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Antibody response dynamics to CoronaVac vaccine and booster immunization in adults and the elderly: A long-term, longitudinal prospective study

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ABSTRACT

Background: The long-term humoral immune response after vaccination varies between vaccines and is dependent on the accuracy of the antibody test. A better understanding of the vaccine immune response may help to define vaccination strategies against coronavirus disease 2019 (COVID-19).
Objective: To investigate the long-term immunological response to CoronaVac vaccine and determinants of breakthrough COVID-19 infection.
Methods: A long-term, prospective cohort study involving vaccinated adult and elderly subjects was conducted to investigate the presence of anti-RBD-specific immunoglobulin (Ig)G, anti-nucleocapsid IgG and anti-spike trimeric protein IgG. Antibody level dynamics and risk factors associated with breakthrough COVID-19 infection were investigated.
Results: In total, 3902 participants were included in this study. Vaccination with two doses of CoronaVac and a booster dose increased the levels of anti-RBD-specific IgG, anti-nucleocapsid IgG and anti-spike trimeric IgG significantly. In adults, anti-nucleocapsid IgG and anti-spike trimeric IgG levels decreased significantly 7 months after the second dose. In adults and the elderly, the levels of anti-spike trimeric IgG and anti-RBD IgG decreased significantly 4 and 6 months after the booster dose, respectively. Previous exposure to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and anti-spike trimeric IgG titres was independently associated with a lower probability of post-vaccination infection.
Conclusions: A significant increase in antibody levels was found after two doses of CoronaVac and a booster dose. Antibody titres declined significantly 7 months post-vaccination in participants who did not receive a booster dose. Higher levels of antibodies and previous SARS-CoV-2 infection were associated with protection against breakthrough COVID-19.

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Introduction

The coronavirus 2019 (COVID-19) pandemic continues worldwide [1]. Several vaccines against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) have been developed, with different efficacies and antibody response profiles [2]. An inactivated SARS-CoV-2 vaccine (CoronaVac, Sinovac Life Sciences, Beijing, China) has been available in Brazil since September 2021, and is the most commonly used vaccine for immunization in older people and healthcare workers [3].

In phase I and II trials, CoronaVac induced seroconversion in 97% of adults and 96.9–100% of elderly subjects [4,5]. Phase III trials found that the efficacy of CoronaVac for preventing symptomatic cases, hospitalization and COVID-19-related deaths was 50.7%–83.5%, 83.7–87.5% and 86.3–100%, respectively [6,7]. In a stepped-wedge randomized trial, the effectiveness of CoronaVac for preventing symptomatic cases, COVID-19-related hospitalizations and COVID-19-related deaths was 80.5%, 95% and 94.9%, respectively [8].

Although vaccination plays a central role in reducing the severity of COVID-19, the vaccine response is often less effective in elderly people, probably due to immunosenescence [9,10]. Lower immunogenicity induced by COVID-19 vaccines has been associated with older age, immunocompromise and chronic diseases [9,11,12]. The immune response may also vary between vaccines and depend on the accuracy of antibody testing [12,13]. Assessing vaccine immunogenicity in different age groups with sensitive tests is crucial to understand immune response kinetics and determinants. This study aimed to prospectively analyse antibody responses to two doses of CoronaVac, followed or not by a third (booster) dose, in adults and elderly subjects using high-performance serological tests, and to investigate demographic and clinical determinants of COVID-19.

Methods

Study design and participants

This study was a prospective, observational cohort study embedded in a larger project named 'Project S' (NCT0474782), a stepped-wedge cluster-randomized trial designed to assess the effectiveness of CoronaVac among residents in the urban area of Serrana, São Paulo State, Brazil. In Project S, all residents were eligible, and two doses of CoronaVac were offered 4 weeks apart to adults aged ≥18 years according to the location of their homes in a cluster. From February to April 2021, 26,891 participants were enrolled, 4493 of whom were aged ≥60 years; this represented 81.3% of the adults and 60.9% of the urban population of Serrana. All participants had blood drawn to assess the presence of antibodies against SARS-CoV-2 before vaccination [8]. Four months after the second dose of CoronaVac, Project S participants were invited to join the current observational study. All persons aged ≥60 years were eligible for inclusion; for adults aged 18–59 years, an age-stratified sample was defined (see online supplementary material). This study was initiated on 4 July 2021, participants were recruited from 4 July to 1 August 2021, and data collection was stopped on 22 May 2022.

In September 2021, a booster dose of CoronaVac was recommended by the National Immunization Program for people aged ≥60 years and healthcare workers; in November 2021, all adults were considered for the booster dose. Importantly, the booster dose was taken by the participants as part of the Brazilian National Immunization Program and not as a procedure of the current study.

This study was conducted according to the precepts of the Declaration of Helsinki, and was approved by the Ethics Committee of Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto–University of São Paulo. All participants provided written informed consent specific to the current study.

Clinical data

All clinical data were recorded in a clinical interview supervised by a physician at the time of the first dose of CoronaVac in the main trial (Project S). The presence of chronic diseases such as hypertension, asthma, diabetes, obesity, heart disease, kidney disease, liver disease, immunosuppression and lung disease was ascertained based on self-report.

Procedures and sample processing

Participants were vaccinated with two doses of CoronaVac with a 4-week interval. Blood samples were collected at five time points: immediately before vaccination (baseline sample collected on the day of the first dose of CoronaVac as part of Project S), and 4 (T1, 121.2 ± 8.9 days), 7 (T2, 206.9 ± 8.9 days), 10 (T3, 303.9 ± 8.7 days) and 13 (T4, 417.2 ± 9.0 days) months after the second dose of CoronaVac. At baseline, antibody titres for receptor binding domain (RBD)-specific immunoglobulin (Ig)G (Elecsys Anti-SARS-CoV-2 S) and nucleocapsid IgG (Elecsys Anti-SARS-CoV-2) were quantified to assess the presence of antibodies against SARS-CoV-2 before vaccination. At T1–T4, antibody titres for RBD-specific IgG (Elecsys Anti-SARS-CoV-2 S), spike trimeric protein IgG (LIAISON SARS-CoV-2 TrimericS IgG) and nucleocapsid IgG (Elecsys Anti-SARS-CoV-2) were quantified. Detailed information on serological testing is provided in the online supplementary material.

Case definition

COVID-19 surveillance was based on enhanced public health surveillance systems (e-SUS and SIVEP-Gripe). Participants with one or more symptoms (cough, fever, muscle pain, headache, nausea, vomiting, diarrhoea, dysgeusia, anosmia, dyspnea, coryza, nasal congestion, sore throat or fatigue) for at least 2 days had access to free SARS-CoV-2 reverse transcriptase-polymerase chain reaction (RT-PCR) testing, with results available the next working day. Confirmed SARS-CoV-2 infection was defined by a positive RT-PCR result [14]. Patients with confirmed SARS-CoV-2 infection were followed for 28 days or until hospital discharge or death [14]. Disease severity was classified using the World Health Organization clinical progression scale [15]. Confirmed COVID-19 cases were included from 2 weeks after the second dose for each patient until June 2022. For participants with more than one episode of confirmed COVID-19, only the first infection was considered in the analysis.

Statistical analysis

Immunogenicity was analysed, for each time period and by age group, using geometric mean of titres (GMT), geometric mean titre ratio between post-vaccination and baseline values (rGMT), seroconversion rate and seropositivity rate. GMT and rGMT were calculated as anti-logarithms of the mean of the log-transformed titre. Ninety-five percent confidence intervals (CI) were calculated as anti-logarithm transformation of the upper and lower limits for a two-sided CI for the mean of log-transformed titres. In addition, percentages of seroconversion and seropositivity were calculated with their associated 95% CI by the Clopper–Pearson method. Detailed information about the quantification limit of each test, seroconversion and seropositivity are provided in the online supplementary material.

Comparisons between adults (age 18–59 years) and elderly people (≥60 years) were performed for each time point using the Mann–Whitney test for continuous variables and Fisher's exact test for categorical variables. In addition, as antibody titre data had an asymmetric, non-normal distribution, paired Wilcoxon test was used to compare continuous variables between time points in each age group.

Logistic regression models were used to evaluate risk factors associated with confirmed cases of COVID-19. This analysis was carried out

Table 1
Demographic and clinical characteristics by age group.

	Age <60 years		Age ≥60 years		Total		*P*-value[a]
	(*n*=2247)		(*n*=1655)		(*n*=3902)		
Sex, *n* (%)							
Female	1264	(56.2%)	850	(51.4%)	2114	(54.2%)	**0.003**
Male	983	(43.7%)	805	(48.6%)	1788	(45.8%)	
Comorbidities, *n* (%)							
Diabetes	129	(5.7%)	390	(23.6%)	519	(13.3%)	**<0.001**
Heart disease	52	(2.3%)	149	(9.0%)	207	(5.3%)	**<0.001**
Hypertension	332	(14.8%)	746	(45.6%)	1052	(27.0%)	**<0.001**
Other[b]	240	(10.7%)	280	(16.9%)	520	(13.3%)	**<0.001**
At least one	539	(24.0%)	945	(57.1%)	1484	(38.0%)	**<0.001**
Previous exposure, *n*/*n* total (%)							
Up to baseline	543/2208	(24.6%)	280/1614	(17.3%)	823/3822	(21.5%)	**<0.001**
Third dose, *n*/*n* total (%)							
Up to T2	67/1598	(4.2%)	1205/1256	(95.9%)	1272/2854	(44.6%)	**<0.001**
Up to T3	1166/1207	(96.6%)	970/991	(97.9%)	2136/2198	(97.2%)	0.053
Up to T4	774/786	(98.5%)	641/644	(99.5%)	1415/1430	(98.9%)	0.066

T2, 7 months after the second vaccine dose and 1 month after the booster dose in elderly participants; T3, 10 months after the second vaccine dose; T4, 13 months after the second vaccine dose.

[a] *P*-value for comparison between age groups.

[b] Liver disease, kidney disease, asthma, chronic diseases, obesity, immunosuppression and/or lung disease.

in three different periods employing COVID-19 cases for T1–T2, T2–T3 and T3–T4. For each period, univariable analyses were performed using age, gender, presence of at least one chronic disease, diabetes, hypertension, previous exposure to COVID-19, and the last obtained serum antibody titre as independent variables. Variables with $P \leq 0.20$ on univariable analysis were selected for multi-variable analysis. Additionally, univariable and multi-variable analysis for COVID-19 illness for different periods was performed, excluding patients with previous infections and patients who developed COVID-19 infection. The final model was obtained with all independent variables with $P<0.05$ and the variable 'age' regardless of statistical significance. The results of logistic regression models are expressed as unadjusted and adjusted odds ratios (OR), with respective 95% CI.

All statistical tests were two-sided, and $P<0.05$ was considered to indicate statistical significance. Analyses were conducted using R (R Core Team 2020).

Results

Participant characteristics and time-point evaluations

Participant baseline characteristics are presented in Table 1. In total, 3902 participants (42.4% aged ≥60 years) were included 121.2 ± 8.9 days after their second dose of CoronaVac. The proportion of females was slightly higher among adults than among elderly people ($P=0.003$). Elderly participants had higher prevalence rates for chronic diseases (e.g. diabetes, heart disease, hypertension), and for reporting at least one chronic disease ($P<0.001$). A higher frequency of previous exposure to SARS-CoV-2 was observed among adults ($P<0.001$).

All participants received two doses of CoronaVac before study entry; 1205 (95.9%) elderly participants received a booster dose 41.2 ± 6.1 days before T2; and 1166 (96.6%) adults received a booster dose 62.4 ± 17.9 days before T3 (73.9% of them received a booster dose). Of the 3902 study participants, 3822 had a blood sample assessed at baseline, 3902 at T1, 2854 at T2, 2198 at T3, and 1430 at T4 (Figure S1, see online supplementary material). Eighty participants had no serological data available at baseline due to blood processing issues.

Seropositivity and seroconversion dynamics

Pre- and post-vaccination serological titres, seropositivity and seroconversion for anti-RBD IgG and anti-nucleocapsid IgG are summarized in Tables S1 and S2, respectively (see online supplementary material). Table S3 (see online supplementary material) summarizes post-vaccination serological titres and seropositivity for anti-spike trimeric IgG.

The overall proportion of anti-RBD IgG seropositivity before vaccination was 21.5%, with a significantly higher positivity rate in adults than in elderly participants (24.6% vs 17.3%; $P<0.001$). Four months after the second dose, overall seropositivity increased to 99.1%, again significantly higher in adults than in elderly participants (99.7% vs 98.2%; $P<0.001$). After 7, 10 and 13 months, overall seropositivity was 99.7%, 99.9% and 100%, respectively, with no significant difference between adults and elderly participants. Adults had a similar rate of seroconversion at 4 months (82.3% vs 84.4%; $P=0.087$) and lower rates at 7, 10 and 13 months (82.1% vs 86.2%, $P=0.004$; 84.5% vs 88.1%, $P=0.017$; 84.9% vs 88.6%, $P=0.028$, respectively) compared with elderly participants.

For anti-nucleocapsid IgG, when compared with elderly participants, a higher proportion of adults were positive before vaccination (23.6% vs 17.1%; $P<0.001$) and 4 months after the second dose (81.0% vs 53.5%; $P<0.001$). Of note, at 7 months, elderly participants presented a significantly higher proportion of seropositivity than adults (94.0% vs 70.6%; $P<0.001$). Later, adults had a higher proportion of seropositivity at 10 months than elderly participants ($P=0.030$), and there was no significant difference between adults and elderly participants at 13 months ($P=0.131$). The seroconversion rate in adults, compared with elderly participants, was significantly higher at 4 months (68.2% vs 41.8%; $P<0.001$), lower at 7 months (53.9% vs 83.4%; $P<0.001$), not significantly different at 10 months (83.6% vs 81.3%; $P=0.154$), and lower at 13 months (83.9% vs 88.3%; $P=0.020$).

Anti-spike trimeric IgG was only assessed after vaccination. When compared with elderly participants, adults presented a significantly higher proportion of seropositivity at 4 months (72.7% vs 56.4%; $P<0.001$), lower at 7 months (59.5% vs 95.6%; $P<0.001$), higher at 10 months (98.9% vs 87.2%; $P<0.001$), and similar at 13 months (97.2% vs 95.6%; $P=0.149$).

Antibody kinetics

Overall, anti-RBD IgG GMT increased progressively and significantly from 0.7 U/mL at baseline to 74.7 U/mL at 4 months, 135.6 U/mL at 7 months, 226.7 U/mL at 10 months, and 239.9 U/mL at 13 months after complete-schedule vaccination ($P<0.001$; Table S1, see online supplementary material). When comparing different time points in the same

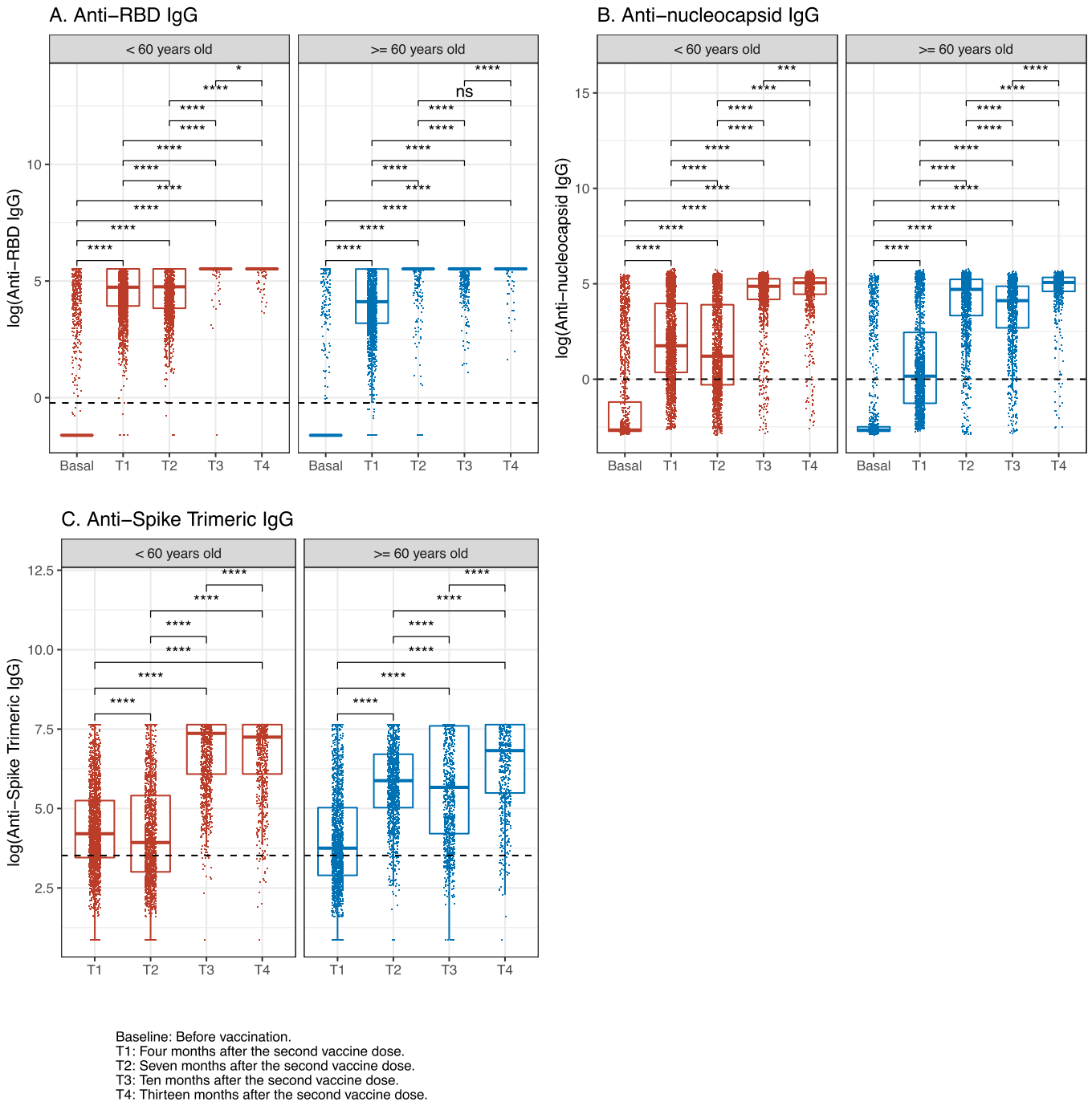

Figure 1. Antibody titres over time according to age group.

age group (Figures 1 and 2), adults had a significant increase in anti-RBD IgG GMT from pre-vaccination to 4 months (0.9 U/mL vs 95.1 U/mL; $P<0.001$), followed by a significant decrease at 7 months (93.1 U/mL; $P<0.001$), a significant increase at 10 months (244.6 U/mL; $P<0.001$), and a significant decrease at 13 months (242.7 U/mL; $P=0.032$). For elderly participants, there was a significant increase in GMT for anti-RBD IgG from pre-vaccination to 4 months and to 7 months (0.6 U/mL vs 53.8 U/mL vs 218.7 U/mL; $P<0.001$), a significant decrease at 10 months (206.6 U/mL; $P<0.001$), and an increase at 13 months (236.6 U/mL; $P<0.001$). Adults had a significantly higher increase in anti-RBD IgG serum titres from 4 months to baseline (rGMT 107.6 vs 89.1; $P<0.001$), whereas rGMT at 7, 10 and 13 months was significantly lower in adults than in elderly participants (110.1 vs 355.5, $P<0.001$; 305.8 vs 376.3, $P<0.001$; 286.1 vs 457.6, $P<0.001$, respectively; Table S1, see online supplementary material).

Anti-nucleocapsid IgG levels (Table S2, see online supplementary material) in adults increased significantly from pre-vaccination to 4 months, decreased at 7 months, and increased at 10 and 13 months [0.3 cut-off index (COI) vs 7.3 COI vs 5 COI vs 75.9 COI vs 90.4 COI; $P<0.001$]. In elderly participants, antibody titres increased significantly from pre-vaccination to 4 and 7 months, decreased at 10 months, and increased at 13 months (0.2 COI vs 2.2 COI vs 51.0 COI vs 32.3 COI vs 108.1 COI; $P<0.001$). The rGMT in adults compared with elderly participants was significantly higher at 4 months (25.4 vs 10.2; $P<0.001$), lower at 7 months (17.7 vs 230.7; $P<0.001$), higher at 10 months (282.0 vs 163.5; $P<0.001$), and higher at 13 months (286.1 vs 590.8; $P<0.001$).

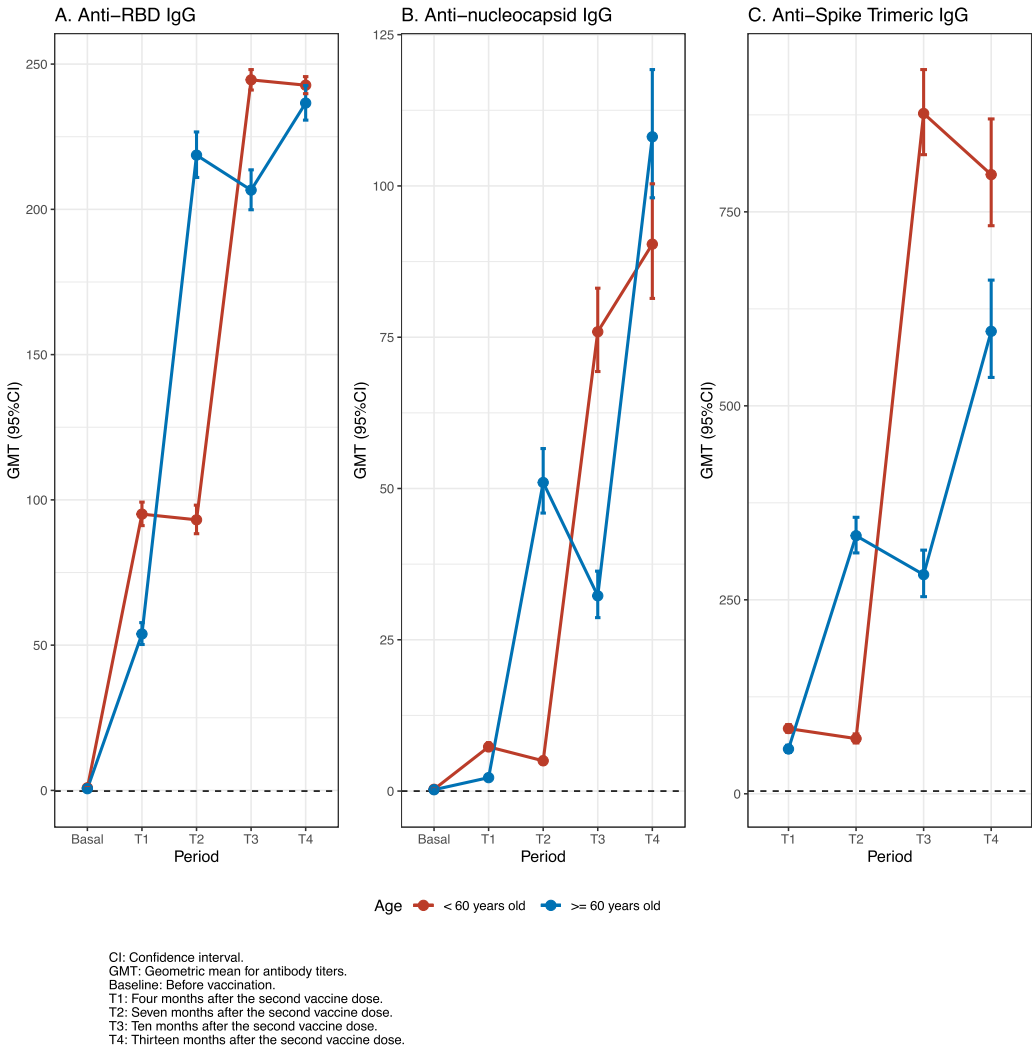

Figure 2. Geometric mean titres (95% confidence interval) over time according to antibody and age group.

For anti-spike trimeric IgG (Table S3, see online supplementary material), in adults, there was a significant decrease in GMT from 4 to 7 months, an increase at 10 months and a decrease at 13 months [84.1 units of bound antibody (BAU)/mL vs 71.1 BAU/mL vs 876.8 BAU/mL vs 798.0 BAU/mL; $P<0.001$], whereas there was a significant increase in GMT in elderly participants from 4 to 7 months, similar levels at 10 months ($P=0.145$) and a significant increase at 13 months (57.7 BAU/mL vs 332.6 BAU/mL vs 283.6 BAU/mL vs 596.7 BAU/mL; $P<0.001$). In comparison with elderly participants, adults presented a more elevated GMT at 4 months (84.1 BAU/mL vs 57.7 BAU/mL; $P<0.001$), lower GMT at 7 months (71.1 BAU/mL vs 332.6 BAU/mL; $P<0.001$), and higher GMT at 10 months (876.8 BAU/mL vs 283.6 BAU/mL) and 13 months (798.0 BAU/mL vs 596.7 BAU/mL; $P<0.001$).

Booster dose and antibody titre kinetics

Table 2, Figure S2 and Table S4 (see online supplementary material) summarize antibody kinetics according to age group and receipt of a booster dose. In the overall population, at 7 months (T2), individuals who had received a booster dose had a higher GMT for anti-RBD IgG (223.3 U/mL vs 90.8 U/mL; $P<0.001$), anti-nucleocapsid IgG (53.2 COI vs 4.7 COI; $P<0.001$) and anti-spike trimeric IgG (349.5 BAU/mL vs 67.3 BAU/mL; $P<0.001$) than those who had only received two doses of CoronaVac (i.e. had not received a booster dose). In participants who did not receive a booster dose, antibody titres declined significantly after 7 months post-vaccination.

Seven months after the second dose, when the booster dose had been allowed only for elderly people and healthcare workers, among participants who received the booster dose, there was no difference between adults and elderly participants for anti-RBD IgG (216.9 U/mL vs 223.7 U/mL; $P=0.872$) and anti-nucleocapsid IgG (39.7 COI vs 54.1 COI; $P=0.283$), but adults presented a higher GMT for anti-spike trimeric IgG than elderly participants (498.7 BAU/mL vs 342.7 BAU/mL; $P=0.003$) (Table 2). Ten months after the second dose, when the booster had been allowed for all adults, levels of anti-RBD IgG, anti-nucleocapsid IgG and anti-spike trimeric IgG were significantly higher in adults who had received a booster dose than in elderly participants (246.2 U/mL vs 207.6 U/mL, $P<0.001$; 78 COI vs 32.7 COI, $P<0.001$; 892.1 BAU/mL vs 278.2 BAU/mL, $P<0.001$).

Factors influencing vaccination response

Before vaccination, 823 (21.5%) participants had contact with SARS-CoV-2, and 711 (18.2%) participants had confirmed COVID-19 after the second dose. On multi-variable analysis, based on the antibody titres at 4 months, age <60 years was independently associated with higher probability of post-vaccination infection (OR 3.99; $P<0.001$), whereas previous exposure to SARS-CoV-2 (OR 0.06; $P=0.007$) and antibody titres (anti-spike trimeric IgG: OR 0.73; $P=0.003$) was independently associ-

Table 2
Geometric mean for antibody titres (GMT) according to age group and receipt of a booster dose.

	Third dose	Age <60 years			Age ≥60 years			**Total**			***P*-value**[a]
		n	GMT	(95% CI)	*n*	GMT	(95% CI)	*n*	GMT	(95% CI)	
Participants with third dose in T2											
Anti-RBD IgG	No	1531	89.8	(85.0–94.7)	51	127.7	(92.5–176.4)	1582	90.8	(86.1–95.8)	**<0.001**
	Yes	67	216.9	(193.5–243.0)	1205	223.7	(216.1–231.5)	1272	223.3	(216.1–230.8)	0.872
Anti-nucleocapsid IgG	No	1531	4.6	(4.1–5.1)	51	12.5	(5.6–28.0)	1582	4.7	(4.2–5.3)	**<0.001**
	Yes	67	39.7	(24.2–65.1)	1205	54.1	(48.8–59.9)	1272	53.2	(48.1–58.8)	0.283
Anti-spike trimeric IgG	No	1531	65.3	(60.2–70.9)	51	164.4	(97.8–276.3)	1582	67.3	(62.0–73.0)	**<0.001**
	Yes	67	498.7	(630.6–689.6)	1205	342.7	(320.1–366.8)	1272	349.5	(326.9–373.7)	**0.003**
Participants with third dose in T3											
Anti-RBD IgG	No	41	201.5	(169.2–239.8)	21	165.9	(101.7–270.7)	62	188.6	(155.3–229.2)	0.546
	Yes	1166	246.2	(242.9–249.6)	970	207.6	(201.0–214.5)	2136	227.9	(224.1–231.7)	**<0.001**
Anti-nucleocapsid IgG	No	41	34.5	(17.3–68.4)	21	18.3	(5.3–63.5)	62	27.8	(15.2–50.8)	0.255
	Yes	1166	78.0	(71.3–85.4)	970	32.7	(29.0–36.8)	2136	52.6	(48.7–56.7)	**<0.001**
Anti-spike trimeric IgG	No	41	536.7	(313.4–918.8)	21	565.6	(235.2–1360.0)	62	546.3	(348.8–855.6)	0.809
	Yes	1166	892.1	(838.6–949.0)	970	278.2	(250.1–309.5)	2136	525.6	(493.0–560.2)	**<0.001**

CI, confidence interval; T2, 7months after the second vaccine dose; T3, 10 months after the second vaccine dose.
[a] *P*-value for comparison between age groups.

Table 3
Univariable and multi-variable analyses for coronavirus disease 2019 (COVID-19) in different periods.

Variables	T1–T2			T2–T3			T3–T4		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Univariable									
Age (<60 years)	**3.10**	**(1.76–5.47)**	**<0.001**	1.22	(0.94–1.59)	0.138	1.41	(0.76–1.71)	0.526
Gender (male)	1.10	(0.70–1.72)	0.692	0.79	(0.61–1.03)	0.077	0.68	(0.45–1.03)	0.066
Comorbidities	0.81	(0.50–1.30)	0.382	1.00	(0.77–1.30)	0.996	0.88	(0.58–1.34)	0.563
Diabetes	0.85	(0.42–1.71)	0.640	1.12	(0.78–1.59)	0.543	0.72	(0.38–1.36)	0.307
Hypertension	0.67	(0.39–1.17)	0.156	1.00	(0.75–1.33)	0.998	1.12	(0.72–1.72)	0.621
COVID-19 case up to T#	**0.04**	**(0.01–0.29)**	**0.001**	**0.43**	**(0.30–0.62)**	**<0.001**	**0.19**	**(0.10–0.38)**	**<0.001**
log (Anti-RBD) at T#	**0.79**	**(0.68–0.91)**	**0.001**	**0.81**	**(0.73–0.91)**	**<0.001**	0.88	(0.59–1.32)	0.536
log (Anti-N) at T#	**0.82**	**(0.74–0.91)**	**<0.001**	**0.89**	**(0.85–0.94)**	**<0.001**	0.94	(0.85–1.04)	0.223
log (Anti-S) at T#	**0.68**	**(0.58–0.81)**	**<0.001**	**0.83**	**(0.77–0.90)**	**<0.001**	**0.77**	**(0.69–0.87)**	**<0.001**
Multi-variable									
Age (<60 years)	**3.99**	**(2.23–7.12)**	**<0.001**	1.02	(0.74–1.41)	0.900	**1.95**	**(1.22–3.13)**	**0.005**
COVID-19 case up to T#	**0.06**	**(0.01–0.48)**	**0.007**	**0.51**	**(0.34–0.76)**	**0.001**	**0.76**	**(0.66–0.88)**	**<0.001**
log (Anti-S) at T#	**0.73**	**(0.59–0.90)**	**0.003**	**0.88**	**(0.79–0.97)**	**0.008**	**0.22**	**(0.11–0.43)**	**<0.001**

Anti-N, anti-nucleocapsid IgG; Anti-S, anti-spike trimeric IgG; CI, confidence interval; OR, odds ratio; #, equal to T1 for T1–T2, equal to T2 for T2–T3, and equal to T3 for T3–T4.

ated with lower probability of post-vaccination infection (Table 3). At 7 months, previous exposure to SARS-CoV-2 (OR 0.51; *P*=0.001) and antibody titres (anti-spike trimeric IgG: OR 0.88; *P*=0.008) continued to be independently associated with lower probability of post-vaccination infection adjusted by age. At 10 months, age <60 years was independently associated with higher probability of post-vaccination infection (OR 1.95; *P*=0.005), whereas previous exposure to SARS-CoV-2 (OR 0.76; *P*<0.001) and antibody titres (anti-spike trimeric IgG: OR 0.22; *P*<0.001) continued to be independently associated with lower probability of post-vaccination infection. Table S5 shows univariable analyses by age group. In a multi-variable analysis not including previous exposure to SARS-CoV-2 as an independent variable (Table S6, see online supplementary material), age <60 years continued to be independently associated with higher probability of post-vaccination infection at 4 and 10 months, whereas antibody titres of anti-spike trimeric IgG were independently associated with lower probability of post-vaccination infection at 4, 7 and 10 months.

Additional analysis of antibody titre kinetics in participants who received only two doses of CoronaVac

An additional analysis of antibody kinetics was performed considering only those participants who had received two doses of CoronaVac, excluding at each time point the participants who had received a booster dose or developed COVID-19 during the study (Table S7 and Figure S3, see online supplementary material). In these analyses, the pattern remained the same (i.e. there was an increase in antibody titres from pre-vaccination to 4 months and a decrease at 7 months). Of note, there was also an increase in antibody levels at 10 and 13 months. As the participants had not received the booster dose or had symptomatic COVID-19, this increase is attributed to asymptomatic SARS-CoV-2 infection. It is important to mention that the number of participants with only two doses was small at 10 and 13 months (*n*=71 and 21, respectively). Additionally, the 10-month collection was at the end of January 2022, during the Omicron wave in Brazil.

Discussion

In this real-world study, adult and elderly recipients of CoronaVac achieved high seroconversion and seropositivity rates for anti-RBD over 13 months following vaccination. Seropositivity was slightly lower (1.5%) in elderly participants than in adults at 4 months, but not in the following months, probably due to the booster dose.

Previous studies have shown similar seropositivity rates with CoronaVac in the short term. In a phase 3 trial, the seropositivity rate reached 89.7% 14 days after the second dose [6]. In a real-world study, CoronaVac had IgG seropositivity of 77.4% 3 weeks after the second dose and 47.3% after 16 weeks [12]. Of note, in the present study, seropos-

itivity in adults was high after two doses of CoronaVac. The different results could be explained by diagnostic test sensitivity, and reinforce the need to use highly accurate tests and not rely solely on point-of-care serological tests [16].

Four months after the second dose of CoronaVac, titres for anti-RBD IgG, anti-nucleocapsid IgG and anti-spike trimeric IgG were significantly lower in elderly participants than in adults. A reduced immune response in elderly participants has been described previously as a consequence of immunosenescence [10]. In elderly people, the decrease in immune response has been described for several COVID-19 vaccines, such as BNT162b2 [9,17] and CoronaVac [18].

At 7 months, anti-RBD IgG titres in adults remained stable, whereas anti-nucleocapsid IgG and anti-spike trimeric IgG titres decreased significantly. A similar pattern was observed at 10 months for elderly participants and 13 months for adults (i.e. the titres increased after the booster dose, but some antibody levels decreased). The decrease in antibody levels between 4 and 7 months after the second dose, and approximately 4–6 months after the booster dose, although small and not for all antibodies, indicates waning of the immune response. In a prospective study with 120 participants, antibody levels to the BNT162b2 vaccine declined significantly approximately 12 weeks after the second dose [19]. In Chile, two studies using different serological tests showed that antibody production declines over time in individuals vaccinated with CoronaVac and BNT162b2, being less pronounced for BNT162b2 [12,20]. Previous studies showed a substantial decrease in the humoral response 6 months after the second dose of BNT162b2, especially in men, older individuals and immunosuppressed individuals [21,22].

As a consequence of the booster dose, all antibody titres increased in adults and elderly participants, reinforcing its importance in increasing the immune response. In the present study, three doses of CoronaVac increased antibody levels significantly, although some studies have shown higher increases with heterologous vaccine regimens [20,23–25]. More studies are needed to assess the effectiveness of different booster administration strategies.

Although the immune response to natural infection induces antibody production for different virus antigens, spike and nucleocapsid proteins are the dominant antigens [26]. Based on this and the mechanism of action of the CoronaVac vaccine (inactive virus), the antibody titres for RBD-specific IgG, spike trimeric protein IgG and nucleocapsid IgG were assessed in the current study as they provide different information and involve different mechanisms. The spike protein is more specific for diagnostic purposes and has been used most frequently [16,27]. The nucleocapsid protein is a less variable portion, but tests may lead to false-positive results [26]. Batra et al. demonstrated that a high concentration of IgG against the nucleocapsid protein was a prognostic factor for the clinical course of disease, being independently associated with admission to an intensive care unit and longer hospital stay [28]. Recent development of tests targeting the trimeric form of the spike protein could have better correlation with neutralization activity for testing other domains related to virus internalization mechanism [29,30]. The use of a chimeric protein, consisting of the nucleocapsid and spike-1 protein components of SARS-CoV-2, has been indicated as a potential vaccine candidate [31].

This study also showed that previous infections and high levels of antibody were associated with decreased risk of having COVID-19, whereas age <60 years was a risk factor for COVID-19. High levels of binding and neutralizing antibodies after COVID-19 vaccines were correlated with reduced risk of symptomatic infection [32,33]. These data reinforce the importance of approved COVID-19 vaccines and the booster dose in reducing the risk of symptomatic infection.

This study has limitations. First, neutralizing antibodies were not evaluated. Second, as comorbidities were based on self-report, the authors could not confirm their existence or quantify their severity or the degree of associated immunosuppression. Finally, as samples were not diluted and several samples reached the upper limit of the test, seroconversion and the increase in antibody level may have been underestimated.

Conclusion

This study provides additional data on the vaccine immune response and highlights the importance of using highly accurate tests for the evaluation of vaccine strategies in a real-world study. The results demonstrate a significant immune response after two doses of CoronaVac, and a significant increase in antibody levels after a booster dose in adults and elderly participants. A decline in antibody titres was observed at 7 months post-vaccination in participants who had not received a booster dose, and approximately 4–6 months after the booster dose. Higher levels of antibodies and previous SARS-CoV-2 infection were associated with protection against breakthrough COVID-19.

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Ethical approval

Ethical approval for this study was obtained from the Ethics Committee of Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto–University of São Paulo.

Data sharing

Anonymous participant data will be available upon request to the corresponding author (marcosborges@fmrp.usp.br). Proposals will be reviewed and approved by the sponsor, researcher and staff on the basis of scientific merit and absence of competing interests. After the proposal has been approved, data can only be shared through a secure online platform after a data access and a confidentiality agreement are signed.

Author contributions

MCB, GJV, NNF, PMMG, SCSV and DTC contributed to the conception and design of the study. MCB, GJV, NNF, PMMG, GRM, SCSV, LBS, MAALSA, PEB, SK, HABG, JDF, MAL, PHMP and DTC were involved in the acquisition of data. MCB, GJV, NNF, PMMG, GRM, SK, BALF, RTC, LBS, MAALSA, PEB, SK, HABG, JDF, MAL and PHMP contributed to the analysis and interpretation of data. MCB and GJP drafted the manuscript. MCB, GJV, NNF, PMMG, GRM, SK, BALF, RTC, SCSV, LBS, MAALSA, PEB, SK, HABG, JDF, MAL, PHMP and DTC edited the manuscript. All authors critically reviewed the manuscript and approved the final version. All authors had full access to all data in the studies, and had final responsibility for the decision to submit for publication

Conflict of interest statement

MCB, GJV, NNF, PMMG, GRM, BALF and RTC received research support from Instituto Butantan during the conduct of this study. SCSV, LBS, MAALSA, PEB, SK, HABG, JDF, MAL, PHMP and DTC are employees of Instituto Butantan.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2023.04.003.

References

[1] Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* 2020;**20**:533–4.

[2] Feikin DR, Higdon MM, Abu-Raddad LJ, Andrews N, Araos R, Goldberg Y, et al. Duration of effectiveness of vaccines against SARS-CoV-2 infection and COVID-19 disease: results of a systematic review and meta-regression. *Lancet* 2022;**399**:924–44.

[3] Ministério da Saúde do Brasil *Vacinômetro COVID-19*. Rio de Janeiro: Ministério da Saúde do Brasil; 2021.

[4] Wu Z, Hu Y, Xu M, Chen Z, Yang W, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021;**21**:803–12.

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

[6] Tanriover MD, Doganay HL, Akova M, Guner HR, Azap A, Akhan S, et al. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet* 2021;**398**:213–22.

[7] Palacios R, Patino EG, de Oliveira Piorelli R, Conde M, Batista AP, Zeng G, et al. Double-blind, randomized, placebo-controlled phase III clinical trial to evaluate the efficacy and safety of treating healthcare professionals with the adsorbed COVID-19 (inactivated) vaccine manufactured by Sinovac – PROFISCOV: a structured summary of a study protocol for a randomised controlled trial. *Trials* 2020;**21**:853.

[8] Borges MC, Palacios R, Brango HA, Conde MTRP, Patiño EG, Batista AP, et al. Projeto S: a stepped-wedge randomized trial to assess CoronaVac effectiveness in Serrana, Brazil. Available at: http://doi.org/10.2139/ssrn.3973422.

[9] Muller L, Andree M, Moskorz W, Drexler I, Walotka L, Grothmann R, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 coronavirus disease 2019 vaccination. *Clin Infect Dis* 2021;**73**:2065–72.

[10] Gustafson CE, Kim C, Weyand CM, Goronzy JJ. Influence of immune aging on vaccine responses. *J Allergy Clin Immunol* 2020;**145**:1309–21.

[11] Lee A, Wong SY, Chai LYA, Lee SC, Lee MX, Muthiah MD, et al. Efficacy of COVID-19 vaccines in immunocompromised patients: systematic review and meta-analysis. *BMJ* 2022;**376**:e068632.

[12] Saure D, O'Ryan M, Torres JP, Zuniga M, Santelices E, Basso LJ. Dynamic IgG seropositivity after rollout of CoronaVac and BNT162b2 COVID-19 vaccines in Chile: a sentinel surveillance study. *Lancet Infect Dis* 2022;**22**:56–63.

[13] Jaaskelainen AJ, Kuivanen S, Kekalainen E, Ahava MJ, Loginov R, Kallio-Kokko H, et al. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. *J Clin Virol* 2020;**129**:104512.

[14] Ferreira NN, Garibaldi PMM, Moraes GR, Moura JC, Klein TM, Machado LE, et al. The impact of an enhanced health surveillance system for COVID-19 management in Serrana. *Brazil. Public Health Pract* 2022;**4**:100301.

[15] WHO Working Group on the Clinical Characterisation Management of COVID-19 infectionA minimal common outcome measure set for COVID-19 clinical research. *Lancet Infect Dis* 2020;**20**:e192–7.

[16] Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP, Johnston JC, et al. Diagnostic accuracy of serological tests for COVID-19: systematic review and meta–analysis. *BMJ* 2020;**370**:m2516.

[17] Collier DA, Ferreira I, Kotagiri P, Datir RP, Lim EY, Touizer E, et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature* 2021;**596**:417–22.

[18] Seyahi E, Bakhdiyarli G, Oztas M, Kuskucu MA, Tok Y, Sut N, et al. Antibody response to inactivated COVID-19 vaccine (CoronaVac) in immune-mediated diseases: a controlled study among hospital workers and elderly. *Rheumatol Int* 2021;**41**:1429–40.

[19] 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.

[20] Vargas L, Valdivieso N, Tempio F, Simon V, Sauma D, Valenzuela L, et al. Serological study of CoronaVac vaccine and booster doses in Chile: immunogenicity and persistence of anti-SARS-CoV-2 spike antibodies. *BMC Med* 2022;**20**:216.

[21] Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning immune humoral response to BNT162b2 COVID-19 vaccine over 6 months. *N Engl J Med* 2021;**385**:e84.

[22] Tober-Lau P, Schwarz T, Vanshylla K, Hillus D, Gruell H, et al., Group ECS Long-term immunogenicity of BNT162b2 vaccination in older people and younger health-care workers. *Lancet Respir Med* 2021;**9** e104–e5.

[23] Pozzetto B, Legros V, Djebali S, Barateau V, Guibert N, Villard M, et al. Immunogenicity and efficacy of heterologous ChAdOx1-BNT162b2 vaccination. *Nature* 2021;**600**:701–6.

[24] Marra AR, Miraglia JL, Malheiros DT, Guozhang Y, Teich VD, da Silva Victor E, et al. Effectiveness of heterologous COVID-19 vaccine booster dosing in Brazilian healthcare workers, 2021. *Clin Infect Dis* 2023;**76**:e360–6.

[25] Leung NHL, Cheng SMS, Martin-Sanchez M, Au NYM, Ng YY, Luk LLH, et al. Immunogenicity of a third dose of BNT162b2 to ancestral SARS-CoV-2 & Omicron variant in adults who received two doses of inactivated vaccine. *Clin Infect Dis* 2022.

[26] Jiang HW, Li Y, Zhang HN, Wang W, Yang X, Qi H, et al. SARS-CoV-2 proteome microarray for global profiling of COVID-19 specific IgG and IgM responses. *Nat Commun* 2020;**11**:3581.

[27] Mekonnen D, Mengist HM, Derbie A, Nibret E, Munshea A, He H, et al. Diagnostic accuracy of serological tests and kinetics of severe acute respiratory syndrome coronavirus 2 antibody: a systematic review and meta-analysis. *Rev Med Virol* 2021;**31**:e2181.

[28] Batra M, Tian R, Zhang C, Clarence E, Sacher CS, Miranda JN, et al. Role of IgG against N-protein of SARS-CoV2 in COVID19 clinical outcomes. *Sci Rep* 2021;**11**:3455.

[29] Bonelli F, Blocki FA, Bunnell T, Chu E, De La OA, Grenache DG, et al. Evaluation of the automated LIAISON((R)) SARS-CoV-2 TrimericS IgG assay for the detection of circulating antibodies. *Clin Chem Lab Med* 2021;**59**:1463–7.

[30] Fenwick C, Croxatto A, Coste AT, Pojer F, Andre C, Pellaton C, et al. Changes in SARS-CoV-2 spike versus nucleoprotein antibody responses impact the estimates of infections in population-based seroprevalence studies. *J Virol* 2021;**95** e01828-20.

[31] Kumar A, Ladha A, Choudhury A, Ikbal AMA, Bhattacharjee B, Das T, et al. The chimera of S1 and N proteins of SARS-CoV-2: can it be a potential vaccine candidate for COVID-19? *Expert Rev Vaccines* 2022;**21**:1071–86.

[32] Gilbert PB, Montefiori DC, McDermott AB, Fong Y, Benkeser D, Deng W, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* 2022;**375**:43–50.

[33] Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;**27**:1205–11.