOSTER SHOT

Booster shot CoronaVac	Booster shot Pfizer-BioNTech	Booster shot AstraZeneca
56% to 80%	56% to 90%	56% to 93%

8

RESULTS

1ª ENTREGA DE RESULTADOS ESTUDIO DOSIS DE REFUERZO

INCREASED EFFECTIVINESS AGAINST HOSPITALIZATION

14 DAYS AFTER THE BOOSTER SHOT

Booster shot CoronaVac	Booster shot Pfizer-BioNTech	Booster shot AstraZeneca
84% to 88%	84% to 87%	84% to 96%

9

CONCLUSION

1ª 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 decission to initiate a boosting program among people immunized with inactivated vaccines.

10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7.2 Dose de reforço da CoronaVac aumenta mais de 12 vezes o nível de anticorpos de quem tomou duas doses da vacina

Pesquisadores chilenos, americanos e chineses constataram que a dose de reforço da CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac, aumenta em mais de 12 vezes o nível de anticorpos de quem tomou as duas doses do imunizante há pelo menos cinco meses. Os resultados do estudo "A booster dose of an inactivated vaccine increases neutralizing antibodies and T cell responses against SARS-CoV-2" foram publicados nesta quarta (17) na plataforma de preprints medRxiv.

"Após a dose de reforço, a capacidade de neutralização dos anticorpos aumentou ainda mais do que a relatada duas semanas após a segunda dose. Observamos que, quatro semanas após a dose de reforço, a capacidade neutralizante aumentou mais de 12 vezes em comparação com a resposta cinco meses após a segunda dose, e aumentou mais de duas vezes em comparação com os níveis registrados duas semanas após a segunda dose", afirmam os pesquisadores, do Instituto Milênio de Imunologia e Imunoterapia, da Pontifícia Universidade Católica do Chile; do Instituto de Imunologia La Jolla, da Universidade da Califórnia em San Diego, nos Estados Unidos; e da Sinovac.

O estudo foi realizado com 129 voluntários que receberam a primeira dose da CoronaVac de janeiro a março de 2021, e a segunda com um intervalo de 28 dias. Decorridos cinco meses, os voluntários tomaram a dose de reforço. A capacidade de neutralização de anticorpos foi avaliada em 77 voluntários.

Em adultos entre 18 e 59 anos de idade, a capacidade de neutralização dos anticorpos circulantes atingiu seu máximo quatro semanas após a dose de reforço, aumentando mais de 18 vezes em comparação com os níveis registrados cinco meses após a segunda dose, e mais de quatro vezes em comparação com os níveis registrados duas semanas após a segunda dose. A soropositividade nesse grupo chegou a 100% quatro semanas após a segunda dose.

Em um esquema normal de imunização de duas doses com intervalo de 28 dias, o pico na capacidade de neutralização dos anticorpos é atingido duas semanas após a segunda dose. Entre maiores de 60 anos, que correspondiam a 53,2% dos voluntários, os pesquisadores observaram que após a dose de reforço houve um aumento de mais de nove vezes na capacidade neutralizante em relação à resposta observada cinco meses após a segunda dose.

Publicado em: 23/11/2021

A booster dose of an inactivated vaccine increases neutralizing antibodies and T cell responses against SARS-CoV-2.

Bárbara M Schultz[1,#], Felipe Melo-González[1,#], Luisa F Duarte[1,#], Nicolás MS Gálvez[1,#], Gaspar A Pacheco[1,#], Jorge A Soto[1,#], Roslye V Berríos-Rojas[1,2], Liliana A González[1,2], Daniela Moreno-Tapia[1,2], Daniela Rivera-Pérez[1,2], Guillermo Hoppe-Elsholz[1,2], Carolina Iturriaga[3], Mariana Ríos[1,2], Omar P Vallejos[1,2], Marcela Urzua[3], Yaneisi Vázquez[1,2], María S Navarrete[4], Álvaro Rojas[4], Daniela Weiskopf[5], Alessandro Sette[5,6], Gang Zeng[7], Weining Meng[7], CoronaVac03CL Study Group, José V González-Aramundiz[8], Pablo A González[1,2], Katia Abarca[1,3], Alexis M Kalergis*[1,2,9], Susan M Bueno*[1,2]

Affiliations:

[1]Millennium Institute on Immunology and Immunotherapy, Santiago, Chile. [2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. [3]Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. [4]Departamento de Enfermedades Infecciosas del Adulto, División de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. [5]Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), La Jolla, CA 92037, USA. [6]Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego (UCSD), La Jolla, CA 92037, USA. [7]Sinovac Biotech, Beijing, China.

[8]Departamento de Farmacia, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile. [9]Departamento de Endocrinología, Facultad de Medicina, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.

[#] These authors contributed equally to this work.

*Corresponding authors.

Keywords:

CoronaVac; Phase 3 clinical trial; SARS-CoV-2; COVID-19; Vaccines, Booster, Third dose.

Abstract

Numerous vaccines have been generated to decrease the morbidity and mortality of COVID-19. CoronaVac® is an inactivated SARS-CoV-2 vaccine approved by the World Health Organization (WHO) to prevent COVID-19 that has safety and immunogenicity profiles described in different clinical trials. We previously reported an increase in levels of neutralizing antibodies two- and four-weeks after administering two doses of CoronaVac® in a two-week interval (0-14 day) vaccination schedule, as compared to pre-immune sera in adults in the Chilean population that are participating in a phase 3 clinical trial. Here we report the levels of antibodies directed against the Receptor Binding Domain of the SARS-CoV-2 spike protein comparing their neutralizing capacities and the cellular response at five months after the second dose and four weeks after a booster (third) dose in volunteers immunized with two doses of CoronaVac® in a four-week interval (0-28 day) vaccination schedule. We observed a decrease in the levels of anti-SARS-CoV-2 antibodies with neutralizing capacities five months after the second dose (GMU 39.0 95% confidence interval (CI)□(32.4-47.0), which increased up to 12 times at four weeks after the booster dose (GMU 499.4, 95% CI=370.6-673.0). Equivalent results were observed in adults aged 18-59 years old and individuals ≥60 years old. In the case of cellular response, we observed that activation of specific $CD4^+$ T cell increases in time and reaches its maximum at four weeks after the booster dose in both groups. Our results support the notion that a booster dose of the SARS-CoV-2 inactivated vaccine increases the levels of neutralizing antibodies and the specific cellular response in adults of both groups, which is likely to boost the protective capacity of these vaccines against COVID-19.

Introduction

The ongoing pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has promoted the rapid development of safe, immunogenic, and effective vaccines against SARS-CoV-2 to be used by the general population, which have successfully reduced the transmission of the disease burden. CoronaVac® is an inactivated SARS-CoV-2 vaccine developed by Sinovac Life Sciences Co., Ltd. (Beijing, China) and is among the current vaccines approved by the WHO to combat COVID-19 [1,2]. Phase 1 and 2 clinical trials in China demonstrated that this vaccine induces cellular and humoral response upon immunization [3–5]. Furthermore, an ongoing phase 3 clinical trial in Chile has described that two- and four-weeks after the second dose of CoronaVac® there is an increase in the levels of IgG and neutralizing antibodies in adults aged 18-59 years old and ≥ 60 years old [5][6]. In addition, the vaccination promotes the activation of the cellular immune response against SARS-CoV-2 antigens in a 0-14 immunization schedule [5], being an effective vaccine to prevent COVID-19 [7,8]. In Chile, 91.5% of the target population has received the first vaccine dose, and 88.7% were fully vaccinated in October 2021 in a 0-28 vaccination schedule [9]. Although neutralizing antibody titers present in the serum of vaccinated people are thought to be highly predictive of immune protection [10], these titers decrease in time [6,11,12]. Besides this, vaccine-induce antibodies have lower levels of neutralization against highly transmissible variants of the virus as compared to the original vaccine strain, potentially decreasing the effectiveness of these vaccines as new variants emerge [13,14]. For these reasons, the use of booster doses was

approved in adults in August 2021 in Chile, in high-risk populations and subjects with more than five months after the second dose applied in a 0-28-day vaccination schedule [15]. Notably, a previous study performed in adults between 18-59 years old demonstrates that a booster dose of CoronaVac®, applied after six months to individuals previously receiving two doses of this vaccine, increases the levels of antibodies 3-5-fold as compared to those levels observed four weeks after the second dose [12]. Here, we further extend these results by reporting the levels of neutralizing antibodies and specific T cells against SARS-CoV-2 in adults ≥18 years old who participated in phase 3 clinical trial carried out in Chile, who were vaccinated in a 0-28-day vaccination schedule with a booster (third) dose five months after the second dose.

Materials and methods

Patients and sample collection

Blood samples were obtained from volunteers recruited in the clinical trial CoronaVac03CL (clinicaltrials.gov #NCT04651790) carried out in Chile starting January 2021. The Institutional Scientific Ethical Committee of Health Sciences reviewed and approved the study protocol at the Pontificia Universidad Católica de Chile (#200708006). Trial execution was approved by the Chilean Public Health Institute (#24204/20) and was conducted according to the current Tripartite Guidelines for Good Clinical Practices, the Declaration of Helsinki [16], and local regulations. Informed consent was obtained from all volunteers upon enrollment. Volunteers receive two doses of CoronaVac® (3 µg or 600SU of inactivated SARS-

CoV-2 inactivated along with alum adjuvant) in a four-week interval (0–28-day immunization schedule) and then a booster dose five months after the second dose. A complete inclusion and exclusion criteria list has been reported. On November 11st 2021, one hundred and eighty-six volunteers in the immunogenicity branch received the booster dose, and the antibodies against RBD with neutralizing capacities were quantified in 77 volunteers who had completed all their previous visits in one of the centers of the study (**Figure 1A**). Blood samples were obtained from all the volunteers before administration of the first dose (pre-immune), two weeks after the second dose, four weeks after the second dose, twenty weeks (or five months) after the 2nd dose, and four weeks after the booster (third) dose (**Figure 1B**).

Procedures

To assess the presence of antibodies against RBD with neutralizing capacities, blood samples from 77 volunteers that had completed all their study visits, including one month after the booster dose of CoronaVac®, were measured. The neutralizing capacities of circulating antibodies were evaluated by a surrogate virus neutralization test (sVNT) (Genscript Cat#L00847-A). Samples were serially two-fold diluted starting at a 4-fold until reaching a 512-fold dilution. Assays were performed according to the instructions of the manufacturer and as reported previously [5]. Neutralizing antibody titers were determined as the last fold dilution with a cut-off over 30% of inhibition. Samples with a percentage of inhibition ≤30 at lowest dilution (1:4) were assigned as seronegative with a titer of 2. A sample was considered seropositive when its titer is higher than the pre-immune titer. The

percentage of inhibition was determined as: 100 * [OD450nm value of negative control - OD450nm value of sample] / [OD450nm of negative control]. A standard curve was used to plot the neutralization response in the samples as international units (IU) by using the WHO International Standard for SARS-CoV-2 antibody (NIBSC code 20/136), which was prepared according to the manufacturer's instructions [17]. Data were analyzed using a sigmoidal curve model with log concentration transformed, and the final concentration for each sample was the average of the product of the interpolated IU from the standard curve and the sample dilution factor required to achieve the OD450 value that falls within the linear range. Samples with undetermined concentration at the lowest dilution tested (1:4) were assigned the lower limit of quantification (16.4 IU). The Geometric Mean Units (GMU) or titers (GMT) were represented in the **Figure 2** and **Supplementary Figure 1,** respectively**,** and **Table 1** for comparisons among the visits.

ELISPOT and flow cytometry assays were performed to evaluate the cellular immune response, stimulating PBMCs with four Mega Pools (MPs) of peptides derived from the proteome of SARS-CoV-2 [18]: peptides from the S protein of SARS-CoV-2 (MP-S), the remaining proteins of the viral particle (excluding S protein peptides) (MP-R), and of peptides from the whole proteome of SARS-CoV-2 (MP-CD8-A and MP-CD8-B) [18]. Positives and negative controls were held for each assay. The number of Spot Forming cells (SFC) for IFN-γ and IL-4 were determined by ELISPOT, and the expression of Activation-Induced Markers (AIM+) by T cells was evaluated by flow cytometry. Assays were performed according to the instructions of the manufacturer and as reported previously [5]. Further details

on the ELISPOT assay, antibodies used for flow cytometry, and the respective protocols can be found in the **Supplementary Table 1**.

Statistical analyses

Statistical differences for the immunogenicity results considered one-way ANOVAs mixed-effects analysis for comparisons between the booster dose and the other visits performed on the logarithms of the data. The significance level was set at 0.05 for all the analyses. All data were analyzed with GraphPad Prism 9.0.1.

Results

One hundred and twenty-nine volunteers from the immunogenicity branch, who received the booster dose of the CoronaVac®, were included in this study. The first dose of the vaccine was inoculated from January - March of 2021, and the second dose was inoculated 28 days after the first one. Of them, we evaluated the neutralization capacity of circulating antibodies in 77 volunteers at five different time points indicated previously by sVNT and 33 of the same volunteers by ELISPOT and flow cytometry (**Figure 1B**).

In a normal 0-28-day schedule, the peak in the neutralizing capacity of the antibodies is reached at two weeks after the second dose (GMT 25.8, 95% CI=19.5-34.2) (**Supp. Figure 1**), decreasing at four weeks after the second dose (GMT 16.6, 95% CI=13.1-21.0). However, this neutralizing capacity present an important decreased five months after the second dose (GMT 3.5, 95% CI=3.0-4.1), which is in line with previous reports where the immunity against SARS-CoV-2 wanes six months after infection or vaccination [19,20]. As expected, after the

booster dose, the neutralizing capacity of the antibodies increased even more than the one reported two weeks after the second dose. When we expressed the neutralizing capacity in arbitrary units of WHO (**Figure 2**) we observed that four weeks after the booster dose the neutralizing capacity increased more than 12-fold (GMU 499.4, 95% CI=370.6-673.0), as compared to the response at five months after the second dose (GMU 39.0 ± 32.4-47.0) and more than 2-fold as compared to the two weeks after the second dose (GMU 168.0 ± 126.8-222.5) (**Figure 2A**).

In adults between 18-59 years old, the neutralizing capacity of circulating antibodies reach its maximum four weeks after the booster dose (GMU 918.8 ± 623.4-1354) increasing more than 18-fold as compared to five months after the second dose (48.9 ± 37.6-63.5) and more than 4-fold as compared with two weeks after the second dose (GMU 220.2 ± 150.7-321.7) (**Figure 2B**). Seropositivity in this group reach 100% four weeks after the second dose (**Table 1**). 53.2% of the total volunteer analyzed here were adults ≥60 years. As seen in **Figure 2C**, the neutralizing capacity of circulating antibodies in this population also reached its peak at two weeks after the second dose (GMU 134.1 ± 89.2-201.6), decreasing at four weeks after the second dose (GMU 104.1 ± 71.8-151.0), and reaching its minimum at five months after the second dose (GMU 32.4 ± 25.1-41.8). In this group, we also observed an increase of more than 9-fold (GMU 300.5 ± 203.5-443.6) in the neutralizing capacity as compared to the response observed five months after the second dose (GMU 32.4). The seropositivity rate reached 49.4% in the total vaccine group and 35.7% in adults ≥60 years at five months after the second dose, which increased to 97.4% and 95.2%, respectively, four weeks after

the booster dose (**Table 1**). The seropositivity rate achieved at four weeks after the booster dose was the highest when compared with the other visits in the study in the total vaccinated group and in both groups analyzed.

Here we also report cellular responses following the booster dose of CoronaVac®, which is the first report of T cell responses in subjects vaccinated with a third dose of CoronaVac® to our knowledge. We did observe a significantly further increase in $CD4^+$ T cell activation in both age groups following the third booster dose by flow cytometry (**Figure 3**) but we did not see a further increase in IFN-γ production upon stimulation with S and R MPs by ELISPOT at that time point (**Supp. Figure 2**). In addition, $CD4^+$ T cell activation was still significantly increased 5 months after the 2^{nd} dose in both age groups, suggesting that the 0-28 schedule can stimulate $CD4^+$ cell responses over time. Moreover, we observed a significant increase in $CD8^+$ AIM^+ T cells following the third dose as compared to the time point 2 weeks following the second booster but not as compared to the pre-immune, whereas we did not observe a significant increase in IFN-γ upon stimulation with CD8 MPs at any time point, suggesting that CoronaVac promotes a reduced $CD8^+$ T cell responses, even after a third dose. Thus, although humoral responses decrease over time following vaccination with CoronaVac®, $CD4^+$ T cell responses stay significantly increased as compared to the pre-immune and the booster dose increases at least their activation.

Discussion

Although there was an adequate neutralization titer of anti-SARS-CoV-2 antibodies after two doses of CoronaVac® in the 0-28 schedule, with a 65.9% of effectiveness of preventing COVID-19 [8], the GMT waned in time, which was observed five months after the second dose. Due to this decrease in neutralizing capacity, a booster dose of CoronaVac® was evaluated in a clinical study in China, showing promising results in humoral immune responses [12]. The evaluation of the neutralization capacities reported here shows that after the booster dose, the neutralizing titers and seroconversion rates increase in the whole group even higher than two weeks after the second dose where was observed the peak in neutralization. As the neutralizing antibody titers correlate with protection against SARS-CoV-2 infection [10], these results likely imply a better outcome and protection against illness, as reported in previous studies performed in Israel that showed a decrease in the transmission and the severe disease by COVID-19 twelve or more days after booster inoculation [21]. Another study, performed with a booster dose of CoronaVac®, showed that an additional dose induced a good neutralization against SARS-CoV-2 WT strain and against variants four weeks after the booster dose, generating a long-lasting humoral response that was due to an enhancement of the memory immune response generated by B cells [22].

Adults ≥60 years old produced lower levels of antibodies with neutralizing capacities than the whole group during this study, which was also described in Bueno et al. [5]. This result is in line with previous data reported for a population vaccinated in Chile [6], a study among hospital workers who received two doses of CoronaVac® [23], and with the mRNA-1273 vaccine [24]. In this sense, our results are equivalent to those described in a phase 1/2 of the clinical trial with

CoronaVac®, showing that the neutralizing antibody titers in this group decrease at five months after the second dose and that a booster dose is required 6-8 months after the first vaccination to rapidly increased and steadily the neutralizing antibody titers [25].

In the case of cellular response, other studies have shown that Pfizer BNT162b2 and mRNA-1273 induce durable $CD4^+$ T cell activation and cytokine production up to six months following vaccination but it remains to be elucidated whether $CD4^+$ AIM^+ T cells and cytokine production further increase following a booster dose of these vaccines [26,27]. In contrast to these vaccines, CoronaVac® delivers not only the Spike protein but other viral antigens, which may explain why vaccinated individuals still display $CD4^+$ AIM^+ T cells five months after the second dose, without even a third dose.

Our report shows that the booster dose with CoronaVac® in a 0-28 schedule induces a higher production of antibodies with neutralizing capacities, which are higher than the levels observed with 2- and 4-weeks after the first doses, generating an increased humoral response even in adults ≥60 years old. Besides this, our results suggest that a third dose of CoronaVac® supports $CD4^+$ T cell activation, which may confer either protection or enhanced immune responses against the virus and prevent severe disease following SARS-CoV-2 exposure.

Limitations

This study presents some limitations, such as the reduced sample size for the assays. The assessment of total antibody response against Spike proteins and other SARS-CoV-2 proteins would also add additional information about the

humoral immune response against SARS-CoV-2 after the booster dose. Due to the limit of quantification of the technique, samples with undetermined concentration at the lowest dilution tested (1:4) were assigned the lower limit of quantification (16.4 IU) and other neutralization assays, such as conventional neutralization test, would confirm our results with the surrogate neutralization test used in this study.

Funding

This work was supported by: The Ministry of Health, Government of Chile supported the funding of the CoronaVac03CL Study; The Confederation of Production and Commerce (CPC), Chile, supported the funding of the CoronaVac03CL Study; the NIH NIAID under Contract No. 75N93021C00016 supports AS and Contract No. 75N9301900065 supports AS, DW. The Millennium Institute on Immunology and Immunotherapy, ANID - Millennium Science Initiative Program ICN09_016 (former P09/016-F) supports SMB, KA, PAG and AMK; The Innovation Fund for Competitiveness FIC-R 2017 (BIP Code: 30488811-0) supports SMB, PAG and AMK; SINOVAC contributed to this study with the investigational vaccine and experimental reagents.

Competing interests

ZG and MW are SINOVAC employees and contributed to the conceptualization of the study (clinical protocol and eCRF design) and did not participate in the analysis or interpretation of the data presented in the manuscript. All other authors declare no conflict of interest. A.S. is a consultant for Gritstone, Flow Pharma, Arcturus,

Immunoscape, CellCarta, OxfordImmunotech and Avalia. Jolla Institute for Immunology (LJI) has filed for patent protection for various aspects of T cell epitope and vaccine design work. All other authors declare no conflict of interest. The authors declare this study received the investigational product (placebo and vaccines) from the company SINOVAC Biotech. SINOVAC employees contributed to the conceptualization of the study (clinical protocol and eCRF design) but did not participate in either the analysis or interpretation of the data shown in this manuscript.

Acknowledgments

We would like to thank the support of the Ministry of Health, Government of Chile; Ministry of Science, Technology, Knowledge, and Innovation, Government of Chile; and The Ministry of Foreign Affairs, Government of Chile, and the Chilean Public Health Institute (ISP). We also would like to thank Rami Scharf, Jessica White, Jorge Flores and Miren Iturriza-Gomara from PATH for their support on experimental design and discussion. We also thank the Vice Presidency of Research (VRI), the Direction of Technology Transfer and Development (DTD), the Legal Affairs Department (DAJ) of the Pontificia Universidad Católica de Chile. We are also grateful to the Administrative Directions of the School of Biological Sciences and the School of Medicine of the Pontificia Universidad Católica de Chile for their administrative support. Members of the CoronaVac03CL Study Group are listed in the Supplementary Appendix (SA). We would also like to thank the members of the independent data safety monitoring committee (members in the SA) for their oversight, and the subjects enrolled in the study for their

participation and commitment to this trial. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. Contract No. 75N9301900065 to A.S, D.W.

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Figures

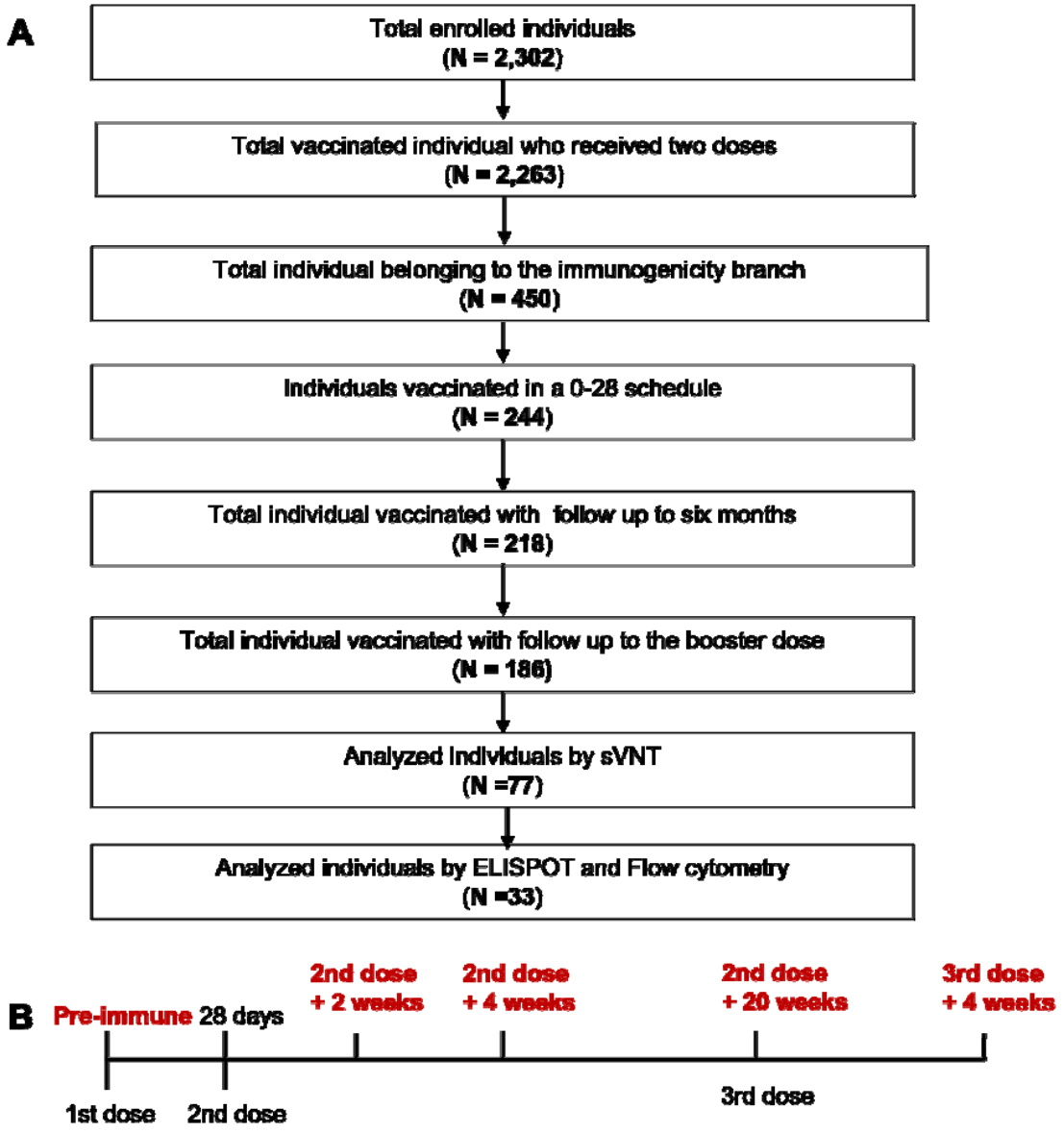


Figure 1: Study profile, enrolled volunteers and cohort included in this study on October 31st, 2021. 77 of the 450 vaccinated individuals belonging to the immunogenicity branch of the clinical trial conducted in Chile were selected of one of the centers of the study (the CL1-Marcoleta) for immunogenicity 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.

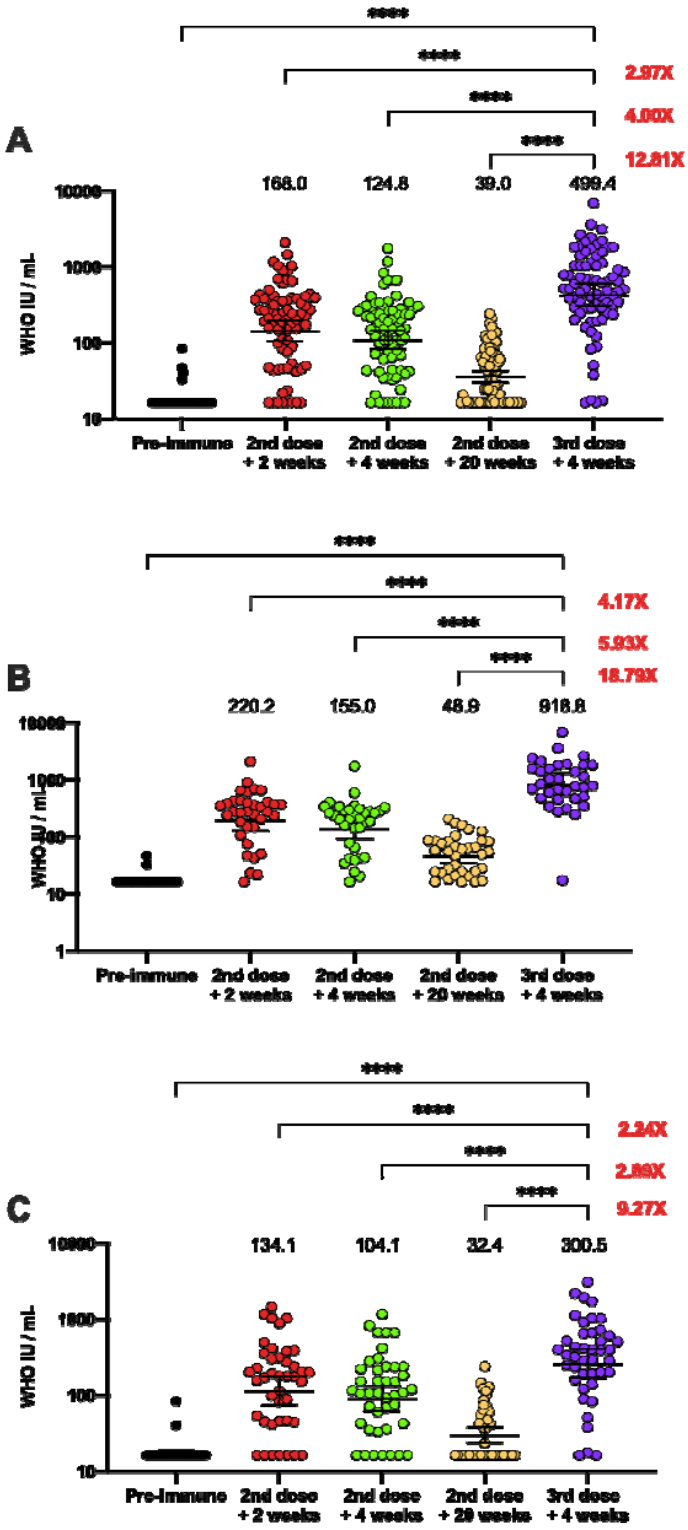


Figure 2: Quantification of circulating antibodies inhibiting the interaction between the S1-RBD and hACE2 in volunteers that received the booster dose twenty weeks after the second dose, in a 0–28-day vaccination schedule. Inhibiting antibody titer is expressed as international units by using a WHO 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.

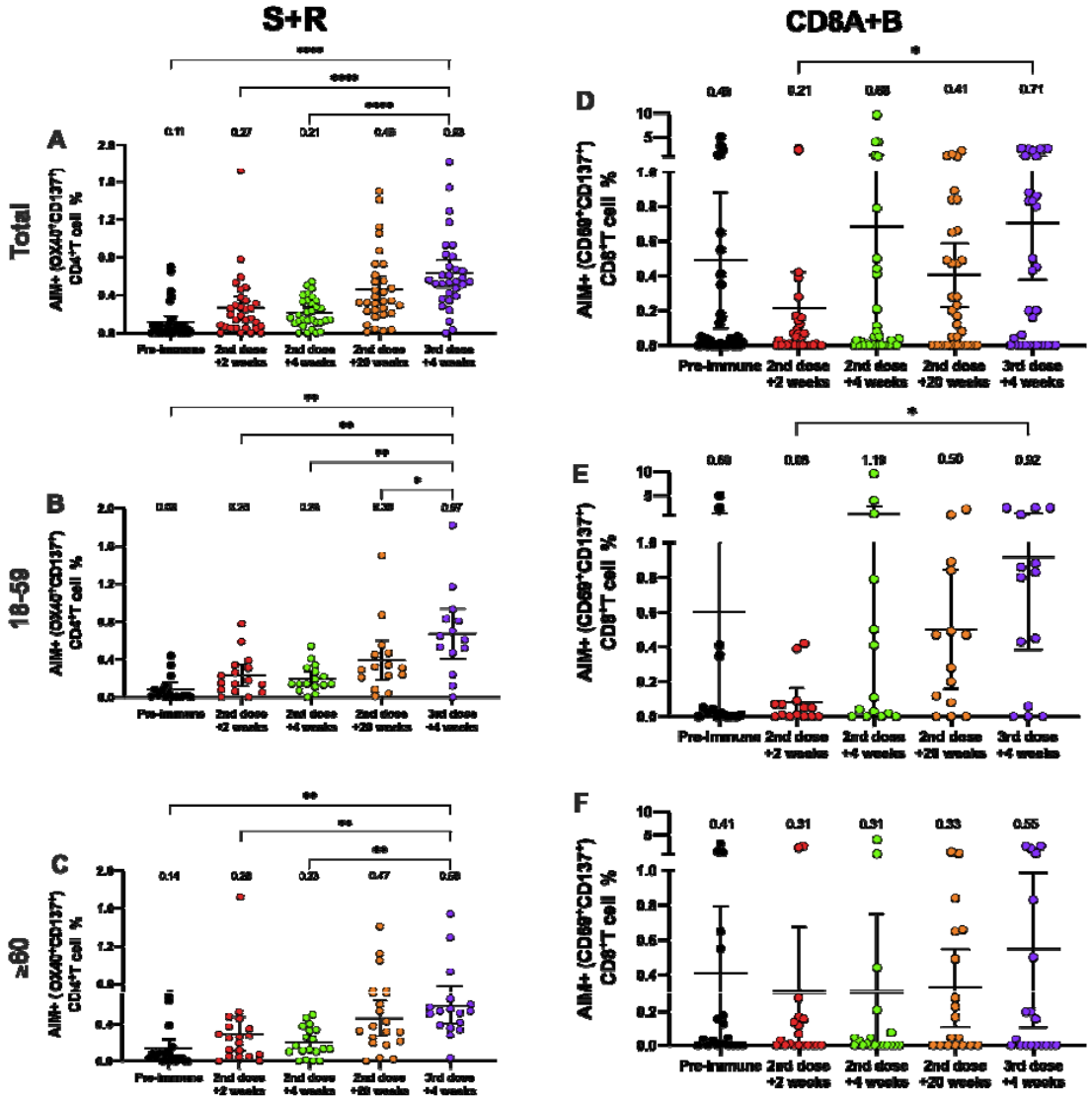


Figure 3: Changes in activation-induced markers (AIMs) expression in T cells through flow cytometry upon stimulation with Mega Pools of peptides derived from SARS-CoV-2 in volunteers immunized with CoronaVac with the booster dose, given twenty weeks after the second dose, in a 0–28-day vaccination schedule. The percentage of activated $CD4^+$ (AIM^+ [$OX40^+$, $CD137^+$]) and $CD8^+$ (AIM^+ [$CD69^+$, $CD137^+$]) T cells was determined by flow cytometry, upon

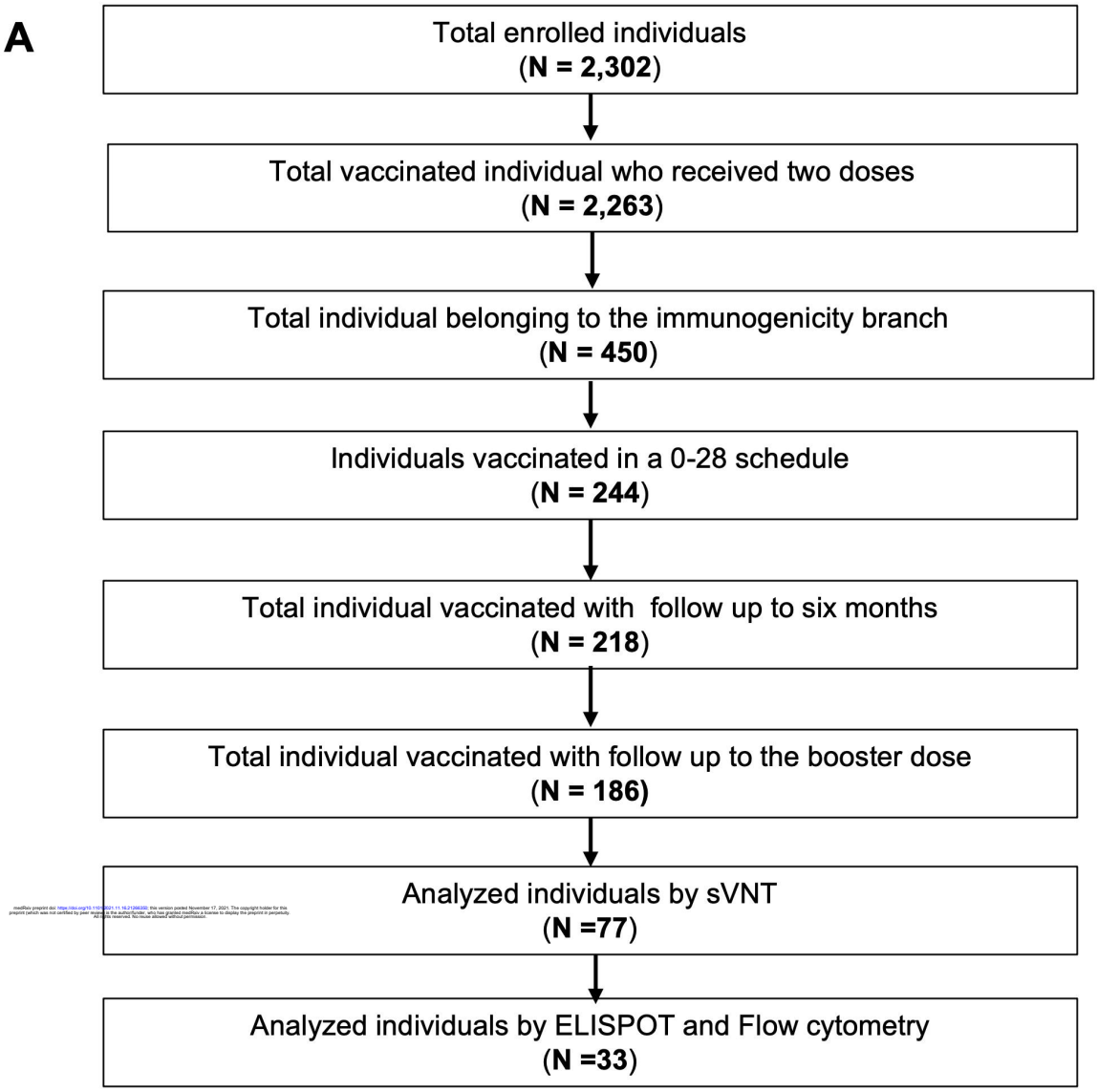
stimulation for 24h with MP-S+R (**A-C**) and MP-CD8A+B (**D-E**) in samples obtained at pre-immune, two weeks after the 2nd dose, four weeks after the 2nd dose, twenty weeks the 2nd dose, and four weeks after the 3rd dose. Results were obtained from a total of 33 volunteers (**A-D**), 14 were of them were adults between 18-59 years old (**B-E**), and 19 of them were ≥ 60 years old (**C-F**). Data shown represent means + 95%CI. Data from flow cytometry was normalized against DMSO and analyzed separately by One-way ANOVA with mixed effect analysis. *P<0.05; **p<0.005; ****p<0.0001.

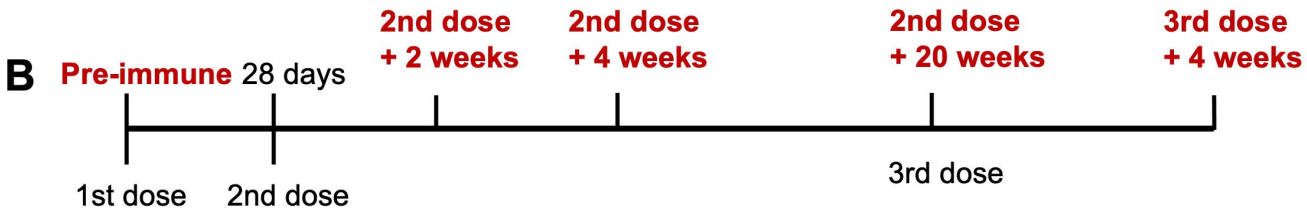
Table

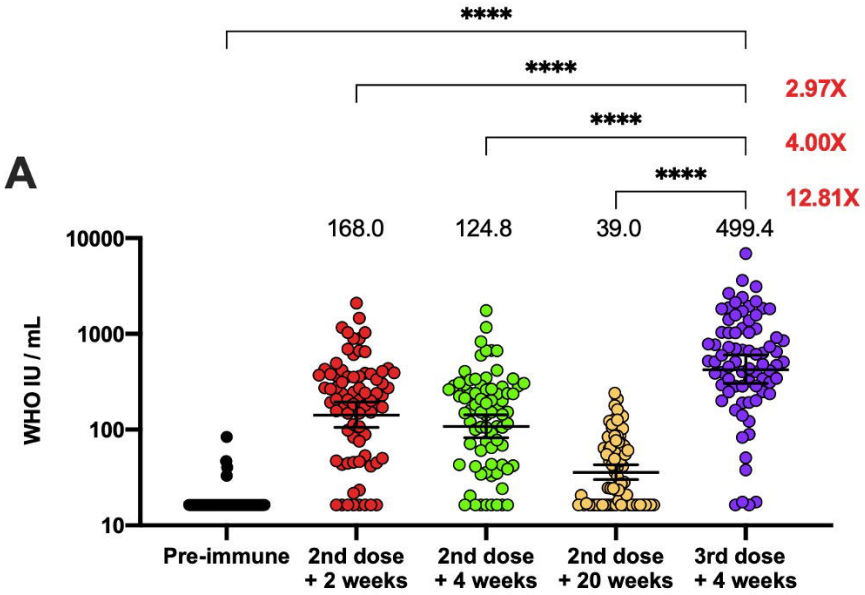
Table 1: Seropositivity rates, Geometric Median Titer (GMT), and Geometric Median Units (GMU) of circulating neutralizing antibodies against SARS-CoV-2 RBD.

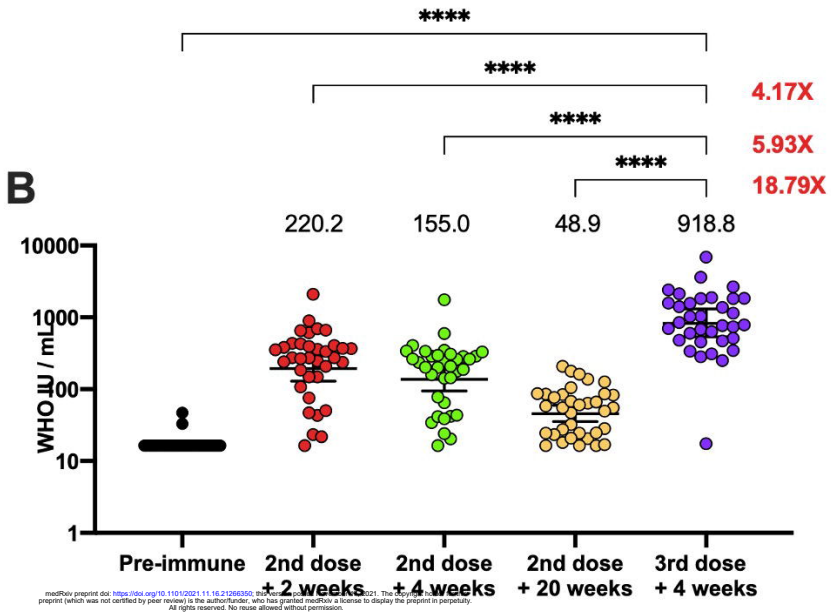
Age group	Indicators	2w after 2nd dose	4w after 2nd dose	5m after 2nd dose	4w after 2nd dose
Total Vaccine	Seropositivity n/N	72/77	73/77	38/77	75/77
	(%)	93.5	94.8	49.4	97.4
	GMU	168.0	124.8	39.0	499.4
	95% CI	126.8-222.5	96.3-161.7	32.4-47.0	370.6-673.0
	GMT	25.8	16.6	3.5	53.0
	95% CI	19.5-34.2	13.1-21.0	3.0-4.1	40.8-68.8
18-59	Seropositivity n/N	35/36	36/36	24/36	36/36
	(%)	97.2	97.2	66.7	100
	GMU	220.2	155.0	48.9	918.8
	95% CI	150.7-321.7	108.0-222.6	37.6-63.5	623.4-1354
	GMT	33.3	19.1	4.3	82.8
	95% CI	23.4-47.3	14.0-26.1	3.4-5.4	59.7-114.8
≥60	Seropositivity n/N	38/41	39/42	15/42	40/42
	(%)	90.5	92.9	35.7	95.2
	GMU	134.1	104.1	32.4	300.5
	95% CI	89.2-201.6	71.8-151.0	25.1-41.8	203.5-443.6
	GMT	20.8	14.7	2.9	36.5
	95% CI	13.6-31.9	10.3-21.0	2.4-3.5	25.3-52.7

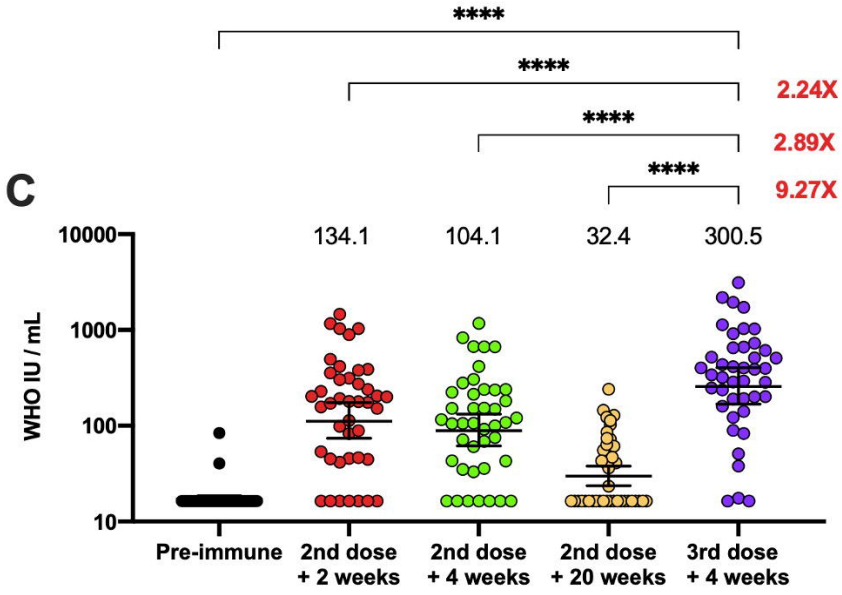
GMT: Geometric mean titer; GMU: Geometric mean units.

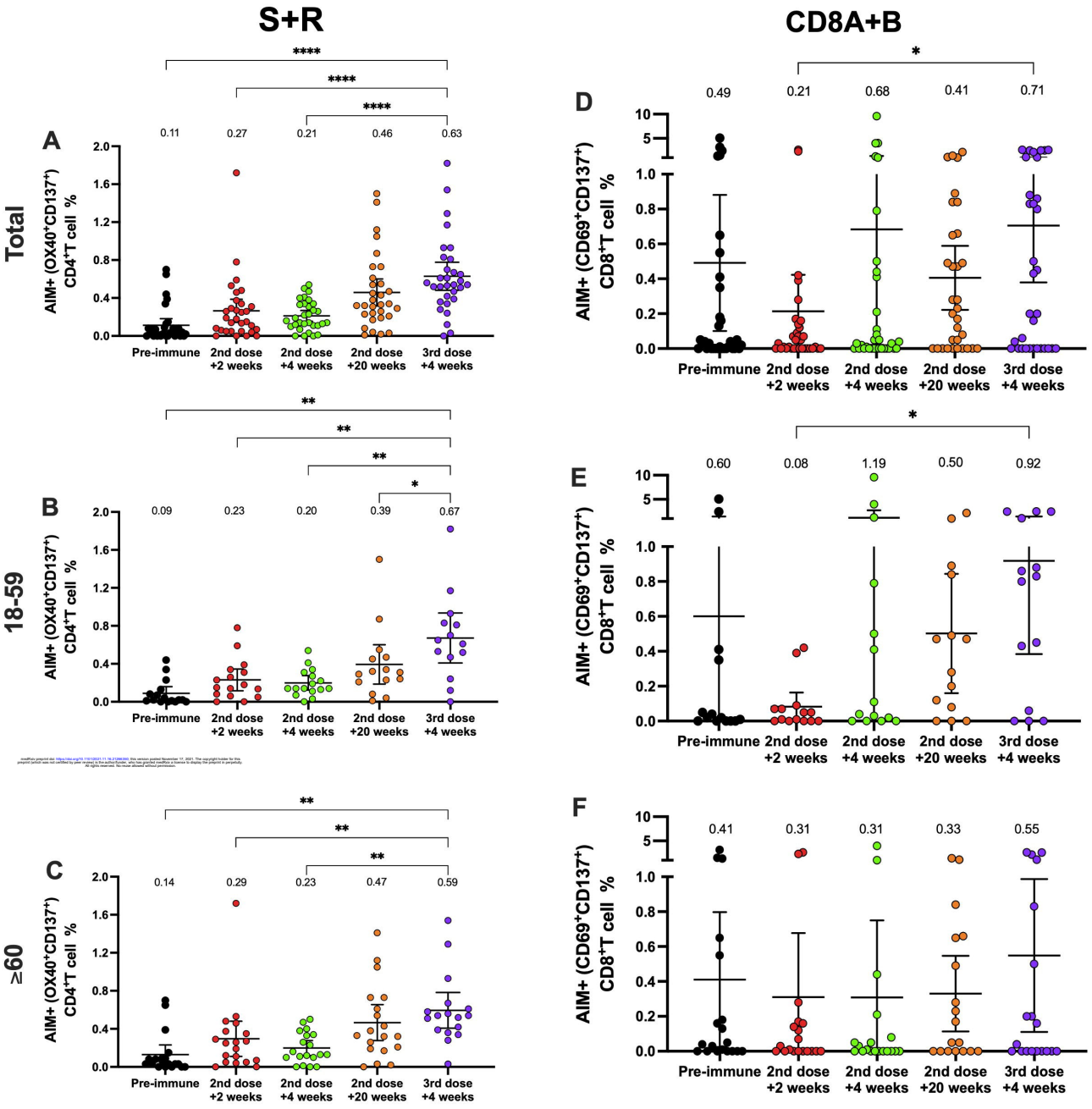











S+R
CD8A+B
Total
18-59
≥60
A
B
C
D
E
F
AIM+ (OX40+CD137+) CD4+T cell %
AIM+ (CD69+CD137+) CD8+T cell %
Pre-immune
2nd dose +2 weeks
2nd dose +4 weeks
2nd dose +20 weeks
3rd dose +4 weeks
0.11 0.27 0.21 0.46 0.63
0.09 0.23 0.20 0.39 0.67
0.14 0.29 0.23 0.47 0.59
0.49 0.21 0.68 0.41 0.71
0.60 0.08 1.19 0.50 0.92
0.41 0.31 0.31 0.33 0.55

7.3 Dose de reforço da CoronaVac administrada oito meses após a segunda dose aumenta em até cinco vezes os níveis de anticorpos neutralizantes

Uma pesquisa publicada na revista médica britânica The Lancet Infectious Diseases mostrou que a dose de reforço da CoronaVac, vacina do Butantan e da Sinovac, é capaz de aumentar de três a cinco vezes a produção de anticorpos neutralizantes em indivíduos adultos, incluindo idosos com mais de 60 anos. O estudo foi conduzido por pesquisadores chineses da Universidade Fudan, da Sinovac e dos Centros de Controle e Prevenção de Doenças de Nanquim e Hebei.

Na primeira análise, 271 participantes com idades entre 18 e 59 anos imunizados com a CoronaVac receberam a dose de reforço oito meses após a segunda dose, resultando em um aumento de três a cinco vezes nos títulos de anticorpos neutralizantes (NAb) contra o SARS-CoV-2 em comparação com os títulos de anticorpos neutralizantes após a segunda dose.

Um segundo levantamento feito entre 303 adultos com 60 anos ou mais, que também receberam a dose de reforço oito meses após a segunda dose, mostrou que as concentrações de anticorpos neutralizantes aumentaram de 42,9 GMT (ou títulos médios geométricos) no dia 28 após a segunda dose para 158,5 GMT no dia 28 após a dose de reforço – um aumento de 3,7 vezes.

De acordo com os pesquisadores, "nosso estudo descobriu que um esquema de duas doses de CoronaVac gerou boa memória imunológica. A dose de reforço administrada oito meses após a segunda dose foi altamente eficaz em relembrar uma resposta imune específica de SARS-CoV-2, levando a um aumento significativo nos níveis de anticorpos".

Além disso, a pesquisa indica que uma dose de reforço homóloga (com a mesma vacina) pode fornecer imunidade de longa duração e níveis elevados de proteção.

Publicado em: 08/12/2021

Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials

Gang Zeng, Qianhui Wu*, Hongxing Pan*, Minjie Li*, Juan Yang*, Lin Wang, Zhiwei Wu, Deyu Jiang, Xiaowei Deng, Kai Chu, Wen Zheng, Lei Wang, Wanying Lu, Bihua Han, Yuliang Zhao†, Fengcai Zhu†, Hongjie Yu†, Weidong Yin†*

Summary

Background Large-scale vaccination against COVID-19 is being implemented in many countries with CoronaVac, an inactivated vaccine. We aimed to assess the immune persistence of a two-dose schedule of CoronaVac, and the immunogenicity and safety of a third dose of CoronaVac, in healthy adults aged 18 years and older.

Methods In the first of two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials, adults aged 18–59 years in Jiangsu, China, were initially allocated (1:1) into two vaccination schedule cohorts: a day 0 and day 14 vaccination cohort (cohort 1) and a day 0 and day 28 vaccination cohort (cohort 2); each cohort was randomly assigned (2:2:1) to either a 3 μg dose or 6 μg dose of CoronaVac or a placebo group. Following a protocol amendment on Dec 25, 2020, half of the participants in each cohort were allocated to receive an additional dose 28 days (window period 30 days) after the second dose, and the other half were allocated to receive a third dose 6 months (window period 60 days) after the second dose. In the other phase 2 trial, in Hebei, China, participants aged 60 years and older were assigned sequentially to receive three injections of either 1·5 μg, 3 μg, or 6 μg of vaccine or placebo, administered 28 days apart for the first two doses and 6 months (window period 90 days) apart for doses two and three. The main outcomes of the study were geometric mean titres (GMTs), geometric mean increases (GMIs), and seropositivity of neutralising antibody to SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank accession number MT407649.1), as analysed in the per-protocol population (all participants who completed their assigned third dose). Our reporting is focused on the 3 μg groups, since 3 μg is the licensed formulation. The trials are registered with ClinicalTrials.gov, NCT04352608 and NCT04383574.

Findings 540 (90%) of 600 participants aged 18–59 years were eligible to receive a third dose, of whom 269 (50%) received the primary third dose 2 months after the second dose (cohorts 1a-14d-2m and 2a-28d-2m) and 271 (50%) received a booster dose 8 months after the second dose (cohorts 1b-14d-8m and 2b-28d-8m). In the 3 μg group, neutralising antibody titres induced by the first two doses declined after 6 months to near or below the seropositive cutoff (GMT of 8) for cohort 1b-14d-8m (n=53; GMT 3·9 [95% CI 3·1–5·0]) and for cohort 2b-28d-8m (n=49; 6·8 [5·2–8·8]). When a booster dose was given 8 months after a second dose, GMTs assessed 14 days later increased to 137·9 (95% CI 99·9–190·4) for cohort 1b-14d-8m and 143·1 (110·8–184·7) 28 days later for cohort 2b-28d-8m. GMTs moderately increased following a primary third dose, from 21·8 (95% CI 17·3–27·6) on day 28 after the second dose to 45·8 (35·7–58·9) on day 28 after the third dose in cohort 1a-14d-2m (n=54), and from 38·1 (28·4–51·1) to 49·7 (39·9–61·9) in cohort 2a-28d-2m (n=53). GMTs had decayed to near the positive threshold by 6 months after the third dose: GMT 9·2 (95% CI 7·1–12·0) in cohort 1a-14d-2m and 10·0 (7·3–13·7) in cohort 2a-28d-2m. Similarly, in adults aged 60 years and older who received booster doses (303 [87%] of 350 participants were eligible to receive a third dose), neutralising antibody titres had declined to near or below the seropositive threshold by 6 months after the primary two-dose series. A third dose given 8 months after the second dose significantly increased neutralising antibody concentrations: GMTs increased from 42·9 (95% CI 31·0–59·4) on day 28 after the second dose to 158·5 (96·6–259·2) on day 28 following the third dose (n=29). All adverse reactions reported within 28 days after a third dose were of grade 1 or 2 severity in all vaccination cohorts. There were three serious adverse events (2%) reported by the 150 participants in cohort 1a-14d-2m, four (3%) by 150 participants from cohort 1b-14d-8m, one (1%) by 150 participants in each of cohorts 2a-28d-2m and 2b-28d-8m, and 24 (7%) by 349 participants from cohort 3-28d-8m.

Interpretation A third dose of CoronaVac in adults administered 8 months after a second dose effectively recalled specific immune responses to SARS-CoV-2, which had declined substantially 6 months after two doses of CoronaVac, resulting in a remarkable increase in the concentration of antibodies and indicating that a two-dose schedule generates good immune memory, and a primary third dose given 2 months after the second dose induced slightly higher antibody titres than the primary two doses.

Lancet Infect Dis 2021

Published Online December 7, 2021 https://doi.org/10.1016/S1473-3099(21)00681-2

See Online/Comment https://doi.org/10.1016/S1473-3099(21)00696-4

For the Mandarin translation of the abstract see Online for appendix 1

*Contributed equally

†Joint supervisors

Sinovac Biotech, Beijing, China (G Zeng PhD, Le Wang MSc, W Yin MBA); **School of Public Health, Fudan University, Key Laboratory of Public Health Safety, Ministry of Education, Shanghai, China** (Q Wu MPH, J Yang PhD, X Deng MSc, W Zheng BSc, W Lu BSc, Prof H Yu PhD); **Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China** (H Pan MSc, K Chu MSc, Prof F Zhu MD); **Hebei Provincial Center for Disease Control and Prevention, Shijiazhuang, Hebei, China** (M Li MSc, Z Wu MSc, B Han MSc, Prof Y Zhao MSc); **Sinovac Life Sciences, Beijing, China** (Li Wang MSc, D Jiang MSc); **Shanghai Institute of Infectious Disease and Biosecurity, Fudan University, Shanghai, China** (J Yang, Prof H Yu); **Department of Infectious Diseases, Huashan Hospital, Fudan University, Shanghai, China** (Prof H Yu)

Correspondence to: Yuliang Zhao, Hebei Center for Disease Control and Prevention, Shijiazhuang 050021, China **yuliang_zh1@163.com**

or

Fengcai Zhu, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210000, China
jszfc@vip.sina.com
or
Hongjie Yu, School of Public Health, Fudan University, Key Laboratory of Public Health Safety, Ministry of Education, Shanghai 200032, China
yhj@fudan.edu.cn
or
Weidong Yin, Sinovac Biotech, Beijing 100085, China
yinwd@sinovac.comv

Funding National Key Research and Development Program, Beijing Science and Technology Program, and Key Program of the National Natural Science Foundation of China.

Introduction

More than 20 vaccines have been approved for use in response to the COVID-19 pandemic,[1] with over 6·33 billion doses administered globally as of Oct 3, 2021.[2] Following primary vaccination with vaccines including BNT162b2[3–5] (Pfizer–BioNTech's mRNA vaccine), mRNA-1273[4,6] (Moderna's mRNA vaccine), and ChAdOx1 nCoV-19[7,8] (AstraZeneca's non-replicating adenoviral vectored vaccine), neutralising antibody titres and vaccine effectiveness against symptomatic illness have been observed to decrease over time, particularly against the delta (B.1.617.2) variant of SARS-CoV-2, which has become the predominant strain across the globe.[9] A booster dose given 6–8 months after the second dose of BNT162b2,[10] mRNA-1273,[11] and NVX-CoV2373[12] (Novavax's protein subunit vaccine) greatly increased neutralising antibody concentrations, and thus increased neutralisation capacity against the delta variant. Booster vaccination with BNT162b2 was initiated in Israel in response to a surge of COVID-19 cases caused by the delta variant;[13] interim results show that the booster dose significantly reduces rates of confirmed infection and severe illness.[14]

CoronaVac (Sinovac Life Sciences, Beijing, China), an inactivated vaccine against COVID-19, has been authorised for conditional use in China,[15] and is included

Research in context

Evidence before this study

We used the terms "SARS-CoV-2", "COVID-19", "vaccine", and "clinical trial" to search PubMed and Europe PMC on Sept 29, 2021, without language or date restrictions, to identify seven research articles on the immune persistence of currently approved vaccines or the immunogenicity of additional doses in the general population. Previous research reported that neutralising antibody responses elicited by mRNA vaccines (BNT162b2, developed by Pfizer and BioNTech, and mRNA-1273, developed by Moderna), adenovirus-vectored vaccines (ChAdOx1 nCoV-19, developed by Oxford and AstraZeneca, and Ad26.COV2-S, developed by Janssen), an inactivated vaccine (CoronaVac, developed by SinoVac), and a protein subunit vaccine (NVX-CoV2373, developed by Novavax) persisted for 6–8 months after full-schedule vaccination and declined to varying degrees. Neutralising antibodies against variants of concern started at lower concentrations than they did against the original alpha variant and waned substantially, especially against the beta (B.1.351) variant, whereas neutralising antibody concentrations against other variants of concern were less affected. Neutralisation capacity against the delta (B.1.617.2) variant, mediated by a homologous third dose given 6–8 months after the second dose of mRNA-1273, BNT162b2, or ChAdOx1 nCoV-19, increased multifold and was similar to or higher than the level against the ancestral SARS-CoV-2 after the second dose. Several clinical trials have explored heterologous vaccination schedules with ChAdOx1 nCoV-19 and BNT162b2, BNT162b2 and Ad26.COV2-S, CoronaVac and ChAdOx1 nCoV-19, and CoronaVac and Convidecia (adenovirus type-5-vectored vaccine, developed by CanSino), showing that heterologous vaccination can induce robust immune responses in adults aged 18 years and older. These results indicate flexibility in deploying COVID-19 vaccines in mix-and-match schedules.

Added value of this study

Our phase 2 trial among adults aged 18–59 years provides preliminary evidence of 6-month immune persistence after two two-dose schedules (14-day and 28-day intervals) of CoronaVac and immunogenicity and safety of a third dose of CoronaVac given 2 months or 8 months after the second dose. Neutralising antibody titres induced by two doses of CoronaVac (3 μg formulation) declined to near or below the lower limit of seropositivity after 6 months. A third dose given 8 months after the second dose led to a strong boost in immune response (a three-fold to five-fold increase in neutralising antibody titres 28 days after the second dose). Our phase 2 trial in healthy adults aged 60 years and older found that neutralising antibody titres declined to low concentrations 6 months after the second dose but rapidly rebounded after a third dose given at 8 months after the second dose (an approximate three-fold increase in neutralising antibody titre). Seropositivity after an 8-month third dose was 98–100% regardless of age group. No safety concerns were seen with a third dose; reactogenicity of the vaccine was indistinguishable from reactogenicity of aluminium hydroxide placebo. This study provided data on immune persistence after primary immunisation with CoronaVac, and immunogenicity and safety of a third homologous dose in adults aged 18 years or older.

Implications of all the available evidence

The rapid and robust rebound in immunity induced by a third dose of CoronaVac showed that primary vaccination with two doses induced immune memory in adults aged 18 years and older. A third dose was immunogenic and markedly increased neutralising antibody titres when given 8 months after the second dose. Therefore, a third dose might provide additional benefit, including longer-lasting immunity and higher level of protection, over a two-dose schedule, but such determinations need longer-term study and real-world studies of vaccine effectiveness.

in WHO's emergency use listing.[16] This vaccine has been administered in 26 countries, including China,[1] and is increasing the global supply through COVAX.[17] In China, 2·21 billion doses of COVID-19 vaccines have been administered as of Oct 3, 2021,[18] the vast majority of which are inactivated vaccines. Evidence from real-world studies of CoronaVac in two-dose schedules in Chile,[19] Brazil,[20] and China[21,22] shows that the vaccine effectively prevents laboratory-confirmed COVID-19, with greater effectiveness against more severe outcomes, including in settings with circulation of variants of concern. However, persistence of CoronaVac vaccine-induced immunity is unknown, and the immunogenicity and safety of a booster dose has not been determined.

To fill this knowledge gap, we aimed to assess immune persistence after primary immunisation with CoronaVac, and immunogenicity and safety of a third homologous dose, in two population groups: adults aged 18–59 years and adults aged 60 years or older.

Methods

Study design and participants

Our study is built upon two single-centre, double-blind, randomised, placebo-controlled, phase 2 clinical trials of CoronaVac. One trial was initiated in Suining County, Jiangsu province, China, by Jiangsu Provincial Center for Disease Control and Prevention (CDC) on May 3, 2020, among healthy adults aged 18–59 years, and the other was initiated in Renqiu, Hebei province, China, by Hebei Provincial CDC, on June 12, 2020, among healthy adults aged 60 years and older. The designs of the phase 2 trials have been published previously.[23,24] Briefly, key exclusion criteria for trial enrolment included suspected or laboratory-confirmed SARS-CoV-2 infections and known allergy to any vaccine component. A complete list of exclusion criteria is in the protocol (appendix 2 pp 74–76; appendix 3 pp 38–39).

For the trial in adults aged 18–59 years, eligible participants were initially recruited and randomly allocated (1:1) to vaccination cohorts with two-dose schedules, either 14 days apart (cohort 1) or 28 days apart (cohort 2). Within each cohort, participants were randomly allocated (2:2:1) to either a 3 μg group, a 6 μg group, or a placebo group. For the trial in adults aged 60 years and older, eligible participants were assigned (2:2:2:1) sequentially to receive two doses 28 days apart of either 1·5 μg, 3 μg, or 6 μg vaccine or placebo (cohort 3). Randomisation codes for each vaccination schedule cohort were generated individually and randomly assigned using block randomisation developed with SAS version 9.4. Adults aged 18–59 years were assigned with a block size of five and adults aged 60 years and older were assigned with a block size of 14. Concealed random group allocations and blinding codes were kept in signed and sealed envelopes. Investigators, participants, and laboratory staff were masked to group assignment. The randomisation code was assigned to each participant in sequence in the order of enrolment by investigators, who were involved in the rest of the trial.

1·5 μg, 3 μg, or 6 μg doses of CoronaVac (Vero cell, inactivated CN02 strain of SARS-CoV-2 with 1·5, 3, or 6 μg per 0·5 mL of aluminium hydroxide adjuvant) or placebo (0·5 mL of aluminium hydroxide adjuvant) in prefilled syringes were administered by intramuscular injection into the deltoid muscle. To evaluate the immunogenicity of primary vaccination, blood samples were taken before vaccination and at day 28 after the second dose. Interim results of these data have been published.[23,24] For the trial in adults aged 18–59 years, the protocol was amended on Dec 25, 2020, to evaluate the immunogenicity of an additional dose (appendix 2 p 3). The amended protocol was updated on ClinicalTrials.gov. According to the order of the blocks, half of the participants were sequentially allocated to receive an additional dose of the vaccine or placebo at 28 days after the second dose (with a 30-day window period; hereafter cohort 1a-14d-2m and cohort 2a-28d-2m, with 14d and 28d representing the interval in days between the first two doses, and 2m denoting the actual median interval in months between the second and third doses), and the other half were allocated to receive a booster dose 6 months after the second dose (with a 60-day window period; hereafter cohort 1b-14d-8m and cohort 2b-28d-8m, with 8m denoting the actual median interval in months between the second and third doses). For the trial in adults aged 60 years and older, a booster dose was given 6 months after the second dose (with a 90-day window period; cohort 3-28d-8m) per the original protocol (appendix 3 p 41–42). Key exclusion criteria for third doses are shown in appendix 4 (p 3). Written informed consent was obtained from participants both before enrolment and before administration of a third dose of a vaccine in eligible participants. The clinical trial protocol and informed consent forms for the study in adults aged 18–59 years were approved by the Jiangsu Ethics Committee (JSJK2020-A021–02), and those for the study in adults aged 60 years and older were approved by Hebei CDC Ethics Committee (IRB2020–006).

Essential steps and timing for each visit specified in the protocol are shown in appendix 4 (p 4). Participants in each cohort received homologous third doses, vaccine or placebo. Participants were to be withdrawn from the trial if they had an unacceptable adverse event as judged by the investigators and defined by the Guidelines of the National Medical Products Administration for Adverse Event Classification Standards for Clinical Trials of Preventive Vaccines (2019), an unacceptable health status as judged by the investigators, or abnormal clinical manifestations as judged by the investigators, or at the participant's request or for any other reason judged necessary by the investigator. The trial would be suspended under the following conditions as judged by the investigators: occurrence of one or more grade 4 local or systemic adverse reactions related to vaccination or more than 15% of the participants

For more on **the amendment to the NCT04352608 trial** see https://clinicaltrials.gov/ct2/show/NCT04352608

See **Online** for appendix 4

See **Online** for appendix 2

See **Online** for appendix 3

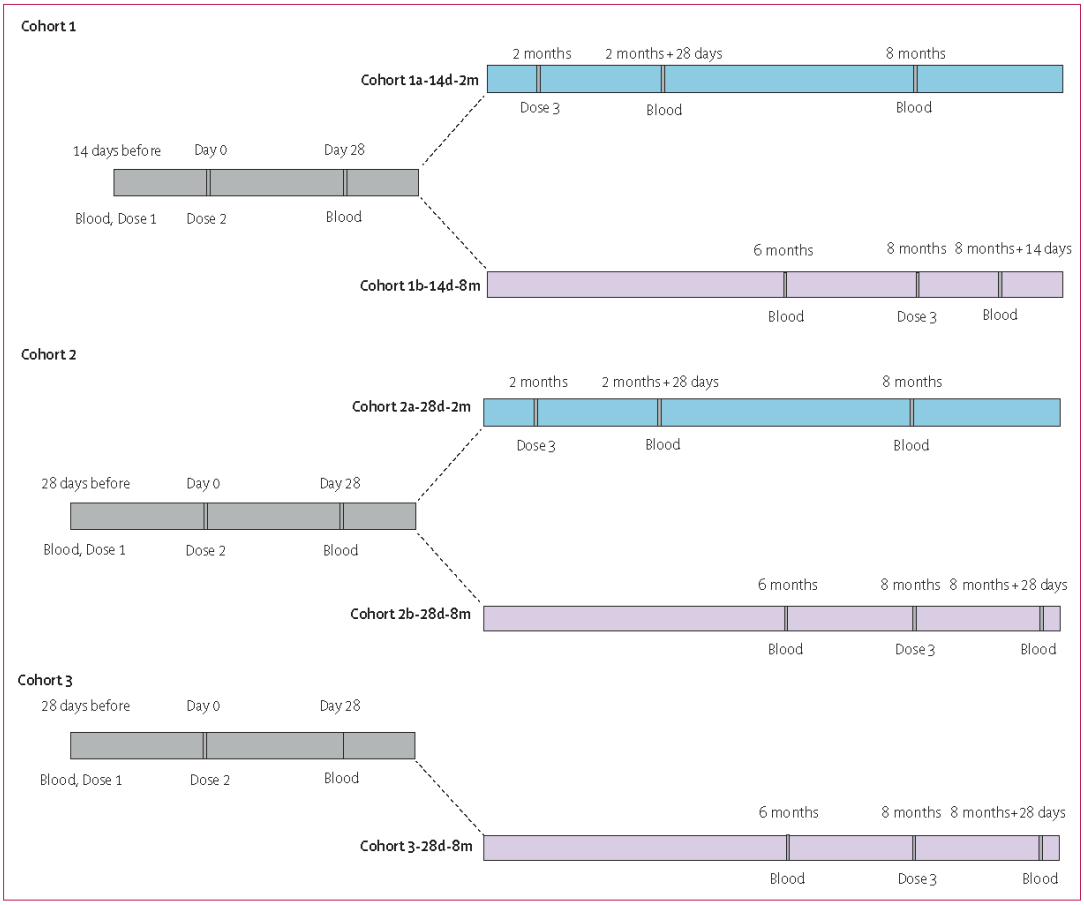


Figure 1: Trial process timeline
Blood=blood sample taken.

having grade 3 or above adverse reactions, including local reactions, systemic reactions, and vital sign changes. During the trial periods, no active surveillance for natural infection with SARS-CoV-2 was done by this study. SARS-CoV-2 occurring in study participants was required to be reported to the investigator. Under the China Government's COVID-19 prevention and control policy of zero tolerance for local transmission, all infections are identified in a timely manner and reported by local health departments for contact tracing, isolated treatment, and quarantine of close contacts and testing for SARS-CoV-2 RNA.

For participants who received their third dose 28 days after the second dose (cohort 1a-14d-2m and cohort 2a-28d-2m), blood samples were collected on day 28 and month 6 after the third dose to evaluate immunogenicity and immune persistence of the third dose. For participants who received their third dose 6 months after the second dose (cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m), blood samples were collected at month 6 after the second dose to evaluate the immune persistence of the second dose, and on day 28 after the third dose to assess immunogenicity of the third dose (with the exception of cohort 1b-14d-8m, in which samples were collected on day 14 after the third dose; figure 1; appendix 4 p 4).

Safety information after the third dose was obtained by the same methods as for the first two doses, as described previously.[23] Participants were required to record injection-site adverse events (eg, pain, redness, and swelling), or systemic adverse events (eg, allergic reactions, cough, and fever) on diary cards for 7 days after their third dose. For days 8–28, unsolicited adverse reactions were collected by spontaneous reporting from participants in all cohorts. We planned to collect serious adverse events until 6 months after the third dose for participants in cohorts 1 and 2, and until 1 year after third dose for participants in cohort 3. The cut-off day of this report was 6 months after the second dose for participants in cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m, and 6 months after the third dose for

www.thelancet.com/infection Published online December 7, 2021 https://doi.org/10.1016/S1473-3099(21)00681-2

participants in cohort 1a-14d-2m and cohort 2a-28d-2m. Reported adverse events were graded according to China National Medical Products Administration guidelines.[23] Serious adverse events were coded by the Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class. The existence of causal associations between adverse events and vaccination was determined by the investigators.

Immunological assessment methods and related procedures are described in appendix 4 (p 5). Neutralising antibodies against infectious SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank accession number MT407649.1) were quantified using a microcytopathogenic effect assay.[23] Several measures were taken to control the quality of the microcytopathogenic effect assay, including virus back-titration for each batch of tests to determine whether the amount of virus was within the range of 32–320 tissue culture infectious dose ($TCID_{50}$) per 50 µL.[25] Two types of positive antibody control, a negative antibody control, a serum toxicity control, and a cell control were included for each test. Blood samples taken at baseline and 28 days after the second dose had been tested previously, and the neutralising antibody titres were comparable between the group aged 18–59 years and those aged 60 years or older.[23,24]

Blood samples taken 6 months after the second dose or 14 days, 28 days, or 6 months after the third dose were tested in our analyses. However, neutralising antibody titres of sera obtained on day 28 after the third dose from participants in the older age group were approximately two-fold higher (352·8 [95% CI 266·4–441·1] in cohort 3-28d-8m) than titres from participants in the younger age group (143·1 [95% CI 110·8–184·7] in cohort 2b-28d-8m) who had been immunised with the same vaccination schedule. To verify the stability and reliability of the neutralising antibody test results, we retested a convenient random sample of specimens from 100 adults in the younger age group and 100 adults in the older age group. In the group of younger adults, neutralising antibody titres were consistent between the first test and the retest. Accordingly, the results of the first test were used in our analysis for this population. In the group of older adults, neutralising antibody titres were significantly lower in the retests than they were in the first tests. Considering the acceptable results of serum samples in younger adults and older adults in the retests, and the consistence of our procedures with the protocol after evaluation, we used the retest results of the 100 adults in the older age group, which we believe to be more reliable, in our analyses. Due to repeated freezing and thawing, and insufficient quantity of sera, we were unable to retest specimens from the other adults in the older age group. A detailed description of retest procedures and results for the older adults is provided in appendix 4 (pp 9–11).

Outcomes

The primary immunological outcomes of the two phase 2 trials have been reported previously;[23,24] here, we report the results of prespecified secondary and exploratory immunological outcomes. Secondary immunological outcomes included geometric mean titres (GMTs), geometric mean increases (GMIs), and seropositivity of neutralising antibodies to infectious SARS-CoV-2 28 days after the third dose (for cohort 1a-14d-2m and cohort 2a-28d-2m). Exploratory immunological outcomes included GMTs and seropositivity at 6 months after the second dose (for cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m) and at 14 days (for cohort 1b-14d-8m) or 28 days (for cohort 2b-28d-8m and cohort 3-28d-8m) after the third dose. The additional outcome of GMTs and seropositivity at 6 months after the third dose for cohort 1a-14d-2m and cohort 2a-28d-2m was a post-hoc analysis. To assess the immunogenicity of a third dose, we included the participants who received their assigned third doses and had available antibody results on day 28 after the third dose (day 14 after the third dose for cohort 1b-14d-8m); defined as the per-protocol analysis set of third doses. To assess the immune persistence of primary two-dose series we included participants who completed 6-month follow-up after two doses for cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m; to assess the immune persistence of primary three-dose series we included participants who completed 6-month follow-up after three doses for cohort 1a-14d-2m and cohort 2a-28d-2m; defined as the immune persistence analysis set. We defined seropositivity as a titre of 8 or greater for neutralising antibodies to infectious SARS-CoV-2. Primary safety endpoints included any adverse reactions within 28 days after dose three in all cohorts. Secondary safety endpoints were serious adverse events occurring from the first dose to 6 months after the third dose in all vaccination cohorts. A complete list of outcomes is provided in appendix 4 (pp 6–7). Given that the 3 µg dose is the licensed formulation, and owing to space constraints, we mainly present results for the 3 µg group in the main text and provide detailed results for other intervention groups in tables and appendix 4.

Statistical analysis

The sample size was determined following requirements of the National Medical Products Administration, China's regulatory authority for vaccines. We assessed immunological endpoints in the per-protocol population, which included all participants who completed their assigned third doses and had antibody results available according to the protocol. In addition, we assessed the immune persistence of primary immunisation in the immune-persistence analysis set, which included participants who completed 6-month follow-up after two doses for cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m and who completed 6-month follow-up after three doses for cohort 1a-14d-2m and cohort 2a-28d-2m. Serious adverse

	1·5 µg group	3 µg group	6 µg group	Placebo group
Cohort 1a-14d-2m (a third dose at month 2 after the second dose)				
Number of participants	NA	55	58	26
Age, years	NA	45·2 (9·1)	44·7 (8·6)	44·3 (8·6)
Male	NA	29 (53%)	20 (34%)	10 (38%)
Female	NA	26 (47%)	38 (66%)	16 (62%)
Cohort 1b-14d-8m (a third dose at month 8 after the second dose)				
Number of participants	NA	55	56	30
Age, years	NA	40·4 (10·3)	42·4 (8·8)	44·8 (6·9)
Male	NA	24 (44%)	27 (48%)	12 (40%)
Female	NA	31 (56%)	29 (52%)	18 (60%)
Cohort 2a-28d-2m (a third dose at month 2 after the second dose)				
Number of participants	NA	54	50	26
Age, years	NA	42·5 (8·6)	40·7 (9·4)	44·0 (7·7)
Male	NA	34 (63%)	26 (52%)	14 (54%)
Female	NA	20 (37%)	24 (48%)	12 (46%)
Cohort 2b-28d-8m (a third dose at month 8 after the second dose)				
Number of participants	NA	52	50	28
Age, years	NA	44·3 (9·5)	43·1 (9·9)	45·7 (9·7)
Male	NA	23 (44%)	26 (52%)	11 (39%)
Female	NA	29 (56%)	24 (48%)	17 (61%)
Cohort 3-28d-8m (a third dose at month 8 after the second dose)				
Number of participants	85	90	81	47
Age, years	66·3 (4·4)	66·4 (4·4)	66·3 (4·4)	67·1 (4·7)
Male	41 (48%)	44 (49%)	37 (46%)	27 (57%)
Female	44 (52%)	46 (51%)	44 (54%)	20 (43%)

Data are n (%) or mean (SD). NA=not applicable.

Table 1: Baseline demographic characteristics in the safety population of participants who received the third dose

events were evaluated in the safety population, which included all participants who received at least one dose of study vaccine from the beginning of the vaccination schedule. Safety assessments for the third dose were done in a safety population data set of all participants who received a third dose.

The demographics of participants who received the third dose were summarised for vaccination cohorts, and Pearson χ^2 test or Fisher's exact test were used to analyse categorical outcomes. We calculated 95% CIs for all categorical outcomes using the Clopper-Pearson method. We calculated GMTs and corresponding 95% CIs on the basis of the standard normal distribution of log-transformed antibody titres. For the third dose given at 28 days after the second dose (cohort 1a-14d-2m and cohort 2a-28d-2m), GMIs were calculated using antibody titres before vaccination and at 28 days after the third dose (taking prevaccination as baseline). For the booster dose given 6 months after the second dose (cohort 1b-14d-8m, cohort 2b-28d-8m, and cohort 3-28d-8m), GMIs were calculated using antibody titres before (ie, 6 months after the second dose) and at 28 days or 14 days after the third (booster) dose (taking pre-booster as baseline). ANOVA models with log-transformation (per GMT and GMI as above) were used to detect differences among groups. Post-hoc generalised liner mixed models (GLMM) were done to compare antibody concentrations induced by the third dose among participants in the four groups in cohorts 1 and 2, accounting for age, sex, dose group, vaccine schedule, interactions of dose and schedule, sampling time, and a random intercept for each participant.

Comparisons were done between groups by group t-tests with log-transformation and Bonferroni correction done as a post-hoc test if variance was significant. Hypothesis testing was two-sided, and we considered p values of less than 0·05 to be significant. We used R software version 3.6.0 for all analyses. The clinical trial is supervised by an independent data monitoring committee that consists of an independent statistician, a clinician, and an epidemiologist. Detailed information on the members is provided in appendix 4 (p 8). The trials are registered with ClinicalTrials.gov, NCT04352608 and NCT04383574.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

On May 3, 2020, 600 participants aged 18–59 years were enrolled into the phase 2 trial, of whom 540 (90%) were eligible and allocated to receive third doses (appendix 4 pp 13–14). Of these 540 participants, 139 (26%) participants were allocated to cohort 1a-14d-2m and 130 (24%) participants were allocated to cohort 2a-28d-2m; cohort 1a-14d-2m received a third dose at a median of 2 months (IQR 56–56 days) and cohort 2a-28d-2m received a third dose at a median of 2 months (IQR 51–51 days) after the second dose. 135 (97%) of 139 participants from cohort 1a-14d-2m and 124 (95%) of 130 participants from cohort 2a-28d-2m completed blood sampling to assess immune persistence for 6 months after dose three. Separately, 147 (25%) of the 600 participants assigned to cohort 1b-14d-8m and 138 (23%) assigned to cohort 2b-28d-8m were followed up for 6 months after the second dose, and 141 participants in cohort 1b-14d-8m (26% of the 540 participants eligible for a third dose) and 130 participants in cohort 2b-28d-8m (24% of the 540 participants eligible for a third dose) received a third dose at month 8 after the second dose for immunogenic evaluation (figure 1).

On June 12, 2020, 350 participants aged 60 years and older were enrolled in the phase 2 trial and 303 (87%) were allocated to receive third doses at month 8 after the second dose (appendix 4 p 15). 98 (32%) of the 303 participants were included in the immunogenicity analysis as described in the Methods (two participants were excluded due to protocol violation). The demographic characteristics of these 98 participants were similar to the other participants in the same age group

Figure 2: **Level of neutralising antibodies to infectious SARS-CoV-2 in adults aged 18–59 years**
Dots are reciprocal neutralising antibody titres for individuals in the per-protocol population. Numbers above the bars are GMTs, and the error bars indicate the 95% CI. The dotted horizontal line represents the seropositivity threshold. Titres lower than the limit of detection (1/4) are presented as half the limit of detection. Numbers above the short horizontal lines are p values of comparisons between 3 µg group and 6 µg group. GMT=geometric mean titre.

(appendix 4 pp 9–11). All participants in the older age group were included in the safety analyses.

No natural infections were reported in any cohort. There were 141 minor protocol deviations in cohort 1b-14d-8m, including 141 participants given third doses 9–11 days outside of the prespecified time window, which did not result in exclusion of participants from the analysis (appendix 3 p 12). Mean ages of participants were between 40·4 years (SD 10·3) and 45·7 years (9·7) in cohorts 1 and 2 (adults aged 18–59 years old), and between 66·3 years (SD 4·4) and 67·1 years (4·7) in cohort 3 (adults aged 60 years and older; table 1). At baseline, none of the participants in any cohort had detectable neutralising antibodies (figures 2, 3).

A third dose of CoronaVac given at month 2 after the second dose moderately increased neutralising antibody levels induced by the first two doses. In the 3 µg group, the GMT in cohort 1a-14d-2m on day 28 after dose 2 was 21·8 (95% CI 17·3–27·6) and on day 28 after dose 3 was 45·8 (35·7–58·9), and in cohort 2a-28d-2m GMT on day 28 after dose 2 was 38·1 (95% CI 28·4–51·1) and on day 28 after dose 3 was 49·7 (39·9–61·9; figure 2; table 2). GMIs of neutralising antibodies from baseline to 28 days after the third dose were 22·9 (95% CI 17·8–29·4) for cohort 1a-14d-2m and 24·8 (19·9–31·0) for cohort 2a-28d-2m (table 2). Seropositivity rates in all vaccination groups in cohorts 1a-14d-2m and 2a-28d-2m were above 95% at 28 days after three doses (table 2).

Results of immune persistence analysis from cohort 1a-14d-2m and cohort 2a-28d-2m show that, by 6 months after the third dose, the GMT was approximately 10 and seropositivity remained above 50% (appendix 4 pp 16–17). GMTs in cohort 1a-14d-2m on day 28 (p=0·0053) and at month 6 (p=0·039) after the third dose were significantly higher in the 6 µg group than in the 3 µg group, whereas there was no significant difference between the two doses at either timepoint in cohort 2a-28d-2m (appendix 4 pp 16–17).

Regardless of the interval between the first two doses, neutralising antibody titres declined to below the

	1·5 µg group	3 µg group	6 µg group	Placebo	p value*	p value†
Cohort 1a-14d-2m						
Seropositivity	NA	53/54 (98%; 90·11–99·95)	57/58 (98%; 90·76–99·96)	0/26 (0·00–13·23)	<0·0001	1·00
GMT (95% CI)	NA	45·8 (35·7–58·9)	74·2 (59·0–93·3)	2·0 (2·0–2·0)	<0·0001	0·0053
GMI (95% CI)	NA	22·9 (17·8–29·4)	37·1 (29·5–46·6)	1·0 (1·0–1·0)	<0·0001	0·0052
Cohort 1b-14d-8m‡						
Seropositivity	NA	53/53 (100%; 93·28–100·00)	55/55 (100%; 93·51–100·00)	0/30 (0·00–11·57)	<0·0001	1·00
GMT (95% CI)	NA	137·9 (99·9–190·4)	175·1 (138·8–221·0)	2·0 (2·0–2·0)	<0·0001	0·23
GMI (95% CI)	NA	35·1 (24·3–50·7)	36·9 (28·5–47·8)	1·0 (1·0–1·0)	<0·0001	0·82
Cohort 2a-28d-2m						
Seropositivity	NA	52/53 (98%; 89·93–99·95)	48/48 (100%; 92·60–100·00)	0/25 (0·00–13·72)	<0·0001	1·00
GMT (95% CI)	NA	49·7 (39·9–61·9)	51·9 (41·3–65·3)	2·0 (2·0–2·0)	<0·0001	0·78
GMI (95% CI)	NA	24·8 (19·9–31·0)	26·0 (20·7–32·7)	1·0 (1·0–1·0)	<0·0001	0·78
Cohort 2b-28d-8m						
Seropositivity	NA	49/49 (100%; 92·75–100·00)	48/48 (100%; 92·60–100·00)	0/27 (0·00–12·77)	<0·0001	1·00
GMT (95% CI)	NA	143·1 (110·8–184·7)	215·7 (162·6–286·2)	2·0 (2·0–2·0)	<0·0001	0·03
GMI (95% CI)	NA	21·2 (15·3–29·2)	30·4 (21·5–43·0)	1·0 (1·0–1·0)	<0·0001	0·24
Cohort 3-28d-8m§						
Seropositivity	27/28 (96%; 81·65–99·91)	29/29 (100%; 88·06–100·00)	27/28 (96%; 81·65–99·91)	0/13 (0·00–24·71)	<0·0001	0·49
GMT (95% CI)	99·6 (62·0–159·9)	158·5 (99·0–253·7)	178·9 (125·2–255·6)	2·0 (2·0–2·0)	<0·0001	0·37
GMI (95% CI)	28·2 (16·8–47·4)	39·7 (23·6–66·6)	44·2 (27·2–71·9)	0·9 (0·7–1·1)	<0·0001	0·77

Data are n/N (%; 95% CI) unless otherwise stated. ANOVA model with log-transformation (per GMT and GMI as above) was used to detect the difference among groups. Comparison between groups was conducted by group t-test with log-transformation. GMT=geometric mean titre. GMI=geometric mean increase. NA=not applicable. *p values are for comparisons among all groups. †p values are for comparisons between the 3 µg group and the 6 µg group. ‡Immunogenicity was assessed on day 14 after the third dose. §p values for comparisons between the 1·5 µg group and the 3 µg group were 0·49 for seropositivity, 0·18 for GMTs, and 0·37 for GMIs; p values for comparisons between the 1·5 µg group and the 6 µg group were 1·00 for seropositivity, 0·06 for GMTs, and 0·22 for GMIs.

Table 2: **Immunogenicity assessment on day 28 after the third dose**

seropositive cutoff by 6 months after the second dose (GMT 3·9 [95% CI 3·1–5·0] in cohort 1b-14d-8m and 6·8 [5·2–8·8] in cohort 2b-28d-8m; figure 2). In the immune persistence analysis set, at month 6 after the second dose, ten (17%) of 59 participants in cohort 1b-14d-8m and 19 (35%) of 54 participants in cohort 2b-28d-8m were seropositive (appendix 4 pp 18–19).

In post-hoc analyses, after administering a booster dose at 8 months after the second dose, GMTs increased to 137·9 (95% CI 99·9–190·4) in cohort 1b-14d-8m 14 days later, and to 143·1 (110·8–184·7) in cohort 2b-28d-8m 28 days later (figure 2). Neutralising antibody concentrations 14 days after dose 3 were approximately five-fold higher than neutralising antibody concentrations on day 28 after the second dose in cohort 1b-14d-8m (from a GMT of 27·4 to 137·9 in the 3 µg group and from a GMT of 30·4 to 175·1 in the 6 µg group), and in cohort 2b-28d-8m, neutralising antibody titres 28 days after the third dose were approximately three-fold higher than neutralising antibody titres 28 days after the second dose (from a GMT of 45·9 to 143·1 in the 3 µg group; table 2, figure 2). Seropositivity on day 14 after the third dose in cohort 1b-14d-8m and on day 28 after the third dose in cohort 2b-28d-8m was 100% for both doses (table 2). GMIs from before to after the booster dose were 35·1 (95% CI 24·3–50·7) in cohort 1b-14d-8m and 21·2 (15·3–29·2) in cohort 2b-28d-8m (table 2).

In GLMM models, neutralisation titres decreased with increasing age (appendix 4 p 21). Immune responses induced by 6 µg doses were better than those induced by 3 µg doses, and a third dose significantly raised antibody levels compare with 28 days after dose 2. The vaccination

schedule used in cohort 2b-28d-8m produced the best immunogenicity (appendix 4 p 21).

In the immune persistence analysis of cohort 3-28d-8m, in the 3 μg group, neutralising antibody titres had declined to below the seropositive cutoff at 6 months after the second dose (from 40·8 [95% CI 33·8–49·3] at day 28 after dose 2 to 3·4 [2·9–4·1]), and 17 (18%) of 98 participants were seropositive (appendix 4 p 20). A booster dose given 8 months after the second dose increased the GMT to 158·5 (95% CI 96·9–259·2) 28 days after the booster dose (figure 3, table 2). The GMI from before to after the booster dose was 39·7 (95% CI 23·6–66·6; table 2). GMTs on day 28 after the third dose were highest in the 6 μg group (p<0·0001) and similar between the 3 μg group and the 1·5 μg group (p=0·18; table 2).

Severities of solicited local and systemic adverse reactions reported within 28 days after the third dose were grade 1–2 in all vaccination cohorts in both trials. The most common reported reaction was injection-site pain (table 3; appendix 4 pp 22–28). Taking the 3 μg group as an example, the incidences of adverse reactions within 28 days after the third dose in primary three-dose regimens were five (9%) of 55 participants in cohort 1a-14d-2m and three (6%) of 54 participants in cohort 2a-28d-2m; not higher than the incidence of adverse reactions within 28 days after each previous dose (table 3; appendix 4 pp 22–23, 25–26). The overall incidence of any adverse reaction within 28 days after the booster dose (3 μg) was ten (18%) of 55 participants in cohort 1b-14d-8m, eight (15%) of 52 in cohort 2b-28d-8m, and five (6%) of 90 in cohort 3-28d-8m (table 3; appendix 4 p 24, 27–28).

Serious adverse events were reported in one (2%) of 60 participants in the 3 μg group and two (3%) of 60 participants in the 6 μg group in cohort 1a-14d-2m, in two (3%) of 60 participants in the 3 μg group and two (3%) of 60 in the 6 μg group in cohort 1b-14d-8m, and in no participant in the 30 μg group and one (2%) of 60 in the 6 μg group in each of cohorts 2a-28d-2m and 2b-28d-8m (appendix 4 pp 29–30). No participant in the placebo group reported a serious adverse event. From the beginning of immunisation to 28 days after dose 3 in cohort 3-28d-8m, ten (10%) of 100 participants in the 1·5 μg group, five (5%) of 101 in the 3 μg group, seven (7%) of 99 in the 6 μg group, and two (4%) of 49 in the placebo group had non-fatal serious adverse events (appendix 4 pp 30–31). No serious adverse event in either trial was considered by the investigators to be related to vaccination, and no prespecified trial-halting rules were met.

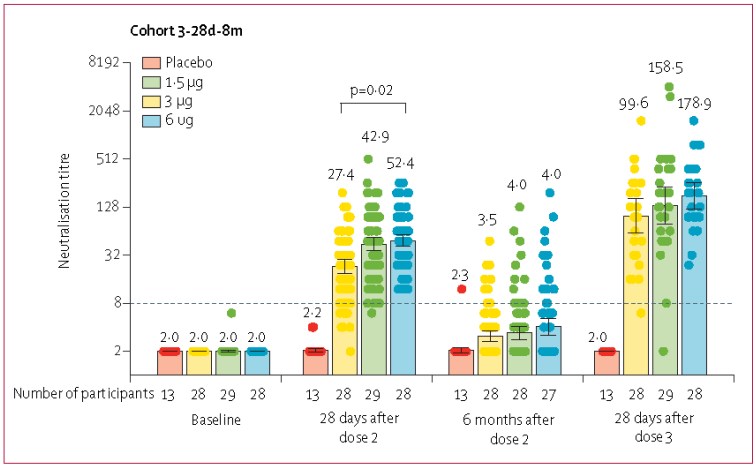


Figure 3: **Level of neutralising antibodies to infectious SARS-CoV-2 in adults aged 60 years and older**
Dots are reciprocal neutralising antibody titres for individuals in the per-protocol population. Numbers above the bars are GMTs, and the error bars indicate the 95% CI. The dotted horizontal line represents the seropositivity threshold. Titres lower than the limit of detection (1/4) are presented as half the limit of detection. Numbers above the short horizontal lines are p values of comparisons between 1.5 μg group, 3 μg group, and 6 μg group. Only the p values indicating significant difference are marked. GMT=geometric mean titre.

Discussion

Our study showed that the initial neutralising antibody response from two doses of CoronaVac declined to near or below the lower limit of seropositivity after 6 months. A third dose of CoronaVac (3 μg) given 8 months after the second dose led to a strong boost in immunity, with neutralising GMTs increasing to approximately 140 among adults aged 18–59 years and 159 among adults aged 60 years and older 14–28 days after the booster dose. These increases correspond to roughly three-fold to five-fold increases in neutralising antibody titres compared with titres 28 days after a second dose. Seropositivity 28 days after a third dose at 8 months was 98–100% regardless of age group. By contrast, a third dose given 2 months after the second dose induced much lower neutralising antibody titres. Reactogenicity of the third dose was indistinguishable from reactogenicity of the previous two doses, regardless of age group.

Decreases over time of vaccine-induced neutralising antibodies against ancestral SARS-CoV-2 have been observed with other COVID-19 vaccines, but at a much lower magnitude. For example, following vaccination with Moderna's mRNA-1273 vaccine, neutralising antibodies declined but remained detectable among all participants on days 90 and 180 after a second dose.[6,26] SARS-CoV-2 spike protein-specific memory B cells are detectable in most patients with COVID-19 and in people who are naive to SARS-CoV-2 after receiving two doses of COVID-19 vaccines.[27,28] This study is the first to show that the antibody response mediated by a third dose of CoronaVac given 2 months after the second dose rebounded only moderately and degraded to near the seropositive threshold after 6 months. This observation is probably because the interval between the two doses was short and the memory B cells were immature. However, a third dose of CoronaVac given 8 months after the second dose appears to effectively augment the potency, breadth, and likely duration of anamnestic responses against SARS-CoV-2.[29] Compared

	Cohort 1a-14d-2m			Cohort 1b-14d-8m			Cohort 2a-28d-2m			Cohort 2b-28d-8m			Cohort 3-28d-8m			
	3 µg (N=55)	6 µg (n=58)	Placebo (N=26)	3 µg (N=55)	6 µg (N=56)	Placebo (N=30)	3 µg (N=54)	6 µg (N=50)	Placebo (N=26)	3 µg (N=52)	6 µg (N=50)	Placebo (N=28)	1·5 µg (N=85)	3 µg (N=90)	6 µg (N=81)	Placebo (N=47)
Any adverse reaction																
Grade 1	5 (9%)	5 (9%)	0	10 (18%)	13 (23%)	3 (10%)	3 (6%)	1 (2%)	0	7 (13%)	10 (20%)	1 (4%)	3 (4%)	3 (3%)	3 (4%)	2 (4%)
Grade 2	1 (2%)	1 (2%)	0	1 (2%)	0	1 (3%)	0	0	0	1 (2%)	1 (2%)	1 (4%)	1 (1%)	2 (2%)	2 (2%)	1 (2%)
Systemic diseases and injection site adverse reactions																
Injection site pain	3 (5%)	5 (9%)	0	8 (1%)	9 (16%)	0	1 (2%)	1 (2%)	0	6 (12%)	7 (14%)	0	1 (1%)	2 (2%)	2 (2%)	1 (2%)
Injection site swelling	0	0	0	0	0	1 (3%)	0	0	0	1 (2%)	0	0	0	0	0	0
Injection site itch	0	0	0	0	1 (2%)	2 (7%)	0	0	0	1 (2%)	0	0	0	0	0	0
Injection site erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1%)	0	0
Fever	0	0	0	0	1 (2%)	0	0	0	0	1 (2%)	1 (2%)	1 (4%)	0	0	0	0
Fatigue	0	0	0	0	1 (2%)	0	1 (2%)	0	0	1 (2%)	2 (4%)	0	0	1 (1%)	0	1 (2%)
Respiratory, thoracic, and mediastinal disorders																
Cough	0	1 (2%)	0	0	2 (4%)	0	2 (4%)	0	0	0	0	0	1 (1%)	0	1 (1%)	1 (2%)
Runny nose	0	0	0	0	0	0	0	0	0	0	1(2%)	0	0	0	1(1%)	0
Oropharyngeal pain	0	0	0	0	1 (2%)	0	0	0	0	1 (2%)	0	0	0	0	0	0
Laryngeal stimulation	0	0	0	1 (2%)	0	0	0	0	0	0	0	0	0	0	0	0
Nervous system disorders																
Dizziness	0	0	0	0	1 (2%)	0	0	0	0	0	0	0	1 (1%)	1 (1%)	0	0
Headache	0	1 (2%)	0	1 (2%)	2 (4%)	1 (3%)	0	0	0	1 (2%)	1 (2%)	1 (4%)	0	0	1 (1%)	0 (0%)
Gastrointestinal disorders																
Diarrhoea	1 (2%)	0	0	0	0	0	1 (2%)	0	0	1 (2%)	0	0	0	0	0	0
Nausea	1 (2%)	1 (2%)	0	1 (2%)	0	0	1 (2%)	0	0	0	2 (4%)	0	1 (1%)	1 (1%)	0	0
Musculoskeletal and connective tissue disorders																
Muscle pain	1 (2%)	0	0	0	1 (2%)	0	0	0	0	0	0	0	0	0	0	0
Myalgia	0	0	0	0	0	0	0	0	0	0	1 (2%)	0	1 (1%)	0	0	0
Skin and subcutaneous tissue disorders																
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1%)	0
Eye disorders																
Periorbital oedema	0	0	0	0	1 (2%)	0	0	0	0	0	0	0	0	0	0	0

Data are n (%), representing the total number of participants who had adverse reactions (ie, adverse events related to vaccination).

Table 3: **Adverse reactions within 28 days after the third dose**

www.thelancet.com/infection Published online December 7, 2021 https://doi.org/10.1016/S1473-3099(21)00681-2

with the 3 µg formulation of CoronaVac, which is approved for use, the 1·5 µg formulation produced similar neutralising antibody titres by day 28 after the third dose for adults aged 60 years and older. Whether the 1·5 µg formulation could serve as a booster dose needs further study due to the small sample size in the analysis of this dose (28 participants).

Significant rebound in antibody concentration induced by homologous booster doses has been reported for other vaccines. Neutralisation titres against ancestral SARS-CoV-2 increased approximately four-fold after a homologous booster dose compared with titres following primary series with BNT162b2,[10] mRNA-1273,[11] and NVX-CoV2373,[12] with similarly long intervals (6–8 months) between the booster dose and primary vaccination. A nine-fold increase in spike protein-binding antibody was observed after a 6-month homologous booster dose of Ad26.COV2-S.[30]

Heterologous prime–boost regimens appear to induce higher levels of immune response than homologous booster doses. Vaccination with mRNA vaccines and adenovirus-vectored vaccines[31,32] or inactivated vaccines and adenovirus-vectored vaccines[33] have shown strong short-term immune responses and tolerable reactogenicity. Wanlapakorn and colleagues[34] found that CoronaVac and AZD1222 vaccine recipients had higher neutralising antibody activity against the original wild-type virus and the beta (B.1.351) variant of concern than did recipients of two doses of CoronaVac or AZD1222, suggesting that heterologous immunisation might be considered an alternative to homologous boosting for immunisation programmes. Long-term effectiveness of boosting remains unevaluated because of the newness of COVID-19 vaccine booster dosing.

SARS-CoV-2 continues to evolve and produce variants, among which the delta variant has become predominant.[9] Although we did not perform neutralisation testing in vitro against emerging variants of concern, high neutralising antibody titres against the ancestral strain are believed to be important for protection against novel circulating SARS-CoV-2 variants that potentially can lead to immune escape.[35] Several studies have reported in-vitro neutralisation titres against variants for CoronaVac, but results varied greatly. Vacharathit and colleagues, using a live-virus microneutralisation assay, identified 22-fold and 32-fold reductions in neutralising antibodies against the beta and delta variants, respectively, compared with ancestral SARS-CoV-2.[36] Wang and colleagues reported a three-fold reduction in neutralising antibody titres against the beta variant, using a pseudovirus neutralisation assay.[37] Another study reported 5·7-fold, 4·3-fold, and 3·7-fold reductions of neutralising antibody titres against beta, gamma (P.1), and delta variants, respectively.[29] Of note, it is difficult to directly compare these estimates because of the differences in study design and laboratory methods.[38] Determining the neutralisation ability of CoronaVac to emerging variants and evaluating the protection level in risk groups such as immunosuppressed individuals or elderly people are important research endeavours.

Decreased effectiveness of mRNA vaccines against SARS-CoV-2 infection with circulating variants has been seen in real-world studies in the USA, but effectiveness against hospitalisation was sustained.[39,40] Two doses of CoronaVac showed good effectiveness in a setting with co-circulating alpha and gamma variants in Chile: the vaccine was 66% effective against COVID-19 and nearly 90% effective against severe outcomes.[19] A test-negative case-control study done in Brazil showed that the adjusted vaccine effectiveness against hospital admission was above 55% in older adults during a time of extensive transmission of the gamma variant.[20] During local outbreaks caused by the delta variant in China, two studies with small sample sizes showed that inactivated vaccines were 70·2% effective against illness of moderate or worse severity[41] and could lower the risk of progressing to severe disease by 88%.[22] Protection against variants and persistence in protection with CoronaVac need to be continually evaluated in real-world studies.

Interim protection results from booster programmes in Israel showed that booster doses effectively reduced breakthrough infections, including breakthroughs of the delta variant.[14] Considering sustained protection of primary immunisation with COVID-19 vaccines against severe outcomes[42] and equity in vaccine deployment, WHO currently prioritises completion of primary immunisation over booster dose strategies to protect more people from COVID-19 due to global shortage of supply of COVID-19 vaccines,[43] although the US Centers for Disease Control and Prevention has issued booster recommendations for specific populations.[44]

During the trials, participants were masked to study group assignment and participants in placebo groups could be vaccinated immediately after completion of the phase 2 trial for adults aged 18–59 years and completion of follow-up for 28 days after the booster dose for adults aged 60 years and older. Since strict non-pharmaceutical interventions have been maintained to date across mainland China, the risk of infection was very low for participants in the placebo group. Maintenance of the placebo groups until the end of the trial was approved by Jiangsu Ethics Committee (JSJK2020-A021–02) and Hebei CDC Ethics Committee (IRB2020–006).

Our study has several limitations. First, establishment of SARS-CoV-2 spike protein-specific immune memory, in addition to inducing durable antibodies, might be important for a successful COVID-19 vaccine. For example, T-cell immunity elicited by inactivated vaccines might contribute to protection.[45,46] However, T-cell responses and neutralisation tests in vitro against emerging variants were not assessed in our study, and these need to be further explored. Second, we report the results of interim analyses, and long-term follow-up is ongoing to identify a satisfactory duration of immunity

induced by the booster dose and to assess longer-term safety. Third, a population at greatest risk of immunosenescence (ie, adults aged 80 years and older) was not evaluated in this study. Larger, multicentre studies will be needed to assess primary outcomes among subpopulations for whom our study had relatively small proportions. Fourth, although neutralising antibodies are related to protection, actual protection from infection with current and emerging variants will need to be monitored with real-world observational studies. Further research to identify correlates of protection and to determine whether different vaccines have different correlates is important.

In conclusion, our study found that a two-dose schedule of CoronaVac generated good immune memory. Although neutralising antibody titres decreased to near or below the lower limit of seropositivity 6 months after the second dose, a third dose given 8 months after the second dose was highly effective at recalling a SARS-CoV-2-specific immune response, leading to a significant rebound in antibody levels. Our study indicates that a homologous booster dose might provide longer-lasting immunity and higher levels of protection than a two-dose schedule, but additional study is needed to monitor neutralisation ability and effectiveness against variants.

Contributors

GZ, QW, HP, ML, JY, YZ, FZ, HY, and WY designed the study and contributed to data collection, data analysis, data interpretation, and writing of the manuscript. GZ, QW, HP, and ML verified the data. ZW, KC, LeW, and BH collected data and revised the manuscript. DJ and LiW did the laboratory assays and revised the manuscript. XD, WZ, and WL analysed the data and revised the manuscript. All authors had full access to all of the data (including statistical reports and tables) in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors had final responsibility for the decision to submit the manuscript for publication.

Declaration of interests

HY received research funding from Sanofi Pasteur, GlaxoSmithKline, Yichang HEC Changjiang Pharmaceutical Company, and Shanghai Roche Pharmaceutical Company; none of this research funding is related to development of COVID-19 vaccines. GZ, LeW and WY are employees of Sinovac Biotech and LiW and DJ are employees of Sinovac Life Sciences. All other authors declare no competing interests.

Data sharing

The individual participant-level data that underlie the results reported in this Article (text, tables, figures, and appendices) will be shared after de-identification. This clinical trial is ongoing, and all the individual participant data cannot be available until the immune persistence evaluation is done. The data will be available immediately after publication and finalisation of the completed clinical study report for at least 1 year. Supporting clinical documents, including the study protocol and statistical analysis plan, and the informed consent form will be available immediately following the publication of this Article for at least 1 year. Information on how to access supporting clinical documents is available online for adults aged 18–59 years at http://www.jshealth.com/ and for adults aged 60 years and older at http://www.hebeicdc.cn/kygz/22506.jhtml. Researchers who provide a scientifically sound proposal will be allowed access to the de-identified individual participant data. Proposals should be sent to the corresponding authors. These proposals will be reviewed and approved by the sponsor, investigators, and collaborators on the basis of scientific merit. To gain access, data requestors will need to sign a data access agreement.

Acknowledgments

The study was supported by grants from National Key Research and Development Program (2020YFC0849600), Beijing Science and Technology Program (Z201100005420023), and Key Program of the National Natural Science Foundation of China (82130093). We thank Dr Lance Rodewald from Chinese Center for Disease Control and Prevention for his comments and English language editing.

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8. Raros efeitos adversos

8.1 CoronaVac é a vacina com menos efeitos adversos dentre as que estão em uso no Brasil, revelam estudos

Um estudo publicado na revista científica Lancet Infectious Diseases revelou que a CoronaVac, vacina do Butantan e da farmacêutica chinesa Sinovac contra a Covid-19, causa efeitos adversos em apenas 29% a 33%, e todos muito leves (como dor no braço ou fadiga passageira). Esse é um ótimo indicador, que atesta o alto perfil de segurança do imunizante, e um dos menores índices de efeitos adversos entre todas as vacinas aprovadas até o momento para uso emergencial pela Organização Mundial de Saúde.

O estudo foi realizado por pesquisadores dos Centros de Controles de Doenças das províncias de Hangzhou, Nanjing e Jiangsu, na China, cientistas da Academia Chinesa de Ciências e pesquisadores da Sinovac, com 744 voluntários que participaram dos ensaios clínicos de fase 1 e 2 da CoronaVac. Na fase 1, 29% dos voluntários relataram ter experimentado reações adversas, principalmente dor no local da aplicação e fadiga, no período de 14 dias após receber a vacina. Na fase 2, apenas 33% dos voluntários relataram efeitos adversos. Menos de 5% dos voluntários em ambas etapas tiveram sintomas de febre, dor de cabeça ou náusea.

No Brasil, dados sobre a segurança da vacina do Butantan foram obtidos em ensaios clínicos de fase 3 com 9 mil voluntários em 2020. As manifestações indesejadas foram muito leves e não foi necessária atenção médica maior. No Projeto S, estudo clínico realizado pelo Butantan na cidade de Serrana, foram administradas 54.882 doses na população adulta do município e não houve relato de evento adverso grave relacionado à vacinação. Durante a aplicação da primeira dose do imunizante em Serrana, foram 4,4% de relatos de reações adversas e apenas 0,02% considerados de grau 3 (mialgia e cefaleia), porque interferiram nas atividades diárias. Já na segunda dose, houve somente 0,2% de relatos de efeitos adversos, nenhum considerado de grau 3 ou superior. Outro indicador que atesta a segurança da CoronaVac é que, até hoje, a área de Farmacovigilância

do Butantan não recebeu nenhum relato de trombose associado à vacinação – um dos efeitos adversos já relatados em outras vacinas contra a Covid-19.

Tais resultados contrastam com as conclusões observadas em estudos com as demais vacinas contra a Covid-19 – embora não seja possível comparar diretamente os resultados de pesquisas, pois os grupos estudados são diferentes, assim como as metodologias de análise. Entre 70% e 75% dos norte-americanos que tomaram vacinas feitas com a tecnologia do RNA mensageiro (mRNA) relataram experimentar efeitos adversos, percentual que subiu para 86% a 88% entre pacientes britânicos que tomaram a vacina AstraZeneca/Oxford, feita com a tecnologia de vetor viral. Já no caso da vacina da Janssen, também de vetor viral, entre 35% e 62% dos entrevistados relataram efeitos adversos.

A tecnologia empregada na CoronaVac, de vírus inativado, é uma das mais estudadas e seguras do mundo. O vírus é replicado e, posteriormente, morto. Assim, não é capaz de se multiplicar no corpo e adoecer o organismo, mas consegue desencadear a produção de anticorpos e produzir resposta imunológica.

Vacinas feitas com a tecnologia do RNA mensageiro (mRNA)

Um estudo recentemente publicado no jornal da Associação Americana de Medicina sobre a percepção de efeitos adversos das vacinas das farmacêuticas americanas Pfizer ou Moderna, feitas com a tecnologia do RNA mensageiro (mRNA), foi feito com 3,6 milhões de norte-americanos que tomaram a primeira dose, e 1,9 milhão que tomaram a segunda dose. A maioria dos participantes relatou ter experimentado reação no local da injeção (70% dos que tomaram a primeira dose, e 75% dos que receberam a segunda dose) ou reação sistêmica (50% após a primeira dose, e 69,4% após a segunda dose) durante os primeiros sete dias após a vacinação. As reações mais

frequentemente após a primeira dose da vacina foram dor no local da injeção (67,8%), fadiga (30,9%), cefaleia (25,9%) e mialgia (19,4%). O relato de efeitos adversos foi maior após a segunda dose para ambas as vacinas, particularmente para reações como fadiga (53,9%), dor de cabeça (46,7%), mialgia (44%), calafrios (31,3%), febre (29,5%) e dos nas articulações (25,6%).

Vacinas feitas com vetor viral

Um estudo publicado na The Lancet analisou a percepção de efeitos adversos de 560 adultos que receberam a vacina elaborada pela farmacêutica anglo-sueca AstraZeneca e por pesquisadores da Universidade Oxford. Entre aqueles que receberam duas doses, após a primeira dose foram relatadas reações locais em 88% dos participantes no grupo de 18 a 55 anos, 73% no grupo de 56 e 69 anos, e 61% no grupo de 70 anos e mais. Foram relatadas reações sistêmicas em 86% dos participantes no grupo de 18 a 55 anos, 77% no grupo de 56 a 69 anos, e 65% no grupo de 70 anos ou mais. Fadiga, dor de cabeça, febre e mialgia foram as reações adversas sistêmicas mais comumente relatadas.

Além disso, o Centro de Controle de Doenças dos Estados Unidos realizou um levantamento com 3.356 norte-americanos que tomaram a dose única da vacina da farmacêutica Janssen. No grupo de 18 e 59 anos, um total de 62% relataram ter experimentado um ou mais efeitos adversos, sendo os principais deles fadiga (43,8%), dor de cabeça (44,4%), mialgia (39,1%), náusea (15,5%) e febre (12,8%). Já no grupo com mais de 60 anos, 35% tiveram algum efeito adverso, como fadiga (29,7%), dor de cabeça (30,4%), mialgia (24%), náusea (10,8%) e febre (3,1%).

Publicado em: 11/08/2021

Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial

Yanjun Zhang, Gang Zeng*, Hongxing Pan*, Changgui Li*, Yaling Hu, Kai Chu, Weixiao Han, Zhen Chen, Rong Tang, Weidong Yin, Xin Chen, Yuansheng Hu, Xiaoyong Liu, Congbing Jiang, Jingxin Li, Minnan Yang, Yan Song, Xiangxi Wang, Qiang Gao†, Fengcai Zhu†*

Summary

Background With the unprecedented morbidity and mortality associated with the COVID-19 pandemic, a vaccine against COVID-19 is urgently needed. We investigated CoronaVac (Sinovac Life Sciences, Beijing, China), an inactivated vaccine candidate against COVID-19, containing inactivated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), for its safety, tolerability and immunogenicity.

Methods In this randomised, double-blind, placebo-controlled, phase 1/2 clinical trial, healthy adults aged 18–59 years were recruited from the community in Suining County of Jiangsu province, China. Adults with SARS-CoV-2 exposure or infection history, with axillary temperature above 37·0°C, or an allergic reaction to any vaccine component were excluded. The experimental vaccine for the phase 1 trial was manufactured using a cell factory process (CellSTACK Cell Culture Chamber 10, Corning, Wujiang, China), whereas those for the phase 2 trial were produced through a bioreactor process (ReadyToProcess WAVE 25, GE, Umea, Sweden) . The phase 1 trial was done in a dose-escalating manner. At screening, participants were initially separated (1:1), with no specific randomisation, into two vaccination schedule cohorts, the days 0 and 14 vaccination cohort and the days 0 and 28 vaccination cohort, and within each cohort the first 36 participants were assigned to block 1 (low dose CoronaVac [3 μg per 0·5 mL of aluminium hydroxide diluent per dose) then another 36 were assigned to block 2 (high-dose Coronavc [6 μg per 0·5 mL of aluminium hydroxide diluent per dse]). Within each block, participants were randomly assigned (2:1), using block randomisation with a block size of six, to either two doses of CoronaVac or two doses of placebo. In the phase 2 trial, at screening, participants were initially separated (1:1), with no specific randomisation, into the days 0 and 14 vaccination cohort and the days 0 and 28 vaccination cohort, and participants were randomly assigned (2:2:1), using block randomisation with a block size of five, to receive two doses of either low-dose CoronaVac, high-dose CoronaVac, or placebo. Participants, investigators, and laboratory staff were masked to treatment allocation. The primary safety endpoint was adverse reactions within 28 days after injection in all participants who were given at least one dose of study drug (safety population). The primary immunogenic outcome was seroconversion rates of neutralising antibodies to live SARS-CoV-2 at day 14 after the last dose in the days 0 and 14 cohort, and at day 28 after the last dose in the days 0 and 28 cohort in participants who completed their allocated two-dose vaccination schedule (per-protocol population). This trial is registered with ClinicalTrials.gov, NCT04352608, and is closed to accrual.

Findings Between April 16 and April 25, 2020, 144 participants were enrolled in the phase 1 trial, and between May 3 and May 5, 2020, 600 participants were enrolled in the phase 2 trial. 743 participants received at least one dose of investigational product (n=143 for phase 1 and n=600 for phase 2; safety population). In the phase 1 trial, the incidence of adverse reactions for the days 0 and 14 cohort was seven (29%) of 24 participants in the 3 ug group, nine (38%) of 24 in the 6 μg group, and two (8%) of 24 in the placebo group, and for the days 0 and 28 cohort was three (13%) of 24 in the 3 μg group, four (17%) of 24 in the 6 μg group, and three (13%) of 23 in the placebo group. The seroconversion of neutralising antibodies on day 14 after the days 0 and 14 vaccination schedule was seen in 11 (46%) of 24 participants in the 3 μg group, 12 (50%) of 24 in the 6 μg group, and none (0%) of 24 in the placebo group; whereas at day 28 after the days 0 and 28 vaccination schedule, seroconversion was seen in 20 (83%) of 24 in the 3 μg group, 19 (79%) of 24 in the 6 μg group, and one (4%) of 24 in the placebo group. In the phase 2 trial, the incidence of adverse reactions for the days 0 and 14 cohort was 40 (33%) of 120 participants in the 3 μg group, 42 (35%) of 120 in the 6 μg group, and 13 (22%) of 60 in the placebo group, and for the days 0 and 28 cohort was 23 (19%) of 120 in the 3 μg group, 23 (19%) of 120 in the 6 μg group, and 11 (18%) of 60 for the placebo group. Seroconversion of neutralising antibodies was seen for 109 (92%) of 118 participants in the 3 μg group, 117 (98%) of 119 in the 6 μg group, and two (3%) of 60 in the placebo group at day 14 after the days 0 and 14 schedule; whereas at day 28 after the days 0 and 28 schedule, seroconversion was seen in 114 (97%) of 117 in the 3 μg group, 118 (100%) of 118 in the 6 μg group, and none (0%) of 59 in the placebo group.

Lancet Infect Dis 2021; 21: 181–92

Published Online November 17, 2020 https://doi.org/10.1016/S1473-3099(20)30843-4

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For the Chinese translation of the abstract see Online for appendix 1

*Joint first authors

†Contributed equally

Department of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China (Prof Y Zhang PhD); **Sinovac Biotech, Beijing, China** (G Zeng PhD, Ya Hu MSc, W Han MSc, W Yin, MBA, Yu Hu MPH); **Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China** (H Pan MSc, K Chu MSc, R Tang BA, Prof J Li PhD, Prof F Zhu MD); **National Institutes for Food and Drug Control, Beijing, China** (Prof C Li PhD, Z Chen MSc); **Suining County Center for Disease Control and Prevention, Suining, Jiangsu Province, China** (X Chen BA, X Liu BA, C Jiang BA, Y Song BA); **CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China** (M Yang PhD, Prof X Wang PhD) **and Sinovac Life Sciences, Beijing, China** (Q Gao MSc)

Correspondence to:
Dr Qiang Gao, Sinovac Life Sciences, Beijing 100085, China
gaoq@sinovac.com

or

Dr Fengcai Zhu, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, China
jszfc@vip.sina.com

Interpretation Taking safety, immunogenicity, and production capacity into account, the 3 μg dose of CoronaVac is the suggested dose for efficacy assessment in future phase 3 trials.

Funding Chinese National Key Research and Development Program and Beijing Science and Technology Program.

Introduction

The on-going COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to high morbidity and mortality worldwide.[1] Globally, as of Oct 28, 2020, 43·3 million laboratory-confirmed cases of SARS-CoV-2 infection have been reported, resulting in 1·15 million deaths.[2]

Although physical distancing, quarantine, and isolation were effective in limiting the number of people becoming infected during the pandemic in the short term, the absence of immunity in the population leave them susceptible to further waves of SARS-CoV-2 infection. Health-care workers, older people (aged >60 years), and those with underlying health conditions are at particularly high risk.[3,4] The shortage of an effective treatment for COVID-19 has led to quick action in the development of potential vaccines against the disease.

Since the outbreak began, researchers around the world have been trying to develop vaccines for COVID-19, with more than 198 vaccines currently in preclinical or clinical development.[5] Frenetic efforts towards the development of a vaccine have led to several candidate vaccines, derived from multiple platforms and progressing to the clinical evaluation stage, including inactivated vaccines, live virus vaccines, recombinant protein vaccines, vectored vaccines, and DNA or RNA vaccines.[6–14] Development of various vaccine platforms and strategies in parallel is essential because little is known of the nature of protective immune responses to COVID-19 and which vaccine strategies will be most successful is unclear.

CoronaVac (Sinovac Life Sciences, Beijing, China) is an inactivated vaccine candidate against COVID-19 that has shown good immunogenicity in mice, rats, and non-human primates with vaccine-induced neutralising antibodies to SARS-CoV-2, which could neutralise ten representative strains of SARS-CoV-2.[15] Moreover, the results indicated CoronaVac provided partial or complete protection in macaques from severe interstitial pneumonia after a SARS-CoV-2 challenge, without observable antibody-dependent enhancement of infection, which support progression to clinical trials in humans.[15]

Methods

Study design and participants

In this single-centre, double-blind, randomised, placebo-controlled, phase 1/2 clinical trial, participants were recruited from the community to assess two two-dose regimens of CoronaVac. The study was run at Jiangsu Provincial Center for Disease Control and Prevention (CDC) in Suining County, Jiangsu province, China. The phase 1 trial was dose-escalation study. In phase 1, participants were recruited and allocated sequentially

Research in context

Evidence before this study

We searched PubMed and the American Medical Association website on Aug 13, 2020, for published research articles, with no language or date restrictions, using the search terms of "SARS-CoV-2", "COVID-19", "vaccine", and "clinical trial". The search results showed that the COVID-19 pandemic resulted in an unprecedented race to develop an effective vaccine. We identified preclinical data on three immunisations using two different doses of CoronaVac (3 μg and 6 μg per dose), an inactivated whole virus vaccine against COVID-19 developed by Sinovac Life Sciences (Beijing, China), providing partial or complete protection in macaques against SARS-CoV-2 challenge, without observable antibody-dependent enhancement of infection. We also identified a phase 2 clinical trial of another inactivated vaccine developed by Sinopharm (Beijing, China), which showed the incidence of adverse reactions was 19·0% within 28 days after two doses of vaccine (5 μg in 0·5 mL of diluent) in a day 0 and 21 vaccination schedule, and the seroconversion rates of the neutralising antibody detected by plaque reduction neutralisation test was 97·6% at 14 days after a day 0 and 21 vaccination schedule. The clinical study of CoronaVac can further provide safety and immunogenic evidence for the inactivated vaccine.

Added value of this study

In this first in-human study of CoronaVac, we used a phase 1/2 study design to screen the safety of two doses and two vaccination schedules in a dose-escalation study in a small cohort before expanding the study to a larger cohort to explore the immunogenicity of the vaccine in healthy adults. The immune response in the phase 2 study was substantially higher than in the phase 1 study, which might be due to the difference in preparation process of vaccine batches used in phase 1 and 2 resulting in a higher proportion of intact spike protein on the purified inactivated SARS-CoV-2 virions in the vaccine used in phase 2 than that used in phase 1.

Implications of all the available evidence

Data from this study support the approval of emergency use of CoronaVac in China, and three phase 3 clinical trials that are ongoing in Brazil, Indonesia, and Turkey.

(1:1), with no specific randomisation, to one of two vaccination schedules, with either a 14-day interval (the day 0 and 14 vaccination cohort) or a 28-day interval (the day 0 and 28 vaccination cohort) between doses. Within each cohort, the first 36 participants (block 1) were randomly assigned to either the low dose vaccine or placebo, and then after 7 days of follow-up for safety after the first dose, another 36 (block 2) were randomly assigned to either high-dose vaccine or placebo. Phase 2 was initiated after all participants in phase 1 has finished a 7-day safety observation period after the first dose. As in phase 1, participants were recruited and allocated (1:1) with no specific randomisation to one of the two vaccination-schedule cohorts, and then randomly assigned within each cohort to either low-dose vaccine, high-dose vaccine, or placebo.

Participants were eligible if they were healthy and aged 18–59 years. The key exclusion criteria were high-risk epidemiology history within 14 days before enrolment (eg, travel or residence history in Wuhan city and surrounding areas or other communities with case reports; contact history with someone infected with SARS-CoV-2); SARS-CoV-2 specific IgG or IgM positive in serum; positive PCR test for SARS-CoV-2 from a pharyngeal or anal swab sample; axillary temperature of more than 37·0°C; and known allergy to any vaccine component. A complete list of exclusion criteria is in the protocol.

Written informed consent was obtained from each participant before enrolment. The clinical trial protocol and informed consent form were approved by the Jiangsu Ethics Committee (JSJK2020-A021–02). This study was conducted in accordance with the requirements of Good Clinical Practice of China and the International Conference on Harmonisation.

Randomisation and masking

In both phase 1 and 2, no specific randomisation was used when allocating participants to the vaccinations schedule cohorts. In phase 1, participants in blocks 1 and 2 in each schedule cohort were randomly assigned (2:1) to either CoronaVac or placebo, and in phase 2, participants in each schedule cohort were randomly assigned (2:2:1) to either low-dose CoronaVac, high-dose CoronaVac, or placebo. The randomisation codes for each vaccination schedule cohort were generated individually, using block randomisation with a block size of six in phase 1 and a block size of five in phase 2, using SAS software (version 9.4). The randomisation code was assigned to each participant in sequence in the order of enrolment, and then the participants received the investigational products labelled with the same code. The vaccine and the placebo are identical in appearance. All participants, investigators, and laboratory staff were masked to treatment allocation.

Procedures

The phase 1 clinical trial was run in a dose-escalation manner. First, participants in block 1 were given the low dose of vaccine, and only after a successful safety observation 7 days after the first dose was the trial able to proceed and participants in block 2 be given the high dose of vaccine. The criteria that had to be met from the 7-day safety observation were that no life-threatening adverse events occur, no more than 15% of vaccinated participants report severe adverse events, and no other safety concerns in the opinion of the data monitoring committee (DMC) occur. The same conditions needed to be met 7 days after the first dose in block 2 of the phase 1 trial before the study could proceed to the phase 2 trial.

CoronaVac is an inactivated vaccine candidate against COVID-19, created from African green monkey kidney cells (Vero cells) that have been inoculated with SARS-CoV-2 (CN02 strain). At the end of the incubation period, the virus was harvested, inactivated with β-propiolactone, concentrated, purified, and finally absorbed onto aluminium hydroxide. The aluminium hydroxide complex was then diluted in a sodium chloride, phosphate-buffered saline, and water solution before being sterilised and filtered ready for injection. The placebo is just the aluminium hydroxide diluent solution with no virus. Both the vaccine and placebo were prepared in a Good Manufacturing Practice-accredited facility of Sinovac Life Sciences (Beijing, China) that is periodically inspected by the Chinese National Medical Products Administration committee for compliance. Vaccine of 3 μg and 6 μg in 0·5 mL of aluminium hydroxide diluent per dose and placebo in ready-to-use syringes were administered intramuscularly according to the dosing schedule of either day 0 and day 14, or day 0 and day 28, depending on the cohort. These vaccine doses had been found to be sufficient for protection against SARS-CoV-2 challenge in macaques.[15] Cultivation technology by cell factory system (CellSTACK Cell Culture Chamber 10, Corning, Wujiang, China) was used in the preparation of the vaccine used in the phase 1 trial. However, for the phase 2 trial, we used a highly automated bioreactor (ReadyToProcess WAVE 25, GE, Umea, Sweden) to produce the vaccine to increase vaccine production capacity. After the immunogenicity results of the trial were obtained, we discovered that the change in manufacture of the vaccine optimised the cell culture and resulted in higher intact spike protein content of the vaccine batch for the phase 2 trial, which was unexpected. However, we were not aware of this antigen-level difference between the vaccine batches for the phase 1 and 2 trials when we obtained the ethical approval for the trials.

For the first 7 days after each dose, participants were required to record the injection-site adverse events (eg, pain, redness, swelling), or systemic adverse events (eg, allergic reaction, cough, fever) on paper diary cards. From day 8 to day 28 after each dose (and day 8 to day 14 for the first dose of the days 0 and 14 vaccination cohort), safety data were collected by spontaneous report from the participants combined with the regular visit (which occurred on day 8 and day 28 after each dose, and on

For the **protocol** see http://www.jscdc.cn/jkfw/kygz/202009/t20200930_69600.html

day 8 and day 14 for the first dose in the days 0 and 14 vaccination schedule cohort). Serious adverse events were collected through the trial and will be collected until 6 months after the last dose. The reported adverse events were graded according to the China National Medical Products Administration guidelines.[16] The causal association between adverse events and vaccination was determined by the investigators.

In the phase 1 trial, blood and urine samples were taken on day 3 after each dose and tested to investigate any abnormal changes of the haematology and biochemistry indexes. 7 days after each dose, blood and urine samples were taken to measure serum inflammatory factors including IL-2, IL-6, and TNF-α using the solid phase sandwich ELISA method to explore the underlying pathological immune responses. Blood samples were collected at days 0 (baseline), 7, 14, 21, 28, and 42 from participants in the day 0 and 14 vaccination cohort, and days 0, 28, 35, 42, and 56 from participants in the days 0 and 28 vaccination schedule cohort, to determine the levels of neutralising antibodies, receptor-binding domain (RBD)-specific IgG, S-specific IgG, and IgM. Additionally, T-cell responses were determined via IFN-γ detection on day 14 after each dose.

In the phase 2 trial, blood samples were collected on day 0, 28, and 56 from participants in the days 0 and 14 cohort, and on day 56 from participants in the days 0 and 28 cohort, to determine the levels of neutralising antibodies and RBD-specific IgG.

The neutralising antibodies to live SARS-CoV-2 (virus strain SARS-CoV-2/human/CHN/CN1/2020, GenBank number MT407649.1) were quantified using a micro cytopathogenic effect assay[17] with a minimum four-fold dilution, and neutralising antibodies to pseudovirus[18] were quantified with a minimum ten-fold dilution. The S-specific IgG and IgM were detected using the chemiluminescence qualitative kit (Auto Biotechnology, Zhengzhou, China). These antibody detection tests were done by the National Institute for Food and Drug Control (Beijing, China).

Additionally, antibody titres for RBD-specific IgG were quantified using the in-house ELISA kit from Sinovac, with a minimum 160-fold dilution. T-cell response was determined with the ELISpot method using a commercial kit (Human IFN γ ELISpotPRO [3420-2AST-10, AID]; Mabtech, Stockholm, Sweden). Further information on all methods is in the appendix 2 (pp 1–3). Additionally, in a post-hoc analysis, we tested serum samples from 117 convalescent patients who had previously had COVID-19 collected in the hospitals for neutralising antibodies to live SARS-CoV-2 using the same method as for the detection of serum neutralising antibodies to live SARS-CoV-2 in the phase 1 and 2 trials, to give a comparison of the vaccine-induced and infection-induced humoral immunity. Written informed consent was obtained from all these convalescent patients.

See Online for appendix 2

Outcomes

The primary safety endpoint was any adverse reactions within 28 days after each dose of study drug. Secondary safety endpoints were any abnormal changes in laboratory measurements at day 3 and in serum inflammatory factors 7 days after each dose of study drug. The secondary safety endpoints were prespecified only in the phase 1 trial.

The primary immunogenic endpoint was the seroconversion of neutralising antibodies to live SARS-CoV-2 at day 14 after the last dose in the days 0 and 14 vaccination cohort, or day 28 after the last dose in the days 0 and 28 vaccination cohort. Secondary immunogenic endpoints were geometric mean titres (GMTs) of neutralising antibodies to live SARS-CoV-2, RBD-specific IgG, S-specific IgG, and IgM. Exploratory endpoints were T-cell responses and, post hoc, GMTs of neutralising antibodies to psuedovirus. Seroconversion of antibodies was defined as a change from seronegative at baseline to seropositive or a four-fold titre increase if the participant was seropositive at baseline. The positive cutoff of the neutralising antibodies to live SARS-CoV-2 was 1/8, neutralising antibodies to pseudovirus was 1/30, and RBD-specific IgG was 1/160. Regarding the ELISpot measured T-cell response, the results were expressed as the number of spot-forming cells (SFCs) per 100 000 cells.

Other secondary endpoints are listed in the appendix 2 (p 4), including 6 month outcomes that are not available yet, which will be reported elsewhere.

Statistical analysis

We assessed the safety endpoints in the safety population, which included all participants who received at least one dose of study drug. We assessed immunogenic endpoints in the per-protocol population, which included all participants who completed their assigned two-dose vaccination schedule and with available antibody results.

We did not determine the sample size on the basis of a statistical power calculation, but followed the requirement of the National Medical Products Administration in China—ie, recruitment of at least of 20–30 participants in phase 1 and 500 participants in phase 2.

We used the Pearson χ^2 test or Fisher's exact test for the analysis of categorical outcomes. We calculated 95% CIs for all categorical outcomes using the Clopper-Pearson method. We calculated GMTs and corresponding 95% CIs on the basis of standard normal distribution of the log-transformed antibody titre. We used the ANOVA method to compare the log-transformed antibody titre. When the comparison among all three groups showed significant difference, we then did pairwise comparisons. Hypothesis testing was two-sided and we considered p values of less than 0·05 to be significant.

An independent data monitoring committee consisted of one independent statistician, one clinician, and one epidemiologist was established before commencement of the study. Safety data were assessed and

reviewed by the committee to ensure the suspension criteria of the dose-escalation part of phase 1 were not met and allow the further proceeding of the clinical trial.

We used SAS (version 9.3) for all analyses. This trial is registered with ClinicalTrials.gov, NCT04352608.

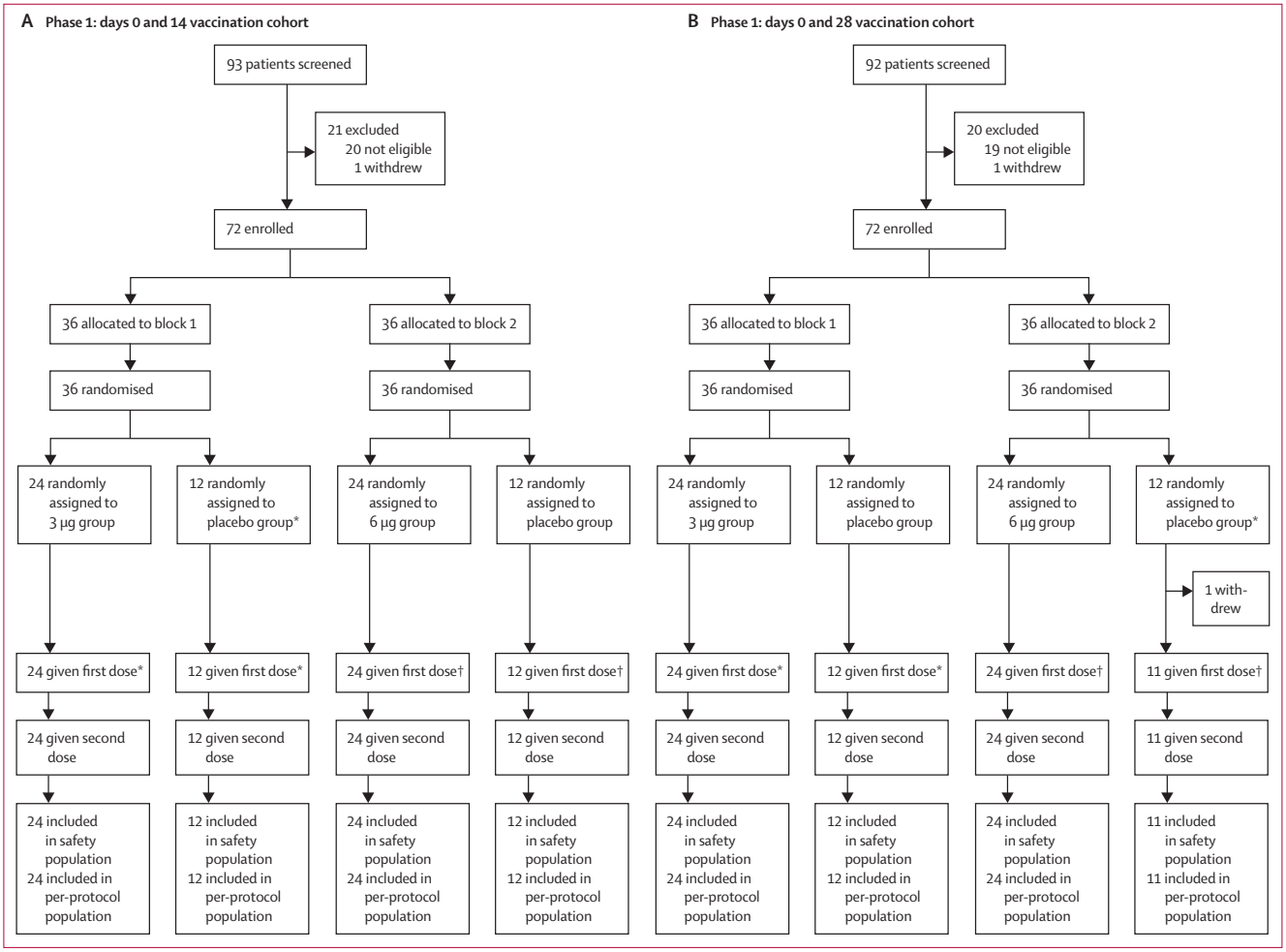
Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All the authors have full access to all the data in the study and the corresponding authors had final responsibility for the decision to submit for publication.

Results

Between April 16 and April 25, 2020, 185 individuals were screened and 144 participants were enrolled in the phase 1 trial, and between May 3 and May 5, 2020, 662 individuals were screened and 600 participants were enrolled in the phase 2 trial. 743 participants received at least one dose of the investigational product (143 for phase 1 and 600 for phase 2) and were included in the safety population (figure 1). 143 participants in phase 1 and 591 participants in phase 2 were eligible for the immunogenic evaluation (per-protocol population; figure 1). Baseline demographic characteristics of the participants in the safety population at enrolment were similar among the treatment groups in terms of sex, nationality, and mean age (table 1).

In the phase 1 trial, the overall incidence of adverse reactions was seven (29%) of 24 participants in the 3 µg group, nine (38%) of 24 in the 6 µg group, and two (8%) of 24 in the placebo group in the days 0 and 14 vaccination cohort; and three (13%) of 24 in the 3 µg group, four (17%) of 24 in the 6 µg group, and three (13%) of 23 in the placebo group in the days 0 and 28 vaccination



(Figure 1 continues on next page)

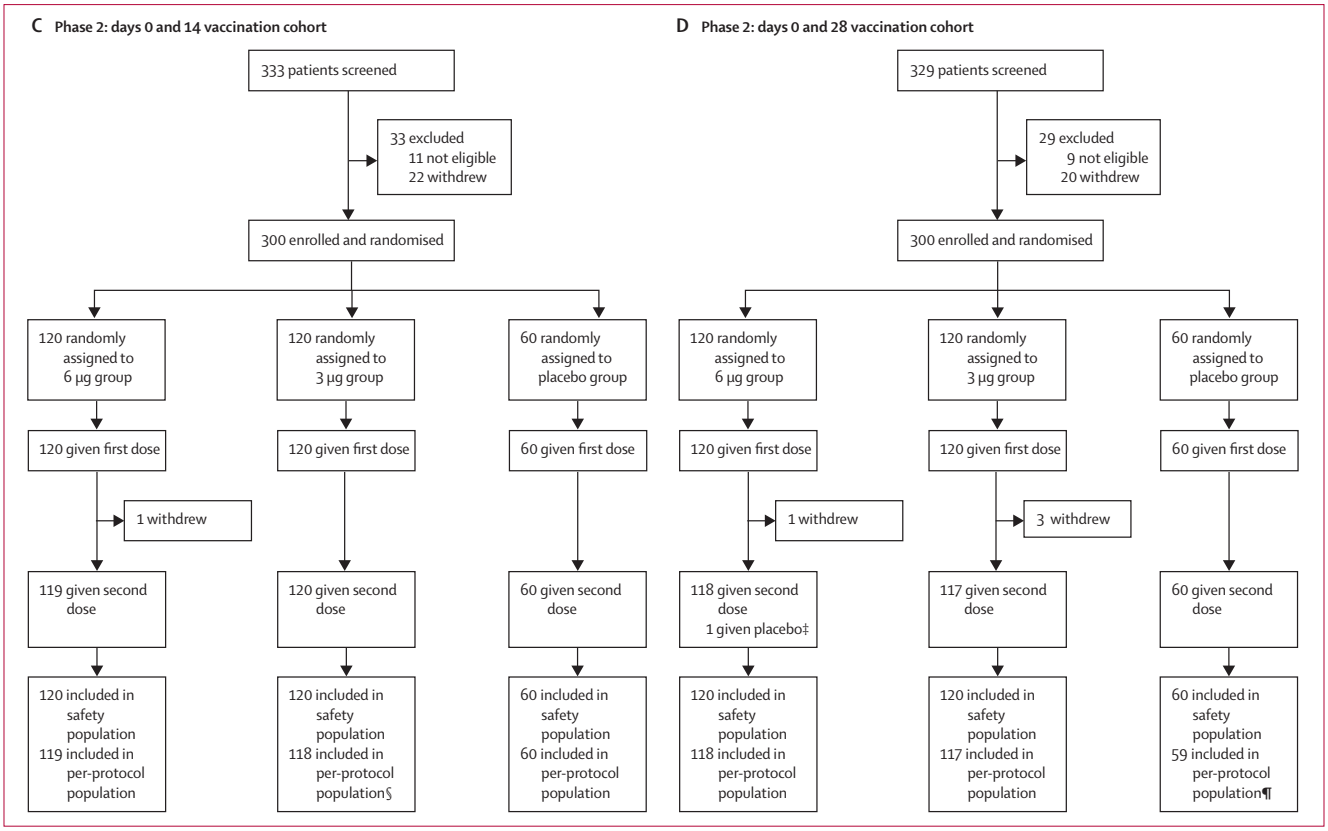


Figure 1: **Study profile**

*7 days after first dose, safety observation was done, and safety criteria were met, as determined by the data monitoring committee, participants in block 2 were then given their first dose of vaccine. †7 days after first dose of study drug in block 2, if safety criteria were met as determined by the data monitoring committee, participants enrolled in phase 2 were started on study treatment. ‡A participant in the 6 µg group was mistakenly given placebo rather than vaccine at the second dose; therefore, this participant was included in the 6 µg group dataset in the overall safety evaluation but not in the immunogenicity analysis. §Two participants did not have available antibody results, and so were not included in the immunogenicity analysis. ¶One participant did not have available antibody results, and so was not included in the immunogenicity analysis.

	3 µg group	6 µg group	Placebo group	Overall
Days 0 and 14 vaccination cohorts, pooled				
Participants	144	144	84	372
Sex				
Female	77 (53%)	86 (60%)	44 (52%)	207 (56%)
Male	67 (47%)	58 (40%)	40 (48%)	165 (44%)
Han nationality	144 (100%)	144 (100%)	84 (100%)	372 (100%)
Age, years	42·4 (10·2)	42·8 (9·0)	42·4 (8·8)	42·6 (9·4)
Days 0 and 28 vaccination cohorts, pooled				
Participants	144	144	83	371
Sex				
Female	75 (52%)	70 (49%)	45 (54%)	190 (51%)
Male	69 (48%)	74 (51%)	38 (46%)	181 (49%)
Han nationality	144 (100%)	144 (100%)	83 (100%)	371 (100%)
Age, years	41·8 (9·4)	41·2 (10·2)	44·1 (9·1)	42·1 (9·7)

Data are n, n (%), or mean (SD).

Table 1: **Baseline demographic characteristics for the safety population, phases 1 and 2 combined**

cohort, with no significant difference seen among the three groups for both vaccination schedules (figure 2; appendix 2 pp 5–6). The most common symptom was injection-site pain, which was reported by four (17%) participants in the 3 µg group, five (21%) in the 6 µg, and one (4%) in the placebo group in the days 0 and 14 vaccination cohort and three (13%) in the 3 µg group, three (13%) in the 6 µg group, and three (13%) in the placebo group in the days 0 and 28 vaccination cohort. Most adverse reactions were mild (grade 1) in severity and participants recovered within 48 h. Only one case of acute hypersensitivity with manifestation of urticaria 48 h after the first dose of study drug was reported in the 6 µg group (one [4%] of 24) in the days 0 and 14 vaccination cohort, which was graded as severe and considered to be possibly related to vaccination. The participant was given chlorphenamine and dexamethasone and recovered within 3 days, and no similar reaction was observed after the second dose of vaccine. No vaccine-related serious adverse events were noted within 28 days of vaccination (figure 2; appendix 2 pp 4–5).

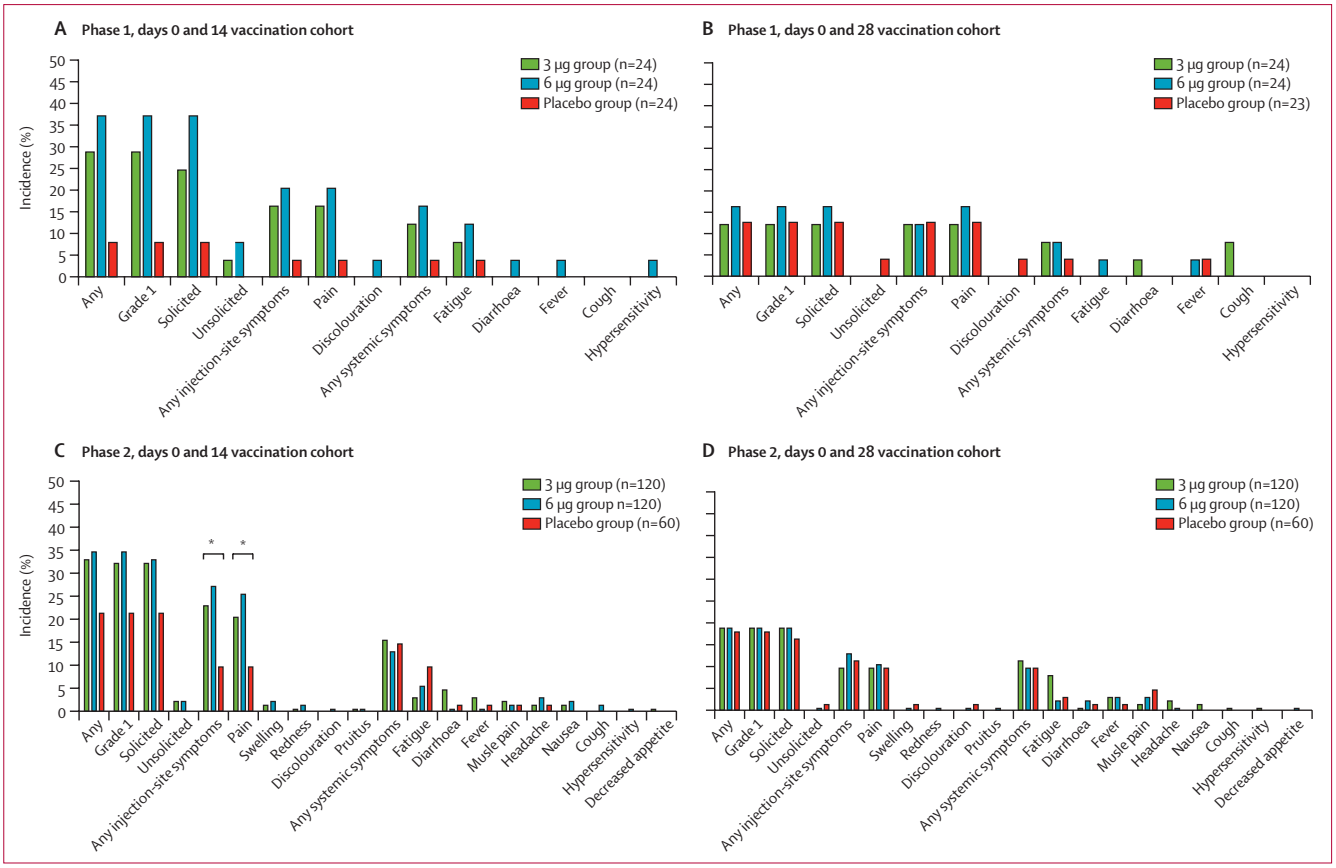


Figure 2: **Incidence of adverse reactions reported within 28 days after second dose of study drug, in the days 0 and 14 vaccination cohort in phase 1 (A) and phase 2 (C) and in the days 0 and 28 vaccination cohort in phase 1 (B) and phase 2 (D)**

Adverse reactions refer to the adverse events related to the vaccination. Rare injection-site symptoms reported only in the days 0 and 14 vaccination cohort are not shown in the figure and are listed in appendix 2 along with all adverse reactions after the first and second dose (pp 4–13). *The p value of comparison among three groups is significant for the incidence of any injection-site symptoms (p=0·02) and injection-site pain (p=0·04).

Additionally, ten (7%) of 143 participants in phase 1 had a clinically significant increase of laboratory indicators at day 3 after vaccination (appendix 2 pp 15–16), but none was considered to be related to the vaccination. No significant increases in inflammatory factors in serum were detected at day 7 after each dose (appendix 2 pp 17–18).

At baseline, none of the participants in the phase 1 trial had any detectable neutralising antibodies to live SARS-CoV-2. The seroconversion rates of neutralising antibodies were 11 (46%) of 24 participants in the 3 μg group (GMT 5·6 [95% CI 3·6–8·7]) versus 12 (50%) of 24 participants in the 6 μg group (7·7 [5·2–11·5]) versus none of 24 participants in the placebo group (2·0 [2·0–2·0]) at 14 days after the second dose, and six (25%) participants in the 3 μg group (5·4 [3·6–8·1] versus 20 (83%) in the 6 μg group (15·2 [11·2–20·7]) versus none in the placebo group (2·0 [2·0–2·0]) at 28 days after the second dose in the days 0 and 14 vaccination cohort; and 19 (79%) of 24 participants in the 3 μg group (16·0 [10·4–24·7]) versus 20 (83%) of 24 in the 6 μg group (25·9 [14·6–46·1) versus none of 23 in the placebo group (2·0 [2·0–2·0]) at 14 days after the second dose, and 20 (83%) in the 3 μg group (19·0 [13·2–27·4] versus 19 (79%) in the 6 μg group (29·6 [17·9–48·9]) versus one (4%) in the placebo group (2·2 [1·8–2·8]) at 28 days after the second dose in the days 0 and 28 vaccination cohort (table 2, figure 3; appendix 2 p 19). The seroconversion rates of RBD-specific IgG were 20 (83%) of 24 participants in the 3 μg group (GMT 465·8 [95% CI 277·6–781·7] versus 24 (100%) of 24 participants in the 6 μg group (987·0 [647·8–1504·0]) versus two (8%) of 24 participants in the placebo group (84·8 [78·0–92·1]) at 14 days after the second dose, and 21 (88%) in the 3 μg group (465·8 [288·1–753·1]) versus 24 (100%) in the 6 μg group (1395·9 [955·2–2039·7]) versus two (8%) in the placebo group (89·8 [76·1–105·9]) at 28 days after the second dose in the days 0 and 14 vaccination cohort; and 24 (100%) of 24 participants in the 3 μg group (1365·1 [881·4–2086·4]) versus 24 (100%) of 24 participants in the 6 μg group (2152·7 [1446·1–3204·6])

	3 μg group	6 μg group	Placebo group	p value*
Phase 1				
Days 0 and 14 vaccination cohort				
Neutralising antibodies to live SARS-CoV-2				
Day 14	11/24 (45·8%; 25·6–67·2)	12/24 (50·0%; 29·1–70·9)	0/24 (0·0%; 0·0–14·3)	0·77
Day 28	6/24 (25·0%; 9·8–46·7)	20/24 (83·3%; 62·6–95·3)	0/24 (0·0%; 0·0–14·3)	<0·0001
RBD-IgG				
Day 14	20/24 (83·3%; 62·6–95·3)	24/24 (100%; 85·8–100)	2/24 (8·3%; 1·0–27·0)	0·11
Day 28	21/24 (87·5%; 67·6–97·3)	24/24 (100%; 85·8–100)	2/24 (8·3%; 1·0–27·0)	0·23
Days 0 and 28 vaccination cohort				
Neutralising antibodies to live SARS-CoV-2				
Day 14	19/24 (79·2%; 57·9–92·9)	20/24 (83·3%; 62·6–95·3)	0/23 (0·0%; 0·0–14·8)	1·00
Day 28	20/24 (83·3%; 62·6–95·3)	19/24 (79·2%; 57·9–92·9)	1/23 (4·4%; 0·1–22·0)	1·00
RBD-IgG				
Day 14	24/24 (100%; 85·8–100)	24/24 (100%; 85·8–100)	0/23 (0·0%; 0·0–14·8)	1·00
Day 28	24/24 (100%; 85·8–100)	24/24 (100%; 85·8–100)	0/23 (0·0%; 0·0–14·8)	1·00
Phase 2				
Days 0 and 14 vaccination cohort				
Neutralising antibodies to live SARS-CoV-2				
Day 14	109/118 (92·4%; 86·0–96·5)	117/119 (98·3%; 94·1–99·8)	2/60 (3·3%; 0·4–11·5)	0·030
Day 28	111/118 (94·1%; 88·2–97·6)	117/118 (99·2%; 95·4–100)	0/60 (0·0%; 0·0–6·0)	0·066
RBD-IgG				
Day 14	111/115 (96·5%; 91·3–99·0)	118/118 (100%; 96·9–100)	0/56 (0·0%; 0·0–6·4)	0·058
Day 28	111/114 (97·4%; 92·5–99·5)	118/118 (100%; 96·9–100)	0/57 (0·0%; 0·0–6·3)	0·12
Days 0 and 28 vaccination cohort				
Neutralising antibodies to live SARS-CoV-2				
Day 28	114/117 (97·4%; 92·7–99·5)	118/118 (100%; 96·9–100)	0/59 (0·0%; 0·0–6·1)	0·12
RBD-IgG				
Day 28	116/117 (99·2%; 95·3–100)	117/117 (100%; 96·9–100)	4/59 (6·8%; 1·9–16·5)	1·00

Data are n/N (%; 95% CI). Timepoints refer to the number of days since the second dose of vaccine in the schedule. RBD=receptor binding domain. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. *p values are for comparisons between the 3 μg and 6 μg groups.

Table 2: **Seroconversion rates of neutralising antibodies to live SARS-CoV-2 and RBD-specific IgG**

versus none of 23 participants (80·0 [80·0-80·0]) in the placebo group at 14 days after the second dose, and 24 (100%) in the 3 μg group (1045·7 [721·6–1515·5]), versus 24 (100%) in the 6 μg group (1917·9 [1344·8–2735·2]) versus none in the placebo group (80·0 [80·0–80·0]) 28 days after the second dose in the days 0 and 28 vaccination cohort (table 2, figure 3; appendix 2 p 19). The dynamic changes of RBD-specific IgG, S-specific IgG, S-specific IgM, and neutralising antibodies to pseudovirus are shown in the appendix 2 (pp 19–23), showing that the antibody levels did not significantly increase until after the second dose of vaccine.

At 14 days after the second dose of study drug, the average IFN-γ-positive SFCs per 100 000 cells were 7·4 (95% CI 3·9 to 11·1) in the 3 μg group, 3·9 (1·0 to 6·7) in the 6 μg group, and 1·5 (0·2 to 2·9) in the placebo group for the days 0 and 14 vaccination cohort; and 3·4 (0·9 to 5·7) in the 3 μg group, 1·2 (0·5 to 1·8) in the 6 μg group, and 1·2 (–0·1 to 2·5) in the placebo group for the days 0 and 28 vaccination cohort (appendix 2 pp 25–26).

In the phase 2 trial, the overall incidence of adverse reactions were 40 (33%) of 120 in the 3 μg group, 42 (35%) of 120 in the 6 μg group, and 13 (22%) of 60 in the placebo group for the days 0 and 14 vaccination cohort and 23 (19%) of 120 in the 3 μg group, 23 (19%) of 120 in the 6 μg group, and 11 (18%) of 60 in placebo group in the days 0 and 28 vaccination cohort, with no significant difference between the three groups for both schedules. However, the p value of comparison among the three groups was significant for the incidence of any injection-site symptoms (p=0·02) and injection-site pain (p=0·04; figure 2; appendix 2 pp 7–10). The most common symptom was injection-site pain, which occurred in 25 (21%) of 120 participants in the 3 μg group, 31 (26%) of 120 in the 6 μg group, and six (10%) of 60 in the placebo group for the days 0 and 14 vaccination cohort, and 12 (10%) of 120 in the 3 μg group, 13 (11%) of 120 in the 6 μg group, and six (10%) of 60 in the placebo group in the days 0 and 28 vaccination cohort. Most adverse reactions were mild (grade 1) in severity and the participants recovered within 48 h. No vaccine-related serious adverse events were noted within 28 days of the second dose of vaccine (figure 2; appendix 2 pp 7–10)

In the phase 2 trial, at baseline, none of the participants had any detectable neutralising antibodies. The seroconversion rates of neutralising antibodies to live SARS-CoV-2 were 109 (92%) of 118 participants in the 3 μg group (GMT 27·6 [95% CI 22·7–33·5]) versus 117 (98%) of 119 participants in the 6 μg group (34·5 [28·5–41·8] versus two (3%) of 60 participants in the placebo group (2·3 [2·0–2·5]) at 14 days after the second dose, and 111 (94%) of 118 in the 3 μg group (23·8 [20·5–27·7]) versus 117 (99%) of 118 in the 6 μg group (30·1 [26·1–34·7]) versus none of 60 in the placebo group (2·0 [2·0–2·0]) at 28 days after the second dose in the day 0 and 14 vaccination cohort; and 114 (97%) of 117 participants in the 3 μg group (44·1 [37·2–52·2]) versus 118 (100%) of 118 participants in the 6 μg group (65·4 [56·4–75·9]) versus none of 59 participants in the placebo group (2·0 [2·0–2·1]) at 28 days after the second dose in the days 0 and 28 vaccination cohort (table 2, figure 3). In post-hoc analyses, the neutralising antibody titres after the second dose of vaccine was lower in all participants who received the vaccine than was detected in 117 convalescent asymptomatic patients who had previously had COVID-19

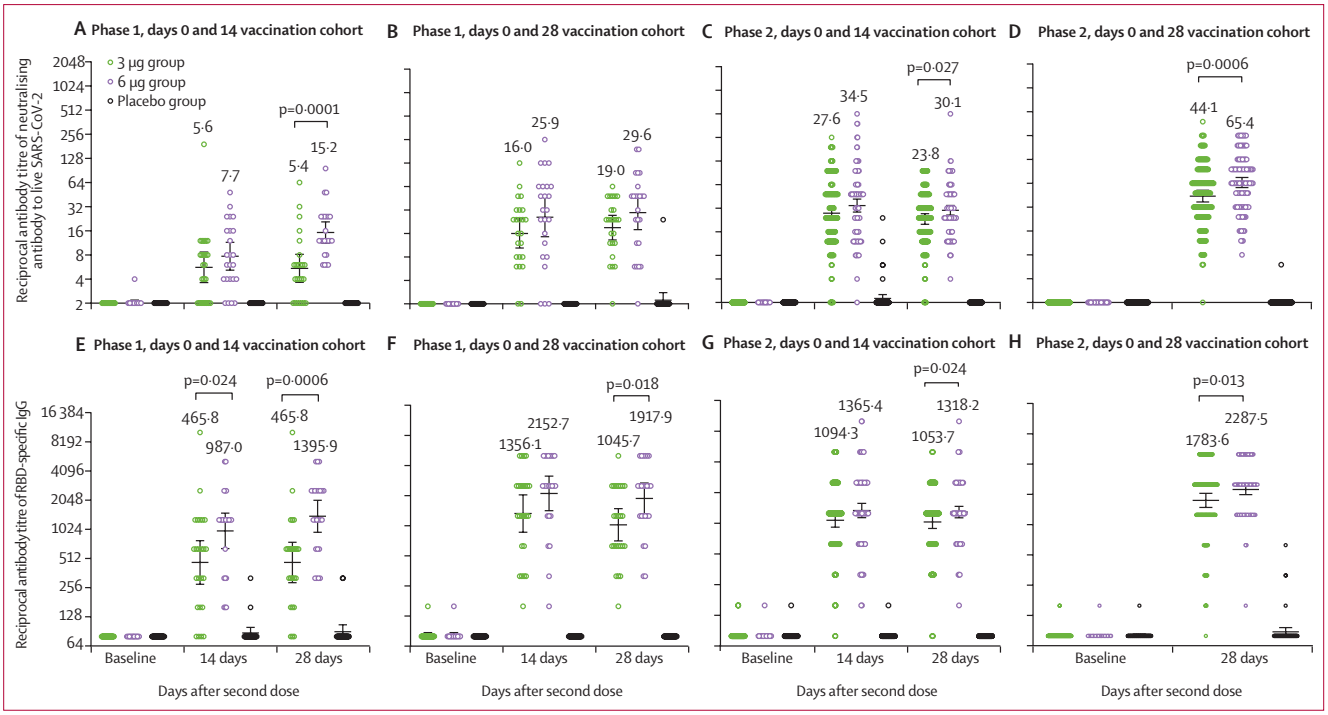


Figure 3: **Antibody titres of neutralising antibodies to live SARS-CoV-2 (A–D) and RBD-specific IgG (E–H) induced after two doses of CoronaVac or placebo given in the days 0 and 14 and days 0 and 28 vaccination cohorts, in the phase 1 and phase 2 trials**

The error bars indicate the 95% CI of the GMT and the spots indicated the individual antibody titres, with the numbers above the spots showing the GMT estimate. Only p values for significant differences are shown on the figure, all p values for all data are in appendix 2 (p 19). GMT=geometric mean titre. RBD=receptor binding domain. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

(GMT 163·7 [95% CI 128·5–208·6]; table 2, figure 3; appendix 2 p 24). The seroconversion rates of RBD-specific IgG were 111 (97%) of 115 participants in the 3 μg group (GMT 1094·3 [95% CI 936·7–1278·4]) versus 118 (100%) of 118 participants in the 6 μg group (1365·4 [1160·4–1606·7]) versus none of 56 participants in the placebo group (81·0 [79·0–83·0]) at 14 days after the second dose and 111 (97%) of 114 in the 3 μg group (1053·7 [911·7–1217·7]) versus 118 (100%) of 118 in the 6 μg group (1318·2 [1156·9–1501·9]) versus none of 57 in the placebo group (80·0 [80·0–80·0]) at 28 days after the second dose in the day 0 and 14 vaccination cohort; and 116 (99%) of 117 in the 3 μg group (1783·6 [1519·3–2093·8]) versus 117 (100%) of 117 in the 6 μg group (2287·5 [2038·2–2567·3]) versus four (7%) of 59 in the placebo group (87·9 [79·7–96·9]) at 28 days after the second dose in the days 0 and 28 vaccination cohort (table 2, figure 3).

Based on the pooled data of the phase 1 and 2 trials (two vaccination cohorts pooled), the correlation coefficient between the neutralising antibody to live SARS-CoV-2 and RBD-specific IgG was 0·85 (95% CI 0·82–0·92) using the antibody titre at 28 days after the second dose of vaccine, and was 0·80 (0·75–0·86) using the titre 14 days after the second. The correlation coefficient between the neutralising antibody to live SARS-CoV-2 and the neutralising antibody to pseudovirus was 0·82 (0·76–0·88) using the antibody titre at 14 days after the second dose (no data taken at day 28). The correlation coefficient between the neutralising antibody to pseudovirus and RBD-specific IgG was 0·73 (0·66–0·80) using the antibody titre at 14 days after the second dose (no data taken at day 28; appendix 2 p 24).

Discussion

We found that two doses of CoronaVac at different concentrations and using different dosing schedules were well tolerated and moderately immunogenic in healthy adults aged 18–59 years. The incidence of adverse reactions in the 3 μg and 6 μg group were similar, indicating no dose-related safety concerns but more long-term follow-up is needed. Furthermore, most adverse reactions were mild, with the most common symptom being injection-site pain, which is in accordance with previous findings for another inactivated COVID-19 vaccine from Sinopharm (Beijing China).[14] Compared with other COVID-19 vaccine candidates, such as viral-vectored vaccines or DNA or RNA vaccines, the occurrence of fever after vaccination with CoronaVac was relatively low.[10,11,13]

Over the course of the phase 1/2 trial, we changed the production process of the vaccine from the use of a cell factory process (which was used in our preclinical and

phase 1 study to generate a 50 L culture of Vero cells) to use of a bioreactor for phase 2. The bioreactor process enabled use to optimise the process for growing cells, with precise control over cell culture parameters like dissolved oxygen, pH, and carbon dioxide and oxygen gas levels. We made this change to increase vaccine production capacity and meet biosafety requirements. Pre-clinical data for each phase trial (data not shown) indicated that the safety profiles of vaccines prepared via the new bioreactor process and old process are similar. Notably, immune responses in phase 2 were much better than those recorded in phase 1, with seroconversion rates over 90% in both the 3 μg and 6 μg groups. To investigate the reason for this change, we did a protein composition analysis of the purified inactivated SARS-CoV-2 virions and found that the bioreactor-produced vaccine had a higher redundancy of intact spike protein (molecular mass approximately 180 kDa) than did the vaccine produced via the cell factory process (appendix 2 p 27). Quantitative analysis showed that the intact spike protein accounted for approximately 3·7% of total protein mass of the vaccine used in phase 1 and approximately 7·0% of total protein mass of the vaccine used in phase 2 trials. Electron microscopic examination of the samples further verified that the average number of spikes per virion of the viral sample used in the phase 2 trial was almost double the number of spikes per virion of the sample used in phase 1 trial (appendix 2 p 27). These observations highlight the importance of developing an optimum manufacturing process and the integration of multi-disciplinary techniques, such as genomics and structural biology to support a new era of precision vaccinology.

The immune response induced by 3 μg and 6 μg of vaccine in 0·5 mL of diluent per dose was similar in this study. As anticipated, after two doses of vaccine, immune responses induced by the days 0 and 28 vaccination schedule were larger than those induced by the days 0 and 14 vaccination schedule, regardless of the dose. However, quick antibody responses could be induced within a relatively short time by using a day 0 and 14 vaccination schedule, which might be suitable for emergency use and is of vital importance during the COVID-19 pandemic. Regarding the days 0 and 28 vaccination schedule, a more robust antibody response was generated and longer persistence could be expected than with the days 0 and 14 schedule, which supports potential routine use of the vaccine according to this schedule when the epidemic risk of COVID-19 is low. However, the actual immune persistence of the two schedules needs to be verified in future studies.

In the phase 2 trial, the level of neutralising antibodies included by the vaccine at day 28 after the last dose of vaccine ranged from a GMT of 23·8 to 65·4, depending on the vaccination schedule, which was lower than those of convalescent patients who previously had COVID-19 with an average GMT level of 163·7, tested by the same method in the same laboratory.[19] However, we still think that CoronaVac could provide satisfying protection against COVID-19 on the basis of the following three reasons. First, from the experiences of other vaccines, such as the enterovirus 71 and varicella vaccines, most of the surrogate endpoints based on neutralising antibody titres have ranged from 8 to 24.[20,21] Second, our preclinical study[15] indicated that the neutralising antibody titres of 1/24 elicited in macaque models conferred complete protection against SARS-CoV-2. Third, although several studies have found that antibody responses generated from natural infection with coronaviruses (eg, SARS-CoV-2, severe acute respiratory syndrome coronavirus, and Middle East respiratory syndrome coronavirus) might decrease substantially over time,[22–24] reinfection in these patients has rarely been reported,[25–27] which indicates that immunological memory might have an important role of prevention of re-infections. Therefore, the antibody level itself might not be the key for a successful COVID-19 vaccine, but rather the establishment of a recallable specific immune response to SARS-CoV-2. Furthermore, the efficacy of the investigational vaccine and its surrogate endpoint need to be determined in a future phase 3 trial. Additionally, comparability of our serum antibody results with those of other COVID-19 vaccine studies is restricted.

Two participants in the placebo group in the phase 1 trial and four in the placebo group in the phase 2 trial had seroconversion of anti-RBD IgG after vaccination, and one participant given placebo in the phase 1 trial and two in the phase 2 trial had seroconversion of neutralising antibodies after vaccination.

CoronaVac was well tolerated and induced humoral responses against SARS-CoV-2, which supported the approval of emergency use of CoronaVac in China, and three phase 3 clinical trials that are ongoing in Brazil (NCT04456595), Indonesia (NCT04508075), and Turkey (NCT04582344). Taking safety, immunogenicity, and production capacity into account, the low dose of 3 μg of CoronaVac in 0·5 mL of diluent, with a day 0 and 14 vaccination schedule, is being investigated in these ongoing trials. And the days 0 and 28 vaccination schedule with 3 μg of Coronavac in 0·5 mL of diluent will also be investigated in future phase 3 clinical trials. The protective efficacy of CoronaVac remains to be determined.

Our study had several limitations. First, we did not assess the T cell responses in the phase 2 trial; however, the response of type 1 T-helper cells and type 2 T-helper cells induced by CoronaVac will be studied in the ongoing phase 3 study in Brazil (NCT04456595). Second, we only reported immune response data for healthy adults, and did not include individuals from more susceptible groups in our study population (eg, older individuals [aged ≥60 years] or with comorbidities); and data on immune persistence is not yet available, which need to be further studied. Third, the calculated p values presented in this study cannot support any powerful statistical conclusions, and are only for reference and so

should be interpreted with caution. Additionally, the T-cell responses measured by ELISpot were low in participants who were given vaccine, which provided no clear evidence that the vaccine induced T-cell responses. The assessment of immune reactions mediated by CD8 cells was not included in our study design, because inactivated vaccines are not thought to induce CD8 T-cell responses. Finally, the change in the manufacturing of vaccine batches for the phase 2 trial resulted in a higher level of the spike antigen contained in the vaccine than was used in the phase 1 trial. Although the change in manufacturing process was planned, the difference in antigenicity of the vaccines was not anticipated, and could potentially bring additional risks for the recipients of the vaccine. Fortunately, the safety profiles of the vaccines in the phase 1 and 2 trials were similar, although the vaccines for the phase 2 trial had substantially stronger immunogenicity than did the vaccines for phase 1 trial. However, the comparisons between the vaccine batches were also not an a-priori defined outcome or sufficiently powered.

In summary, CoronaVac was well tolerated and induced humoral responses against SARS-CoV-2, which suppored the approval of emergency use of CoronaVac in China and in three phase 3 studies. The protective efficacy of CoronaVac remains to be determined.

Contributors

YZ, GZ, HP, and CL were co-first authors of this manuscript. FZ was the principal investigator and HP was the coprincipal investigator of this trial. FZ, GZ, RT, and QG designed the trial and study protocol. YZ, YaH, and WH contributed to the literature search. All authors had access to data and GZ, FZ, and HP verified the data. WH, JL, XW wrote the first draft the manuscript. FZ, YZ, GZ, WY, YaH, and MY contributed to the data interpretation and revision of the manuscript. YuH monitored the trial. XC, XL, CJ, and YS were responsible for the site work including the recruitment, follow up, and data collection, and KC was the site coordinator. CL and ZC were responsible to the laboratory analysis.

Declaration of interests

QG is an employee of Sinovac Life Sciences. GZ, YaH, WH, WY, and YuH are employees of Sinovac Biotech. All other authors declare no competing interests.

Data sharing

The individual participant-level data that underlie the results reported in this Article will be shared after deidentification (text, tables, figures, and appendices). This clinical trial is ongoing, and all the individual participant data cannot be available until after the immune persistence assessments have been done. The data will be available immediately after publication and finalisation of the complete clinical study report for at least 6 months. Supporting clinical documents including study protocol, statistical analysis plan, and the informed consent form will be available immediately after publication of this Article for at least 1 year. Information on how to access the supporting clinical documents is available online. Researchers who provide a scientifically sound proposal will be allowed to access the de-identified individual participant data. Proposals should be sent to the corresponding authors, at jszfc@vip.sina.com or gaoq@sinovac.com. These proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. To gain access, data requestors will need to sign a data access agreement.

Acknowledgments

This study was funded by the National Key Research and Development Program (2020YFC0849600) and Beijing Science and Technology Program (Z201100005420023).

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JAMA Insights

Reactogenicity Following Receipt of mRNA-Based COVID-19 Vaccines

Johanna Chapin-Bardales, PhD, MPH; Julianne Gee, MPH; Tanya Myers, PhD, MSc

In December 2020, 2 mRNA-based COVID-19 vaccines (Pfizer-BioNTech and Moderna) were granted Emergency Use Authorization by the US Food and Drug Administration as 2-dose series and recommended for use by the Advisory Committee on Immunization Practices.[1-3] In late February 2021, the US Food and Drug Administration granted Emergency Use Authorization for a third COVID-19 vaccine, a single-dose adenovirus vector-based vaccine from Janssen (Johnson & Johnson).

Supplemental content

In clinical trials of the mRNA-based 2-dose vaccines, participants reported local and systemic reactions (reactogenicity).[4,5] Frequently reported reactions included injection site pain, fatigue, and headache; greater reactogenicity was reported following the second dose.[4,5] Continued monitoring of reactogenicity of COVID-19 vaccines outside of clinical trial settings may provide additional information for health care practitioners and the public about transient local and systemic reactions following COVID-19 vaccination.

V-safe Active Surveillance System

To facilitate rapid assessment of COVID-19 vaccines, in 2020, the Centers for Disease Control and Prevention (CDC) established v-safe, a new active surveillance system for collecting near–real-time data from COVID-19 vaccine recipients in the US. V-safe participants voluntarily self-enroll and receive periodic smartphone text messages to initiate web-based health surveys from the day of vaccination (day 0) through 12 months after the final dose of a COVID-19 vaccine.[6] From day 0 through day 7 after each vaccine dose, participants are asked questions about solicited local and systemic reactions (eg, injection site pain, fatigue, headache). These solicited reactions do not include allergic reactions or anaphylaxis; however, v-safe does allow participants to enter free-text information about their postvaccination experience and asks about adverse health events (eg, received medical care). Medically attended events are followed up on through active telephone outreach; future analyses will address these adverse vaccine experiences. This report describes information on solicited local and systemic reactogenicity reported to v-safe on days 0 to 7 after each dose of vaccine from December 14, 2020, through February 28, 2021. Responses were limited to individuals who were vaccinated by February 21, 2021, to allow a 7-day reporting period after the day of vaccination. Preliminary data from v-safe through January 13, 2021, have been previously reported.[7] This activity was reviewed by the CDC and was conducted consistent with applicable federal law and CDC policy (see Additional Information).

Table. Solicited Local and Systemic Reactions[a] to mRNA-Based COVID-19 Vaccines Reported 0 to 7 Days After Vaccination—Centers for Disease Control and Prevention V-safe Surveillance System, December 14, 2020, to February 28, 2021

Reaction	No. (%)					
	Dose 1			Dose 2		
	Both vaccines (N = 3 643 918)	Pfizer-BioNTech (n = 1 659 724)	Moderna (n = 1 984 194)	Both vaccines (N = 1 920 872)	Pfizer-BioNTech (n = 971 375)	Moderna (n = 949 497)
Any injection site reaction	2 550 710 (70.0)	1 085 242 (65.4)	1 465 468 (73.9)	1 443 899 (75.2)	666 635 (68.6)	777 264 (81.9)
Pain	2 472 373 (67.8)	1 055 604 (63.6)	1 416 769 (71.4)	1 389 629 (72.3)	645 917 (66.5)	743 712 (78.3)
Redness	204 097 (5.6)	56 780 (3.4)	147 317 (7.4)	240 265 (12.5)	57 956 (6.0)	182 309 (19.2)
Swelling	379 539 (10.4)	110 077 (6.6)	269 462 (13.6)	348 986 (18.2)	100 430 (10.3)	248 556 (26.2)
Itching	197 441 (5.4)	62 486 (3.8)	134 955 (6.8)	214 658 (11.2)	60 946 (6.3)	153 712 (16.2)
Any systemic reaction[a]	1 823 068 (50.0)	797 410 (48.0)	1 025 658 (51.7)	1 333 931 (69.4)	623 746 (64.2)	710 185 (74.8)
Fatigue	1 127 638 (30.9)	483 146 (29.1)	644 492 (32.5)	1 034 462 (53.9)	464 659 (47.8)	569 803 (60.0)
Headache	943 607 (25.9)	409 359 (24.7)	534 248 (26.9)	897 005 (46.7)	392 266 (40.4)	504 739 (53.2)
Myalgia	705 100 (19.4)	281 743 (17.0)	423 357 (21.3)	845 314 (44.0)	357 381 (36.8)	487 933 (51.4)
Chills	321 009 (8.8)	116 034 (7.0)	204 975 (10.3)	600 354 (31.3)	220 831 (22.7)	379 523 (40.0)
Fever	314 676 (8.6)	116 951 (7.0)	197 725 (10.0)	566 112 (29.5)	208 976 (21.5)	357 136 (37.6)
Joint pain	317 034 (8.7)	123 319 (7.4)	193 715 (9.8)	492 031 (25.6)	192 926 (19.9)	299 105 (31.5)
Nausea	275 423 (7.6)	114 087 (6.9)	161 336 (8.1)	319 248 (16.6)	127 454 (13.1)	191 794 (20.2)
Vomiting	25 425 (0.7)	9966 (0.6)	15 459 (0.8)	31 056 (1.6)	11 276 (1.2)	19 780 (2.1)
Diarrhea	189 878 (5.2)	83 016 (5.0)	106 862 (5.4)	133 877 (7.0)	60 641 (6.2)	73 236 (7.7)
Abdominal pain	111 044 (3.0)	47 096 (2.8)	63 948 (3.2)	117 494 (6.1)	48 129 (5.0)	69 365 (7.3)
Rash outside of injection site	42 409 (1.2)	17 765 (1.1)	24 644 (1.2)	32 686 (1.7)	13 132 (1.4)	19 554 (2.1)

[a] Systemic reactions do not include allergic reactions or anaphylaxis.

Self-reported Local and Systemic Reactions Among V-safe Participants

By February 21, 2021, more than 46 million persons received at least 1 dose of an mRNA-based COVID-19 vaccine.[8] A total of 3 643 918 persons were enrolled in v-safe and completed at least 1 health survey within 7 days following their first vaccine dose; 1 920 872 v-safe participants reported receiving a second vaccine dose and completed at least 1 daily health survey within 7 days following the second dose. Solicited local and systemic reactions during days 0 to 7 after each dose were assessed.

Most v-safe participants reported an injection site reaction (dose 1: 70.0%; dose 2: 75.2%) or a systemic reaction (dose 1: 50.0%; dose 2: 69.4%) during days 0 to 7 after vaccination (**Table**). The most frequently reported solicited local and systemic reactions after the first dose of COVID-19 vaccine were injection site pain (67.8%), fatigue (30.9%), headache (25.9%), and myalgia (19.4%). Reactogenicity was substantially greater after the second dose for both vaccines, particularly for systemic reactions, including fatigue (53.9%), headache (46.7%), myalgia (44.0%), chills (31.3%), fever (29.5%), and joint pain (25.6%).

A greater percentage of participants who received the Moderna vaccine, compared with the Pfizer-BioNTech vaccine, reported reactogenicity; this pattern was more pronounced after the second dose (Table). When stratified by age (<65 vs ≥65 years), differences in reactogenicity by vaccine remained consistent with overall findings (data not shown). Local and systemic reactions were less commonly reported by v-safe participants 65 years and older compared with those younger than 65 years, but greater reactogenicity after the second dose was observed for both age groups (eFigure in the Supplement). For both doses of both vaccines, the percentage of v-safe participants who reported local and systemic reactions was highest on day 1 after vaccination and declined markedly through day 7.

The frequency of reported reactions was generally consistent with results observed in clinical trials.[4,5] Data from millions of v-safe participants indicate that injection site pain is common after both the first and second doses of either mRNA-based vaccine. Systemic reactions, including fatigue, headache, myalgia, chills, fever, and joint pain, occurred in participants after the first dose, although they were more frequently reported after the second dose among both Pfizer-BioNTech and Moderna vaccine recipients. Persons 65 years and older reported less reactogenicity than younger persons. Limitations of v-safe include voluntary participation via an opt-in smartphone-based system that includes less than 10% of vaccinated persons.

Although local and systemic reactions are expected and often transient, they may have the most immediate influence on patients' perceptions of the vaccination experience. Setting expectations with patients may alleviate some of the potential anxiety elicited by postvaccination reactogenicity. Clinicians should counsel vaccine recipients that these solicited local and systemic reactions are most commonly reported during the first day following their second dose; a short period before symptom resolution can be expected.[9]

ARTICLE INFORMATION

Author Affiliations: CDC COVID-19 Response Team, Atlanta, Georgia.

Corresponding Author: Johanna Chapin-Bardales, PhD, MPH, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30329 (wif3@cdc.gov).

Published Online: April 5, 2021. doi:10.1001/jama.2021.5374

Conflict of Interest Disclosures: Drs Chapin-Bardales, Gee, and Myers reported receiving nonfinancial technical support to build and maintain the v-safe infrastructure for data capture and messaging to participants from Oracle during the conduct of the study.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention (CDC). Mention of a product or company name is for identification purposes only and does not constitute endorsement by the CDC.

Additional Contributions: We thank investigators from the CDC COVID-19 Response Team and the CDC v-safe team, members of the Oracle v-safe development team, and v-safe participants who contributed to these data.

Additional Information: See eg, 45 CFR part 46.102(l)(2); 21 CFR part 56; 42 USC §241(d); 5 USC §552a; 44 USC §3501 et seq.

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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

Maheshi N Ramasamy, Angela M Minassian*, Katie J Ewer*, Amy L Flaxman*, Pedro M Folegatti*, Daniel R Owens*, Merryn Voysey*, Parvinder K Aley, Brian Angus, Gavin Babbage, Sandra Belij-Rammerstorfer, Lisa Berry, Sagida Bibi, Mustapha Bittaye, Katrina Cathie, Harry Chappell, Sue Charlton, Paola Cicconi, Elizabeth A Clutterbuck, Rachel Colin-Jones, Christina Dold, Katherine R W Emary, Sofiya Fedosyuk, Michelle Fuskova, Diane Gbesemete, Catherine Green, Bassam Hallis, Mimi M Hou, Daniel Jenkin, Carina C D Joe, Elizabeth J Kelly, Simon Kerridge, Alison M Lawrie, Alice Lelliott, May N Lwin, Rebecca Makinson, Natalie G Marchevsky, Yama Mujadidi, Alasdair P S Munro, Mihaela Pacurar, Emma Plested, Jade Rand, Thomas Rawlinson, Sarah Rhead, Hannah Robinson, Adam J Ritchie, Amy L Ross-Russell, Stephen Saich, Nisha Singh, Catherine C Smith, Matthew D Snape, Rinn Song, Richard Tarrant, Yrene Themistocleous, Kelly M Thomas, Tonya L Villafana, Sarah C Warren, Marion E E Watson, Alexander D Douglas*, Adrian V S Hill*, Teresa Lambe*, Sarah C Gilbert*, Saul N Faust*, Andrew J Pollard*, and the Oxford COVID Vaccine Trial Group*

Summary

Background Older adults (aged ≥70 years) are at increased risk of severe disease and death if they develop COVID-19 and are therefore a priority for immunisation should an efficacious vaccine be developed. Immunogenicity of vaccines is often worse in older adults as a result of immunosenescence. We have reported the immunogenicity of a novel chimpanzee adenovirus-vectored vaccine, ChAdOx1 nCoV-19 (AZD1222), in young adults, and now describe the safety and immunogenicity of this vaccine in a wider range of participants, including adults aged 70 years and older.

Methods In this report of the phase 2 component of a single-blind, randomised, controlled, phase 2/3 trial (COV002), healthy adults aged 18 years and older were enrolled at two UK clinical research facilities, in an age-escalation manner, into 18–55 years, 56–69 years, and 70 years and older immunogenicity subgroups. Participants were eligible if they did not have severe or uncontrolled medical comorbidities or a high frailty score (if aged ≥65 years). First, participants were recruited to a low-dose cohort, and within each age group, participants were randomly assigned to receive either intramuscular ChAdOx1 nCoV-19 ($2·2\times10^{10}$ virus particles) or a control vaccine, MenACWY, using block randomisation and stratified by age and dose group and study site, using the following ratios: in the 18–55 years group, 1:1 to either two doses of ChAdOx1 nCoV-19 or two doses of MenACWY; in the 56–69 years group, 3:1:3:1 to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY; and in the 70 years and older, 5:1:5:1 to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY. Prime-booster regimens were given 28 days apart. Participants were then recruited to the standard-dose cohort ($3·5–6·5\times10^{10}$ virus particles of ChAdOx1 nCoV-19) and the same randomisation procedures were followed, except the 18–55 years group was assigned in a 5:1 ratio to two doses of ChAdOx1 nCoV-19 or two doses of MenACWY. Participants and investigators, but not staff administering the vaccine, were masked to vaccine allocation. The specific objectives of this report were to assess the safety and humoral and cellular immunogenicity of a single-dose and two-dose schedule in adults older than 55 years. Humoral responses at baseline and after each vaccination until 1 year after the booster were assessed using an in-house standardised ELISA, a multiplex immunoassay, and a live severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) microneutralisation assay (MNA_{80}). Cellular responses were assessed using an ex-vivo IFN-γ enzyme-linked immunospot assay. The coprimary outcomes of the trial were efficacy, as measured by the number of cases of symptomatic, virologically confirmed COVID-19, and safety, as measured by the occurrence of serious adverse events. Analyses were by group allocation in participants who received the vaccine. Here, we report the preliminary findings on safety, reactogenicity, and cellular and humoral immune responses. This study is ongoing and is registered with ClinicalTrials.gov, NCT04400838, and ISRCTN, 15281137.

Findings Between May 30 and Aug 8, 2020, 560 participants were enrolled: 160 aged 18–55 years (100 assigned to ChAdOx1 nCoV-19, 60 assigned to MenACWY), 160 aged 56–69 years (120 assigned to ChAdOx1 nCoV-19: 40 assigned to MenACWY), and 240 aged 70 years and older (200 assigned to ChAdOx1 nCoV-19: 40 assigned to MenACWY). Seven participants did not receive the boost dose of their assigned two-dose regimen, one participant received the incorrect vaccine, and three were excluded from immunogenicity analyses due to incorrectly labelled samples. 280 (50%) of 552 analysable participants were female. Local and systemic reactions were more common in participants given ChAdOx1 nCoV-19 than in those given the control vaccine, and similar in nature to those previously reported

Lancet 2020; 396: 1979–93

Published Online November 19, 2020 https://doi.org/10.1016/S0140-6736(20)32466-1

This online publication has been corrected. The corrected version first appeared at thelancet.com on December 17, 2020, and further corrections have been made on April 8, 2021

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*Contributed equally

Oxford Vaccine Group, Department of Paediatrics (M N Ramasamy DPhil, M Voysey DPhil, P K Aley PhD, S Bibi PhD, E A Clutterbuck PhD, R Colin-Jones MSc, C Dold PhD, K R W Emary BM BCh, S Kerridge MSc, A Lelliott BMBS, N G Marchevsky MSc, Y Mujadidi MSc, E Plested, S Rhead MBChB, N Singh DPhil, C C Smith PhD, M D Snape MD, R Song MD, Prof A J Pollard FMedSci), **The Jenner Institute** (A M Minassian DPhil, K J Ewer PhD, A L Flaxman DPhil, P M Folegatti MD, B Angus MD, G Babbage MPhil, S Belij-Rammerstorfer PhD, M Bittaye PhD, P Cicconi PhD, S Fedosyuk PhD, M Fuskova MSc, M M Hou MRCPCH, D Jenkin MRCP, C C D Joe PhD, A M Lawrie PhD, R Makinson MBiol, T Rawlinson DPhil, A J Ritchie PhD, Y Themistocleous MBChB, M E E Watson PhD, A D Douglas DPhil, Prof A V S Hill FMedSci, T Lambe PhD, Prof S C Gilbert PhD), **and Clinical**

(injection-site pain, feeling feverish, muscle ache, headache), but were less common in older adults (aged ≥56 years) than younger adults. In those receiving two standard doses of ChAdOx1 nCoV-19, after the prime vaccination local reactions were reported in 43 (88%) of 49 participants in the 18–55 years group, 22 (73%) of 30 in the 56–69 years group, and 30 (61%) of 49 in the 70 years and older group, and systemic reactions in 42 (86%) participants in the 18–55 years group, 23 (77%) in the 56–69 years group, and 32 (65%) in the 70 years and older group. As of Oct 26, 2020, 13 serious adverse events occurred during the study period, none of which were considered to be related to either study vaccine. In participants who received two doses of vaccine, median anti-spike SARS-CoV-2 IgG responses 28 days after the boost dose were similar across the three age cohorts (standard-dose groups: 18–55 years, 20 713 arbitrary units [AU]/mL [IQR 13 898–33 550], n=39; 56–69 years, 16 170 AU/mL [10 233–40 353], n=26; and ≥70 years 17 561 AU/mL [9705–37 796], n=47; p=0·68). Neutralising antibody titres after a boost dose were similar across all age groups (median MNA_{80} at day 42 in the standard-dose groups: 18–55 years, 193 [IQR 113–238], n=39; 56–69 years, 144 [119–347], n=20; and ≥70 years, 161 [73–323], n=47; p=0·40). By 14 days after the boost dose, 208 (>99%) of 209 boosted participants had neutralising antibody responses. T-cell responses peaked at day 14 after a single standard dose of ChAdOx1 nCoV-19 (18–55 years: median 1187 spot-forming cells [SFCs] per million peripheral blood mononuclear cells [IQR 841–2428], n=24; 56–69 years: 797 SFCs [383–1817], n=29; and ≥70 years: 977 SFCs [458–1914], n=48).

Interpretation **ChAdOx1 nCoV-19 appears to be better tolerated in older adults than in younger adults and has similar immunogenicity across all age groups after a boost dose. Further assessment of the efficacy of this vaccine is warranted in all age groups and individuals with comorbidities.**

Funding **UK Research and Innovation, National Institutes for Health Research (NIHR), Coalition for Epidemic Preparedness Innovations, NIHR Oxford Biomedical Research Centre, Thames Valley and South Midlands NIHR Clinical Research Network, and AstraZeneca.**

Biomanufacturing Facility (C Green PhD, R Tarrant PhD), **Nuffield Department of Medicine, and Oxford Centre for Clinical Tropical Medicine and Global Health** (H Robinson DipHE), **University of Oxford, Oxford, UK; NIHR Clinical Research Facility, University Hospital Southampton NHS Trust, Southampton, UK** (D R Owens MRCPCH, L Berry BSc, H Chappell MBBS, D Gbesemete MRCPCH, M N Lwin MSc, A P S Munro MRCPCH, M Pacurar MD, J Rand BA, A L Ross-Russell MA, S Saich BA, S C Warren MBBC); **Paediatric Medicine, University of Southampton, Southampton, UK** (K Cathie MD); **National Infection Service, Public Health England, Porton Down, Salisbury, UK** (S Charlton PhD, B Hallis PhD, K M Thomas PhD); **NIHR Oxford Biomedical Research Centre, Oxford, UK** (E A Clutterbuck, C Dold, S Rhead, H Robinson, A D Douglas, Prof A V S Hill, T Lambe, Prof S C Gilbert, Prof A J Pollard); **AstraZeneca BioPharmaceuticals Research and Development, Washington, DC, USA** (E J Kelly PhD); **AstraZeneca BioPharmaceuticals Research and Development , Bethesda, MA, USA** (T L Villafana PhD); **Division of Infectious Diseases, Boston Children's Hospital, Boston, MA, USA** (R Song); **and NIHR Southampton Clinical Research Facility and Biomedical Research Centre, University Hospital Southampton NHS Trust and Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton, UK** (Prof S N Faust FRCPCH)

Correspondence to:
Dr Maheshi N Ramasamy, Department of Paediatrics, University of Oxford, Oxford OX3 9DU, UK
maheshi.ramasamy@paediatrics.ox.ac.uk

Introduction

As of Nov 13, 2020, over 52 million people have been diagnosed with COVID-19 worldwide, with over 1·2 million confirmed deaths.[1] Severe COVID-19 is more common in adults aged 70 years and older and in individuals with comorbidities such as hypertension, diabetes, cardiovascular disease, and chronic respiratory disease.[2] A safe and effective vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) will be an important tool in controlling the global COVID-19 pandemic. Although there are no licensed vaccines against COVID-19, 48 potential vaccine candidates based on a variety of platforms including lipid nanoparticle mRNA, DNA, adjuvanted protein, inactivated virus particles, and non-replicating viral vectors are in clinical trials (of which 11 candidates are in phase 3 trials) and a further 164 candidates are in preclinical testing.[3]

The WHO global target product profile of critical characteristics for prequalification of a COVID-19 vaccine requires candidates to be targeted at the most at-risk groups, including older adults; have a favourable safety profile; provide efficacy as measured by prevention of virologically confirmed disease or transmission, or both; and to provide at least 6 months of protection for individuals at ongoing risk of exposure to SARS-CoV-2.[4] On Sept 25, 2020, the UK Joint Committee on Vaccination and Immunisation (JCVI) gave interim recommendations for the national prioritisation of COVID-19 vaccines.[5] The following groups were provisionally prioritised: first, older adults living in residential care homes and residential care home workers; second, all adults aged 80 years or older and health-care and social-care workers; and third, all adults aged 75 years and older. However, the JCVI acknowledged that this priority ranking could change substantially if the first available vaccines were not considered safe or effective in older adults. Similar recommendations have also been made by the US Advisory Committee on Immunization Practices.[6]

Immunosenescence refers to the gradual deterioration and decline of the immune system brought on by ageing. Age-dependent differences in the functionality and availability of T-cell and B-cell populations are thought to have a key role in the decrease of immune response.[7] There has been a drive to develop vaccines and adjuvant formulations tailored for older adults to overcome this diminished immune response after vaccination. Assessment of immune responses in older adults is therefore essential in the development of COVID-19 vaccines that could protect this susceptible population.

The spike protein of SARS-CoV-2 binds to ACE2 receptors on target cells during viral entry. Analysis of convalescent patients suggests that the spike protein is an immunodominant antigen, eliciting both antibody and T-cell responses.[8] Most COVID-19 candidate vaccines have been developed to induce anti-spike protein immune responses. Clinical trials using several different vaccine platforms including mRNA,[9,10] adenoviral vectored vaccines,[11,12] inactivated virus,[13,14] and adjuvanted

Research in context

Evidence before this study

We searched PubMed for research articles published from database inception until Nov 13, 2020, with no language restrictions, using the terms "SARS-CoV-2", "vaccine", AND "clinical trial". We identified published clinical trial data on eight other vaccine candidates. Two recombinant viral vectored vaccines have been tested in clinical trials. A single dose adenovirus (Ad) 5 vector-based vaccine (CanSino Biological/Beijing Institute of Biotechnology, China) elicited neutralising antibodies and T-cell responses in a dose-dependent manner, but was less immunogenic in individuals older than 55 years. A heterologous prime-boost Ad5/Ad26-vectored vaccine schedule (Gamaleya Research Institute, Russia) generated neutralising antibody and cellular responses in adults younger than 60 years. Two nucleoside-modified mRNA vaccine candidates using a two-dose regimen were tested in adults aged 18–55 years and 65–85 years, and generated neutralising antibodies in both age groups in a dose-dependent manner, although immunogenicity decreased with age (Pfizer/BioNTech, USA). Another mRNA vaccine (Moderna, USA) was given to adults older than 56 years. The vaccine was tolerated, with neutralising antibodies induced in a dose-dependent manner, which increased after a second dose. Neutralising antibody responses with this mRNA vaccine appeared to be similar in adults older than 56 years to those aged 18–55 years who also received the vaccine. Two inactivated viral vaccines have also shown neutralising antibody responses in a dose-dependent manner in adults aged 18–59 years (Wuhan Institute Biological Products/SinoPharm, China) or adults aged 18–59 and 60 years and older (Beijing Institute Biological products/SinoPharm, China), with the second showing lower neutralising antibody titres in older adults after two doses. Finally, a clinical trial of a nanoparticle vaccine composed of adjuvanted trimeric severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike glycoproteins (Novavax, USA) reported results of a two-dose schedule given 3 weeks apart in healthy adults younger than 60 years. This vaccine was well tolerated and induced neutralisation responses that exceeded those measured in serum samples from convalescent symptomatic patients.

Added value of this study

This study is the fifth published clinical trial of a vaccine against SARS-CoV-2 tested in an older adult population (aged 18–55 years, 56–69 years, and ≥70 years). The vaccine was safe and well tolerated, with reduced reactogenicity in older adults. Antibody responses against the SARS-CoV-2 spike protein were induced in all age groups and were boosted and maintained at 28 days after booster vaccination, including in the 70 years and older group. Cellular immune responses were also induced in all age and dose groups, peaking at day 14 after vaccination.

Implications of all the available evidence

The populations at greatest risk of serious COVID-19 include people with coexisting health conditions and older adults. The immune correlates of protection against SARS-CoV-2 have not yet been determined, but neutralising antibodies are thought to be associated with protection, and in a COVID-19 non-human primate challenge model, neutralising antibody responses correlated with protection. These findings have led to the use of neutralisation assays to assess immune responses in recent human COVID-19 vaccine trials. Immunisation with ChAdOx1 nCoV-19 results in development of neutralising antibodies against SARS-CoV-2 in almost 100% of participants including older adults without severe comorbidities, with higher levels in boosted compared with non-boosted groups. Further assessment of the efficacy of this vaccine is warranted in all age groups and individuals with comorbidities.

spike glycoprotein[15] have shown neutralising antibody responses after immunisation.

Replication-deficient adenovirus vectors containing a pathogen-specific transgene have been used as novel vaccines because of their ability to induce strong humoral and cellular responses.[16] However, pre-existing immunity might reduce the immunogenicity of vectors derived from human viruses; hence, use of simian adenoviruses might be preferable. ChAdOx1 nCoV-19 (AZD1222) is a replication-defective chimpanzee adenovirus-vectored vaccine expressing the full-length SARS-CoV-2 spike glycoprotein gene (GenBank accession number MN908947). Vaccination of rhesus macaques with a single dose of ChAdOx1 nCoV-19 generates humoral and cellular immune responses and protects from lower respiratory infection after subsequent challenge with SARS-CoV-2.[17] Preliminary results of a phase 1/2 clinical trial of ChAdOx1 nCoV-19 in adults aged 18–55 years show that the vaccine is well tolerated and generates robust neutralising antibody and cellular immune responses against the spike glycoprotein.[18] Here we present the safety and immunogenicity results of a phase 2 component of a phase 2/3 multicentre study using ChAdOx1 nCoV-19 at two different doses, in adults including those aged 56–69 years and 70 years and older, and in a one-dose or two-dose regimen.

Methods

Study design and participants

In this continuing single-blind, multicentre, randomised, controlled, phase 2/3 trial, the safety and efficacy of the ChAdOx1 nCoV-19 vaccine is being assessed, with sequential age-escalation immunogenicity substudies being done in older age groups. The study is being run at 20 centres in the UK (listed in the appendix [pp 84–87]). Here we report selected results from the phase 2 component of the trial and for which participants were enrolled at two sites in the UK: the Oxford Vaccine Centre, Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford (Oxford) and the NIHR

See Online for appendix

Southampton Clinical Research Facility, University Hospital Southampton NHS Foundation Trust (Southampton). Data on the participants from the phase 3 component will be published elsewhere.

We recruited participants in an age-escalation manner. We recruited adults aged 18–55 years, then adults aged 56–69 years, and then adults aged 70 years and older, without severe or uncontrolled medical comorbidities, as defined in the clinical study plan (appendix pp 48–54), through local advertisements. Participants aged 65 years and older with a Dalhousie Clinical Frailty Score of 4 or higher were excluded.[19]

Participants were enrolled into one of ten different groups. Recruitment was sequential with low-dose groups recruited first and standard-dose cohorts recruited after a protocol amendment was approved on June 5, 2020, that incorporated the new higher dose level. For the first stage of recruitment, participants aged 18–55 years were recruited to the low-dose group. Subsequently we recruited participants aged 56–69 years, and further extension to recruit those aged 70 years and older only occurred after safety review by the independent Data Safety Monitoring Board (DSMB). A minimum of 2 weeks of safety and immunogenicity data were reviewed by the DSMB before recruitment to each successive age cohort. The 18–55 years groups received two doses of vaccine and were randomly assigned to receive either the experimental vaccine or the control vaccine. The 56–69 years and 70 years and older groups were randomly assigned to receive either one dose or two doses of vaccine and were then randomly assigned to receive the experimental vaccine or the control vaccine. The same process was repeated with recruitment and randomisation for the standard-dose cohorts after review by the DSMB. All participants underwent a screening visit in which a full medical history, targeted examination, blood test for SARS-CoV-2 exposure, and a urinary pregnancy test in women of childbearing potential were done. Volunteers who were seropositive to SARS-CoV-2 before enrolment were excluded from participating in all groups, apart from those in the 18–55 years standard-dose cohort. Additionally, all participants included in this phase 2 component of the study, apart from those in the 18–55 years low-dose group, had additional safety tests (blood tests for HIV, hepatitis B and C serology, full blood count, and kidney and liver function tests). Full details of eligibility criteria are in the trial protocol (appendix pp 135–38).

Written informed consent was obtained from all participants, and the trial is being done in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The study was sponsored by the University of Oxford (Oxford, UK) and approved in the UK by the Medicines and Healthcare products Regulatory Agency (reference 21584/0428/001-0001) and the South-Central Berkshire Research Ethics Committee (reference 20/SC/0179). Vaccine use was authorised by Genetically Modified Organisms Safety Committees at each participating site. An independent DSMB reviewed all interim safety reports. A copy of the protocol is included in the appendix (pp 83–212).

Randomisation and masking

Participants were randomly assigned to receive either the ChAdOx1 nCoV-19 vaccine or the quadrivalent MenACWY protein-polysaccharide conjugate vaccine. MenACWY was used as a comparator vaccine rather than a saline placebo to maintain masking of participants who had local or systemic reactions. Participants aged 18–55 years were randomly assigned (1:1) in the low-dose cohort and (5:1) in the standard-dose cohort to receive either ChAdOx1 nCoV-19 or MenACWY. For both 18–55 years cohorts, participants were given two doses of study vaccine. Participants aged 56–69 years were randomly assigned (3:1:3:1) to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY. Participants aged 70 years or older were randomly assigned (5:1:5:1) to one dose of ChAdOx1 nCoV-19, one dose of MenACWY, two doses of ChAdOx1 nCoV-19, or two doses of MenACWY.

Randomisation lists, using block randomisation stratified by age and dose group and study site, were generated by the study statistician (MV). Block sizes were chosen to align with the age group and dose group sizes. Computer randomisation was done with full allocation concealment within the secure web platform used for the study electronic case report form (REDCap version 9.5.22). The trial staff administering the vaccine prepared vaccines out of sight of the participants and syringes were covered with an opaque material until ready for administration to ensure masking of participants. Participants, clinical investigators, and the laboratory team remained masked to group allocation for the duration of the study. However, trial staff administering the vaccine were unmasked.

Procedures

In the previous phase 1/2 study,[18] a single standard dose of 5×10^{10} virus particles of ChAdOx1 nCoV-19 was used, based on previous experience with a ChAdOx1 Middle East respiratory syndrome (MERS) construct. In this study, we assessed a lower dose of $2\cdot2\times10^{10}$ virus particles and a standard dose of $3\cdot5–6\cdot5\times10^{10}$ virus particles in adults of different age cohorts. Due to the need to rapidly produce large numbers of doses of vaccine manufactured using Good Manufacturing Practice to allow timely enrolment into the phase 2/3 clinical trial, two different batches of vaccine were used in this study: one manufactured and vialed by Advent (Pomezia, Italy), and one manufactured by COBRA Biologics (Keele, UK) and vialed by Symbiosis (Stirling, UK). Both were manufactured according to Good Manufacturing Practice and approved by the regulatory agency in the UK, the Medicines and Healthcare

products Regulatory Agency. The 18–55 years standard-dose cohort received vaccine manufactured by COBRA Biologics for both first (ie, prime) and second (ie, boost) doses and all other cohorts received prime and boost doses, as randomised, manufactured by Advent. Analytical assessment of the batches indicates that the batches are comparable. Formal batch-to-batch comparison studies are ongoing and results will be reported when available.

ChAdOx1 nCoV-19 was administered as a single-dose or two-dose regimen (28 days apart) at either the low dose ($2·2\times10^{10}$ virus particles) or the standard dose ($3·5–6·5\times10^{10}$ virus particles). It was administered as a single intramuscular injection into the deltoid, according to specific study standard operating procedures. The MenACWY vaccine was provided by the UK Department of Health and Social Care and administered as per summary of product characteristics at the standard dose.[20] Depending on the batch used for vaccination, the injection volume for the low dose of ChAdOx1 nCoV-19 was either 0·22 mL or 0·5 mL. The injection volume used for the standard dose of ChAdOx1 nCoV-19 and MenACWY was 0·5 mL.

Safety data from animal studies and our previous phase 1/2 clinical trial[18] of ChAdOx1 nCoV-19 were reviewed before recruitment of participants. Volunteers were considered enrolled into the trial at the point of vaccination. Participants were observed in the clinic for a minimum of 15 min after the vaccination procedure in case of any immediate adverse events.

Participants from each group were instructed to complete a diary card to record solicited local and systemic adverse reactions for 7 days after each dose. Protocol-defined solicited local adverse events included injection-site pain, tenderness, warmth, redness, swelling, induration, and itch, and solicited systemic adverse events included malaise, muscle ache, joint pain, fatigue, nausea, headache, chills, feverishness (ie, a self-reported feeling of having a fever), and objective fever (defined as an oral temperature of 38°C or higher). All participants were given an emergency 24-h telephone number to contact the on-call study physician as required. Serious adverse events will be recorded throughout the follow-up period of 1 year after the last dose of vaccine.

Severity of adverse events was graded with the following criteria: mild (transient or mild discomfort for <48 h, no interference with activity, and no medical intervention or therapy required), moderate (mild-to-moderate limitation in activity, and no or minimal medical intervention or therapy required), severe (substantial limitation in activity and medical intervention or therapy required), or potentially life-threatening (requires assessment in emergency department or admission to hospital). All participants in the 56–69 years and 70 years and older groups and participants in the 18–55 years standard-dose group had clinical and immunogenicity assessments at 0, 7, 14, and 28 days after their prime and booster vaccinations. Participants in the 18–55 years low-dose group had clinical and immunogenicity assessments at baseline, immediately before the boost dose, and at 14 and 28 days after their booster vaccination.

Humoral responses at baseline and after vaccination were assessed using Meso Scale Discovery multiplexed immunoassay against spike and receptor binding domain [RBD], a standardised total IgG ELISA against trimeric SARS-CoV-2 spike protein, and a live SARS-CoV-2 microneutralisation assay MNA_{80}, which was done at Public Health England (Porton Down, UK), as described previously.[18] Cellular responses were assessed using an ex-vivo IFN-γ enzyme-linked immunospot (ELISpot) assay to enumerate antigen-specific T cells.[18] Neutralising antibodies to the ChAdOx1 vector were measured using a secreted embryonic alkaline phosphatase (SEAP)-reporter assay, which measures the reciprocal of the serum dilution required to reduce in-vitro expression of vector-expressed SEAP by 50%, 24 h after transduction.[21] Due to the labour-intensive nature of neutralisation assays, we prioritised analysis of samples from the ChAdOx1 nCoV-19 groups, randomly selecting more samples from ChAdOx1 nCoV-19 participants than control samples to be sent for blinded analysis.

Outcomes

The coprimary outcomes of the trial are to assess efficacy as measured by the number of cases of symptomatic, virologically confirmed COVID-19 and safety of the vaccine as measured by the occurrence of serious adverse events. Secondary outcomes include safety, reactogenicity, and immunogenicity profiles of ChAdOx1 nCoV-19 in older adults (aged 56–69 years and ≥70 years), efficacy against severe and non-severe COVID-19, death, and seroconversion against non-spike proteins. A full list of secondary and tertiary outcomes is in the protocol (pp 118–24).

Here we report preliminary results for selected secondary endpoints, comparing local and systemic reactogenicity and cellular and humoral immunogenicity of ChAdOx1 nCoV-19 between different age groups, after one or two doses and at low or standard dose. Efficacy analyses are not included in this report.

Statistical analysis

We present safety endpoints as frequencies (%) with 95% binomial exact CIs. We present immunological endpoints as medians and IQR. Analyses were by group allocation in participants who received the vaccine.

We did comparisons across the three age groups (aged 18–55 years, aged 56–69 years, and aged ≥70 years) using Kruskal-Wallis tests within each dose level of the vaccine (low dose or standard dose) for antibody responses or unadjusted analysis of variance applied to log-transformed values for neutralisation titres. We did comparisons between low-dose and standard-dose groups using Wilcoxon rank sum tests (antibody

response) or independent samples Student's *t* test applied to log-transformed values for neutralisation titres. We present unadjusted p values for a small number of statistical comparisons to avoid issues of multiplicity. To assess the association between responses on different assays, we used unadjusted linear regression to analyse log-transformed values after baseline.

Sample sizes were nominal for these immunogenicity subgroups and no power calculations were done.

We did all statistical analyses using SAS version 9.4 and R version 3.6.1 or later. This study is registered with ClinicalTrials.gov, NCT04400838, and with ISRCTN, 15281137.

Role of the funding source

AstraZeneca reviewed the data from the study and the final manuscript before submission, but the authors retained editorial control. All other funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 30 and Aug 8, 2020, 560 participants were enrolled in the study and randomly assigned to the experimental vaccine or control vaccine group: 160 participants aged 18–55 years (100 assigned to ChAdOx1 nCoV-19, 60 assigned to MenACWY), 160 aged 56–69 years (120 assigned to ChAdOx1 nCoV-19, 40 assigned to MenACWY), and 240 aged 70 years and older (200 assigned to ChAdOx1 nCoV-19, 40 assigned to MenACWY). Full details on randomisation are in figure 1. All participants randomly assigned to treatment were vaccinated. One participant (in the 18–55 years low-dose group) received the incorrect vaccine after randomisation and was excluded from analysis. Seven participants randomly assigned to receive two doses of vaccine chose not to continue with the boost dose and were excluded from further analyses. Three participants were excluded from immunology analyses due to incorrectly labelled samples (either incorrect participant identification numbers or incorrect timepoints noted on the label, or both; figure 1). The baseline characteristics of the participants eligible for inclusion in the analysis in each group are shown in the table. Participants 70 years and older were recruited from the NIHR Southampton Clinical Research Facility, University Hospital Southampton NHS Foundation Trust. All other participants were recruited at the Oxford Vaccine Centre, Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford. Among the analysed population, 280 (50%) of 552 participants were female. 524 (95%) of 552 participants identified as white, and 540 (98%) were non-smokers. A large proportion of health-care workers who were predominantly female were enrolled in the 18–55 years and 56–69 years age groups. The median age in the 18–55 years group was 43·0 years (IQR 33·6–48·0), in the 56–69 years group was 60·0 years (57·5–63·0) and in the 70 years and older group was 73·0 years (71·0–76·0). The median age in the 70 years and older groups ranged from 73 years to 74 years across dosing groups, with the oldest participants aged 83 years.

The following results for local and systemic adverse reactions are all for participants who were randomly assigned to receive two doses of vaccine. Injection-site pain and tenderness were the most common solicited local adverse reactions and occurred most frequently in the first 48 h after vaccination (data for standard-dose regimen shown in figure 2; data for the low-dose groups and control groups are shown in the appendix [pp 7, 9, 19–21]). In those aged 56 years or older, a standard dose of ChAdOx1 nCoV-19, whether the prime or boost vaccination, elicited a greater number of local or systemic reactions than did MenACWY. The difference was less clear with the low-dose vaccine in the 56–69 years and 70 years and older groups, and the number of participants in the control groups was small (appendix p 30). At least one local symptom was reported after the prime vaccination with standard-dose ChAdOx1 nCoV-19 by 43 (88%) of 49 participants in the 18–55 years group, 22 (73%) of 30 in the 56–69 years group, and 30 (61%) of 49 in the 70 years and older group (appendix p 29). Similar proportions of local symptoms were reported after the boost vaccination with the standard dose of ChAdOx1 nCoV-19, with 37 (76%) of 49 participants in the 18–55 years group, 21 (72%) of 29 in the 56–69 years group, and 27 (55%) of 49 in the 70 years and older group reporting at least one local symptom. A similar pattern was seen across the age groups in participants after their prime vaccination with low-dose ChAdOx1 nCoV-19 and after the boost vaccination with the low-dose vaccine, but with fewer total adverse reactions than in the standard-dose groups (appendix pp 7, 9, 19–21). No severe local symptoms were reported by recipients of ChAdOx1 nCoV-19. In the two-dose control groups, across both the low-dose and standard-dose cohorts, local symptoms were reported by 33 (57%) of 58 participants in the 18–55 years group, five (25%) of 20 in the 56–69 years group, and seven (35%) of 20 in the 70 years and older group after the prime vaccination with MenACWY, and by 50 (86%) of 58 in the 18–55 years group, seven (37%) of 19 in the 56–69 years group, and four (20%) of 20 in the 70 years and older group after the boost vaccination with MenACWY (appendix p 29). Data for participants randomly assigned to receive only one dose of vaccine were similar to the data after a prime dose of vaccine in the two-dose groups (data not shown).

Fatigue, headache, feverishness, and myalgia were the most commonly solicited systemic adverse reactions (data for the standard-dose groups are shown in figure 3; data for the low-dose groups and control groups are shown in the appendix [pp 8, 10, 19–21]). At least one systemic symptom was reported after the prime

vaccination with the standard dose of ChAdOx1 nCoV-19 by 42 (86%) of 49 participants in the 18–55 years group, 23 (77%) of 30 in the 56–69 years group, and 32 (65%) of 49 in the 70 years and older group (appendix p 29). The severity of symptoms reported in the standard-dose groups was reduced after the boost vaccination, with only one (1%) of 127 participants reporting a severe reaction compared with seven (5%) of 128 participants after the prime vaccination. At least one systemic adverse reaction after the boost vaccination of standard dose of ChAdOx1

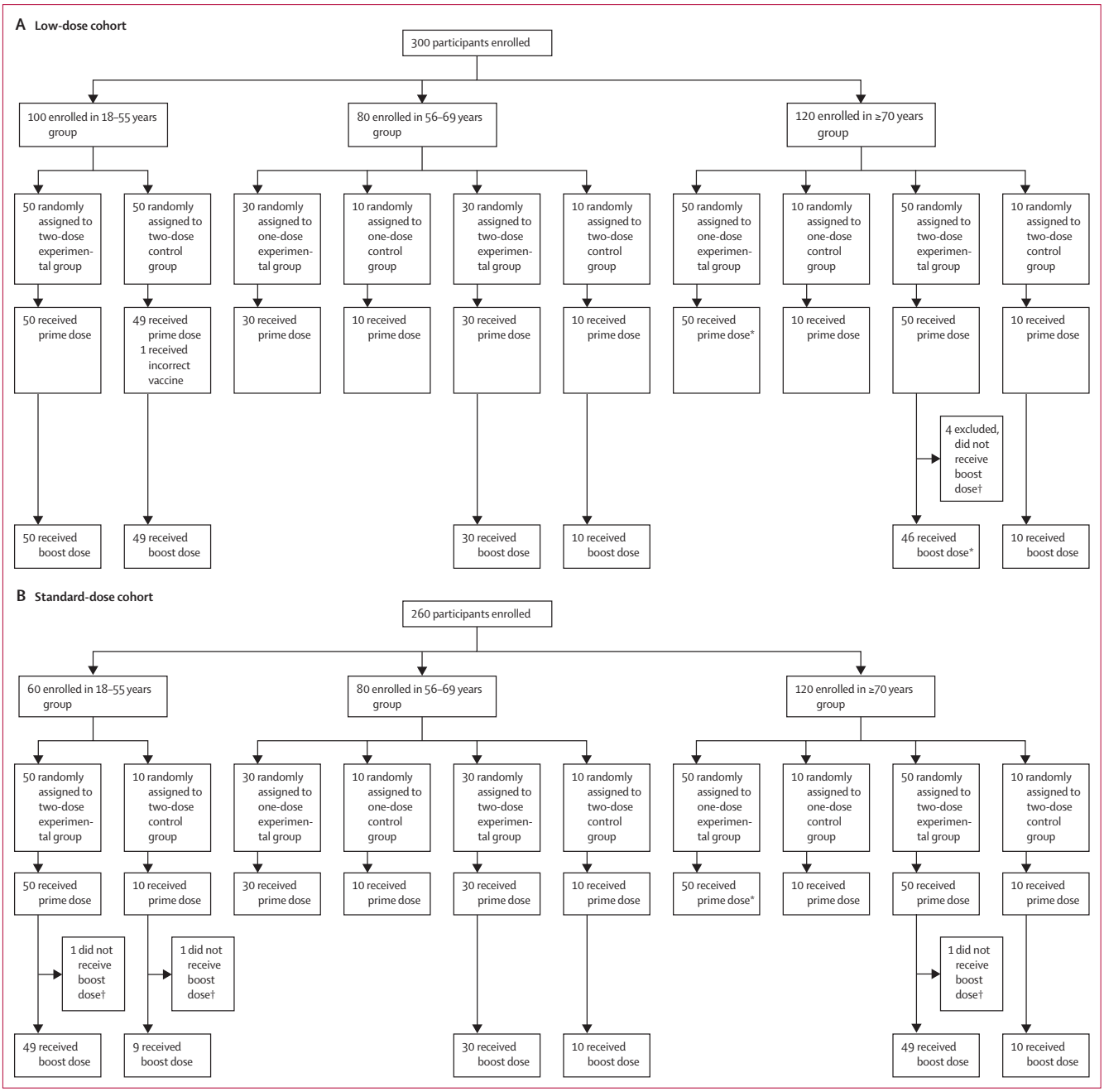

Figure 1: **Study profile for the low-dose (A) and standard-dose (B) cohorts**
*One participant excluded from immunogenicity analyses, due to mislabelling of laboratory sample. †Reasons for not receiving boost dose included that the participant moved away or was unavailable for visits, delay in receiving boost dose, or withdrawal of consent.

	Age 18–55 years		Age 56–69 years				Age ≥70 years			
	ChAdOx1 nCoV-19, two doses	MenACWY, two doses	ChAdOx1 nCoV-19, one dose	MenACWY, one dose	ChAdOx1 nCoV-19, two doses	MenACWY, two doses	ChAdOx1 nCoV-19, one dose	MenACWY, one dose	ChAdOx1 nCoV-19, two doses	MenACWY, two doses
Low dose										
Number enrolled	50	49	30	10	30	10	50	10	46	10
Sex										
Female	35 (70%)	28 (57%)	19 (63%)	4 (40%)	10 (33%)	8 (80%)	24 (48%)	6 (60%)	16 (35%)	6 (60%)
Male	15 (30%)	21 (43%)	11 (37%)	6 (60%)	20 (67%)	2 (20%)	26 (52%)	4 (40%)	30 (65%)	4 (40%)
Age, years, median (IQR, range)	44·5 (39·0–51·0, 22·0–54·0)	42·0 (32·0–48·0, 23·0–55·0)	60·0 (58·9–62·3, 56·0–69·0)	57·8 (56·3–60·8, 56·0–68·0)	60·4 (57·8–66·0, 56·0–69·4)	60·5 (58·3–63·9, 56·7–69·0)	73·5 (71·0–76·0, 69·0–83·0)	73·0 (70·0–74·0, 70·0–81·0)	73·0 (71·0–75·0, 70·0–82·0)	73·0 (71·2–74·0, 70·0–76·0)
BMI, kg/m², median (IQR, range)	24·6 (22·9–28·9, 19·4–45·1)	24·8 (21·6–27·7, 18·0–37·2)	25·0 (23·2–27·3, 20·2–37·6)	25·5 (22·5–27·3, 20·9–34·4)	25·9 (24·0–28·8, 21·3–36·6)	24·0 (23·2–26·0, 22·2–33·2)	26·0 (23·8–28·0, 20·0–36·0)	24·9 (22·3–26·9, 19·3–32·5)	26·0 (23·4–27·7, 19·4–42·1)	26·8 (24·3–29·5, 19·2–35·3)
Smoker	3 (6%)	1 (2%)	0	1 (10%)	2 (7%)	0	1 (2%)	0	1 (2%)	0
Alcohol drinker	44 (88%)	42 (86%)	28 (93%)	9 (90%)	26 (87%)	8 (80%)	43 (86%)	10 (100%)	43 (94%)	9 (90%)
Health-care worker	35 (70%)	26 (53%)	17 (57%)	7 (70%)	12 (40%)	4 (40%)	0	0	0	1 (10%)
Race or ethnicity										
White	48 (96%)	45 (92%)	30 (100%)	9 (90%)	27 (90%)	10 (100%)	50 (100%)	10 (100%)	45 (98%)	10 (100%)
Black or Black British	0	0	0	0	0	0	0	0	0	0
Asian or Asian British	2 (4%)	1 (2%)	0	0	2 (7%)	0	0	0	0	0
Mixed race or ethnicity	0	3 (6%)	0	0	0	0	0	0	1 (2%)	0
Other race or ethnicity*	0	0	0	1 (10%)	1 (3%)	0	0	0	0	0
Comorbidities										
Cardiovascular disease	4 (8%)	10 (20%)	5 (17%)	0	11 (37%)	0	14 (28%)	3 (30%)	16 (35%)	2 (20%)
Respiratory disease	12 (24%)	9 (18%)	7 (23%)	0	7 (23%)	0	6 (12%)	2 (20%)	6 (13%)	1 (10%)
Diabetes	0	0	0	0	0	1 (10%)	1 (2%)	0	2 (4%)	0
Standard dose										
Number enrolled	49	9	30	10	30	10	50	10	49	10
Sex										
Female	23 (47%)	7 (78%)	16 (53%)	3 (30%)	16 (53%)	5 (50%)	25 (50%)	1 (10%)	21 (43%)	2 (20%)
Male	26 (53%)	2 (22%)	14 (47%)	7 (70%)	14 (47%)	5 (50%)	25 (50%)	9 (90%)	28 (57%)	8 (80%)
Age, years, median (IQR, range)	39·0 (30·0–45·0, 19·0–55·0)	43·0 (35·8–50·0, 32·0–54·0)	59·0 (58·0–61·0, 56·0–69·0)	61·5 (57·5–63·8, 57·0–66·0)	59·5 (57·0–61·0, 56·0–67·0)	60·5 (57·9–61·0, 56·0–64·0)	74·0 (72·0–76·0, 70·0–80·0)	74·0 (71·0–75·5, 70·0–78·0)	73·0 (71·0–75·0, 70·0–83·0)	73·5 (72·2–74·8, 71·0–81·0)
BMI, kg/m², median (IQR, range)	26·9 (24·6–30·9, 20·2–39·7)	24·1 (23·8–25·6, 18·6–39·0)	26·7 (25·2–30·0, 18·6–36·8)	28·9 (25·6–30·2, 21·7–31·9)	24·0 (22·4–27·1, 19·9–33·5)	26·1 (23·6–27·7, 20·5–30·2)	25·1 (23·7–28·5, 17·5–32·6)	26·8 (25·8–28·5, 23·0–31·7)	27·1 (24·2–29·2, 20·3–40·2)	25·6 (24·1–29·3, 18·9–32·5)
Smoker	1 (2%)	0	0	0	0	1 (10%)	1 (2%)	0	0	0
Alcohol drinker	45 (92%)	6 (67%)	29 (97%)	10 (100%)	29 (97%)	10 (100%)	39 (78%)	9 (90%)	42 (86%)	9 (90·0%)
Health-care worker	13 (27%)	5 (56%)	10 (33%)	2 (20%)	12 (40%)	5 (50%)	2 (4%)	0	0	0
Race or ethnicity										
White	40 (82%)	7 (78%)	29 (97%)	10 (100%)	26 (87%)	9 (90%)	50 (100%)	10 (100%)	49 (100%)	10 (100%)
Black or Black British	1 (2%)	0	0	0	0	0	0	0	0	0
Asian or Asian British	7 (14%)	2 (22%)	0	0	4 (13%)	1 (10%)	0	0	0	0
Mixed race or ethnicity	0	0	0	0	0	0	0	0	0	0
Other race or ethnicity*	1 (2%)	0	1 (3%)	0	0	0	0	0	0	0
Comorbidities										
Cardiovascular disease	6 (12%)	0	4 (13%)	3 (30%)	4 (13%)	1 (10%)	20 (40%)	3 (30%)	13 (27%)	4 (40%)
Respiratory disease	10 (20%)	1 (11%)	4 (13%)	1 (10%)	3 (10%)	3 (30%)	3 (6%)	0	4 (8%)	0
Diabetes	2 (4%)	0	2 (7%)	2 (20%)	0	0	0	1 (10%)	3 (6%)	1 (10%)

Data are n (%) unless otherwise specified. BMI=body-mass index. *Included Hispanic-Columbian, Indian, Japanese, and White Irish/English.

Table: **Baseline characteristics of prime-boost participants included in the analysis**

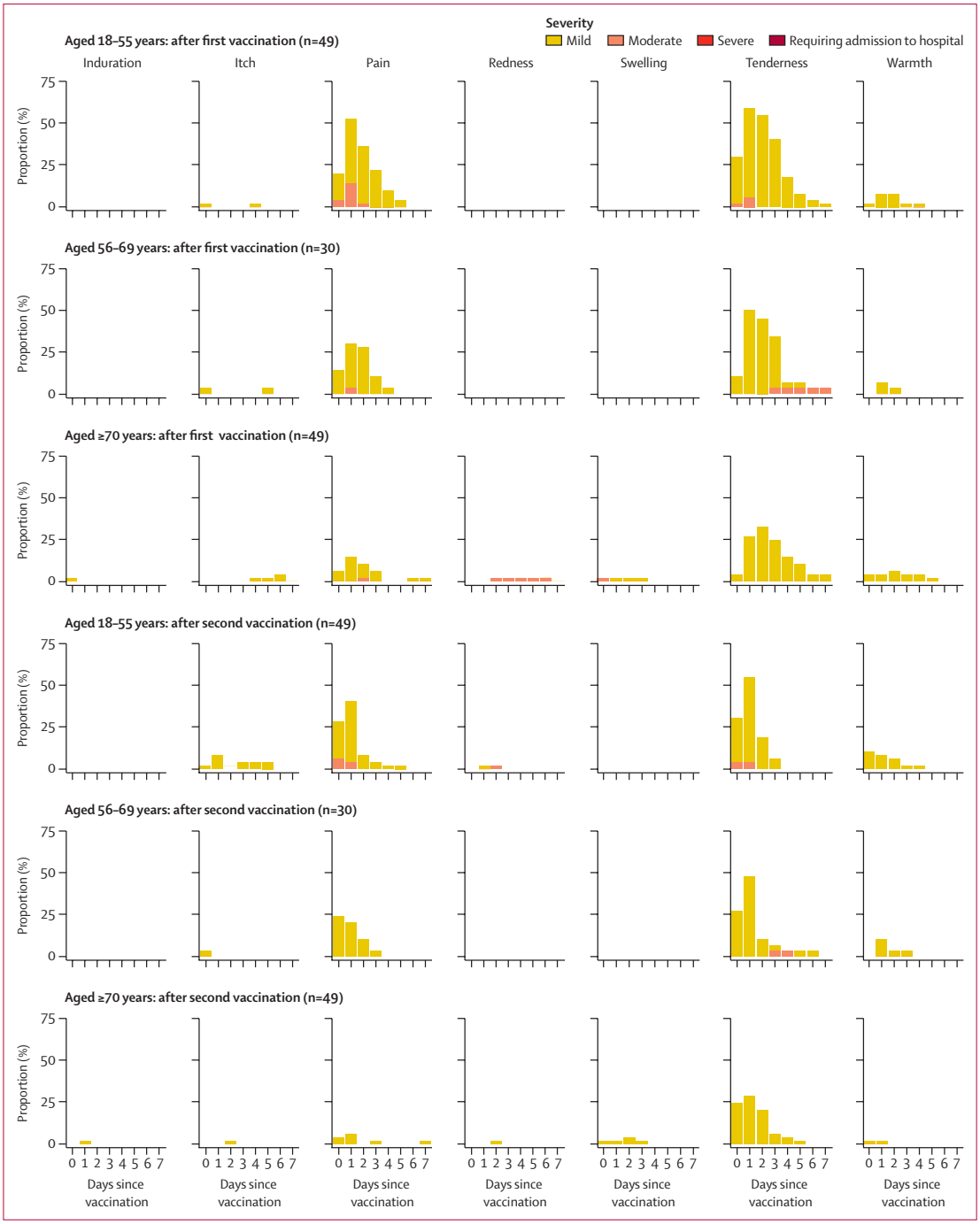

Figure 2: **Solicited local adverse reactions in the 7 days after prime and boost doses of standard-dose vaccine, by age**
Day 0 is the day of vaccination. Participants shown are those randomly assigned to receive two doses, and data are only shown for participants who received both doses of vaccine.

nCoV-19 was reported by 32 (65%) of 49 participants in the 18–55 years group, 21 (72%) of 29 in the 56–69 years group, and 21 (43%) of 49 in the 70 years and older group (appendix p 29). Within 7 days after the prime vaccination with ChAdOx1 nCoV-19, the incidence of objectively measured fever was low in the 18–55 years standard-dose

group (12 [24%] of 49), and no fevers were recorded in either the 56–69 years or 70 years and older standard-dose groups (appendix pp 16–18). No participants of any age who received the standard dose of ChAdOx1 nCoV-19 had objective fever after the boost vaccination. A similar pattern of decreasing reactogenicity with increasing age

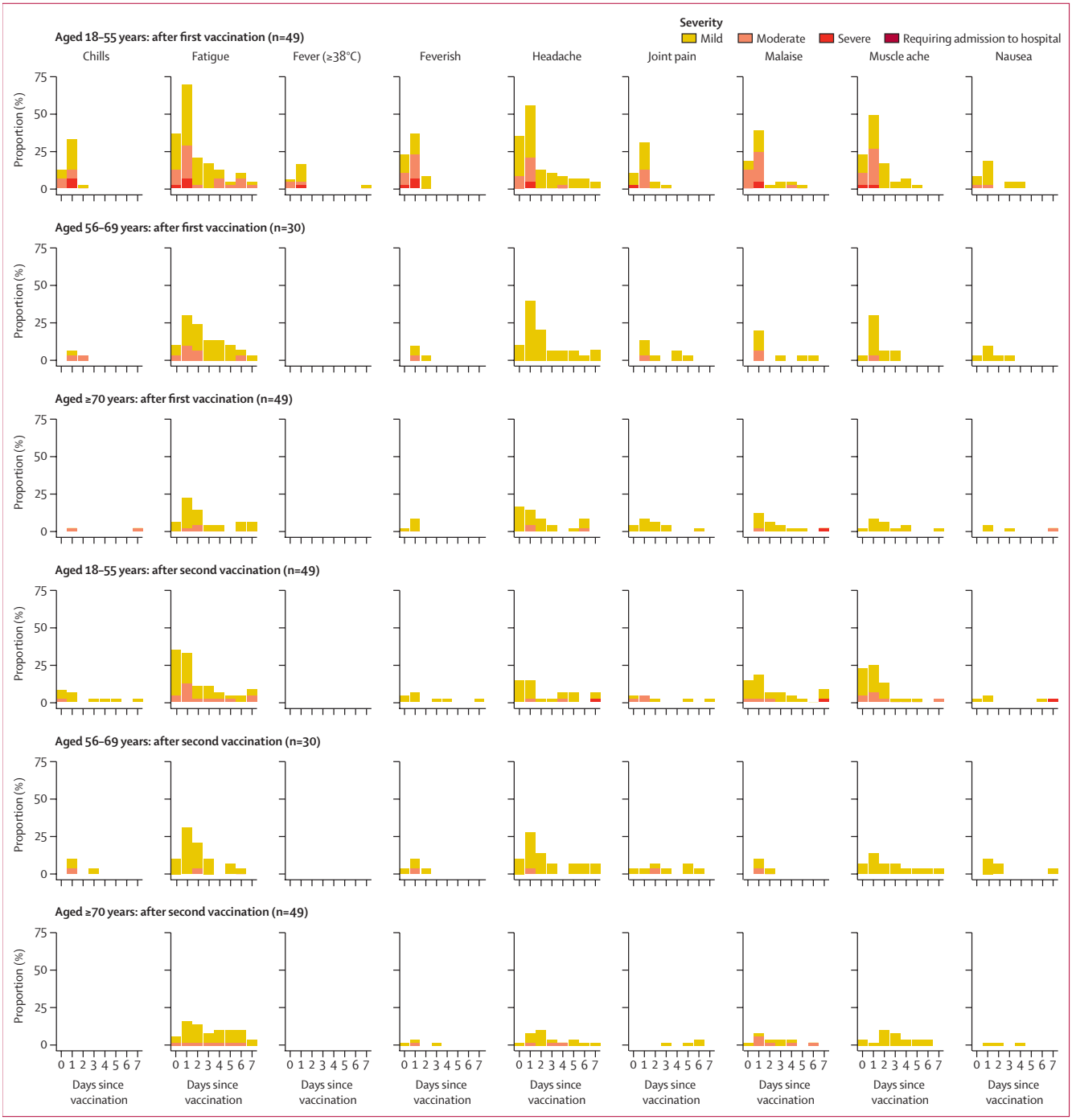

Figure 3: **Solicited systemic adverse reactions in the 7 days after prime and boost doses of standard-dose vaccine, by age**
Day 0 is the day of vaccination. Feverish is self-reported feeling of feverishness, whereas fever is an objective fever measurement (mild: 38·0 to <38·5°C, moderate: 38·5 to <39·0°C, severe: ≥39·0°C). Participants shown are those randomly assigned to receive two doses, and data are only shown for participants who received both doses of vaccine.

was seen in the low-dose groups (appendix pp 7, 8, 19–21). Similar results after the first dose were seen in those who were randomly assigned to receive only one dose of vaccine (data not shown). Data for the control groups are in the appendix (p 10).

As of Oct 26, 2020, 13 serious adverse events have occurred (across all age and vaccine groups), none of which are considered related to either study vaccine as assessed by the investigators (appendix p 31).

Using a multiplex immunoassay that detected total IgG against RBD and trimeric spike protein, we observed that participants who received the prime vaccination of standard-dose ChAdOx1 nCoV-19 had similar anti-spike antibody titres by day 28 after their prime vaccination as those who received a low dose (p=0·12 adjusted for age; figure 4; appendix p 12). At both dose levels, and for all dose groups combined, anti-spike IgG responses at day 28 decreased with increasing age (low-dose groups: 18–55 years, median 6439 arbitrary units [AU]/mL [IQR 4338–10 640], n=49; 56–69 years, 4553 AU/mL [2657–12 462], n=60; ≥70 years, 3565 AU/mL [1507–6345], n=93; p=0·0037; standard-dose groups: 18–55 years, median 9807 AU/mL [IQR 5847–17 220], n=43; 56–69 years, 5496 AU/mL [2548–12 061], n=55; ≥70 years, 4156 [2122–12 595], n=97; p=0·0044). By 28 days after the boost vaccination, similar antibody titres were seen across all two-dose groups, regardless of age or vaccine dose (eg, standard-dose groups: 18–55 years, median 20 713 AU/mL [IQR 13 898–33 550], n=39; 56–69 years, 16 170 AU/mL [10 233–40 353], n=26; and ≥70 years, 17 561 AU/mL [9705–37 796], n=47; p=0·68), and were higher than for those who did not receive a boost vaccination (appendix p 13). Similar results were seen with anti-RBD antibodies (figure 4; appendix p 12) and with an in-house standardised ELISA (appendix pp 12–13). Data for the control group are in the appendix (pp 12–13).

In a live SARS-CoV-2 microneutralisation assay (MNA_{80}), median titres peaked by day 42 in most groups that received two vaccinations (figure 5). There were no significant differences in normalised titres between age groups at day 42 (low-dose groups: 18–55 years, median 161 [IQR 99–233], n=41; 56–69 years, 143 [79–220], n=28; ≥70 years, 150 [103–255], n=34; p=0·90; standard-dose groups: 18–55 years, median 193 [IQR 113–238], n=39; 56–69 years, 144 [119–347], n=20; and ≥70 years, 161 [73–323], n=47; p=0·40). Within each age group, no significant differences were seen in neutralisation titres between low-dose and standard-dose vaccine recipients at the same timepoint (18–55 years p=0·33, 56–69 years p=0·12, ≥70 years p=0·62; figure 5; appendix p 14). Neutralising titres were achieved by 14 days after the boost vaccination in 208 (>99%) of 209 recipients of a boost vaccination. The one participant with a non-neutralising level was in the 70 years and older two-dose low-dose group.

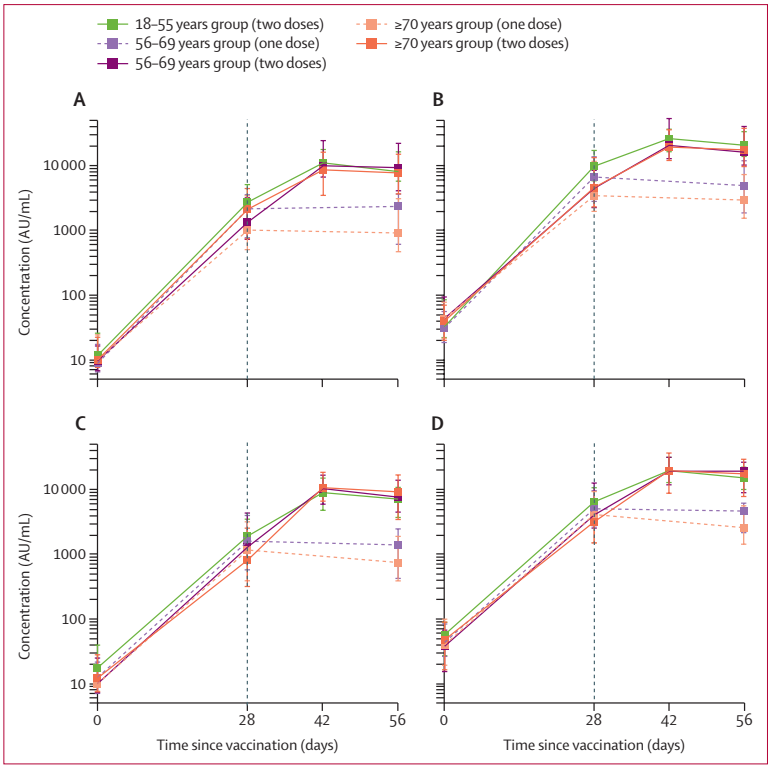

Figure 4: **SARS-CoV-2 IgG response to the receptor binding domain in the standard-dose groups (A) and low-dose groups (C) and the spike protein in the standard-dose groups (B) and the low-dose groups (D), by age**

Datapoints are medians, with whiskers showing the IQRs. Solid lines show participants who were randomly assigned to and received two doses of vaccine and dashed lines indicate participants who were randomly assigned to receive one dose. The vertical black line indicates when participants who received two doses received their boost dose. Data for the control groups are shown in the appendix (p 12). AU=arbitrary units. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

Anti-spike IgG levels after vaccination across all timepoints in those who received two doses of vaccine were highly correlated with neutralising titres in all age groups and for both low-dose and standard-dose vaccines (r^2 from linear regression 0·42–0·75, all p<0·0001; appendix p 32).

IFN-γ ELISpot responses against SARS-CoV-2 spike protein peaked 14 days after the prime vaccination (standard-dose groups: 18–55 years, median 1187 spot-forming cells [SFCs] per million peripheral blood mononuclear cells [PBMCs; IQR 841–2428], n=24; 56–69 years, 797 SFCs [383–1817], n=29; and ≥70 years, 977 SFCs [458–1914], n=48; appendix p 16) and did not increase significantly after the boost vaccination (p=0·46 from paired Student's *t* test of day 28 *vs* day 42; figure 6). ELISpot data were unavailable for the 18–55 years low-dose group because PBMCs were not collected in this group. In those who received two standard doses of vaccine, a significant difference was seen across age groups with those aged 56–69 years having higher responses at day 42 than other age groups receiving the

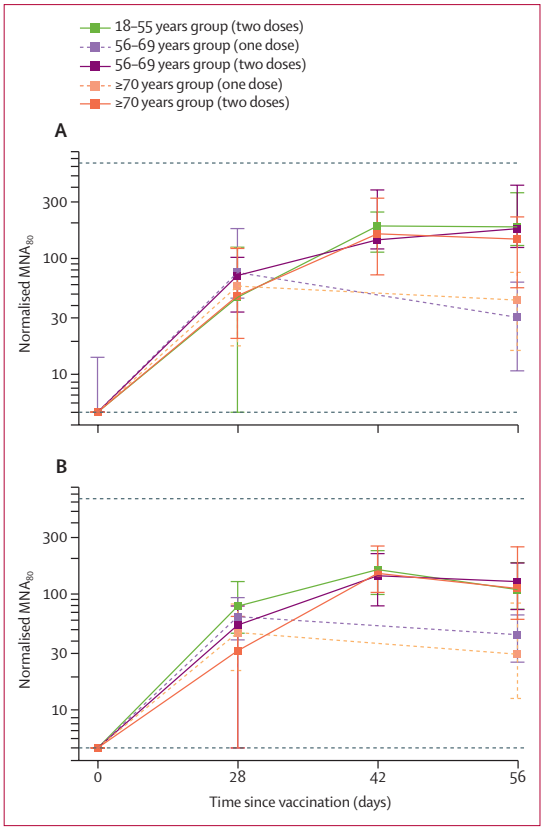

Figure 5: **Neutralising antibody titres measured using a live SARS-CoV-2 microneutralisation assay (MNA_{80}) after prime and boost doses of vaccine in standard-dose groups (A) and low-dose groups (B), by age**
Datapoints are medians, with whiskers showing the IQR. Solid lines show participants who were randomly assigned to and received two doses of vaccine and dashed lines indicate participants who were randomly assigned to receive one dose. Horizontal dotted lines show upper and lower limits of assay (values outside this range set to 640 beyond the upper limit and 5 beyond the lower limit). Data for the control groups are shown in the appendix (p 14).
To normalise data across assay runs, a reference sample was included in all assay runs and test samples normalised to this value by generating log_{10} ratios.
SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

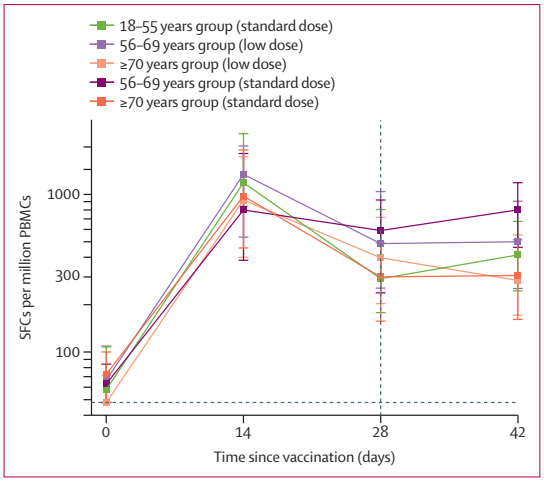

Figure 6: **IFN-γ ELISpot response to peptides spanning the SARS-CoV-2 spike insert after prime and boost doses of vaccine for all participants who were given two doses of vaccine, by age group and vaccine dose**
ELISpot data were unavailable for the 18–55 years low-dose group because PBMCs were not collected in this group. Datapoints are medians, with whiskers showing the IQR. The lower limit of detection is 48 SFCs per million PBMCs (horizontal dotted line). Day 42 samples are from participants who received the boost dose at day 28 (vertical dotted line). Data for both one-dose and two-dose groups, with numbers analysed at each timepoint, are in the appendix (p 15).
ELISpot=enzyme-linked immunospot. PBMC=peripheral blood mononuclear cells. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. SFC=spot-forming cells.

same vaccine regimen (18–55 years, median 413 SFCs per million PBMCs [IQR 245–675], n=23; 56–69 years, 798 SFCs [462–1186], n=28; and ≥70 years, 307 SFCs [161–516], n=47; p<0·0001; appendix p 15).

Anti-ChAdOx1 neutralising antibody titres across different age and dose groups are shown in figure 7. Titres increased with the prime vaccination with ChAdOx1 nCoV-19 in all groups to similar levels, but were not increased further after a boost dose of vaccine at day 28. This observation was in contrast with the anti-SARS-CoV-2 spike protein antibody levels, which were increased 28 days after the boost vaccination (figure 4). Anti-ChAdOx1 neutralising titres immediately before the boost vaccination were negatively correlated with standardised ELISA values 28 days after the boost vaccination (p=0·037; figure 7), but no significant correlation was seen between anti-ChAdOx1 neutralising titres immediately before the boost vaccination and ELISpot responses 14 days after the boost vaccination (p=0·22; figure 7).

Discussion

Our findings show that the ChAdOx1 nCoV-19 vaccine was safe and well tolerated with a lower reactogenicity profile in older adults than in younger adults. Immunogenicity was similar across age groups after a boost vaccination. If these responses correlate with protection in humans, these findings are encouraging because older individuals are at disproportionate risk of severe COVID-19 and so any vaccine adopted for use against SARS-CoV-2 must be effective in older adults.

Most of the reported local and systemic adverse events were mild to moderate in severity, in line with our previous phase 1 study of the ChAdOx1 nCoV-19 vaccine[18] and previously reported studies of ChAdOx1-vectored vaccines.[22–24] Fewer adverse events were reported after the boost vaccination than after the prime vaccination and reactogenicity reduced with increasing age. The lower dose of vaccine was less reactogenic than the standard dose of vaccine across all age groups.

The serious adverse events observed during the trial in these study groups were judged to be unrelated to the study vaccines and occurred at frequencies expected for these conditions in the general population. None of the participants included in this report had any suspected

unexpected serious adverse reactions. In the phase 3 component of the trial, suspected unexpected serious adverse reactions occurred in other groups, and will be reported in detail in a subsequent publication. We carefully monitored suspected unexpected serious adverse reactions and other adverse events to ensure that no pattern of unexplained illnesses emerged that could indicate a safety concern. Independent assessments have led to the recommendation that the trial is safe to continue.

The ChAdOx1 nCoV-19 vaccine induced a specific antibody response to the SARS-CoV-2 spike glycoprotein and RBD at 28 days after a single dose across all age groups, including adults aged 70 years and older. A clear effect of a boost vaccination on antibody titres at day 56 was seen that was unrelated to dose regimen or age group. Similar patterns were observed with neutralising antibody responses, with no difference in the magnitude of the response at day 28 after the prime vaccine regardless of age or vaccine dose, but a booster effect was observed in individuals who received a second dose of vaccine.

Other clinical trials have also assessed safety, tolerability, and immunogenicity of SARS-CoV-2 vaccines in older adults. An adenovirus 5 vector-based vaccine also had reduced reactogenicity in adults aged 55 years and older compared with adults aged 18–54 years after a single dose of vaccine, although immunogenicity was concurrently reduced in this older age group.[11] A two-dose mRNA vaccine has also been shown to be immunogenic in adults older than 56 years with dose-dependent immune responses and similar neutralising antibody titres and cellular immune responses to younger adults.[9] Another two-dose mRNA vaccine has shown immunogenicity in older adults, but absolute neutralising antibody responses in adults aged 65–85 years were lower than in those aged 18–55 years.[10] By contrast with our observations, in both these studies, reactogenicity was more common after the second dose of an mRNA vaccine. A two-dose inactivated virus vaccine has also shown lower absolute neutralising antibody titres in adults aged 60 years and older than in adults aged 18–59 years, but reactogenicity was not formally compared between the first and second doses in this study.[13]

T-cell responses are important in controlling disease in natural infection[8] and therefore generation of a robust cellular immune response is a desirable attribute for a vaccine against SARS-CoV-2. Here, we found that spike-specific T-cell responses measured with ELISpot peaked at 14 days after the prime vaccination, consistent with previous studies of simian adenovirus-vectored vaccines,[25] and were similar in all groups regardless of age and vaccine dose. Spike protein T-cell responses measured with ELISpot have also been reported in studies with other adenovirus-vectored vaccines against SARS-CoV-2,[12] including in adults older than 55 years.[11] Theoretical concerns about vaccine-enhanced disease have led to a

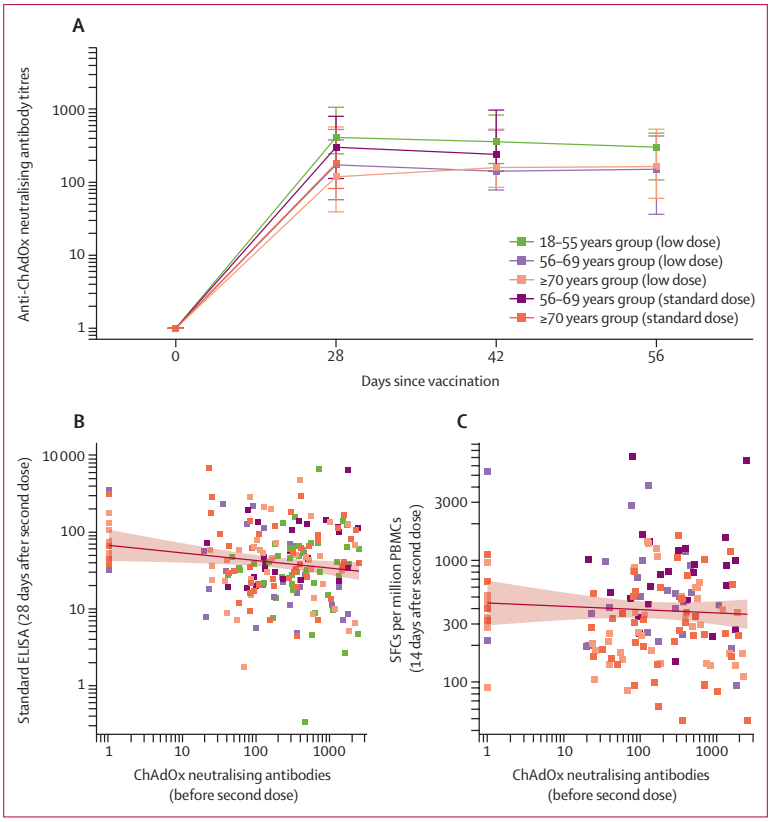

Figure 7: **Anti-ChAdOx1 vector neutralising titres after prime and boost doses of vaccine, by age and vaccine dose, and the correlation between pre-boost dose anti-ChAdOx1 neutralising antibodies and 28 days after boost dose antibody and T-cell responses**

(A) Anti-ChAdOx1 neutralising antibody titres in participants who received ChAdOx1 nCoV-19 vaccine by age and dose: datapoints are medians, with whiskers showing the IQR. Values below the limit of detection were assigned a value of 1. (B) Anti-ChAdOx1 neutralising antibody titre immediately before boost dose of vaccine versus standardised IgG ELISA against SARS-CoV-2 spike 28 days after the boost dose of vaccine with linear regression of logged values (p=0·037). (C) Anti-ChAdOx1 neutralising antibody titres immediately before boost dose of vaccine versus SARS-CoV-2 spike specific T cells measured by IFN-γ ELISpot on day 14 after the boost dose of vaccine with linear regression of logged values (p=0·22). In B and C, each datapoint is one participant and the solid line shows the linear regression, with the shaded area showing the 95% CI from an unadjusted linear regression of anti-vector neutralisation titres against logged ELISA (in B) or ELISpot (in C) response. Data were unavailable at day 56 for the 56–69 years standard-dose group. ELISpot=enzyme-linked immunospot. PBMC=peripheral blood mononuclear cells. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. SFC=spot-forming cells.

view that a type 1 T-helper (Th1)-biased CD4 response is a preferred coronavirus vaccine characteristic.[26] An adjuvanted nanoparticle vaccine has been shown to induce spike-specific CD4 T-cell cytokine responses with a predominantly Th1 profile,[15] as has an mRNA vaccine in small numbers of adults aged 56–70 years and 71 years and older.[9] More detailed investigations of antigen-specific T-cell responses in our study participants are ongoing.

The robust humoral and cellular immune responses obtained in our older adult population were encouraging given that a number of studies have shown that decreasing immune function with age leads to decreased immune responses to vaccines. This fact holds true for vaccines such as for influenza, for which pre-existing

immune memory exists,[27] and vaccines that induce primary immune responses, such as hepatitis B.[28] Other adenovirus-vector platforms against SARS-CoV-2 have either shown reduced immunogenicity in an older age group[11] (although this study was of a single-dose regimen and so not directly comparable with our prime-boost regimen) or have not yet been tested in an older population.[12]

However, our results are consistent with previous studies of adenovirus-vector-based vaccines against respiratory pathogens that evoke humoral and T-cell responses in older adults, including a human adenovirus-vectored respiratory syncytial virus (RSV) vaccine[29] and a simian adenovirus-vectored RSV vaccine.[30] Our results with ChAdOx1 nCoV-19 are also consistent with those of a ChAdOx1-vectored vaccine against influenza that showed good immunogenicity in adults older than 50 years.[22]

Notably, the anti-spike antibody responses in our study increased after a boost vaccination at an interval of 1 month but the neutralising anti-vector antibody responses did not. There was also no difference in anti-vector immunity by age. We observed a small negative correlation between anti-vector antibody titres and anti-spike total IgG, but not T-cell ELISpot responses. Further work is needed to investigate if homologous boosting with adenovirus-vectored vaccines can be done without loss of immunogenicity to the pathogen-specific transgene.

In the absence of a clear serological correlate of protection against SARS-CoV-2, clinical studies have focused on measuring neutralising antibodies because these have been shown to confer protection from challenge in animal models.[9–15] Live virus neutralisation assays are labour intensive and can only be done in specialist laboratories under category 3 biological safety conditions. We found here that anti-spike IgG levels correlate with neutralising antibody titres for all age groups. This finding suggests that, should neutralising antibodies be shown to be protective in humans, routine serological assays could be used for the standardised evaluation of functional antibody by vaccine candidates in clinical trials.

A limitation of this study is its single-blind design. However, all laboratory analyses and clinical assessments reported in this manuscript were done in a blinded fashion. A further limitation is possible variation of severity of local reactions due to the difference in injection volumes between different batches of vaccine in the low-dose group. Ongoing studies in larger groups will investigate the reactogenicity of a booster dose in more detail. Finally, the selection of participants aged 70 years and older, with a median age of 73–74 years between dose groups and with few comorbidities, might not be representative of the general older population, including those living in residential care settings or older than 80 years. Early phase studies in older adults require healthy volunteers to be enrolled for safety assessments, and recruitment to the study occurred during a period of national lockdown when more susceptible individuals were advised by Public Health England to self-isolate. Therefore, we excluded volunteers with substantial comorbidities or clinical frailty. Larger studies are now underway to assess immunogenicity, safety, and efficacy in older adults with a wider range of comorbidities.

Ultimately, licensure of a vaccine relies on the demonstration of efficacy in preventing COVID-19 and safety. Phase 3 studies with ChAdOx1 nCoV-19 are ongoing in the UK, Brazil, and the USA to assess vaccine efficacy and safety. Here we found similar safety and immunogenicity of ChAdOx1 nCoV-19 in older adults compared with younger adults, which could support the use of this vaccine in this older age group, if it is shown to be protective in phase 3 trials.

Contributors

AJP and SCG conceived and designed the trial and AJP is the chief investigator. AJP, AMM, HR, MNR, MV, and PMF contributed to the protocol and design of the study. AVSH and SNF were the study site principal investigators. ALF, CD, EAC, KJE, RM, and TL were responsible for laboratory testing and assay development. MV and NGM did the statistical analysis. SCG and TL were responsible for vaccine development. ADD, CG, and RT were responsible for vaccine manufacture. AJP, AMM, MNR, MV, NGM, and TL contributed to the preparation of the report. AMM, DRO, HR, KJE, MNR, PKA, and PMF contributed to the implementation of the study. All other authors contributed to the implementation of the study and data collection. All authors critically reviewed and approved the final version.

Declaration of interests

Oxford University has entered into a partnership with AstraZeneca for further development of ChAdOx1 nCoV-19 (AZD1222). AstraZeneca reviewed the data from the study and the final manuscript before submission, but the authors retained editorial control. SCG is cofounder of Vaccitech (a collaborator in the early development of this vaccine candidate) and named as an inventor on a patent covering use of ChAdOx1-vectored vaccines (PCT/GB2012/000467) and a patent application covering this SARS-CoV-2 vaccine. TL is named as an inventor on a patent application covering this SARS-CoV-2 vaccine and was consultant to Vaccitech. PMF is a consultant to Vaccitech. AJP is Chair of the UK Department of Health and Social Care's JCVI, but does not participate in policy advice on coronavirus vaccines, and is a member of the WHO Strategic Advisory Group of Experts (SAGE). AVSH is a cofounder of and consultant to Vaccitech and is named as an inventor on a patent covering design and use of ChAdOx1-vectored vaccines (PCT/GB2012/000467). MDS reports grants from Janssen, GlaxoSmithKline, MedImmune, Novavax, and MCM Vaccine and grants and non-financial support from Pfizer outside of the submitted work. CG reports personal fees from the Duke Human Vaccine Institute outside of the submitted work. ADD reports grants and personal fees from AstraZeneca outside of the submitted work. All other authors declare no competing interests.

Data sharing

The study protocol and clinical study plan are provided in the appendix (pp 45–212). Anonymised participant data will be made available when the trial is complete, upon requests directed to the corresponding author. Proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. All data will be made available for a minimum of 5 years from the end of the trial.

Acknowledgments

This Article reports independent research funded by UK Research and Innovation (MC_PC_19055), Engineering and Physical Sciences Research Council (EP/R013756/1), Coalition for Epidemic Preparedness

Innovations, and the National Institute for Health Research (NIHR). We acknowledge support from Thames Valley and South Midland's NIHR Clinical Research Network and the staff and resources of NIHR Southampton Clinical Research Facility, and the NIHR Oxford Health Biomedical Research Centre. PMF received funding from the Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior, Brazil (finance code 001). ALF was supported by the Chinese Academy of Medical Sciences Innovation Fund for Medical Science, China (grant number: 2018-I2M-2-002). KJE is an NIHR Biomedical Research Centre Senior Research Fellow. AJP and SNF are NIHR senior investigators. The views expressed in this publication are those of the authors and not necessarily those of the NIHR or the UK Department of Health and Social Care. We thank the senior management at AstraZeneca for facilitating and funding the Mesoscale antibody assay included in this manuscript. We also thank the volunteers who participated in this study.

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Centers for Disease Control and Prevention

The Janssen COVID-19 Vaccine's Local Reactions, Systemic Reactions, Adverse Events, and Serious Adverse Events

Local Reactions

Local reactions were reported at higher rates by vaccine recipients than placebo recipients. The frequency of any local reaction was higher in participants aged 18 to 59 years than participants aged ≥60 years (59.8% vs 35.4%). Pain at the injection site was the most frequently reported solicited local reaction among vaccine recipients (58.6% of 18-59-year-olds and 33.3% ≥60-year-olds). Erythema and swelling were reported less frequently. No grade 4 local reactions were reported. Overall, the median onset of local reactions in the vaccine group was within two days of vaccination, with a median duration 2 days for erythema and pain and 3 days for swelling. (Table 1)

Table 1. Local reactions in persons aged 18-59 years and persons aged ≥60 years, Janssen COVID-19 vaccine and placebo[a]

	18-59 years		≥60 years	
	Janssen Vaccine N=2036	Placebo N=2049	Janssen Vaccine N=1320	Placebo N=1331
Any Local, n (%)				
Any	1218 (59.8)	413 (20.2)	467 (35.4)	244 (18.3)
Grade 3	18 (0.9)	4 (0.2)	5 (0.4)	2 (0.2)
Pain[b], n (%)				
Any	1193 (58.6)	357 (17.4)	439 (33.3)	207 (15.6)
Grade 3	8 (0.4)	0 (0.0)	3 (0.2)	2 (0.2)
Erythema[c], n (%)				
Any	184 (9.0)	89 (4.3)	61 (4.6)	42 (3.2)
Grade 3	6 (0.3)	2 (0.1)	1 (0.1)	0 (0.0)
Swelling[c], n (%)				
Any	142 (7.0)	32 (1.6)	36 (2.7)	21 (1.6)
Grade 3	5 (0.2)	2 (0.1)	2 (0.2)	0 (0.0)

[a] Solicited local and systemic adverse reactions collected for participants in a safety subset (N=6,736)

[b] Pain – Grade 3: any use of prescription pain reliever or prevented daily activity

[c] Erythema and Swelling – Grade 3: >100mm

Note: No grade 4 local reactions were reported.

Systemic Reactions

Systemic reactions were reported at higher rates by vaccine recipients than placebo recipients. The frequency of systemic reactions was higher in participants aged 18-59 years than participants ≥60 years (61.5% vs 45.3%). For both age groups, fatigue and headache were the most commonly reported systemic reactions. Fever was more common in participants 18-59

years (12.8%) compared to those ≥60 years (3.1%). The majority of systemic reactions were mild or moderate in severity. The most common grade 3 reactions were fatigue and myalgia. No grade 4 reactions were reported. Among vaccine recipients, the median onset of systemic reactions within 2 days of vaccination, with a median duration of 1-2 days. (Table 2)

Table 2. Systemic reactions in persons aged 18-59 years and persons aged ≥60 years, Janssen COVID-19 vaccine and placebo[a]

	18-59 years		≥60 years	
	Janssen Vaccine N=2036	Placebo N=2049	Janssen Vaccine N=1320	Placebo N=1331
Any systemic, n (%)				
Any	1252 (61.5)	745 (36.4)	598 (45.3)	440 (33.1)
Grade 3	47 (2.3)	12 (0.6)	14 (1.1)	9 (0.7)
Fatigue[b], n (%)				
Any	891 (43.8)	451 (22.0)	392 (29.7)	277 (20.8)
Grade 3	25 (1.2)	4 (0.2)	10 (0.8)	5 (0.4)
Headache[b], n (%)				
Any	905 (44.4)	508 (24.8)	401 (30.4)	294 (22.1)
Grade 3	18 (0.9)	5 (0.2)	5 (0.4)	4 (0.3)
Myalgia[b], n (%)				
Any	796 (39.1)	248 (12.1)	317 (24.0)	182 (13.7)
Grade 3	29 (1.4)	1 (<0.1)	3 (0.2)	5 (0.4)
Nausea[c], n (%)				
Any	315 (15.5)	183 (8.9)	162 (12.3)	144 (10.8)
Grade 3	3 (0.1)	3 (0.1)	3 (0.2)	3 (0.2)
Fever[d], n (%)				
Any	261 (12.8)	14 (0.7)	41 (3.1)	6 (0.5)
Grade 3	7 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)

[a] Solicited local and systemic adverse reactions collected for participants in a safety subset (N=6,736)

[b] Fatigue, Headache, Myalgia – Grade 3: use of prescription pain reliever or prevented daily activity

[c] Nausea – Grade 3: prevented daily activity

[d] Fever – Grade 3: ≥39.0 – ≤40.0°C or ≥102.1 – ≤104.0°F

Note: No grade 4 systemic reactions were reported.

Analgesic/Antipyretics Use

Among vaccine recipients aged 18-59 years, 26.4% reported using antipyretic or analgesic medications, compared to 6.0% of placebo recipients. Among vaccine recipients aged ≥60 years, 9.8% reported using antipyretic or analgesic medications, compared to 5.1% of placebo recipients. The reason for medication use (e.g. fever, pain) was not ascertained.

Unsolicited Adverse Events

Overall, rates of reported unsolicited adverse events were similar in the vaccine and placebo groups (13.1% vs 12.0%). Reports of embolic and thrombotic events had a slight numerical imbalance with 0.06% of vaccine recipients and 0.05% of placebo recipients reporting such events. Risk factors for these events were present in the participants, however vaccine cannot be excluded as a contributing factor. Reports of tinnitus had a numerical imbalance with 6 events in vaccine recipients and no events in placebo recipients. Data are insufficient at this time to determine if there is a casual relationship between the

vaccine and tinnitus. Angioedema demonstrated a numerical imbalance with events reported among 0.2% of vaccine recipients and 0.1% of placebo recipients. Of these, urticaria was reported in 8 vaccine recipients and 3 placebo recipients. Based on temporal and biologic plausibility, reports of urticaria are possibly related to vaccine.

Serious Adverse Events

Serious adverse events were defined as any untoward medical occurrence that resulted in death, was life-threatening, required inpatient hospitalization or prolongation of existing hospitalization, or resulted in persistent disability or incapacity. The proportions of participants who reported at least one serious adverse event, excluding those attributed to COVID-19, were 0.4% in the vaccine group and 0.4% in the placebo group. The most common serious adverse event occurring at higher rates in the vaccine group than the placebo group was appendicitis (6 cases in vaccine group vs. 5 cases in placebo group). Three serious adverse events occurring among vaccine recipients were considered by the U.S. Food and Drug Administration (FDA) as likely related to vaccine: the one report of hypersensitivity reaction to study vaccine, one report of pain at the injection site initially evaluated for brachial neuritis, and one report of systemic reactogenicity.

Data source: FDA briefing document

Page last reviewed: August 12, 2021

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