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SHORT COMMUNICATION

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Antibody response 6 months after the booster dose of Pfizer in previous recipients of CoronaVac

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Abstract

The most widely used vaccines were messenger RNA (mRNA), viral vector, and inactivated virus with two-dose schedules. In Brazil, the CoronaVac (Sinovac) was the first vaccine approved for emergency use, and the third dose was administered, preferably, with the BNT162b2 vaccine. We evaluated antibody levels after 6 months of the booster dose with BNT162B2 in previous recipients of CoronaVac and whether a subsequent severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infection enhances the antibody response. We analyze the humoral response (spike [S] IgM for the SARS-CoV-2 and IgG for the S and nucleocapsid [N] proteins) in samples collected before the third dose and 6 months after the third dose. The presence of antibodies was measured by using Abbott Architect i2000SR. The IgM and IgG antispikes were stimulated mainly 30 days after the third dose (30d/3D), with a decline over time. The IgG anti-N was stimulated predominantly in 90d/3D and 180d/3D. The N IgG levels were 50 and 35 times higher in the positive polymerase chain reaction (PCR) group in 90d/3D and 180d/3D, respectively. The S IgG titers were 1.5 times elevated in the positive PCR group, in 180d/3D. The BNT162b2 boosted the S IgG levels, decreasing after 60 days. The booster shot induced IgM and IgG antibodies against spike protein. Infection after vaccination increased antibodies against protein N.

KEYWORDS
antibodies, BNT162B2 vaccine, booster dose, CoronaVac, COVID-19 vaccine, SARS-CoV-2

1 | INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) coronavirus pandemic[1] initiated a scientific race for the development of vaccines.[2] The most widely used vaccines were messenger RNA (mRNA), viral vector, and inactivated virus with two-dose schedules. With waning immunity after complete vaccination of coronavirus disease 2019 (COVID-19)[3] and some evidence of reduced effectiveness against new variants,[4] many countries offered booster doses. In Brazil, the CoronaVac (Sinovac) was the first vaccine approved for emergency use, and the third dose was administered, preferably, with the BNT162b2 vaccine (Pfizer/BioNTech). Previously, we showed that the third dose with the BNT162b2 vaccine boosted the anti-spike (S) antibody titers in recipients of CoronaVac.[5] In the current cohort, we evaluated the antibody levels after 6 months of the booster dose and whether the omicron wave in Brazil impacted the antibody response.

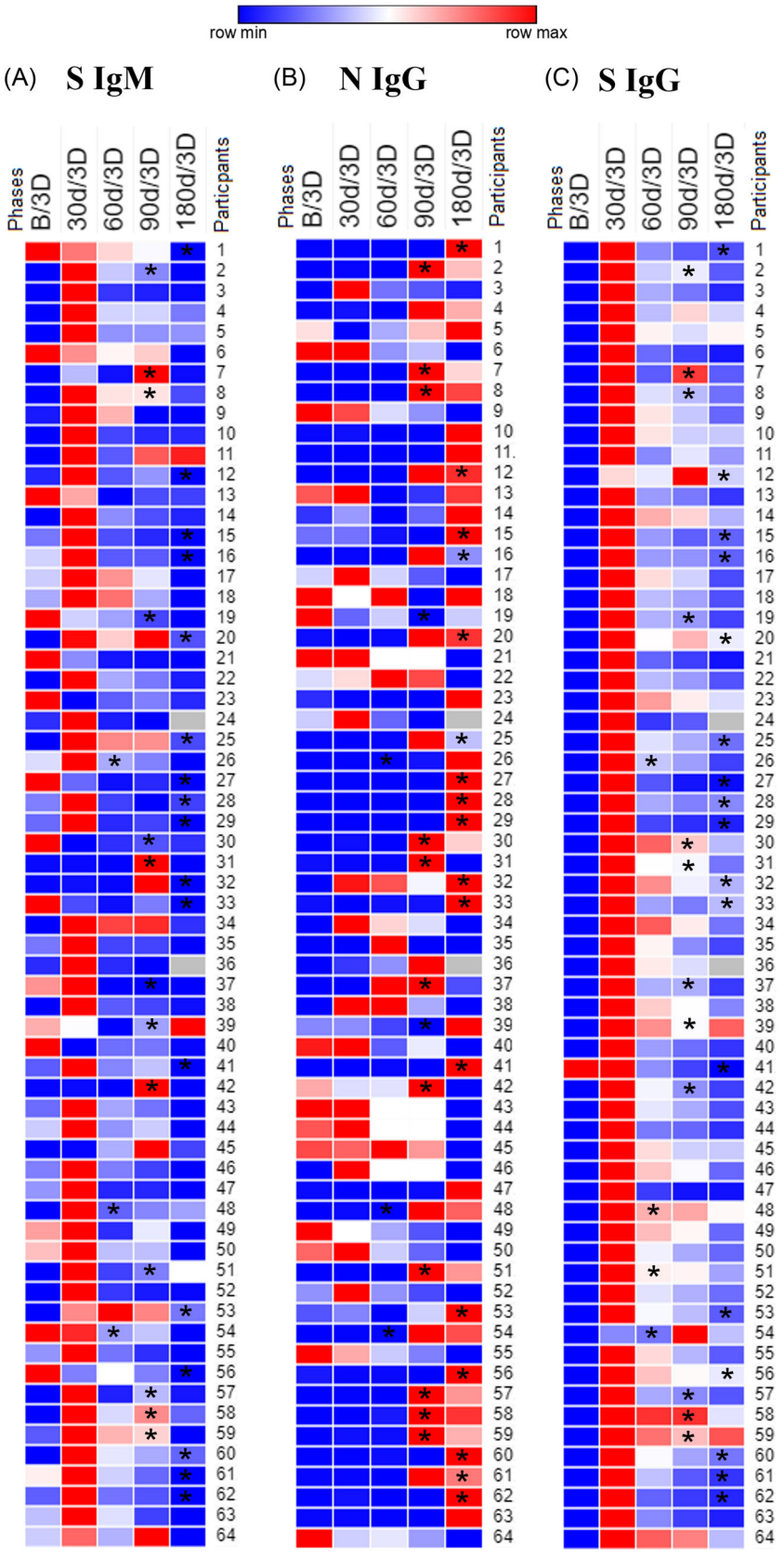

FIGURE 1 Heatmap representing antibody levels before and after the BNT162b2 booster shot in previous recipients of CoronaVac. (A) S IgM, (B) N IgG, and (C) S IgG levels. Antibody responses were evaluated before the third dose (B/3D), 30 (30d/3D), 60 (60d/3D), 90 (90d/3D), and 180 (180d/3D) days after the third dose, in 64 participants. The deeper red color represents the higher relative intensity, the deeper blue color the lower relative intensity, and the intermediary intensity is a white color. In phase 180d/3D, the deeper gray corresponds to the absence of antibody data from two participants.
PCR, polymerase chain reaction. *Represents participants with positive PCR in respective phase.

2 | MATERIALS AND METHODS

We included 64 participants of both sexes, ≥19 years of age, who had received two doses of CoronaVac between January to February 2021, with an interval of 28 days between doses, and a booster dose of BNT162b2 vaccine in October 2021, 8 months after the second dose of CoronaVac. Blood collections and serological tests were performed at Fundação Oswaldo Cruz (Fiocruz, Ceará, Brazil) after an informed consent was obtained from the participants. We performed a longitudinal analysis of the humoral response (IgM for the SARS-CoV-2 spike protein (S) and IgG for the S and nucleocapsid (N) proteins) in samples collected before the third dose (B/3D) and 30 (30d/3D), 60 (60d/3D), 90 (90d/3D) and 180 (180d/3D) days after the third dose. The presence of antibodies was measured by using Abbott Architect i2000SR (Abbott®). The cut-off value was 1.0 index value (S/C) for S IgM antibodies, 50 AU/ml for S IgG, and 1.4 index value for N IgG. The volunteers were monitored for SARS-CoV-2 infection by polymerase chain reaction (PCR) over time. During the study, all participants were tested for SARS-CoV-2 infection by reverse transcription (RT)-PCR regularly. The asymptomatic participants were tested monthly, and the symptomatic participants were tested within 5 days of symptom onset. GraphPad Prism version 5.0 (https://www.graphpad.com) was used for statistical analyses. The data were described as the median and interquartile range or percentage. Analysis of variance was used to analyze the antibody titers over time and Mann–Whitney test in group comparisons. The matrix of antibodies was imported into the Morpheus program (https://software.broadinstitute.org/morpheus/) and the results were illustrated as a three-dimensional dendrogram (heat map). Differences with $p < 0.05$ were considered statistically significant.

3 | RESULTS AND DISCUSSION

Although all participants completed the vaccination schedule, two participants were unable to give a blood sample in 180d/3D. The cohort had a greater representation of female participants, with 81.54% female and 26.09% male. The average age of the cohort was 33.48 (95% confidence interval [CI]: 19–69 years).

We evaluated the antibody levels for S protein (IgM and IgG) and N protein (IgG) before and after the booster shot (Figure 1 and Table 1). The IgM and IgG anti-spike were stimulated mainly in 30d/3D with a significant decline over time ($p < 0.0001$). However, the IgG anti-N was stimulated predominantly in 90d/3D and 180d/3D.

After the BNT162b2, 35 (54.69%) of the participants had a positive PCR result, of which in 3/64 (4.69%) in 60d/3D, 13/64 (20.31%) in 90d/3D, and 19/62 (30.65%) in 180d/3D. The main symptoms reported were cough (73.08%), runny nose (65.38%), sore throat (61.57%), headache (61.54%), body pain (61.54%), low back pain (26.92%), and diarrhea and abdominal pain (19.23%). To evaluate the impact of a SARS-COV-2 infection postbooster dose on the antibody response, the volunteers were divided into two groups, those who had positive PCR after the booster shot and who did not have (Figure 2). There was no statistically significant difference between the groups in terms of S IgM levels (Figure 2A and Table 2). Otherwise, the N IgG levels were 50 and 35 times higher in the positive PCR group in 90/3D and 180/3D, respectively ($p < 0.0001$) (Figure 2B and Table 2). The S IgG titers were 1.5 times elevated in the positive PCR group, in 180/3D ($p = 0.0167$) (Figure 2C and Table 2).

We found that the BNT162b2 booster shot elicits IgM and IgG antibodies against spike protein. BNT162b2 vaccine-elicited IgM antibodies also were reported by Ruggiero et al.[6] They described that S-IgG- and S-IgM-positive sera were more efficient in virus-neutralizing activity, compared to the S-IgM negative. This finding highlights a possible crucial role of IgM in the development of anti-SARS-CoV-2 humoral response, after vaccination.

The administration of CoronaVac in a two-dose schedule followed by a booster dose of BNT162b2 could not contain a SARS-CoV-2 infection in the current cohort, during the omicron wave in Brazil. The omicron peak outbreak in Brazil was between November 2021 and March 2022.[7,8] The cohort received the booster dose of the BNT162b2 vaccine in October 2021, 8 months after the second dose of CoronaVac, and 1 month before the omicron had emerged in Brazil. However, 55% of the participants were infected by SARS-CoV-2 following the booster shot. The S protein is exposed on the surface of virions and mediates the entry into host cells. It is the primary target of the most approved vaccines for COVID-19.[9] Nonetheless, Omicron has 50 mutations in its genetic code, of which

TABLE 1 Median (and IQR) values of the antibody levels before and after the BNT162b2 booster shot in previous recipients of CoronaVac

Phases	S IgM antibodies	*p* Value	N IgG antibodies	*p* Value	S IgG antibodies	*p* Value
B/3D	0.16 (0.06–0.36)	-	0.12 (0.07–0.65)	-	195.9 (77.43–797.5)	-
30d/3D	0.54 (0.23–1.14)	<0.0001	0.18 (0.08–0.70)	0.5753	42 106 (31 040–75 540)	<0.0001
60d/3D	0.24 (0.11–0.49)	<0.0001	0.15 (0.06–0.65)	0.5720	20 264 (11 044–30 671)	<0.0001
90d/3D	0.29 (0.11–0.80)	0.4177	0.60 (0.06–8.66)	0.0135	17 249 (9617–30 671)	0.1790
180d/3D	0.13 (0.07–0.33)	0.0007	2.98 (0.17–7.60)	0.4038	10 229 (6346–18 441)	0.0010

Abbreviations: B/3D, before the third dose; 30d/3D, 30 days after the third dose; 60d/3D, 60 days after the third dose; 90d/3D, 90 days after the third dose; 180d/3D, 180 days after the third dose; IQR, interquartile range; N, nucleocapsid protein; S, spike protein.

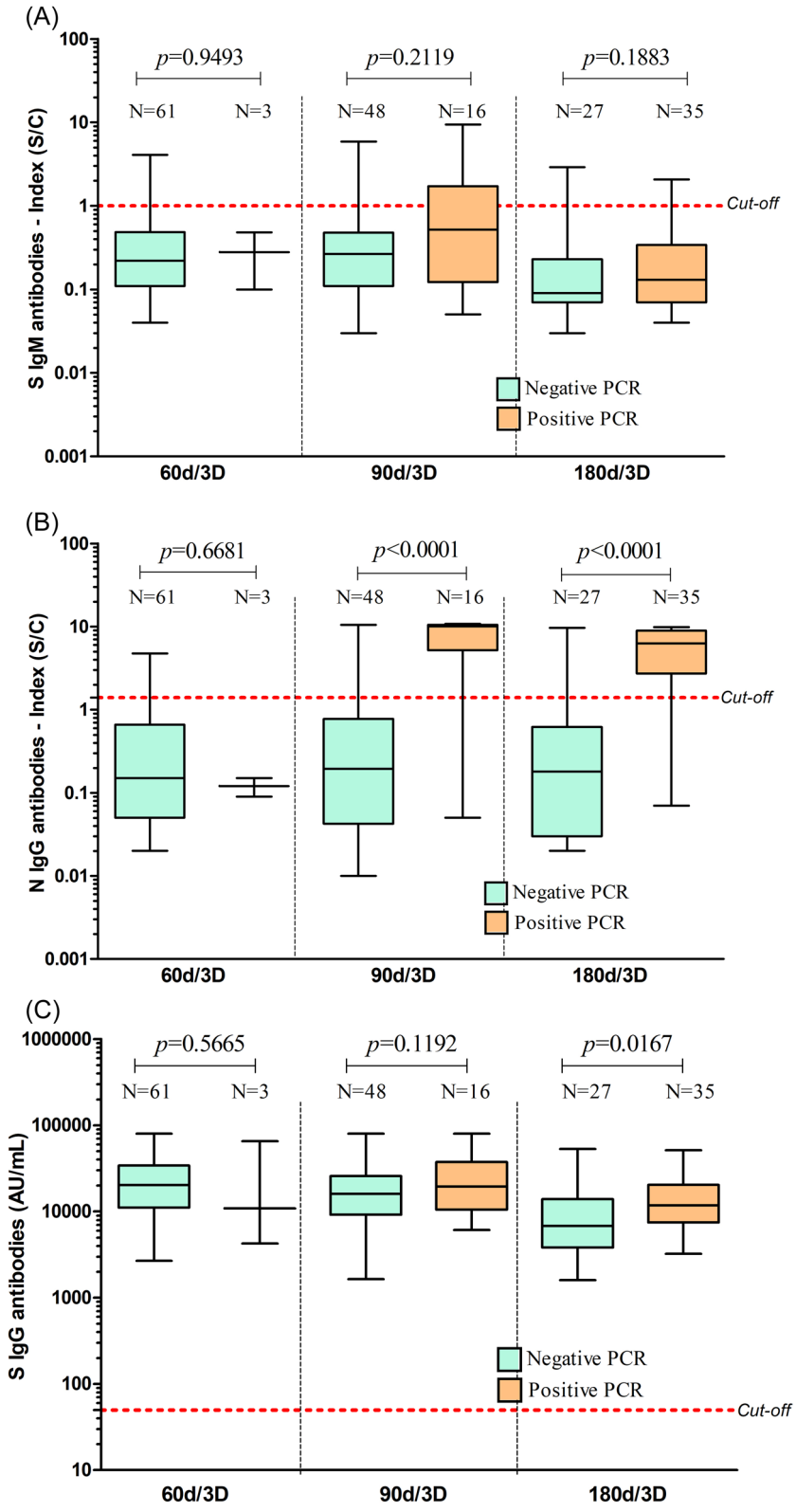

FIGURE 2 Comparison of antibody response before and after the BNT162b2 booster shot in previous recipients of CoronaVac, by PCR positivity status. (A) S IgM levels, (B) N IgG levels, and (C) S IgG levels. Antibody responses were evaluated before the third dose (B/3D), 30 (30d/3D), 60 (60d/3D), 90 (90d/3D), and 180 (180d/3D) days after the third dose. Horizontal black lines in the boxplots represent median levels values and error bars min and max values; the horizontal red dotted lines indicate the cutoff value of the assays. Statistical analysis was performed using the Kruskal–Wallis test with subsequent Dunn's multiple testing correction. N, nucleocapsid protein; S, spike protein; S/C, signal- to- cutoff- ratio.

32 are in the gene encoding the spike protein, facilitating the Omicron transmission,[10] immune evasion, and replication[7] explaining the infection in vaccinated individuals.

The highest S IgM and S IgG levels were founded 30 days after the booster dose. On the other hand, higher N IgG levels were noted 90 and 180 days after the booster shot, when the most of participants were infected. Since mRNA vaccines do not induce a response to the N protein,[7,8] the N IgG appears to be induced mostly by the infection. Interestingly, the infection boosted the N IgG antibodies by up to 50-fold. The viral N protein is a highly conserved

TABLE 2 Median (and IQR) values of the antibody levels after the BNIT162b2 booster shot in previous recipients of CoronaVac, according to postvaccination SARS-COV-2 infection

		S IgM antibodies	*p* Value	N IgG antibodies	*p* Value	S IgG antibodies	*p* Value
Negative PCR	60d/3D	0.22 (0.11–0.49)	-	0.15 (0.05–0.66)	-	20 169 (11 092–34 108)	-
	90d/3D	0.27 (0.11–0.49)	0.8355	0.20 (0.04–0.78)	0.7299	16 045 (9188–25 785)	0.0618
	180d/3D	0.09 (0.07–0.23)	0.0034	0.18 (0.03–0.62)	0.5615	6779 (3825–13 930)	0.0010
Positive PCR	60d/3D	0.28 (0.1–0.48)	-	0.12 (0.09–0.15)	-	10 829 (4276–65 400)	-
	90d/3D	0.52 (0.12–1.71)	0.5022	10.06 (5.21–10.54)	0.0219	19 485 (10 489–37 394)	0.5386
	180d/3d	0.13 (0.07–0.34)	0.0185	6.30 (2.73–8.95)	0.0035	11 751 (7468–20 366)	0.0339

Abbreviations: 60d/3D, 60 days after the third dose, 90d/3D, 90 days after the third dose; 180d/3D, 180 days after the third dose; IQR, interquartile range; N, nucleocapsid protein; PCR, polymerase chain reaction; S, spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

nucleoprotein, with functions associated with RNA transcription and viral replication, and is abundantly expressed during infection.[11] Thus, the high replication ability of Omicron could have allowed a repeated exposure of the N protein to the immune cells. The N protein is highly immunogenic, explaining the high levels of N antibodies found.[12]

The BNT162b2 boosted the S IgG levels, however, waned 60 days after the booster dose. Since not all vaccine-stimulated B-lymphocytes are maintained as memory cells,[13] the decline of antibodies following vaccination is expected. Successful vaccination depends on the induction of long-term immunological memory.[14] The infected participants exhibited S IgG levels 1.5 times higher than uninfected, 180 days after the booster shot. Thus, this finding suggests an immune memory for at least 6 months after the booster dose.[15]

In summary, a booster BNT162B2 shot in previous recipients of CoronaVac induced IgM and IgG antibodies against spike protein. Although immunization with two doses of CoronaVac and a booster dose with the BNT162b2 vaccine provided limited protection against symptomatic disease during the last wave of COVID-19 in Brazil, the vaccines induced an immune memory 6 months after the booster shot. In addition, a SARS-CoV-2 infection after vaccination boosted the antibodies against nucleocapsid protein.

AUTHOR CONTRIBUTIONS

Maria Francilene Souza Silva and Marcela Helena Gambim Fonseca conceived the work and contributed to the design of the study and the writing of the manuscript. Maria Francilene Souza Silva, Ana Carolina Matias Dinelly Pinto, Fátima de Cássia Evangelista de Oliveira, Fernanda Montenegro de CarvalhoAraújo, and Ludmilla Freire Caetano were responsible for the recruitment, follow-up, data collection, laboratory analysis, and data processing work. Maria Francilene Souza Silva made the graphs and figures. Fernanda Montenegro de Carvalho Araújo supervised the project. All authors were involved in writing, reviewing, and editing, and approved the final manuscript version.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The study was approved by the Ethics Committee of the Hospital Geral Dr. César Cals, through CAAE 39691420.7.0000.5049. Informed consent was obtained from all the individual participants.

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