


RESEARCH ARTICLE

Open Access



Serological study of CoronaVac vaccine and booster doses in Chile: immunogenicity and persistence of anti-SARS-CoV-2 spike antibodies

Leonardo Vargas^{1,2†}, Nicolás Valdivieso^{1†}, Fabián Tempio³, Valeska Simon¹, Daniela Sauma¹, Lucía Valenzuela⁴, Caroll Beltrán⁴, Lorian Castillo-Delgado⁵, Ximena Contreras-Benavides⁵, Mónica L. Acevedo⁶, Johanna Acevedo⁷, Rafael I. Gonzalez^{8,9}, Fernando Valiente-Echeverría⁶, Ricardo Soto-Rifo⁶, Mario Roseblatt^{1,2,10}, Mercedes Lopez^{2,3*}, Fabiola Osorio^{7,11*} and María Rosa Bono^{1,2*} 

Abstract

Background: Chile was severely affected by COVID19 outbreaks but was also one of the first countries to start a nationwide program to vaccinate against the disease. Furthermore, Chile became one of the fastest countries to inoculate a high percentage of the target population and implemented homologous and heterologous booster schemes in late 2021 to prevent potential immunological waning. The aim of this study is to compare the immunogenicity and time course of the humoral response elicited by the CoronaVac vaccine in combination with homologous versus heterologous boosters.

Methods: We compared the immunogenicity of two doses of CoronaVac and BNT162b2 vaccines and one homologous or heterologous booster through an ELISA assay directed against the ancestral spike protein of SARS-CoV-2. Sera were collected from individuals during the vaccination schedule and throughout the implementation of homologous and heterologous booster programs in Chile.

Results: Our findings demonstrate that a two-dose vaccination scheme with CoronaVac induces lower levels of anti-SARS-CoV-2 spike antibodies than BNT162b2 in a broad age range (median age 42 years; interquartile range (IQR) 27-61). Furthermore, antibody production declines with time in individuals vaccinated with CoronaVac and less noticeably, with BNT162b2. Analysis of booster schemes revealed that individuals vaccinated with two doses

[†]Leonardo Vargas and Nicolás Valdivieso contributed equally to this work.

*Correspondence: melopez@uchile.cl; fabiolaosorio@med.uchile.cl; mrbono@uchile.cl

¹ Laboratory of Immunology, Biology Department, Faculty of Sciences, Universidad de Chile, Las Palmeras 3425, 7800003 Santiago, Chile

³ Laboratory of Cancer Immunoediting, Immunology Program, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Av. Independencia 1027, 8380453 Santiago, Chile

¹¹ Laboratory of Immunology and Cellular Stress, Immunology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Av. Independencia 1027, 8380453 Santiago, Chile

Full list of author information is available at the end of the article



of CoronaVac generate immunological memory against the SARS-CoV-2 ancestral strain, which can be re-activated with homologous or heterologous (BNT162b2 and ChAdOx1) boosters. Nevertheless, the magnitude of the antibody response with the heterologous booster regime was considerably higher (induction fold BNT162b2: 11.2x; ChAdOx1; 12.4x; CoronaVac: 6.0x) than the responses induced by the homologous scheme. Both homologous and heterologous boosters induced persistent humoral responses (median 122 days, IQR (108-133)), although heterologous boosters remained superior in activating a humoral response after 100 days.

Conclusions: Two doses of CoronaVac induces antibody titers against the SARS-CoV-2 ancestral strain which are lower in magnitude than those induced by the BNT162b2 vaccine. However, the response induced by CoronaVac can be greatly potentiated with a heterologous booster scheme with BNT162b2 or ChAdOx1 vaccines. Furthermore, the heterologous and homologous booster regimes induce a durable antibody response which does not show signs of decay 3 months after the booster dose.

Keywords: COVID-19, Vaccination, Booster, Antibodies

Background

Chile is one of the several countries severely threatened by the COVID-19 pandemic in 2020, but that had prompt access to vaccines for a large number of individuals since early 2021. The first SARS-CoV-2 vaccine authorized in Chile for emergency use by the Health Ministry (MIN-SAL) was the Pfizer-BioNTech vaccine (BNT162b2) on December 16, 2020, and Sinovac's CoronaVac vaccine on January 20, 2021 (Institute of Public Health, ISP). World Health Organization (WHO) listed CoronaVac for emergency use on June 1, 2021 [1], which is currently administered in 48 countries [2]. In Chile, vaccination with CoronaVac began on February 1, 2021, with people over 55 years old, people with specific pathologies, and essential services personnel. Progressively, the vaccination scheme extended to younger people (target population over 18 years old: 15,200,840). In this first phase of vaccination, the CoronaVac vaccine was predominantly used across the population. Real-world data indicated that the two-dose vaccination scheme with CoronaVac in Chile showed a 65.9% vaccine effectiveness, 90.3% for prevention of ICU admission, and 86.3% for prevention of COVID-19 related death [3]. To date, more than 86,8% of the Chilean population received their complete vaccination schedule with any available vaccines, and about 77% of the target population received CoronaVac (Data obtained from the Chilean Ministry of health (MIN-SAL) and department of statistics and health information (DEIS) [4].

However, around mid-2021, immunological studies reported a decline of antibody levels in vaccinated individuals. These studies predicted a reduction in antibody titers directed against SARS-CoV-2 over time, highlighting the requirement of an additional immunization [5]. In this context, a group of countries, including Israel [6] and Chile, authorized a booster vaccine dose. On August 11, 2021, the vaccination with booster doses began for people who had received two doses of CoronaVac in Chile.

Interestingly, Chile implemented a heterologous booster schedule for most individuals including BNT162b2 and the ChAdOx1 vaccine from AstraZeneca as the most used boosters. The vaccines used in this study have different formulations and origins. CoronaVac is an inactivated virus vaccine produced by the Chinese company Sinovac that uses the ancestral strain of the SARS-CoV-2 virus [7]. This vaccine is the most widely used globally, and several studies have shown that it induces the production of neutralizing antibodies in about 60% of individuals. The BNT162b2 vaccine, developed by Pfizer-BioNTech, is an encapsulated mRNA vaccine that encodes a domain of the spike protein of the SARS-CoV-2 virus, and it is around 95% effective in preventing COVID-19 [8]. The ChAdOx1 vaccine developed by Oxford-AstraZeneca is produced with a recombinant adenoviral vector that cannot replicate. Vaccination with two doses of ChAdOx1 vaccine reduces SARS-CoV-2 virus infection by 86% [9]. These schemes offer an important opportunity to assess the magnitude of the immunological response to homologous and heterologous booster schedules within the same population. Furthermore, this issue is relevant considering that immunological studies of heterologous booster schedules using CoronaVac as the first immunization vaccine have not been extensively documented.

This study describes the production of IgG antibodies directed against the ancestral SARS-CoV-2 spike protein induced by the two-dose scheme of the CoronaVac vaccine in a Health Service of the Hospital La Florida, Santiago. Our data shows that detectable levels of specific antibodies appear in most vaccinated individuals. By comparing the humoral responses to CoronaVac and BNT162b2 vaccines over time, we found that the antibody production elicited by CoronaVac declined 6 months after vaccination, whereas people vaccinated with two doses of BNT162b2 maintained a noticeably higher level of antibodies over time. Next, we analyzed the impact of the booster doses of CoronaVac,

BNT162b2, or ChAdOx1 vaccines, administered to individuals vaccinated with the two-dose scheme with CoronaVac 6 months earlier. Our data show that the three types of boosters produce a noticeable increase in anti-spike IgG antibody production 30 days after the booster administration, which was more strongly noticed in individuals vaccinated with the heterologous booster regime. Antibody responses measured 100 days after the booster dose revealed that the heterologous regime induced higher and persistent anti-SARS-CoV-2 spike antibodies compared to the homologous regime.

In summary, our results show that the CoronaVac vaccine produces memory against the SARS-CoV-2 that can be greatly potentiated with a heterologous booster strategy.

Methods

Ethics statement

Hospital Clínico Universidad de Chile approved the study on health worker personnel (Protocol ID Number 1151/20 and Protocol ID Number 074-2020). Hospital Clínico Metropolitano La Florida “Dra. Eloisa Díaz I” was included in the ethical protocols of the University of Chile as part of the COVID-19 research program of ANID grant 0752. Samples obtained from non-health worker individuals were approved by Facultad de Ciencias, Universidad de Chile (Protocol ID 2123-FCS-UCH and consent approval). Samples were collected from February 2021 to January 2022. All patients and healthy controls were required to understand the study and sign with informed consent.

Design of study groups

We obtained blood samples from different individuals; healthcare personnel volunteers from Hospital La Florida, and adult healthy volunteers (over 18 years old). These were divided in four different groups: group 1 to study the immune response following two CoronaVac doses in healthcare personnel, group 2 that was designed to compare CoronaVac and BNT162b2 vaccination, group 3 to analyze the homologous and heterologous booster response (6 months after CoronaVac vaccination), and group 4 to study the persistence of the humoral response after > 100 days following the homologous and heterologous booster.

In this study, only individuals who experienced COVID-19 symptoms were tested by PCR to confirm prior SARS-CoV2 infection (10 participants from group 1 out of 349 participants).

This study is composed of four groups covering the period ranging from the beginning of the vaccination program in February 2021 and months after the administration of the booster doses in August 2021 (depicted

as timelines in Fig. 1A). Group 1 corresponds to clinical staff from the Hospital Clínico Metropolitano La Florida “Dra. Eloisa Díaz” (104 individuals), which were among the first cohort in Chile to be vaccinated as a priority group with the CoronaVac vaccine. In this group of individuals, the antibody response to the first and second dose of the CoronaVac vaccine was assessed.

Group 2 corresponds to 158 individuals from a median age 39 years (IQR 27–61) vaccinated with CoronaVac and BNT162b2 vaccines. A comparison of IgG production against spike SARS-CoV-2 protein induced by the vaccines was performed, and antibody evolution was followed over time by ELISA. To better compare the initial response (13–45 days) with long-term response (more than 80 days) to CoronaVac and BNT162b2 vaccine, we disaggregated data in two groups as shown on Fig. 3C.

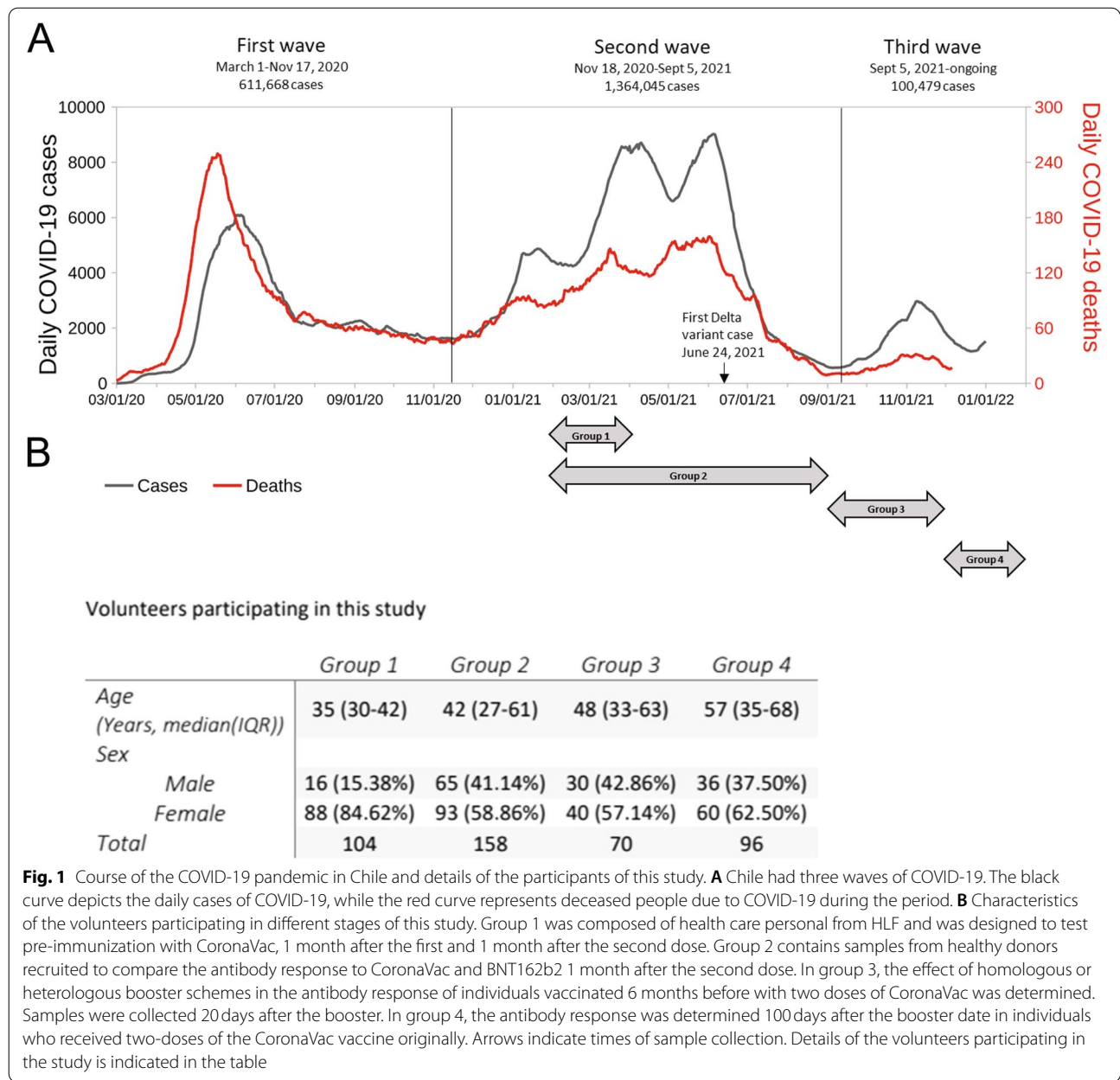
Group 3 corresponds to 43 individuals vaccinated with the two-dose scheme of CoronaVac that received a booster dose with either CoronaVac, BNT162b2, or the ChAdOx1 vaccine. This group determined the magnitude of the antibody response to the homologous and heterologous booster schemes 30 days after the booster. Finally, group 4 corresponds to 96 individuals vaccinated with the two-dose scheme of CoronaVac that received a booster dose with either CoronaVac, BNT162b2, or the ChAdOx1 vaccine and analyzed 100 days after the booster. Figure 1B describes the characteristics of the volunteers who participated in each stage of the study. In total, 349 individuals participated, of which 65.6% were women, and 34.3% were men. The median age of the volunteers was 38 years (IQR 30–57 years). Some individuals participated in group 2 and the longitudinal booster study. Thus, the number of samples is higher than the number of participants.

Isolation of human blood samples

Blood samples were obtained from healthcare personnel volunteers from Hospital La Florida and adult healthy volunteers (over 18 years old). Serum was collected after whole blood centrifugation and stored at -80°C for further analysis.

ELISA

The ELISA was performed as detailed [10] and adapted from the group of Kramer [11]. Briefly, 96-well ELISA plates were coated overnight at 4°C with $50\mu\text{l}$ per well of a $2\mu\text{g/ml}$ solution of resuspended SARS-CoV-2 Spike protein (Recombinant SARS-CoV-2S protein S1 from the original Wuhan SARS-CoV-2 virus, Biolegend 796906) on PBS. Then, the coating solution was removed, and the wells were blocked for 1 h at room temperature with $150\mu\text{l}$ of 3% skim milk prepared in PBS-0.1% Tween-20 (TPBS). After this period, $100\mu\text{l}$ per well of serial



dilutions (from 1/200 to 1/1600) of the sera prepared in 1% skim milk in 0.1% TPBS was added and incubated for 2 h at room temperature. The plates were washed three times, added 100 µl per well of HRP-conjugated anti-human IgG (HRP Donkey anti-human IgG Clone: Poly24109, Biolegend), and incubated for 1 h at room temperature. Plates were washed three times, after which 50 µl of TMB substrate solution (BD Biosciences) was added per well to reveal the reaction, which was stopped by adding 50 µl per well of 1M orthophosphoric acid. Optical density at 450 nm was measured on a Molecular Devices Emax ELISA plate reader.

This test was developed with samples from hospitalized COVID-19 patients as positive controls (13 samples) and pre-pandemic or negative samples (54 samples) for negative controls [10] and developed as reported [11]. Sera were diluted serially from 1/200 to 1/1600, and the area under the curve (AUC) was determined. These values were equivalent to the antibody titer (see [Quantification and statistical analysis](#)). We established the negative limit of the test (AUC = 70 ± 51; mean ± SD) from the analysis of 54 samples from people who had no history of COVID-19.

Our data confirmed that the ELISA test we performed with the SARS-CoV-2 spike protein of the original coronavirus recognizes the variants of concern that have entered Chile at that time including Alpha, Gamma, and Delta (www.vigilancia.ispch.gob.cl).

Neutralization assay

HIV-1-based SARS-CoV-2 pseudotype production

Pseudotyped viral particles were produced by transient transfection of HEK293T cells using polyethylenimine (PEI) and plasmids pNL4.3-ΔEnv-Firefly and pCMV14-3X-Flag-SARS-CoV-2 SΔ19C (lineage A) in a 1:1 ratio as we described [12]. The viral particles were diluted with 50% in fetal bovine serum (Sigma-Aldrich) and stored at -80 °C. Viral stock was quantified with the HIV-1 Gag p24 Quantikine ELISA kit (R&D Systems).

Neutralizations assays were performed as we previously reported [12]. Briefly, inactivated serum samples were diluted in DMEM with 10% fetal bovine serum (serial dilutions from 1:4 to 1:8748) and incubated with 5 ng of p24 HIV-1-based SARS-CoV-2 pseudotyped particles during 1 h at 37 °C, and 1×10^4 HEK-ACE2 cells were added to each well. HEK293T cells incubated with the pseudotyped virus were used as a negative control. Cells were lysed 48 h later, and firefly luciferase activity was measured using the Luciferase Assay Reagent (Promega) in a Glomax 96 Microplate luminometer (Promega). Then, the percentage of neutralization for each dilution was calculated as previously described. All statistical analyses were performed using GraphPad Prism version 8.0.1 [12].

Quantification and statistical analysis

For the ELISA assay, the background value was established at OD 0.100, and area under the curve (AUC) was calculated from serum dilutions. To obtain a correlation between AUC and antibody titers, we used estimated values of antibody titers from 212 samples, which were determined by twofold serial dilutions ranging from 1/200–1/102,400, and we established a curve according to Padé's approximation (with $R^2 = 0.9636$). Normal distribution of data was assessed using Shapiro-Wilk test. Differences between clinical groups were calculated using a one-way ANOVA with Freedman or Kruskal-Wallis test followed with Dunn's multiple comparisons test. Differences between the two groups were calculated using the unpaired two-tailed *t*-test or Mann-Whitney test. Simple linear regression was performed, and correlations were analyzed by calculating nonparametric Spearman's correlation. Statistical analyses were performed using GraphPad Prism 9.1.0, and statistical significance was represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

Results

The course of the humoral response to the CoronaVac vaccine

Figure 1A shows the course of the COVID-19 pandemic in Chile from March 2020 to December 2021 (data obtained from the Chilean Ministry of Health/Department of Statistics and Health; MINSAL/DEIS [4], which illustrates three waves of COVID-19. The first wave began on April 20, 2020, and ended in August 2020. The second wave, mainly caused by gamma and lambda variants, started on November 1, 2020, and ended in September 2021, and it was more extensive in terms of accumulated cases (1,360,987), cases entering into intensive care units (ICU) (33,026), and deaths (27,237) (MINSAL/DEIS) [4].

The drop in active cases began mid-June 2021 and coincided with the drop in cases throughout South America (MINSAL/DEIS, our world in data). Finally, the delta variant entered the country on June 24, 2021, and became predominant as of October of this year causing the third wave. However, this variant had a minor impact in Chile in terms of accumulated cases (180,286), ICU cases (2858), and deaths (2245) (MINSAL/DEIS). Additionally, in the last days of November, the entry of the first case of the omicron variant was reported.

This study is composed of four groups of individuals that were analyzed across entire the vaccination and booster programs in 2021, starting February 2021 and ending in January 2022 (depicted as timelines in Fig. 1A). The details of the individuals participating in this study for are found in Fig. 1B.

Serological analysis of CoronaVac before immunization and post-first and post-second dose

To evaluate the effect of the CoronaVac vaccine on antibody titers in individuals potentially exposed to the SARS-CoV-2 virus, we first focused our study on clinical staff from group 1, who treated COVID-19 patients in the first wave of the disease in Chile. We analyzed the serum of these individuals by ELISA to detect IgG antibodies directed against the spike protein of SARS-CoV-2 virus. We considered a weak response in the ELISA test those values from the area under the curve (AUC) ranging between 120 (upper negative limit of the test) and 300 which corresponds to an antibody titer of 1/1000 approximately and that corresponded to a reference value for convalescent plasma treatment [13]. To analyze the SARS-CoV-2 antibody response course in this group, we analyzed the antibody response in three-time points. The first serum sample was obtained 1–3 days before the first dose of the vaccine were referred to as pre-vaccine (Pre-Vax; Fig. 2);

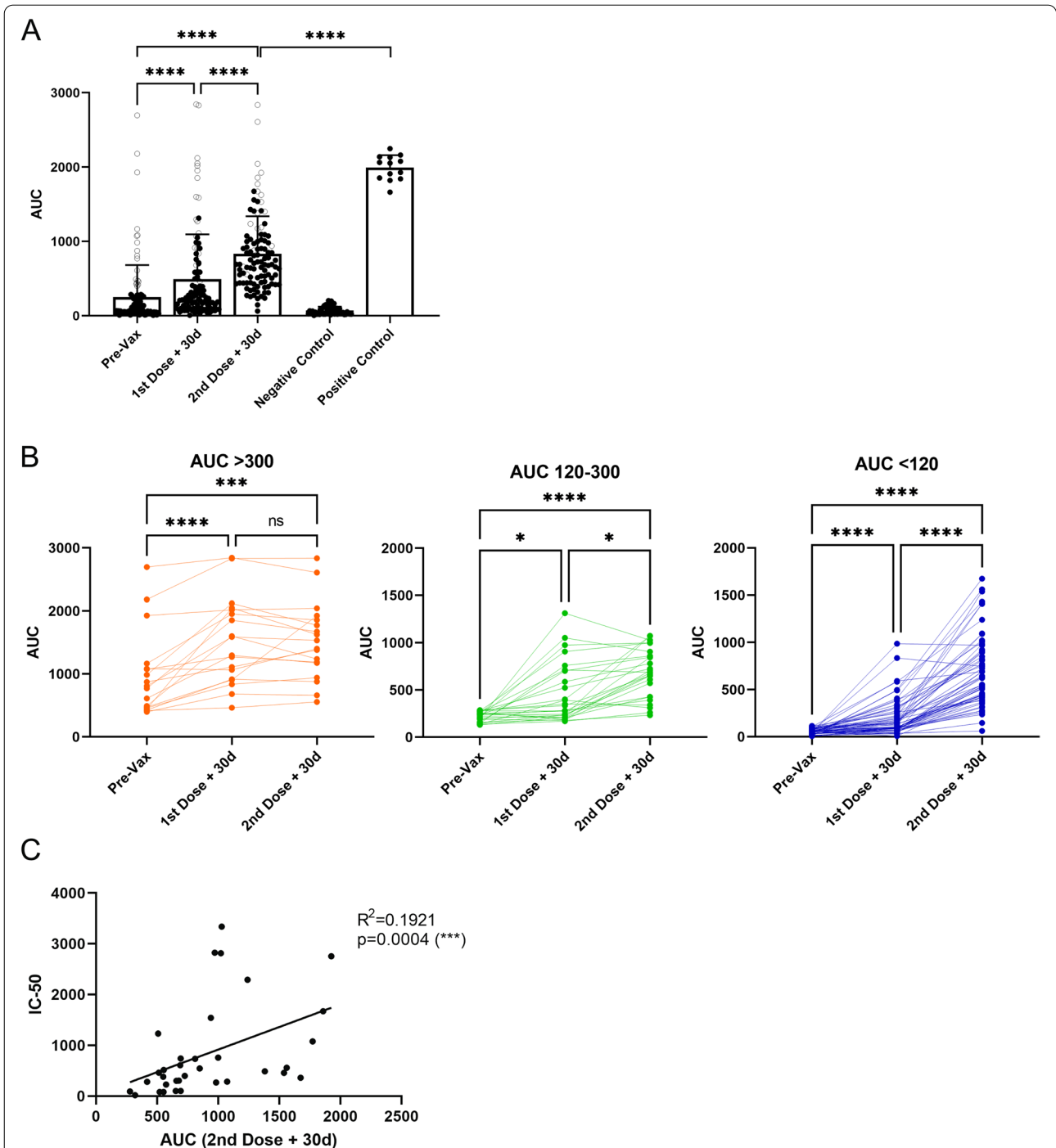


Fig. 2 Seropositivity anti-SARS-CoV-2 spike protein before and after the first and second dose of the vaccine CoronaVac. Health Care services volunteers from study group 1 received a complete CoronaVac vaccination scheme. Serum samples were collected as indicated: Pre-Vax, prior to the first dose, first dose +30d, 30 days after the first dose, second dose +30d, 30 days after the second dose. **A** Data from 104 volunteers are shown before vaccination, 30 days after the first, and 30 days after the second dose. The gray circles show the values of people who may have contracted the disease before vaccination (AUC > 300). Black circles are from the other 86 samples being negative or weakly positive. **B** Data shown in **A** were disaggregated into three groups within AUC values before vaccination: 18 Individuals with an AUC > 300 (positive) are shown in orange, 26 with an AUC 120–300 in green, and 60 individuals AUC < 120 in blue. **C** Serum neutralization capacity in vaccinated participants 30 days after the second dose was correlated with correspondent AUC. Each dot represents a single serum sample. **** $p < .0001$, *** $p < .001$, * $p < .05$

the second sample was obtained 1–3 days before the second immunization (referred to as “First dose +30d,” Fig. 2), and the third sample was collected 1 month after the second dose (referred to as “Second dose +30d,” Fig. 2). Regarding previous SARS-CoV-2 infection, the individuals who participated in this study were laboratory staff and primary clinical caregivers in contact with COVID-19 patients which may have been exposed to SARS-CoV-2 infection. Many individuals in this group reported not knowing whether they had been exposed to SARS-CoV-2 since they could have experienced the asymptomatic disease.

Of a total of 104 people tested, 18 had high antibody titers ($AUC > 300$) before being vaccinated, suggesting that these individuals were infected with SARS-CoV-2 in the first pandemic wave (Fig. 2A, empty circles). Of these 18 individuals, only two did not improve antibody titers with vaccination (Fig. 2B orange lines). For the remaining 16 individuals (orange lines), the first dose of the vaccine led to an increase in anti-SARS-CoV-2 spike IgG production. Interestingly, there were no statistical differences when comparing the level of antibodies induced by the first and the second dose of the vaccine (Fig. 2B, orange circles; values indicated in Additional file 1: Fig. S1).

For individuals who initially had an AUC 120–300 (weak positive response) (Fig. 2B green circles; Additional file 1: Fig. S1), the first dose showed an increase in the level of anti-SARS-CoV-2 spike IgG. Although significant, there was a mild difference between the first and the second doses. Interestingly, the group who initially had an $AUC < 120$ (negative response, Fig. 2B blue circles; Additional file 1: Fig. S1) showed remarkable differences between the first and the second dose of the vaccine. Of the total 104 people, only one person remained unresponsive to the two doses of the vaccine. As such, we conclude that the two-dose vaccination scheme with CoronaVac induces a good antibody response against SARS-CoV-2, which is particularly noticed in individuals who have not been previously exposed to the virus.

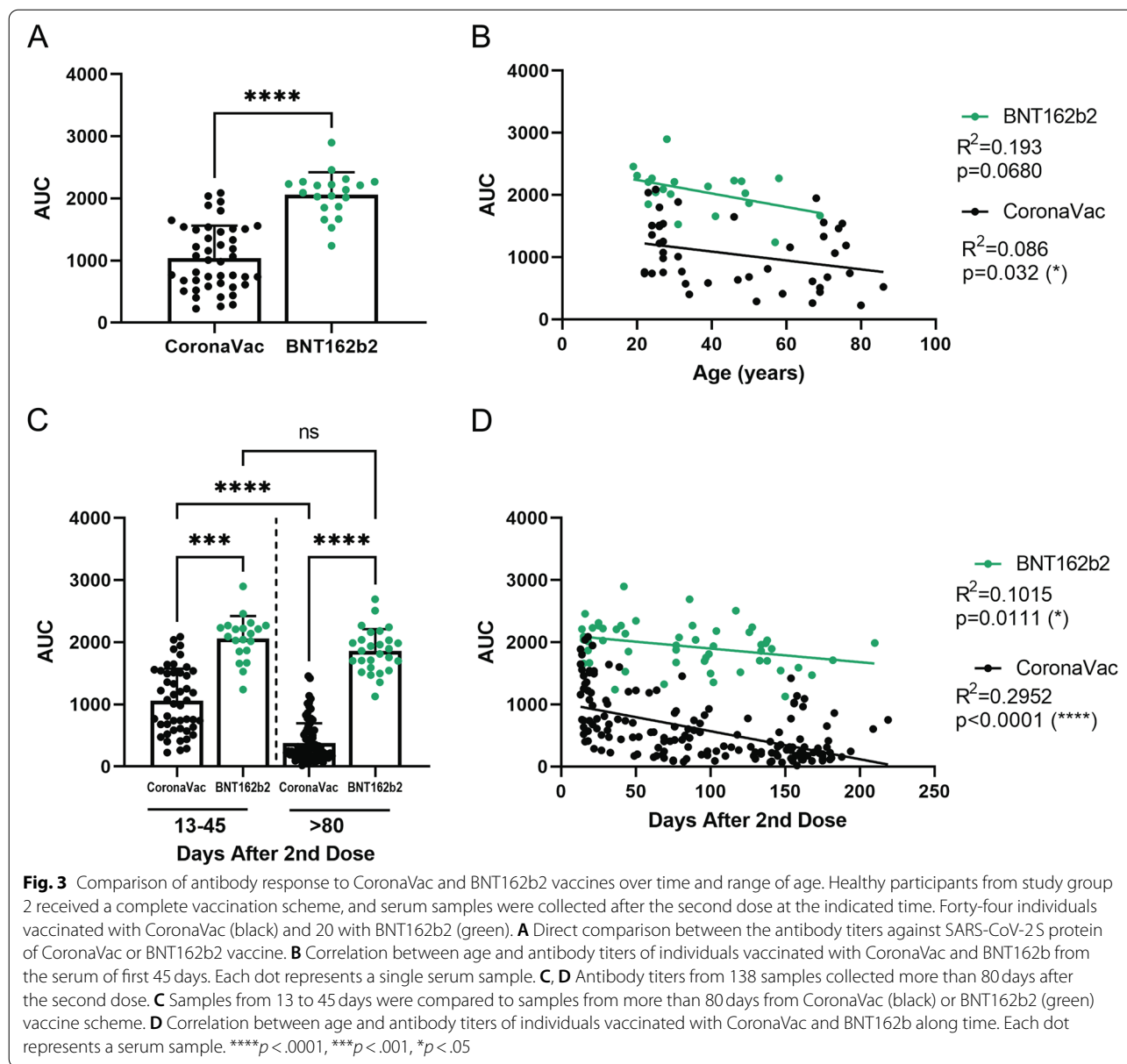
Next, the amount of neutralizing antibodies from 34 samples (5 individuals from group $AUC > 300$; 10 individuals from group $AUC > 120$; 19 individuals from group $AUC < 120$) was determined and results show a significant positive correlation between the AUC values and the IC_{50} of neutralizing antibodies (Fig. 2C). These results demonstrate that the CoronaVac vaccine induces the production of neutralizing antibodies. Furthermore, this data suggests that high titers of total antibodies should represent a greater probability of having neutralizing antibodies against the virus.

Comparison of the humoral immune responses produced by the CoronaVac and BNT162b2 vaccines

The first reports of CoronaVac vaccine immunogenicity were performed in older adults (over 55 years old) [14] since these individuals were among the priority groups for vaccination. In May 2021, individuals under 55 years old began to be vaccinated with BNT162b2 or CoronaVac depending on the availability of the vaccine in Chile. This allowed us to analyze the antibody response 30 to 45 days after the second dose to compare the humoral response elicited by both vaccines. We studied 44 and 20 individuals vaccinated with CoronaVac and BNT162b2 (Pfizer-BioNTech) vaccine, respectively (group 2). Figure 3A shows a comparison of the data from both vaccines in individuals ranging from 18 to 87 years old (median age 36.5 years; IQR: 27–61 years). We observed that the BNT162b2 vaccine induces significantly higher antibody production than the CoronaVac vaccine (2060 ± 361 for BNT162b2 and 1041 ± 520 for CoronaVac). Given that people vaccinated with CoronaVac were mainly older than 55 years in Chile and those vaccinated with BNT162b2 were people between 18 and 54 years old, we compared and plotted antibody production according to the age of the individuals and the type of vaccine they received. Figure 3B shows a significant negative correlation ($p = 0.032$, black circles) for antibody production with increasing age for the CoronaVac vaccine. In contrast, a similar (but not statistically significant, green circles) trend is shown for the BNT162b2 vaccine. These results show that the BNT162b2 vaccine induces twice the amount of IgG against SARS-CoV-2 spike protein compared to CoronaVac, independent of the age of the individuals.

Overtime evolution of the humoral response to CoronaVac and BNT162b2 vaccines

We sought to determine how antibody levels vary with these two vaccines over time. For this purpose, we analyzed samples taken 15 to 200 days after the second dose of CoronaVac or BNT162b2 vaccines. One hundred and fifty-nine samples from individuals vaccinated with CoronaVac and 53 samples from individuals vaccinated with BNT162b2 were analyzed. Figure 3D shows a significant negative correlation for each of these vaccines (CoronaVac $p < 0.0001$; BNT162b2 $p = 0.0111$). The curve slope allows us to infer that around 200 days after the second dose of the CoronaVac vaccine, most individuals vaccinated will present low antibody titers against the SARS-CoV-2 spike protein. In contrast, in individuals vaccinated with the BNT162b2 vaccine, antibodies slightly decrease in most individuals, agreeing with a 6-month longitudinal study reported by Naaber et al.,



which shows that antibodies decline 3–6 months after the second dose [5].

We then disaggregated the data to visualize the results. Comparing the data obtained 13 to 45 days or beyond 80 days after the second dose from both vaccines, we observed a significant loss of antibodies beyond 80 days after the second dose of the CoronaVac vaccine (1057 ± 519 vs. 378 ± 318) compared to the BNT162b2 vaccine (2060 ± 361 vs. 1861 ± 351) (Fig. 3C). These data suggest that the BNT162b2 vaccine is more efficient than the CoronaVac vaccine in inducing and maintaining the production of antibodies against the SARS-CoV-2 virus spike protein.

Analysis of the antibody response of individuals receiving homologous or heterologous booster dose schemes

A total of 44 individuals who were vaccinated with two doses of CoronaVac received, around 180 days after the second dose, a booster dose with the ChAdOx1 vaccine (19 individuals), BNT162b2 vaccine (19 individuals), or CoronaVac vaccine (32 individuals) (timeline scheme depicted in Fig. 4A). Data illustrated in Fig. 4B show that regardless of the type of vaccine used for the booster dose, all individuals significantly enhanced IgG production against the SARS-CoV-2 spike Protein. Values range from 268 ± 218 before the boost to 2245 ± 581 considering any booster, meaning an 7.9-fold change average.

However, when we separated the data based on the type of booster vaccine, we observed that the CoronaVac booster vaccine-induced antibody production, which was noticeable but considerably milder (fold induction: 6.0x) than the antibody production induced by the ChAdOx1 vaccine booster (fold induction: 12.4x) or the BNT162b2 vaccine booster (fold induction: 11.2x). These results demonstrate that the CoronaVac vaccine combined with a booster from CoronaVac or BNT162b2 or ChAdOx1 vaccines enable memory immune responses to be activated, in agreement with recent data from Zeng et al. [15]. These authors showed that a booster with CoronaVac vaccine eight months after the second dose increased neutralizing antibodies against the original virus SARS-CoV-2. However, it is noteworthy to mention that the antibody response induced by the third dose of the CoronaVac vaccine is lower than the two other boosters.

To obtain insights on the extension of the antibody response induced by the homologous and heterologous booster regimes, we measured anti-SARS-CoV-2 spike antibodies in 78 individuals 100 days after the booster dose (median 122 days; IQR: 108–133 days) (Fig. 4C). This analysis revealed that the antibody response elicited by homologous booster with CoronaVac and heterologous boosters with BNT162b2 and ChAdOx1 did not show noticeable signs of immunological waning during the period (Fig. 4C). However, when we compare the level of antibodies of the boosters after 100 days, we see that the homologous booster with CoronaVac produces significantly fewer antibodies than the boosters with BNT162b2 and ChAdOx1. Overall, our results suggest that a heterologous booster scheme using CoronaVac as the basal vaccine with a booster from ChAdOx1 (AstraZeneca) vaccine or BNT162b2 (Pfizer) vaccine re-activates immune memory and elicits a potent and persistent immune response at least over a 3-month period.

Discussion

This work reports the dynamics of anti-spike IgG after SARS-CoV2 vaccination with CoronaVac, a vaccine used globally [16], a comparison with an mRNA vaccine over time, and an assessment of homologous and heterologous booster schemes in Chile using CoronaVac as the basal vaccine. The groups analyzed in this study span the entire vaccination program in Chile, from the beginning

of the vaccination schedule with priority groups, to the implementation of booster schemes in late 2021.

Our data indicate that in individuals not exposed to SARS-CoV-2, a two-dose vaccination scheme with CoronaVac induces a noticeable antibody response against SARS-CoV-2, in agreement with additional reports [15]. Many participants in group 1 with AUC 120–300 (weak response) reached the peak of the humoral response after the first vaccine dose. We speculate/hypothesize that these participants had a previous asymptomatic infection. It is possible that individuals with weak anti-SARS-CoV-2 spike IgG antibodies before vaccination might have immunity to other coronaviruses or produce a weak response to SARS-CoV-2. This group has a quick response to the first dose of the vaccine compared to individuals with no sign of previous response. However, the overall response showed only mild differences in antibody production between the first and the second dose (see Suppl. Fig. 1). Furthermore, there is a positive correlation between the production of neutralizing antibodies and those detected by ELISA (AUC).

When comparing CoronaVac and BNT162b2 vaccines, we found that the BNT162b2 vaccine is more efficient in inducing and maintaining the production of antibodies against the SARS-CoV-2 virus spike protein independent of the age of the individuals. Nevertheless, it is important to note that the number of individuals aged >60 years is underrepresented in the BNT162b2 vaccine group due to the vaccination strategy implemented in Chile. Moreover, we evaluated three different booster schemes in people previously vaccinated with CoronaVac. We found that a homologous booster with CoronaVac or heterologous boosters with ChAdOx1 (AstraZeneca) vaccine or BNT162b2 (Pfizer) vaccine can elicit a humoral immune response against the ancestral strain of the virus. However, our data strongly indicates that heterologous booster regimes greatly potentiate antibody responses compared to a homologous regime. As such, our findings may have relevant implications for the large number of countries currently administering a two-dose scheme of CoronaVac.

Concerning the booster schemes, administration of a homologous booster scheme of CoronaVac has been demonstrated to be immunogenic and safe in a double-blind, randomized, placebo-controlled phase-2 clinical trial [15]. In this context, the homologous and

(See figure on next page.)

Fig. 4 Antibody titers of homologous and heterologous boosters of individuals previously vaccinated with two doses of CoronaVac. Participants from groups 3 and 4 received a complete CoronaVac vaccination scheme and booster after 6 to 8 months with BNT162b2, ChAdOx1, or CoronaVac vaccine. **A** Schema of participant's immunizations. **B** Antibody titer comparison before and 30 days post booster immunization from BNT162b2 (19 individuals), ChAdOx1 (19 individuals), and CoronaVac (32 individuals) from study group 3. **C** Antibody titer comparison 100 days post booster immunization (median 122 days; IQR: 108–133 days) from BNT162b2 (27 individuals), ChAdOx1 (41 individuals), and CoronaVac (29 individuals) from study group 4. Each dot represents a serum sample. **** $p < .0001$, *** $p < .001$, ** $p < .01$

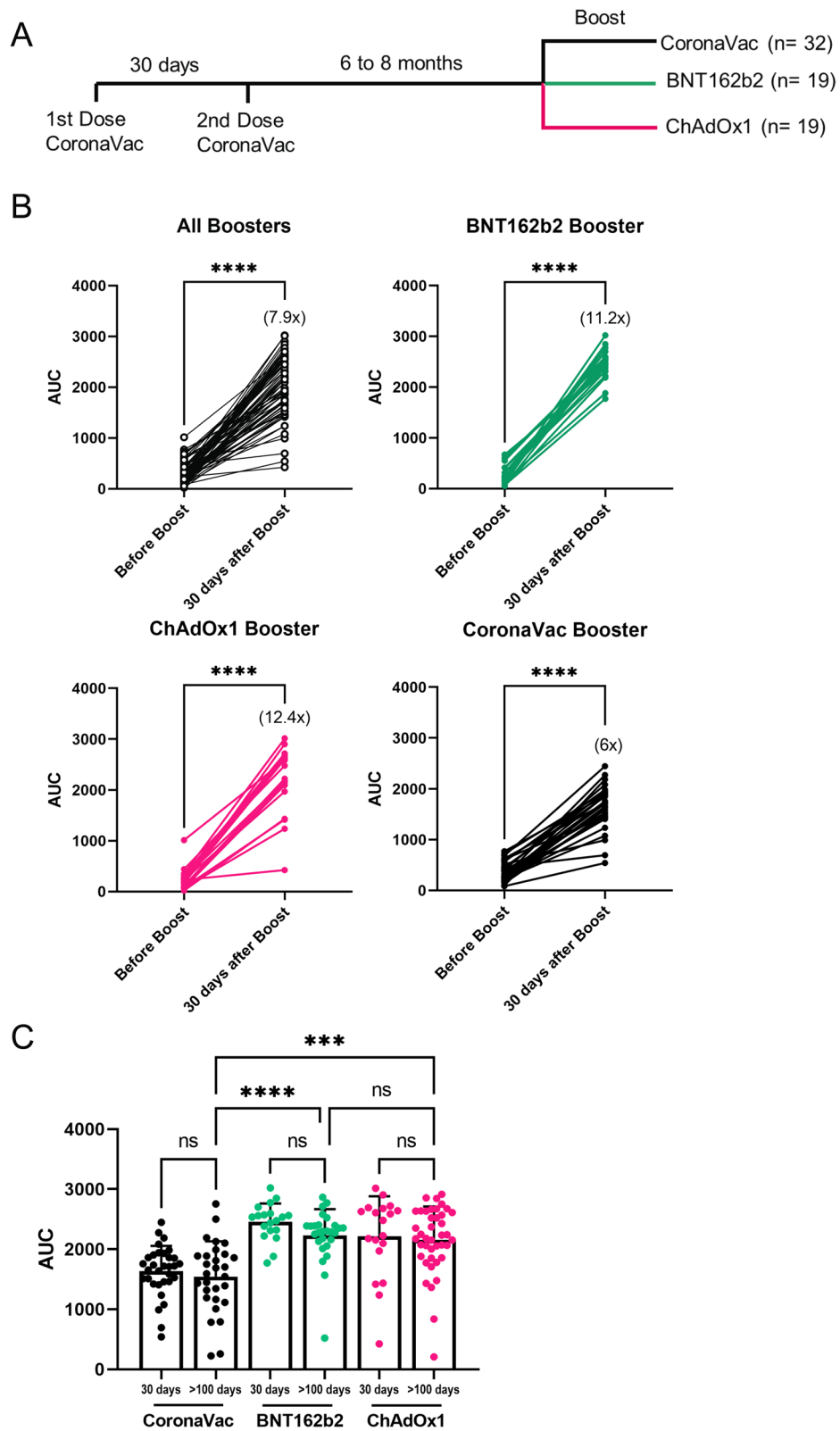


Fig. 4 (See legend on previous page.)

heterologous booster schemes analyzed in this work re-activated anti-spike IgG production in individuals previously vaccinated with the two-dose scheme of CoronaVac. However, it is plausible that the heterologous regime also elicits a combination of primary and tertiary immune responses. Analysis over a more extended period of time (more than 100 days) revealed that heterologous booster schemes are capable of inducing an elevated and long-lasting antibody response compared to two-doses plus a booster of CoronaVac. Thus, these data suggest that the use of heterologous instead of homologous booster regimes may allow to space the subsequent booster doses to achieve long-lasting humoral response and protection against COVID-19. These findings also provide evidence that will allow to prioritize the subsequent booster doses in individuals that have lost optimal anti-SARS-CoV-2 antibodies, such as those with the homologous regime.

It remains to be observed if these heterologous regimes potentiate an immune response that could provide protection (or partial protection) against novel variants. In this context, many questions remain to be addressed. For instance, although we provide data of over 3 months after the booster, it is unclear how long the protection mediated these booster schemes will last or if these strategies will efficiently protect against novel variants such as delta and the recently described omicron [17]. In this regard, a very recent study of a heterologous booster scheme based on CoronaVac + BNT162b2 in the Dominican Republic showed a reduced antibody response towards the Omicron variant [18]. One distinction between that study and the data presented here relates to the timing between the second dose and the booster, which in Chile was implemented after a 6-month interval, whereas in the Dominican Republic study, the heterologous booster scheme was implemented after 4 weeks [18]. As such, the immune response elicited under two different time schemes may differ in terms of the magnitude of antibody production. Thus, future work combined with clinical studies are required to determine the optimal time between vaccine and booster administration. Along these lines, the study of Zeng et al demonstrates that extending the interval of eight months between the second and the homologous booster dose with CoronaVac greatly increases antibody production [15]. Whether this process results in a more optimal generation of antigen-specific, long-lasting plasma cells and T lymphocytes remains to be determined. Interestingly, our study also reports potent responses with the heterologous booster scheme with the ChAdOx1 vaccine, requiring further assessment. In addition, our work is also in line with a very recent report showing

that heterologous booster regimes are superior to homologous booster schemes based on the CoronaVac vaccine in a Brazil study [19].

One limitation of our study is that we assessed antibody production against the spike protein of SARS-CoV-2 but a relevant response mediating long-lasting immunity could also be carried out by T cells, which are not analyzed in this work. However, a recent study with 15 volunteers with no suspected history of COVID-19, vaccinated with two doses of CoronaVac showed humoral and cellular immune response 28 days after the second dose [20].

As such, it is possible that a heterologous booster scheme based on CoronaVac as the basal vaccine could lead to potent immunity, based on the diversity of viral antigens provided by an inactivated virus formulation, followed by a booster with mRNA or adenoviral vector vaccines, which trigger a superior degree of immunogenicity. The long-term immunological effects related to protection against SARS-CoV-2 variants of concerns and variants of interests induced by heterologous booster strategies should be determined with high priority in order to shed light on the future management of the pandemic across the globe.

Conclusions

Our data indicate that whereas two doses of CoronaVac induces lower antibody titers against the SARS-CoV-2 ancestral strain than the BNT162b2 vaccine, this response can be greatly potentiated with a heterologous booster scheme with BNT162b2 or ChAdOx1 vaccines. Furthermore, the heterologous booster regimes induce a durable antibody response that does not show signs of decay three months after the booster dose. As such, these data suggest that a heterologous booster strategy using CoronaVac as a basal vaccine could be considered as an alternative to potentially curb the pandemic for the large number of countries currently administering the vaccine or even in countries that remain with limited access to vaccination.

Abbreviations

AUC: Area under the curve; HRP: Horseradish peroxidase; IC50: Half maximal inhibitory concentration; ICU: Intensive care unit; IgG: Immunoglobulin G; IQR: Interquartile range; PCR: Polymerase chain reaction; ELISA: Enzyme-linked immunosorbent assay; PBS: Phosphate-buffered saline; TMB: 3,3',5,5'-Tetramethylbenzidine; TPBS: Tween 20 phosphate-buffered saline.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12916-022-02406-0>.

Additional file 1: Fig. S1. Values of anti-SARS-CoV-2 spike IgG antibodies before immunization, and post-first and -second dose.

Acknowledgements

We thank all the volunteers who collaborated in this work, particularly the Clinical Laboratory of the La Florida Hospital staff, the staff who work at the Juan Gomez Millas Campus, and many other volunteer's collaborating with the study. Thanks to the Biotechnology Engineering students who attended the 2021 Immunology course for their interest in participating in a real-world experiment.

Authors' contributions

Conceptualization: MRB/FO/ML/LC; data curation: MRB, LV, VS; formal analysis: LV/NV/MLA; funding acquisition: MRB/FO/ML/RSR/FV/MR; investigation: MRB/FO/ML; methodology: LV/NV/MLA/JA; project administration: MRB/LV/VS/FT; resources: MRB/FO/ML/MR/LC/CB/LuV/XC/FT; supervision: MRB; validation: LV/NV/MRB/FO/RSR; visualization: NV/DS/RG; writing—original draft preparation: MRB/FO/DS; writing—review and editing: MRB/FO/ML/DS/MR/LV/VS/NV/FT/MLA/JA/RS/FV/RG/RSR/LuV/CB/LCD/XCB. All authors read and approved the final manuscript.

Funding

This work was supported by a grant from the COVID-19 research program of the National Agency for Research and Development (ANID), grant No 0752 (MRB, FO, ML). MRB, DS, and MR are supported by ANID/BASAL/FB210008, and FONDECYT grants No 1191438 (MRB) and 1180385 (DS). FO is supported by an International Research Scholar grant from HHMI (HHMI#55008744), a FONDECYT grant No. 1200793, and ECOS-CONICYT grant (ECOS180052). CB is supported by a FONDECYT grant No 1181699. RSR is supported by a FONDECYT grant No 1190156. FVE is supported by a FONDECYT grant No 1211547. RG is supported by FONDECYT grants No 11180557 and CEDENNA AFB180001. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Protocol ID Number 1151/20, Protocol ID Number 074-2020 and Protocol ID 2123-FCS-UCH from Hospital Clínico Universidad de Chile and Facultad de Ciencias, Universidad de Chile. All patients and healthy controls were required to understand the study and sign an informed consent.

Consent for publication

Informed consent was collected from all subjects to publish anonymized data.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Laboratory of Immunology, Biology Department, Faculty of Sciences, Universidad de Chile, Las Palmeras 3425, 7800003 Santiago, Chile. ²Centro Ciencia & Vida, Av. Zañartu 1482, Santiago, Chile. ³Laboratory of Cancer Immunoeediting, Immunology Program, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Av. Independencia 1027, 8380453 Santiago, Chile. ⁴Immunogastroenterology Lab., Gastroenterology Unit, Hospital Clínico Universidad de Chile, Faculty of Medicine, Universidad de Chile, Santiago, Chile. ⁵Hospital Clínico Metropolitano La Florida "Dra. Eloisa Diaz I.", Santiago, Región Metropolitana, Chile. ⁶Laboratory of Molecular and Cellular Virology, Virology Program, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Santiago, Chile. ⁷Faculty of Medicine, Universidad del Desarrollo, Santiago, Chile. ⁸Centro de Nanotecnología Aplicada, Universidad Mayor, Santiago, Chile. ⁹Center for the Development of Nanoscience and Nanotechnology, CEDENNA, Santiago, Chile. ¹⁰Faculty of Medicine and Sciences, Universidad San Sebastian, Santiago, Chile. ¹¹Laboratory of Immunology and Cellular Stress, Immunology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Av. Independencia 1027, 8380453 Santiago, Chile.

Received: 24 January 2022 Accepted: 16 May 2022

Published online: 09 June 2022

References

- World Health Organization (WHO). 10 vaccines granted emergency use listing by WHO. Available from: <https://covid19.trackvaccines.org/agency/who/>.
- COVID19 Vaccine Tracker. Sinovac: CoronaVac. Available from: <https://covid19.trackvaccines.org/vaccines/7/>.
- Jara A, Undurraga EA, Gonzalez C, Paredes F, Fontecilla T, Jara G, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med*. 2021;385(10):875–84.
- Ministry of Health of Chile. Department of Statistics and Health Information. Departamento de estadísticas e información de salud. Available from: <https://deis.minsal.cl>.
- Naaber P, Tserel L, Kangro K, Sepp E, Jurjenson V, Adamson A, et al. Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. *Lancet Reg Health Eur*. 2021;10:100208.
- Bar-On YM, Goldberg Y, Mandel M, Bodenheimer O, Freedman L, Alroy-Preis S, et al. Protection against COVID-19 by BNT162b2 booster across age groups. *N Engl J Med*. 2021;385(26):2421–30.
- Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis*. 2021;21(2):181–92.
- Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med*. 2020;383(27):2603–15.
- Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet*. 2020;396(10249):467–78.
- García-González P, Tempio F, Fuentes C, Merino C, Vargas L, Simon V, et al. Dysregulated immune responses in COVID-19 patients correlating with disease severity and invasive oxygen requirements. *Front Immunol*. 2021;12:769059.
- Amanat F, Stadlbauer D, Strohmeyer S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med*. 2020;26(7):1033–6.
- Beltran-Pavez C, Riquelme-Barrios S, Oyarzun-Arrau A, Gaete-Argel A, Gonzalez-Stegmaier R, Cereceda-Solis K, et al. Insights into neutralizing antibody responses in individuals exposed to SARS-CoV-2 in Chile. *Sci Adv*. 2021;7(7):eabe6855.
- Klein SL, Pekosz A, Park HS, Ursin RL, Shapiro JR, Benner SE, et al. Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. *J Clin Invest*. 2020;130(11):6141–50.
- Wu Z, Hu Y, Xu M, Chen Z, Yang W, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis*. 2021;21(6):803–12.
- Zeng G, Wu Q, Pan H, Li M, Yang J, Wang L, et al. Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. *Lancet Infect Dis*. 2022;22(4):483–95.
- Mallapaty S. China's COVID vaccines have been crucial - now immunity is waning. *Nature*. 2021;598(7881):398–9.
- Pulliam JR, van Schalkwyk C, Govender N, von Gottberg A, Cohen C, Groome MJ, et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. *medRxiv*. 2021. <https://doi.org/10.1126/science.abn4947>.
- Perez-Then E, Lucas C, Silva Monteiro V, Miric M, Brache V, Cochon L, et al. Immunogenicity of heterologous BNT162bb2 booster in fully vaccinated individuals with CoronaVac against SARS-CoV-2 variants Delta and Omicron: the Dominican Republic experience; 2021.
- Costa Clemens SA, Weckx LY, Clemens R, Almeida Mendes AV, Ramos Souza A, Silveira MV, et al. Randomized Immunogenicity and Safety Study of Heterologous Versus Homologous COVID-19 Booster Vaccination in

Previous Recipients of Two Doses Of Coronavac COVID-19 Vaccine; 2021. <https://doi.org/10.2139/ssrn.3989848>. Available at SSRN: <https://ssrn.com/abstract=3989848>

20. Escobar A, Reyes-López FE, Acevedo ML, Alonso-Palomares L, Valiente Echeverría F, Soto-Rifo R, et al. Evaluation of the immune response induced by CoronaVac 28-day schedule vaccination in a healthy population group. *Front Immunol.* 2022;12(766278):1–8.

Publisher's Note

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

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

