CORONAVIRUS

Age-dependent impairment in antibody responses elicited by a homologous CoronaVac booster dose

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The emergence of the SARS-CoV-2 Omicron sublineages resulted in increased transmission rates and reduced protection from vaccines. To counteract these effects, multiple booster strategies were used in different countries, although data comparing their efficiency in improving protective immunity remain sparse, especially among vulnerable populations, including older adults. The inactivated CoronaVac vaccine was among the most widely distributed vaccine worldwide and was essential in the early control of SARS-CoV-2–related hospitalizations and deaths. However, it is not well understood whether homologous versus heterologous booster doses in those fully vaccinated with CoronaVac induce distinct humoral responses or whether these responses vary across age groups. We analyzed plasma antibody responses from CoronaVac-vaccinated younger or older individuals who received a homologous CoronaVac or heterologous BNT162b2 or ChAdOx1 booster vaccine. All three evaluated boosters resulted in increased virus-specific IgG titers 28 days after the booster dose. However, we found that both IgG titers against SARS-CoV-2 Spike or RBD and neutralization titers against Omicron sublineages were substantially reduced in participants who received homologous CoronaVac compared with the heterologous BNT162b2 or ChAdOx1 booster. This effect was specifically prominent in recipients >50 years of age. In this group, the CoronaVac booster induced low virus-specific IgG titers and failed to elevate neutralization titers against any Omicron sublineage. Our results point to the notable inefficiency of CoronaVac immunization and boosting in mounting protective antiviral humoral immunity, particularly among older adults, during the Omicron wave. These observations also point to benefits of heterologous regimens in high-risk populations fully vaccinated with CoronaVac.

INTRODUCTION

The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant was a key turning point in the coronavirus disease 2019 (COVID-19) pandemic. Within 2 months of its first report, the BA.1 sublineage became the dominant variant worldwide, followed by the appearance of several additional sublineages. Omicron BA.1 was first displaced by the BA.2 sublineage, subsequently followed by its descendants BA.2.12.1, BA.4, and BA.5 (*1*–*3*). Although specific amino acid changes are shared between all Omicron sublineages, a substantial number of mutations between them were reported in the Spike receptor-binding domain (RBD) region. As a result, these antigenic differences in Omicron sublineages markedly reduced their susceptibility to vaccine-induced neutralizing antibodies, increasing the need for additional vaccination boosters, especially among vulnerable groups (*2*–*6*). Current COVID-19 vaccines, including Moderna mRNA1273, Pfizer/BioNTech BNT162b1, ChAdOx1 nCoV-19, and CoronaVac, are highly effective against hospitalization and death caused by SARS-CoV-2, despite their different formulations (*7*). Whereas the Pfizer/BioNTech BNT162b1 vaccine is based on mRNA encoding the complete stabilized S protein encapsulated within lipid nanoparticles (*8*), the ChAdOx1 nCoV-19 vaccine

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consists of a replication-deficient chimpanzee adenoviral vector encoding the SARS-CoV-2 Spike protein (ChAdOx1-S) (*9*). The CoronaVac is a whole-virus β-propiolactone–inactivated vaccine with aluminum hydroxide adjuvant currently authorized for use in 48 countries (*10*).

Brazil is the second-ranked country in the world in terms of the absolute number of COVID-19–related deaths, with almost 700,000 fatalities to date. Similar to many other countries, Brazil administered CoronaVac widely, which helped control the number of SARS-CoV-2–related hospitalizations and deaths, especially at the beginning of the pandemic (*11*, *12*). However, as SARS-CoV-2 Omicron sublineages emerged with increased transmissibility and extensive escape from previously established immunity, several concerns were raised regarding reduced vaccine effectiveness, particularly for vulnerable populations with suboptimal immunity. Thus, all three vaccines, Pfizer-BioNTech, ChAdOx1 nCoV-19, and CoronaVac, have been administered as booster doses to address both waning immunity and reduced effectiveness against SARS-CoV-2 variants in Brazil and elsewhere (*9*, *12*–*14*). Similar effectiveness of the inactivated vaccine booster and the mRNA vaccine BNT162b2 (Pfizer-BioNTech) booster against COVID-19–related hospitalizations was observed during previous outbreaks with the Delta variant (*15*, *16*). However, given that CoronaVac remains among the top distributed vaccines, studies comparing humoral immune responses from patients primed with CoronaVac followed by boosters with distinct vaccine formulations against Omicron sublineages are largely missing. In addition, studies comparing homologous and heterologous boosters in high-risk, elderly populations are limited (*17*, *18*).

RESULTS

Characterization of the study cohort

To evaluate virus-specific immune responses after different booster vaccines across age groups and to assess the potential risk of vaccine immune evasion by Omicron infection, we assembled a cohort of CoronaVac-vaccinated individuals who received a homologous CoronaVac or a heterologous (BNT162b2 or ChAdOx1) booster vaccine. We investigated antibody titers and vaccine-induced neutralizing responses against the ancestral strain, USA-WA1/2020, B.1.617.2 (Delta variant), and BA.1 (Omicron variant) as well as the Omicron sublineages, BA.2.12.1, XAF (BA.1/BA.2 circulating recombinant), and BA.5, and compared them across age groups. We studied 293 nonhospitalized adult participants who received two doses of the CoronaVac vaccine between 27 November 2021 and 3 February 2022, before and after a BNT162b2, ChAdOx1, or CoronaVac booster dose. Plasma samples were collected before booster administration and 28 days after booster (third dose) administration (Fig. 1A). Immunogenicity endpoints included enzyme-linked immunosorbent assays (ELISAs) and neutralization assays using the authentic virus. Blood samples were collected at the Serviço de Tratamento ao Câncer de Ribeirão Preto, São Paulo, Brazil. Data from a previously analyzed cohort, composed of participants from the Dominican Republic who received two doses of CoronaVac followed by the BNT162b2 booster, were used as a reference (*14*). Study groups were stratified by age (younger adults <50 years and older adults ≥50 years), biological sex, and booster vaccine type. The mean age of the participants was 39.3 ± 15.9 years, and the majority of participants were female (~61%). Participants did not differ with respect to their previous SARS-CoV-2 infection status (i.e., previously infected versus previously uninfected). Basic demographic information, vaccination type, and previous infection status are summarized in table S1.

Defective age-associated SARS-CoV-2 antibody response after a CoronaVac homologous, but not a heterologous, vaccination-booster regimen

Plasma antibody reactivity to full-length ancestral Spike protein (S) and RBD of SARS-CoV-2 S protein was measured at baseline and 28 days after COVID vaccine booster administration (Fig. 1A). Concentrations of immunoglobulin G (IgG) RBD-specific binding antibodies were similar at the baseline for the Brazilian and Dominican Republic cohorts, although a slight, but significant, reduction in virus-specific IgG concentrations against S protein was observed for the Dominican Republic participants (fig. S1A). At 28 days after booster, we detected a significant increase in the virus-specific IgG titers for all groups (BNT162b2, 10.2×, $P < 0.0001$; ChAdOx1, 7.38×, $P < 0.0001$; CoronaVac, 4.29×, $P < 0.0001$). The heterologous booster resulted in an 8.7-fold increase compared with a 4.2-fold increase after a homologous booster regimen (Fig. 1B). However, individuals who received the homologous regimen, CoronaVac prime followed by CoronaVac booster, mounted lower anti-S and anti-RBD titers compared with individuals who received a heterologous regimen (Fig. 1B and fig. S1B). No difference was observed among participants who received CoronaVac prime followed by the BNT162b2 or ChAdOx1-S booster regimen (fig. S1B). Consistently, an unsupervised heatmap assembled using virus-specific antibody titers and the main cohort demographics revealed marked changes in CoronaVac/CoronaVac recipients, especially among older participants compared with young adults (Fig. 1C). Five main clusters of vaccinated participants emerged, and the distribution of participants matched the vaccination booster received and correlated with age groups. Cluster 3 primarily comprised younger participants who received the CoronaVac/ChAdOx1 regimen with the highest virus-specific IgG titers. Clusters 1 and 2 consisted of mainly both younger and older adults who received the CoronaVac/ChAdOx1 or CoronaVac/BNT162b2 regimen with moderate titers of the anti-S and anti-RBD post-booster shot. Clusters 4 and 5 comprised participants with the lowest titers of virus-specific IgG antibodies. The majority of CoronaVac/CoronaVac recipients fell into these clusters, including 77% of the participants ≥50 years old (Fig. 1C).

Concentrations of anti-S and anti-RBD IgG declined with age for participants who received the homologous regimen in contrast to participants who received a heterologous booster regimen; the latter did not present an inverse correlation between age and antibody levels after booster (Fig. 1D). No differences were observed in antibody levels between vaccinated participants of different sexes after stratification by age (fig. S1C).

Further analysis across age groups revealed that the levels of anti-S and anti-RBD IgGs were lower in younger adults who received CoronaVac compared with younger groups that received BNT162b2 or ChAdOx1-S booster. Moreover, IgG levels were substantially lower in participants aged 50 or older receiving homologous boosters than in the younger adult groups or older adult participants with a heterologous regimen (Fig. 1E). No differences were observed in antibody levels between vaccinated participants who received BNT162b2 or ChAdOx1-S booster after stratification

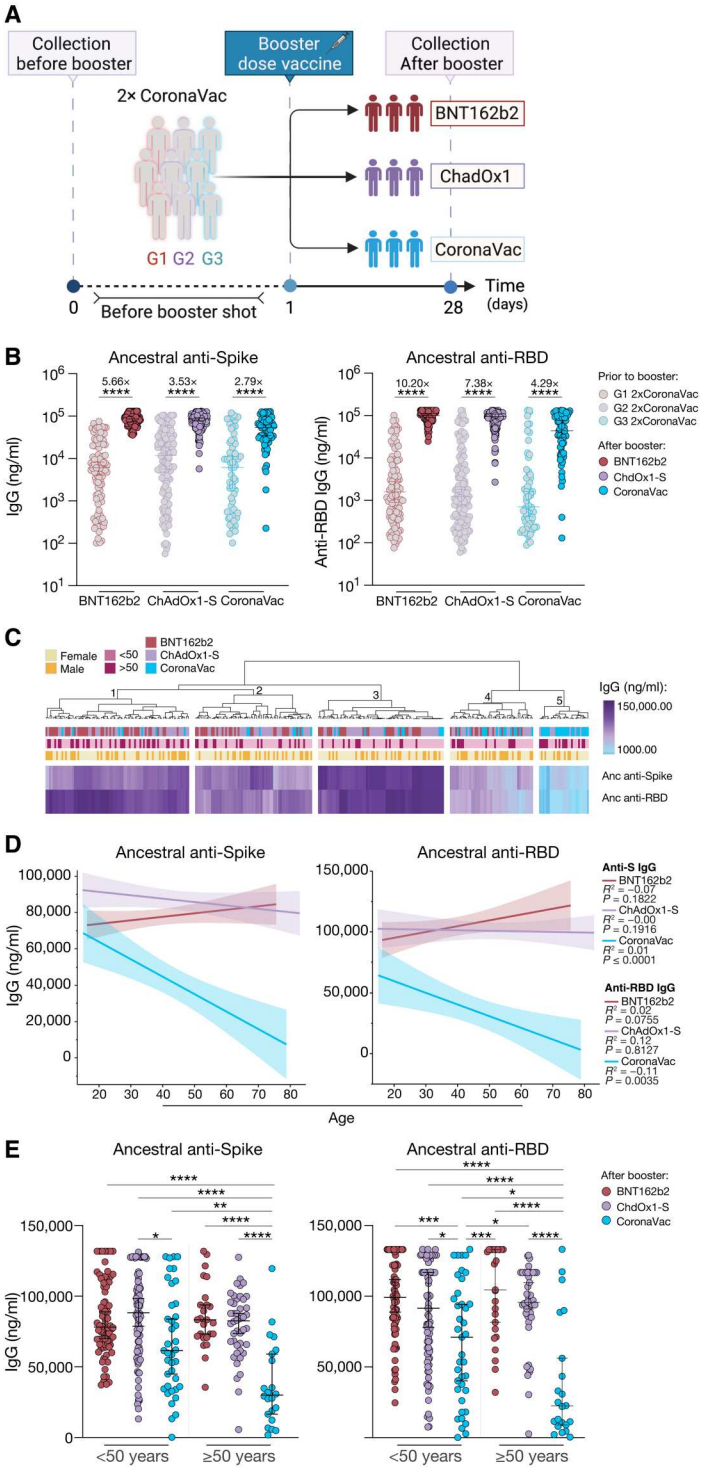

Fig. 1. SARS-CoV-2–specific antibody responses by age after a heterologous or homologous booster regimen in individuals fully vaccinated with CoronaVac. (**A**) Cohort timeline overview indicated by days after the SARS-CoV-2 booster vaccination in the CoronaVac previously vaccinated cohort. Participants received boosters of BNT162b2, ChAdOx1-S, or CoronaVac vaccine, and plasma samples were collected as indicated. Baseline time point 0, before booster vaccination, and 28 days after the booster dose; participants were stratified by booster dose received in three groups (G1, BNT162b2; G2, ChAdOx1-S; G3, CoronaVac). Created with BioRender. (**B**) Plasma reactivity IgG to ancestral S protein and RBD was measured before and after the booster vaccine. S, Spike; RBD, receptor binding domain at baseline and after the booster dose. The median fold change values in antibody titer after booster vaccination are indicated. (**C**) Heatmap comparison of plasma ancestral anti-S and anti-RBD levels within participants CoronaVac-primed followed by BNT162b2, ChAdOx1-S, or CoronaVac. Participants are arranged across rows, and color intensity indicates anti-virus IgG concentration. Analyses were performed 28 days after the booster dose. (**D**) Correlation and linear regression comparisons of virus-specific ancestral anti-S and anti-RBD IgG levels by age after booster vaccination. Regression lines are shown as red (BNT162b2 booster), purple (ChAdOx1-S booster), or blue (CoronaVac booster). Pearson's correlation coefficients and linear regression significance are colored accordingly; shading represents 95% confidence interval. (**E**) Plasma reactivity to ancestral S protein and RBD measured by ELISA at 28 days after booster by age groups. <50 years, vaccinated participants aged 49 or younger; ≥50 years, vaccinated participants aged 50 years or older. Significance was assessed by one-way ANOVA corrected for multiple comparisons using Tukey's method. Before booster dose: G1 (n = 101), G2 (n = 131), and G3 (n = 61). After booster dose: BNT162b2 (n = 101), ChAdOx1-S (n = 131), and CoronaVac (n = 61). Each dot represents a single individual. Horizontal bars represent average ± SD. ****P < 0.0001, ***P < 0.001, **P < 0.01, and *P < 0.05.

by age (Fig. 1E). These observations point to the notable inefficiency in mounting virus-specific antibodies in participants primed with CoronaVac followed by CoronaVac booster, particularly among participants older than 50 years. BNT162b2 and ChAdOx1-S boosters were associated with higher vaccine antibody induction as compared with CoronaVac in both the younger and the ≥50-year-old age group, resulting in a 10.3-/5.8-fold increase (<50 years) and a 9.9-/14.6-fold increase (≥50 years) in anti-RBD IgG titers, compared with the 4.2-fold increase (<50 years) and 7.0-fold increase (≥50 years) for CoronaVac booster recipients (fig. S1D). Together, these data indicate that participants who received a homologous regimen, CoronaVac prime followed by CoronaVac booster, develop lower virus-specific antibody responses, with a remarkable impact in older participants. Of note, these differences appear to be

vaccine specific, rather than resulting from an aging-associated impaired immune response.

Homologous CoronaVac boosters do not improve neutralization responses against Omicron sublineages in older adults

A central premise for current COVID-19 vaccine programs is that neutralizing antibodies correlate with protection against SARS-CoV-2 infection (*19*, *20*). Given our observation that CoronaVac-primed/booster participants aged 50 years or more had lower antibodies compared with other analyzed groups, we hypothesized that this could lead to subprotective neutralizing responses. We next assessed whether different booster strategies also affected neutralizing responses by measuring plasma neutralization activity longitudinally using half-maximal plaque reduction neutralizing assays (PRNT50) against circulating SARS-CoV-2–authentic isolates, including lineage A (ancestral strain, USA-WA1/2020), B.1.617.2 (Delta variant), and BA.1 (Omicron variant), as well as the Omicron sublineages, BA.2.12.1, XAF (BA.1/BA.2-circulating recombinant), and BA.5. Individuals who were fully vaccinated with CoronaVac and who received the BNT162b2 booster displayed the highest increase in neutralization activity (10.8-, 7.0-, 3.5-, 5.4-, 3.8-, and 3.6-fold increase) against ancestral, Delta, BA.1, BA.2.12.1, XAF, and BA.5, respectively, 28 days after the booster shot (Fig. 2A and fig. S2A). The BNT162b2 group was followed by the participants who were fully vaccinated with CoronaVac and who received the ChAdOx1 booster, which showed an increase in neutralization activity (3.4-, 6.5-, 2.2-, 2.4-, 2.7-, and 2.1-fold) against ancestral, Delta, BA.1, BA.2.12.1, XAF, and BA.5, respectively. After the booster shot, CoronaVac/CoronaVac recipients developed neutralizing antibody titers against ancestral strain, Delta, BA.2.12.1, and XAF variants (2.6-, 3.4-, 1.2-, and 1.9-fold increase, respectively). Despite this increase, PRNT50 values were markedly reduced compared with levels induced by the participants who received a heterologous booster. In addition, no statistical differences were observed after booster for CoronaVac/CoronaVac recipients upon assessing neutralizing antibodies titers against BA.1 and BA.5 (Fig. 2A and fig. S2A).

No statistical differences were observed in neutralization titers before the booster shot for the Brazilian cohort (fig. S2B). Stratification by age revealed that, similar to antibody levels, neutralization titers were significantly lower in CoronaVac/CoronaVac recipients of different ages, particularly those age 50 or older, compared with participants who received a heterologous regimen (fig. S2, C and D). Increased PRNT50 values were observed for all participants, independent of age group, against all variants and sublineages tested, who received CoronaVac prime followed by the BNT162b2 or ChAdOx1 booster dose (Fig. 2B). Despite an increase in neutralization levels after booster against the ancestral and Delta variants, older participants who received the homologous regimen did not show increased neutralization titers against BA.1 or additional Omicron sublineages after booster dose (Fig. 2B). With the exception of neutralizing antibodies against the XAF sublineage, younger participants who received the homologous CoronaVac regimen also did not show improved neutralization titers against Omicron sublineages after booster (Fig. 2B). To consider possible interindividual variation, as well as baseline antibody levels, we constructed a random-effects model with random intercept with robust standard errors. This model adjusts for age, sex, days from the second CoronaVac dose, previous infection status (previously infected or not previously infected), and baseline of virus-specific antibodies or PRNT50 levels. These neutralization analyses were consistent with our previous analysis (fig. S3, A and B). To investigate whether the diminished immunogenicity of CoronaVac is a global effect or driven by fewer nonresponder individuals who did not respond appropriately to the vaccine, we extended our analysis by stratifying participants into two groups: responders and nonresponders. Participant stratification was based on anti-S IgG concentrations. Individuals with a ratio of 1 or <1 were indicated as nonresponders. The ratio calculation was based on virus-specific antibody concentrations before and after the booster dose. Although a significantly higher number of nonresponders were observed in both ChAdOx1 and CoronaVac groups in comparison with BNT162b2 participants (fig. S4A), analysis of antibody titers in the responder group is consistent with our previous analysis. Homologous booster induces low levels of virus-specific antibodies in responder adults ≥50 years old, in contrast to heterologous regimens (fig. S4B).

Although previous studies showed that neutralization titers against variants of concern (VOCs) were higher for previously infected individuals when compared with those not previously infected, we did not find such correlation in titers against Omicron sublineages (fig. S5, A and B). Together, these analyses suggest that administration of heterologous vaccine boosters to people fully vaccinated with CoronaVac markedly improves humoral responses, including neutralization titers against currently circulating Omicron VOCs. In addition, these data suggest that homologous CoronaVac boosters do not significantly improve antibody responses against Omicron sublineages, particularly in older individuals.

DISCUSSION

Safe and effective vaccines against SARS-CoV-2 are essential resources to managing the ongoing pandemic. In Brazil, which ranks second in terms of COVID-related deaths (almost 700,000), reduced speed in vaccine distribution rather than hesitancy was associated with increased fatality rate in the earlier phases of the pandemic. In China and several countries in South and Central America, the inactivated COVID vaccine CoronaVac was widely distributed in the early phases in the pandemic. Therefore, even with the declining fatality rate during the Omicron wave (*21*), it is crucial to design booster strategies that continue to induce protective immunity, particularly in the vulnerable population. In this study, we found that booster vaccination induced a 7.5-fold increase in IgG anti-RBD levels, regardless of booster type. However, a heterologous vaccine regimen, composed of a two-dose CoronaVac prime followed by a single BNT162b2 or ChAdOx1 booster, induced higher levels of neutralizing and virus-specific antibodies against VOCs compared with a homologous CoronaVac/CoronaVac regimen. The inactivated vaccine Covaxin, which differs slightly from CoronaVac regarding its adjuvant, is highly effective, with an efficacy of 93% against severe symptomatic COVID-19 disease. Covaxin induces neutralizing antibodies that are sustained for 12 months against ancestral, Alpha, Beta, and Delta variants. However, consistent with our analysis, levels of neutralizing antibodies after vaccination against Omicron BA.1 were reduced compared with other variants or were below the limit of detection (*22*).

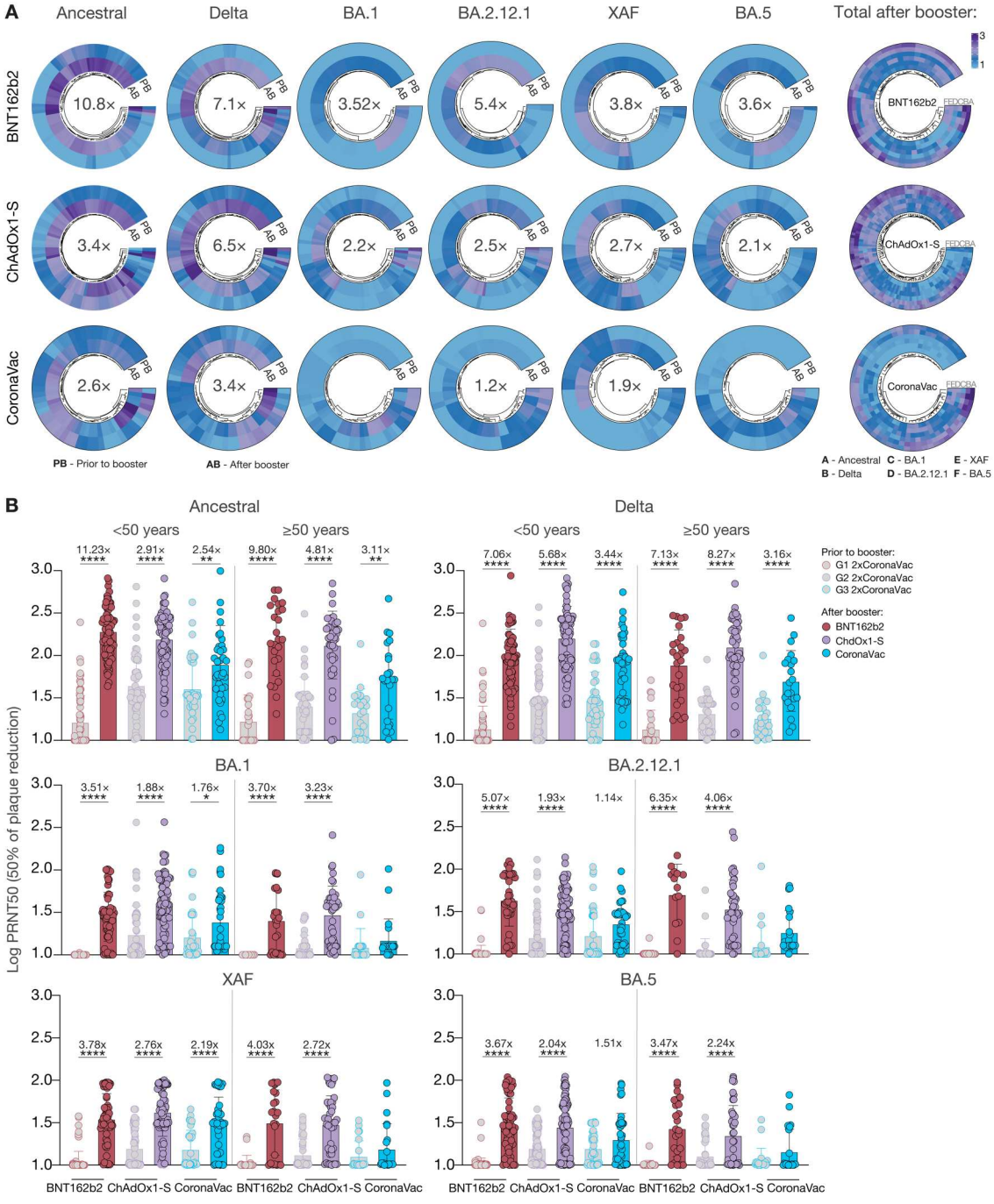

Fig. 2. Neutralization responses after a heterologous or homologous booster regimen in individuals fully vaccinated with CoronaVac. (**A** and **B**) Plasma neutralization capacity against the ancestral strain (WA1, USA), Delta, BA.1, BA.2.12.1, XAF, and BA.5 before and after booster dose. Participants received booster doses of BNT162b2, ChAdOx1-S, or CoronaVac. (**A**) Circular heatmap comparisons of neutralization titers from fully vaccinated CoronaVac participants at baseline and 28 days after booster dose. PB, prior to booster dose; AB, after booster dose. Color intensity indicates log PRNT50 for each specific SARS-CoV-2 variant tested. PRNT50, 50% plaque reduction. The median fold change values in neutralization titers after booster vaccination are indicated inside the circles. Total neutralization titers against ancestral strain, Delta, and BA.1 (BNT162b2, $n = 101$; ChAdOx1-S, $n = 131$; CoronaVac, $n = 61$); BA.2.12.1 (BNT162b2, $n = 49$; ChAdOx1-S, $n = 131$; CoronaVac, $n = 61$); and XAF and BA.5 (BNT162b2, $n = 86$; ChAdOx1-S, $n = 131$; CoronaVac, $n = 61$). (**B**) Plasma neutralization capacity comparisons by age groups. The median fold change values in neutralization titers after booster vaccination are indicated. Neutralization titers for <50-year-old participants against ancestral strain, Delta, and BA.1 (BNT162b2, $n = 76$; ChAdOx1-S, $n = 87$; CoronaVac, $n = 39$); BA.2.12.1 (BNT162b2, $n = 35$; ChAdOx1-S, $n = 87$; CoronaVac, $n = 39$); and XAF and BA.5 (BNT162b2, $n = 35$; ChAdOx1-S, $n = 87$; CoronaVac, $n = 39$). Neutralization titers for ≥50-year-old participants against ancestral strain, Delta, and BA.1 (BNT162b2, $n = 25$; ChAdOx1-S, $n = 44$; CoronaVac, $n = 22$); BA.2.12.1 (BNT162b2, $n = 14$; ChAdOx1-S, $n = 44$; CoronaVac, $n = 22$); and XAF and BA.5 (BNT162b2, $n = 25$; ChAdOx1-S, $n = 87$; CoronaVac, $n = 39$). Significance was assessed using mixed-effect analysis corrected for multiple comparisons using Sidak's method. Each dot represents a single individual. Lines connect the same individual in the two respective time points of sample collection. Horizontal bars represent average ± SD.****$P < 0.0001$, **$P < 0.01$, and *$P < 0.05$.

Age-related decreases in vaccine-induced antibodies and cellular responses were reported by several studies, including those primed with mRNA, viral vectors, or inactivated vaccines. In older populations, lower IgG and IgA anti-S and RBD titers and lower neutralization responses were observed, with notable reductions in participants aged 80 or more after initial vaccination doses (*17*, *18*, *23*–*25*). In contrast to the mRNA BNT162b2, or the viral-vectored ChAdOx1vaccine booster, we observed an inverse correlation between age- and virus-specific antibody responses in recipients of the CoronaVac booster, suggesting that formulation of vaccine boosters may dictate or reinforce age-specific declines in immunity. Differences in antibody responses upon heterologous versus homologous strategies were more pronounced in neutralizing responses against Omicron sublineages, especially among the participants >50 years old. Previous studies have reported increased anti–SARS-CoV-2 S antibodies and higher neutralization titers against ancestral, Delta, and BA.1 induced by the heterologous regimen compared with homologous vaccination, suggesting that heterologous schedules are poised to play an increasingly important role within the global COVID-19 vaccine strategy (*12*, *13*, *26*–*28*). Our findings extend these observations for Omicron sublineages and caution against the use of CoronaVac as a booster strategy. In addition, our analysis supports real-world vaccine efficacy data reporting reduced effectiveness and increased hospitalization or death rates of CoronaVac homologous booster strategy in the elderly when compared with heterologous boosters (51 versus 78%, respectively) (*29*).

Vaccine- or age-related mechanisms underlying these differences remain to be identified. Although neutralizing antibody titers have been extensively correlated and are predictive of symptomatic disease, vaccine effectiveness against severe COVID-19 is determined by not only virus-specific antibody levels but also T cell–mediated immunity among other factors. Our study did not examine cellular immune responses, mucosal immune responses, or the durability of booster-induced immunity. Nevertheless, our findings provide a better understanding of vaccine-induced humoral responses for vulnerable groups during the Omicron wave and could potentially guide future immunization strategies and public health policies.

METHODS

Study design

One hundred ninety-two participants from Brazil and 101 volunteers from the Dominican Republic were followed serially after vaccination. Participants from the Dominican Republic received a BNT162b2 booster dose. Participants from Brazil received the ChAdOx1 or CoronaVac booster. For the Brazilian cohort, we conducted a randomized, participant-blind, immunogenicity study. Participants were randomly assigned to receive one of two different booster vaccines. The computer randomization was conducted using RedCAP according to the recommendations on the guideline for Good Clinical Practice [International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)]. This process was monitored by Centro Avançado de Pesquisa e Estudos para o Diagnóstico (CAPED). Study staff members were aware of vaccine allocations, but the laboratory staff remained blinded until after processing and analyzing raw data. Plasma samples were collected at baseline (before booster, after two doses of CoronaVac) and 28 days after the booster (third dose) administration. Demographic information was aggregated through a systematic review and was used to construct table S1. The clinical data were collected using RedCAP (v5.19.15 @2021 Vanderbilt University) software. Blood acquisition was performed and recorded by a separate team. Vaccinated clinical information and time points of collection information were not available until after processing and analyzing raw data. ELISA and neutralizations were performed blinded. Documented history of prior SARS-CoV-2 infection was confirmed by the absence of SARS-CoV-2–specific antibodies, and information on time window after CoronaVac vaccination is available in table S1.

Ethics statement

This study was approved by the National Research Bioethics Committee of Brazil (CONEP, CAAE 50457721.9.0000.0175) and the National Bioethics Committee of the Dominican Republic (CONABIOS). The participants received two doses of the inactivated whole-virion vaccine CoronaVac followed by a single BNT162b2, ChAdOx-S, or CoronaVac booster dose. The interval between the second dose of CoronaVac and the booster shot was at least 4 weeks. The Brazilian cohort received ChAdOx1-S and CoronaVac boosters, which were administered between 27 November 2021 and 3 February 2022. The Dominican Republic cohort received two doses of CoronaVac followed by the mRNA vaccine BNT162b2 booster, which was administered between 30 July 2021 and 27 August 2021. Informed consent was obtained from all enrolled vaccinated individuals. None of the participants experienced serious adverse effects after vaccination.

Plasma isolation and storage

Whole blood was collected in heparinized cellular preparation tubes (CPT) blood vacutainers (BD; # BDAM362780 or Greiner; REF 455051BR) and kept on gentle agitation until processing. All blood was processed on the day of collection in a single-step standardized method. Plasma samples were collected after centrifugation of whole blood at 600*g* for 20 min at room temperature (RT) without breaks. The undiluted plasma was transferred to 15-ml polypropylene conical tubes, aliquoted, and stored at −80°C for subsequent shipping and analysis. Plasma samples were sourced from participants from the Dominican Republic and Brazil and were shipped to Yale University. The plasma was aliquoted and heat-inactivated at 56°C for 30 min to inactivate complement before microneutralization.

SARS-CoV-2 culture

TMPRSS2-VeroE6 kidney epithelial cells were cultured in Dulbecco′s modified Eagle′s medium (DMEM) supplemented with 1% sodium pyruvate [non-essential amino acids (NEAA)] and 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. The cell line has been tested negative for contamination with mycoplasma. SARS-CoV-2 lineage A (USA-WA1/2020) was obtained from BEI Resources (#NR-52281). Delta and Omicron subvariants, BA.1, BA2.12.1, XAF, and BA.5, were sequenced as part of the Yale Genomic Surveillance Initiative′s weekly surveillance program in Connecticut, USA and then isolated from nasopharyngeal specimens as previously described (*30*). The pelleted virus was then resuspended in phosphate-buffered saline (PBS) and aliquoted for storage at −80°C. Lineage assignments were confirmed using

Pangolin (version 3.1.17), and the respective consensus sequences were submitted to National Center for Biotechnology Information [GenBank accession: ancestral lineage A = MZ468053, Delta = MZ468047, Omicron (BA.1) = OL965559, Omicron (BA.2.12.1) = ON411581, Omicron (XAF, S region derived from BA.2) = OP031604, and Omicron (BA.5) = OP031606]. Viral titers were measured by standard plaque assay using TMPRSS2-VeroE6. Briefly, 300 µl of serial fold virus dilutions were used to infect Vero E6 cells in DMEM supplemented with $NaHCO_3$, 4% FBS, and 0.6% Avicel RC-581. Plaques were resolved at 48 hours after infection by fixing in 10% formaldehyde for 1 hour followed by 0.5% crystal violet in 20% ethanol staining. Plates were rinsed in water to plaque enumeration. All experiments were performed in a biosafety level 3 laboratory with approval from the Yale Environmental Health and Safety office.

SARS-CoV-2–specific antibody measurements

ELISAs were performed as previously described (*30*). Briefly, 96-well MaxiSorp plates (Thermo Fisher Scientific, #442404) were coated with recombinant SARS Cov-2 SARS-CoV-2 S protein (50 µl per well; ACROBiosystems, #SPN-C52H9-100 µg) or RBD (ACROBiosystems, #SPD-C52H3-100 µg) at a concentration of 2 µg/ml in PBS and were incubated overnight at 4°C. The coating buffer was removed, and plates were incubated for 1 hour at RT with 200 µl of blocking solution (PBS with 0.1% Tween-20 and 3% milk powder). Plasma was diluted 1:800 in dilution solution (PBS with 0.1% Tween-20 and 1% milk powder), and 100 µl of diluted serum were added for 2 hours at RT. Human anti-Spike [SARS-CoV-2 Human Anti-Spike (AM006415) (Active Motif #91351)] was serially diluted to generate a standard curve. Plates were washed three times with PBS-T (PBS with 0.1% Tween-20), and 50 µl of horseradish peroxidase anti-Human IgG Antibody (GenScript #A00166; 1:5000) diluted in dilution solution were added to each well for 1 hour. Plates were developed with 100 µl of TMB (3,3',5,5'-tetramethybenzine) Substrate Reagent Set (BD Biosciences #555214) and then read at a wavelength of 450 and 570 nm.

Neutralization assay

Sera from vaccinated individuals were heat-treated for 30 min at 56°C. Sixfold serially diluted plasma, from 1:10 to 1:2430, were incubated with SARS-CoV-2 variants for 1 hour at 37°C. The mixture was subsequently incubated with TMPRSS2-VeroE6 in a 12-well plate for 1 hour for adsorption. Then, cells were overlaid with MEM supplemented with $NaHCO_3$, 4% FBS, and 0.6% Avicel mixture. Plaques were resolved at 40 hours after infection by fixing in 10% formaldehyde for 1 hour followed by staining in 0.5% crystal violet. All experiments were performed in parallel with baseline control sera in an established viral concentration to generate 60 to 120 plaques per well.

Statistical analysis

All analyses were conducted using GraphPad Prism 8.4.3, JMP 15, R, and Morpheus web tool. Multiple group comparisons were analyzed by running parametric [analysis of variance (ANOVA)] statistical tests. Multiple comparisons were corrected using Tukey's test as indicated in the figure legends. Multiple comparisons were corrected using Tukey's or Sidak's method test as indicated in the figure legends. Heatmap of the unsupervised hierarchical cluster of anti-S and anti-RBD was created using the open-source software Morpheus (https://software.broadinstitute.org/morpheus) applying Euclidian distance metric (*31*). Circular heatmaps characterizing the unsupervised hierarchical cluster of log PRNT50 by the average linkage method were created using circlize and ComplexHeatmap R packages (*32*).

Supplementary Materials

This PDF file includes:
Figs. S1 to S5
Table S1

Other Supplementary Material for this manuscript includes the following:
Data file S1
MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. R. Viana, S. Moyo, D. G. Amoako, H. Tegally, C. Scheepers, C. L. Althaus, U. J. Anyaneji, P. A. Bester, M. F. Boni, M. Chand, W. T. Choga, R. Colquhoun, M. Davids, K. Deforche, D. Doolabh, L. du Plessis, S. Engelbrecht, J. Everatt, J. Giandhari, M. Giovanetti, D. Hardie, V. Hill, N. Y. Hsiao, A. Iranzadeh, A. Ismail, C. Joseph, R. Joseph, L. Koopile, S. L. Kosakovsky Pond, M. U. G. Kraemer, L. Kuate-Lere, O. Laguda-Akingba, O. Lesetedi-Mafoko, R. J. Lessells, S. Lockman, A. G. Lucaci, A. Maharaj, B. Mahlangu, T. Maponga, K. Mahlakwane, Z. Makatini, G. Marais, D. Maruapula, K. Masupu, M. Matshaba, S. Mayaphi, N. Mbhele, M. B. Mbulawa, A. Mendes, K. Mlisana, A. Mnguni, T. Mohale, M. Moir, K. Moruisi, M. Mosepele, G. Motsatsi, M. S. Motswaledi, T. Mphoyakgosi, N. Msomi, P. N. Mwangi, Y. Naidoo, N. Ntuli, M. Nyaga, L. Olubayo, S. Pillay, B. Radibe, Y. Ramphal, U. Ramphal, J. E. San, L. Scott, R. Shapiro, L. Singh, P. Smith-Lawrence, W. Stevens, A. Strydom, K. Subramoney, N. Tebeila, D. Tshiabuila, J. Tsui, S. van Wyk, S. Weaver, C. K. Wibmer, E. Wilkinson, N. Wolter, A. E. Zarebski, B. Zuze, D. Goedhals, W. Preiser, F. Treurnicht, M. Venter, C. Williamson, O., G. Pybus, J. Bhiman, A. Glass, D. P. Martin, A. Rambaut, S. Gaseitsiwe, A. von Gottberg, T. de Oliveira, Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature* **603**, 679–686 (2022).
2. Y. Cao, A. Yisimayi, F. Jian, W. Song, T. Xiao, L. Wang, S. Du, J. Wang, Q. Li, X. Chen, Y. Yu, P. Wang, Z. Zhang, P. Liu, R. An, X. Hao, Y. Wang, J. Wang, R. Feng, H. Sun, L. Zhao, W. Zhang, D. Zhao, J. Zheng, L. Yu, C. Li, N. Zhang, R. Wang, X. Niu, S. Yang, X. Song, Y. Chai, Y. Hu, Y. Shi, L. Zheng, Z. Li, Q. Gu, F. Shao, W. Huang, R. Jin, Z. Shen, Y. Wang, X. Wang, J. Xiao, X. S. Xie, BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection. *Nature* **608**, 593–602 (2022).
3. H. Tegally, M. Moir, J. Everatt, M. Giovanetti, C. Scheepers, E. Wilkinson, K. Subramoney, Z. Makatini, S. Moyo, D. G. Amoako, C. Baxter, C. L. Althaus, U. J. Anyaneji, D. Kekana, R. Viana, J. Giandhari, R. J. Lessells, T. Maponga, D. Maruapula, W. Choga, M. Matshaba, M. B. Mbulawa, N. Msomi; NGS-SA consortium, Y. Naidoo, S. Pillay, T. J. Sanko, J. E. San, L. Scott, L. Singh, N. A. Magini, P. Smith-Lawrence, W. Stevens, G. Dor, D. Tshiabuila, N. Wolter, W. Preiser, F. K. Treurnicht, M. Venter, G. Chiloane, C. McIntyre, A. O'Toole, C. Ruis, T. P. Peacock, C. Roemer, S. L. Kosakovsky Pond, C. Williamson, O. G. Pybus, J. N. Bhiman, A. Glass, D. P. Martin, B. Jackson, A. Rambaut, O. Laguda-Akingba, S. Gaseitsiwe, A. von Gottberg, T. de Oliveira, Emergence of SARS-CoV-2 Omicron lineages BA.4 and BA.5 in South Africa. *Nat. Med.* **28**, 1785–1790 (2022).
4. P. Qu, J. Faraone, J. P. Evans, X. Zou, Y. M. Zheng, C. Carlin, J. S. Bednash, G. Lozanski, R. K. Mallampalli, L. J. Saif, E. M. Oltz, P. J. Mohler, R. J. Gumina, S. L. Liu, Neutralization of the SARS-CoV-2 Omicron BA.4/5 and BA.2.12.1 subvariants. *N. Engl. J. Med.* **386**, 2526–2528 (2022).
5. Q. Wang, Y. Guo, S. Iketani, M. S. Nair, Z. Li, H. Mohri, M. Wang, J. Yu, A. D. Bowen, J. Y. Chang, J. G. Shah, N. Nguyen, Z. Chen, K. Meyers, M. T. Yin, M. E. Sobieszczyk, Z. Sheng, Y. Huang, L. Liu, D. D. Ho, Antibody evasion by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4 and BA.5. *Nature* **608**, 603–608 (2022).
6. N. P. Hachmann, J. Miller, A. Y. Collier, J. D. Ventura, J. Yu, M. Rowe, E. A. Bondzie, O. Powers, N. Surve, K. Hall, D. H. Barouch, Neutralization escape by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4, and BA.5. *N. Engl. J. Med.* **387**, 86–88 (2022).
7. A. Jara, E. A. Undurraga, C. Gonzalez, F. Paredes, T. Fontecilla, G. Jara, A. Pizarro, J. Acevedo, K. Leo, F. Leon, C. Sans, P. Leighton, P. Suarez, H. Garcia-Escorza, R. Araos, Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *N. Engl. J. Med.* **385**, 875–884 (2021).

8. F. P. Polack, S. J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. P. Marc, E. D. Moreira, C. Zerbini, R. Bailey, K. A. Swanson, S. Roychoudhury, K. Koury, P. Li, W. V. Kalina, D. Cooper, R. W. Frenck Jr., L. L. Hammitt, O. Tureci, H. Nell, A. Schaefer, S. Unal, D. B. Tresnan, S. Mather, P. R. Dormitzer, U. Sahin, K. U. Jansen, W. C. Gruber; C4591001 Clinical Trial Group, Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* **383**, 2603–2615 (2020).
9. S. A. Costa Clemens, L. Weckx, R. Clemens, A. V. Almeida Mendes, A. Ramos Souza, M. B. V. Silveira, S. N. F. da Guarda, M. M. de Nobrega, M. I. de Moraes Pinto, I. G. S. Gonzalez, N. Salvador, M. M. Franco, R. N. de Avila Mendonca, I. S. Queiroz Oliveira, B. S. de Freitas Souza, M. Fraga, P. Aley, S. Bibi, L. Cantrell, W. Dejnirattisai, X. Liu, J. Mongkolsapaya, P. Supasa, G. R. Screaton, T. Lambe, M. Voysey, A. J. Pollard; RHH-001 study team, Heterologous versus homologous COVID-19 booster vaccination in previous recipients of two doses of CoronaVac COVID-19 vaccine in Brazil (RHH-001): A phase 4, non-inferiority, single blind, randomised study. *Lancet* **399**, 521–529 (2022).
10. M. D. Tanriover, H. L. Doganay, M. Akova, H. R. Guner, A. Azap, S. Akhan, S. Kose, F. S. Erdinc, E. H. Akalin, O. F. Tabak, H. Pullukcu, O. Batum, S. Simsek Yavuz, O. Turhan, M. T. Yildirmak, I. Koksal, Y. Tasova, V. Korten, G. Yilmaz, M. K. Celen, S. Altin, I. Celik, Y. Bayindir, I. Karaoglan, A. Yilmaz, A. Ozkul, H. Gur, S. Unal; CoronaVac Study Group, Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): Interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet* **398**, 213–222 (2021).
11. M. D. T. Hitchings, O. T. Ranzani, M. S. S. Torres, S. B. de Oliveira, M. Almiron, R. Said, R. Borg, W. L. Schulz, R. D. de Oliveira, P. V. da Silva, D. B. de Castro, V. S. Sampaio, B. C. de Albuquerque, T. C. A. Ramos, S. H. H. Fraxe, C. F. da Costa, F. G. Naveca, A. M. Siqueira, W. N. de Araujo, J. R. Andrews, D. A. T. Cummings, A. I. Ko, J. Croda, Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 gamma variant transmission in Manaus, Brazil: A test-negative case-control study. *Lancet Reg. Health Am.* **1**, 100025 (2021).
12. G. Zeng, Q. Wu, H. Pan, M. Li, J. Yang, L. Wang, Z. Wu, D. Jiang, X. Deng, K. Chu, W. Zheng, L. Wang, W. Lu, B. Han, Y. Zhao, F. Zhu, H. Yu, W. Yin, Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: Interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. *Lancet Infect. Dis.* **22**, 483–495 (2022).
13. S. M. S. Cheng, C. K. P. Mok, Y. W. Y. Leung, S. S. Ng, K. C. K. Chan, F. W. Ko, C. Chen, K. Yiu, B. H. S. Lam, E. H. Y. Lau, K. K. P. Chan, L. L. H. Luk, J. K. C. Li, L. C. H. Tsang, L. L. M. Poon, D. S. C. Hui, M. Peiris, Neutralizing antibodies against the SARS-CoV-2 Omicron variant BA.1 following homologous and heterologous CoronaVac or BNT162b2 vaccination. *Nat. Med.* **28**, 486–489 (2022).
14. E. Perez-Then, C. Lucas, V. S. Monteiro, M. Miric, V. Brache, L. Cochon, C. B. F. Vogels, A. A. Malik, E. De la Cruz, A. Jorge, M. De Los Santos, P. Leon, M. I. Breban, K. Billig, I. Yildirim, C. Pearson, R. Downing, E. Gagnon, A. Muyombwe, J. Razeq, M. Campbell, A. I. Ko, S. B. Omer, N. D. Grubaugh, S. H. Vermund, A. Iwasaki, Neutralizing antibodies against the SARS-CoV-2 Delta and Omicron variants following heterologous CoronaVac plus BNT162b2 booster vaccination. *Nat. Med.* **28**, 481–485 (2022).
15. A. Jara, E. A. Undurraga, J. R. Zubizarreta, C. Gonzalez, A. Pizarro, J. Acevedo, K. Leo, F. Paredes, T. Bralic, V. Vergara, M. Mosso, F. Leon, I. Parot, P. Leighton, P. Suarez, J. C. Rios, H. Garcia-Escorza, R. Araos, Effectiveness of homologous and heterologous booster doses for an inactivated SARS-CoV-2 vaccine: A large-scale prospective cohort study. *Lancet Glob. Health* **10**, e798–e806 (2022).
16. M. Mousa, M. Albreiki, F. Alshehhi, S. AlShamsi, N. A. Marzouqi, T. Alawadi, H. Alrand, H. Alsafar, A. Fikri, Similar effectiveness of the inactivated vaccine BBIBP-CorV (Sinopharm) and the mRNA vaccine BNT162b2 (Pfizer-BioNTech) against COVID-19 related hospitalizations during the Delta outbreak in the UAE. *J. Travel Med.* **29**, (2022).
17. P. Naaber, L. Tserel, K. Kangro, E. Sepp, V. Jurjenson, A. Adamson, L. Haljasmagi, A. P. Rumm, R. Maruste, J. Karner, J. M. Gerhold, A. Planken, M. Ustav, K. Kisand, P. Peterson, Dynamics of antibody response to BNT162b2 vaccine after six months: A longitudinal prospective study. *Lancet Reg. Health Eur.* **10**, 100208 (2021).
18. H. Parry, R. Bruton, C. Stephens, C. Bentley, K. Brown, G. Amirthalingam, B. Hallis, A. Otter, J. Zuo, P. Moss, Extended interval BNT162b2 vaccination enhances peak antibody generation. *NPJ Vaccines* **7**, 14 (2022).
19. D. Cromer, M. Steain, A. Reynaldi, T. E. Schlub, A. K. Wheatley, J. A. Juno, S. J. Kent, J. A. Triccas, D. S. Khoury, M. P. Davenport, Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: A meta-analysis. *Lancet Microbe* **3**, e52–e61 (2022).
20. D. S. Khoury, D. Cromer, A. Reynaldi, T. E. Schlub, A. K. Wheatley, J. A. Juno, K. Subbarao, S. J. Kent, J. A. Triccas, M. P. Davenport, Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* **27**, 1205–1211 (2021).
21. V. K. C. Yan, E. Y. F. Wan, X. Ye, A. H. Y. Mok, F. T. T. Lai, C. S. L. Chui, X. Li, C. K. H. Wong, P. H. Li, T. Ma, S. Qin, V. K. C. Wong, T. C. Tsang, S. H. Tsui, W. C. M. Chui, B. J. Cowling, G. M. Leung, C. S. Lau, I. C. K. Wong, E. W. Y. Chan, Effectiveness of BNT162b2 and CoronaVac vaccinations against mortality and severe complications after SARS-CoV-2 Omicron BA.2 infection: A case-control study. *Emerg. Microbes Infect.* **11**, 2304–2314 (2022).
22. N. P. Kumar, V. V. Banurekha, C. P. G. Kumar, A. Nancy, C. Padmapriyadarsini, S. Shankar, L. E. Hanna, M. Murhekar, K. R. U. Devi, S. Babu, Inactivated COVID-19 vaccines: Durability of Covaxin/BBV152 induced immunity against variants of concern. *J. Travel Med.* **29**, (2022).
23. S. Saadat, Z. Rikhtegaran Tehrani, J. Logue, M. Newman, M. B. Frieman, A. D. Harris, M. M. Sajadi, Binding and neutralization antibody titers after a single vaccine dose in health care workers previously infected with SARS-CoV-2. *JAMA* **325**, 1467–1469 (2021).
24. A. M. Borobia, A. J. Carcas, M. Perez-Olmeda, L. Castano, M. J. Bertran, J. Garcia-Perez, M. Campins, A. Portoles, M. Gonzalez-Perez, M. T. Garcia Morales, E. Arana-Arri, M. Aldea, F. Diez-Fuertes, I. Fuentes, A. Ascaso, D. Lora, N. Imaz-Ayo, L. E. Baron-Mira, A. Agusti, C. Perez-Ingidua, A. Gomez de la Camara, J. R. Arribas, J. Ochando, J. Alcami, C. Belda-Iniesta, J. Frias, S. S. G. CombiVac, Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): A multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet* **398**, 121–130 (2021).
25. O. T. Ranzani, M. D. T. Hitchings, M. Dorion, T. L. D'Agostini, R. C. de Paula, O. F. P. de Paula, E. F. M. Villela, M. S. S. Torres, S. B. de Oliveira, W. Schulz, M. Almiron, R. Said, R. D. de Oliveira, P. Vieira da Silva, W. N. de Araujo, J. C. Gorinchteyn, J. R. Andrews, D. A. T. Cummings, A. I. Ko, J. Croda, Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: Test negative case-control study. *BMJ* **374**, n2015 (2021).
26. L. Vargas, N. Valdivieso, F. Tempio, V. Simon, D. Sauma, L. Valenzuela, C. Beltran, L. Castillo-Delgado, X. Contreras-Benavides, M. L. Acevedo, J. Acevedo, R. I. Gonzalez, F. Valiente-Echeverria, R. Soto-Rifo, M. Rosemblatt, M. Lopez, F. Osorio, M. R. Bono, Serological study of CoronaVac vaccine and booster doses in Chile: Immunogenicity and persistence of anti-SARS-CoV-2 spike antibodies. *BMC Med.* **20**, 216 (2022).
27. J. Li, L. Hou, X. Guo, P. Jin, S. Wu, J. Zhu, H. Pan, X. Wang, Z. Song, J. Wan, L. Cui, J. Li, Y. Chen, X. Wang, L. Jin, J. Liu, F. Shi, X. Xu, T. Zhu, W. Chen, F. Zhu, Heterologous AD5-nCOV plus CoronaVac versus homologous CoronaVac vaccination: A randomized phase 4 trial. *Nat. Med.* **28**, 401–409 (2022).
28. E. P. K. Parker, S. Desai, M. Marti, K. L. O'Brien, D. C. Kaslow, S. Kochhar, F. Olayinka, A. Cravioto, H. Nohynek, J. Hombach, A. Wilder-Smith, Emerging evidence on heterologous COVID-19 vaccine schedules—To mix or not to mix? *Lancet Infect. Dis.* **22**, 438–440 (2022).
29. O. T. Ranzani, M. D. T. Hitchings, R. Leite de Melo, G. V. A. de França, C. d. F. R. Fernandes, M. L. Lind, M. S. Scaramuzzini Torres, D. H. Tsuha, L. C. S. David, R. F. C. Said, M. Almiron, R. D. de Oliveira, D. A. T. Cummings, N. E. Dean, J. R. Andrews, A. I. Ko, J. Croda, Effectiveness of an inactivated Covid-19 vaccine with homologous and jeterologous boosters against Omicron in Brazil. *Nat. Commun* **13**, 5536 (2022).
30. C. Lucas, C. B. F. Vogels, I. Yildirim, J. E. Rothman, P. Lu, V. Monteiro, J. R. Gehlhausen, M. Campbell, J. Silva, A. Tabachnikova, M. A. Pena-Hernandez, M. C. Muenker, M. I. Breban, J. R. Fauver, S. Mohanty, J. Huang, S.-C.-G. S. I. Yale, A. C. Shaw, A. I. Ko, S. B. Omer, N. D. Grubaugh, A. Iwasaki, Impact of circulating SARS-CoV-2 variants on mRNA vaccine-induced immunity. *Nature* **600**, 523–529 (2021).
31. J. Starruss, W. de Back, L. Brusch, A. Deutsch, Morpheus: A user-friendly modeling environment for multiscale and multicellular systems biology. *Bioinformatics* **30**, 1331–1332 (2014).
32. Z. Gu, R. Eils, M. Schlesner, Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847–2849 (2016).

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the study. B.A.F., P.V.S., V.d.P.M., L.E.R.Z., and R.A.L. participated in the organization and sample collection of the Brazilian cohort. E.P.-T., M.M., V.B., L.C., and S.H.V. participated in the organization and sample collection of the Dominican Republic cohort. A.M.H., N.F.G.C., K.P., and N.D.G. surveilled, detected, and performed virus sequencing. V.S.M. and C.L. performed experiments and data collection. V.S.M. and C.L. analyzed the data. G.C.B. performed the heatmap analysis. A.A.M. and J.S. performed data analysis. B.A.F., V.S.M., A.I., and C.L. wrote the original draft. R.D.R.M., D.B.S., A.R.P., J.C., I.Y., S.B.O., and A.I.K. reviewed and edited the manuscript. A.I. and C.L. supervised the project. All authors reviewed and approved the manuscript. **Competing interests:** A.I. serves as a consultant for RIGImmune, Xanadu, and Revelar Biotherapeutics. B.A.F. had lecture fees and sponsored travel by AstraZeneca. L.E.R.Z. served on the advisory board for Zodiac and had lecture fees by AstraZeneca, Bayer, Janssen, and Astellas. N.D.G. is a consultant for Tempus Labs to develop infectious disease diagnostic assays. All other authors declare that they have no competing interests.

Data and materials availability statement: All of the background information of participants and data generated in this study are included in Source Data Fig. 1. The genome information and aligned consensus genomes for SARS-CoV-2 variants used in this study are available on National Center for Biotechnology Information [GenBank Accession numbers: ancestral lineage A = MZ468053, Delta = MZ468047, Omicron (BA.1) = OL965559, Omicron (BA.2.12.1) = ON411581, Omicron (XAF) = OP031604, and Omicron (BA.5) = OP031606]. Additional correspondence and requests for materials should be addressed to the corresponding authors.

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Age-dependent impairment in antibody responses elicited by a homologous CoronaVac booster dose

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