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

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Abstract

We evaluated antibody levels after six months of the booster dose with BNT162B2 in previous recipients of CoronaVac and whether a subsequent SARS-COV-2 infection enhances the antibody response. The booster shot induced IgM and IgG antibodies against Spike protein. Infection after vaccination increased antibodies against protein N.

INTRODUCTION

The SARS-CoV-2 coronavirus pandemic (Zhu et al., 2020) initiated a scientific race for the development of vaccines (Graham, 2020). The most widely used vaccines were mRNA, viral vector, and inactivated virus with two-dose schedules. With waning immunity after complete vaccination of COVID-19 (Fonseca, de Souza et al., 2022) and some evidence of reduced effectiveness against new variants (Andrews et al., 2022), 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 antibody titers in recipients of CoronaVac (Fonseca, Pinto et al., 2022). Now, we evaluated in the current cohort, the antibody levels after six months of the booster dose and whether the omicron wave in Brazil impacted the antibody response.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Hospital Geral Dr. César Cals, through CAAE 39691420.7.0000.5049. 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 Fundacao Oswaldo Cruz (Fiocruz, Ceara, Brazil), after an informed consent. 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), 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$^{(r)}$). The cut-off value was 1.0 index value (S/C) for S IgM antibodies, 50AU/ml for S IgG, and 1.4 index value for N IgG. The volunteers were monitored for SARS-CoV-2 infection by PCR over time. The GraphPad Prism version 5.0 (https://www.graphpad.com) was used for statistical analyses. The data were described

as the median and interquartile range (IQR) or percentage. ANOVA 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 3D dendrogram (heat map). Differences with $p<0.05$ were considered statistically significant.

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% 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, Appendix 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 90/3D and 180/3D.

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%), diarrhea and abdominal pain (19.23%). To evaluate the impact of a SARS-COV-2 infection post-booster 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 2, panel A, Appendix 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 2, panel B, Appendix table 2). The S IgG titers were 1.5 times elevated in the positive PCR group, in 180/3D ($p= 0.0167$) (Figure 2, panel C, Appendix 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 and colleagues (Ruggiero et al., 2022). 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 and March 2022 (Duong et al., 2022; Ricardo Valverde, 2022). The cohort received the booster dose of the BNT162b2 vaccine in October 2021, eight 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 (Muller et al., 2022). Nonetheless, Omicron has 50 mutations in its genetic code, of which 32 are in the gene encoding the Spike protein, facilitating the Omicron transmission (Saxena et al., 2022), immune evasion, and replication (Duong et al., 2022) 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, the 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 (Duong et al., 2022; Ricardo Valverde, 2022), 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 nucleoprotein, with functions associated with RNA transcription and viral replication, and is abundantly expressed during infection (Zeng et al., 2020). 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 (Galipeau et al., 2020).

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 (Palm e Henry, 2019), the decline of antibodies following vaccination is expected. Success vaccination depends on the induction of long-term

immunological memory (Siegrist, 2003). The infected participants exhibited S IgG levels 1.5 times higher than uninfected, 180 days after the booster shot. So, this finding suggests an immune memory for at least 6 months after the booster dose (Gray et al., 2021).

Limitations of this study are the absence of cellular immunity and neutralizing antibodies assays and relatively small sample size.

CONCLUSIONS

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 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.

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ETHICS STATEMENT

The study was approved by the Ethics Committee of the Hospital Geral Dr. Cesar Cals, through CAAE 39691420.7.0000.5049.

CONFLITO DE INTEREST

The authors declare that there is no conflict of interest

AUTHOR CONTRIBUTIONS

M.F.S.S. and M.H.G.F. conceived the work, contributed to the design of the study and the writing of the manuscript. M.F.S.S, A.C.M.D.P., F.C.E.O, F.M.C.A and L.F.C. were responsible for the recruitment, follow up, data collection, laboratory analysis and data processing work. M.F.S.S made the graphs and figures. F.M.C.A supervised the project. All authors involved in writing, review and editing, approved the final manuscript version.

DATA AVAILABILITY STATEMENT

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

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Figure 1. Heatmap representing antibody levels before and after the BNT162b2 booster shot in previous recipients of CoronaVac. A) S IgM levels. B) N IgG levels. 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.

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. 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.

Appendix . Additional information about Antibody response six months after the booster dose of Pfizer in previous recipients of CoronaVac.