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RESEARCH ARTICLE

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Neutralizing antibody response after immunization with a COVID-19 bivalent vaccine: Insights to the future

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Abstract

The raising of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants led to the use of COVID-19 bivalent vaccines, which include antigens of the wild-type (WT) virus, and of the Omicron strain. In this study, we aimed to evaluate the impact of bivalent vaccination on the neutralizing antibody (NAb) response. We enrolled 93 volunteers who had received three or four doses of monovalent vaccines based on the original virus ($n = 61$), or a booster

Milena Silva Souza and Jéssica Pires Farias contributed equally to this study and share first authorship.

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shot with the bivalent vaccine ($n = 32$). Serum samples collected from volunteers were subjected to neutralization assays using the WT SARS-CoV-2, and Omicron subvariants. In addition, immunoinformatics to quantify and localize highly conserved NAb epitopes were performed. As main result, we observed that the neutralization titers of samples from individuals vaccinated with the bivalent vaccine were higher for the original virus, in comparison to their capacity of neutralizing the Omicron variant and its subvariants. NAb that recognize epitopes mostly conserved in the WT SARS-CoV-2 were boosted, while those that recognize epitopes mostly present in the Omicron variant, and subvariants were primed. These results indicate that formulation of future vaccines shall consider to target present viruses, and not viruses that no longer circulate.

KEYWORDS
bivalent vaccine, boost, epitope, Omicron, SARS-CoV-2

1 | INTRODUCTION

More than 763 million people have been affected globally by the *Severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2)[1] since its emergence, resulting in more than 6.9 million deaths.[2] The numbers of COVID-19 cases have diminished mainly due to the progress of vaccination[3,4] using antigens based on the wild-type (WT) virus. However, from the late 2020 to early 2023, Alpha, Beta, Gamma, Delta, and Omicron variants of concern (VOC) have emerged with increasing capacity of abrogating several neutralizing antibodies (NAbs) epitopes, particularly the Omicron variant and its subvariants. This led to the development and use of messenger RNA-based bivalent COVID-19 vaccines, which code antigens of the original virus strain as well as of the Omicron variant or Omicron subvariants (BA.1 or BA.4 and BA.5).[5,6] Although such new vaccines have been shown to be effective in preventing symptomatic infections,[6] the World Health Organization (WHO) recognizes that the risk of new VOCs emergence remains.[7] In this study, we aimed to evaluate the impact of vaccinating with the Pfizer COVID-19 bivalent (COMINARTY Original/Omicron BA.4-5 COVID-19 vaccine) on the induction of NAb responses. We enrolled 93 volunteers that had received three or four doses of monovalent vaccines ($n = 61$), and individuals who received a booster shot with the bivalent vaccine ($n = 32$) (Figure 1A). Demographic information (Supplementary Material S1) as well as serum samples were collected. Serum neutralization assays were carried out using the WT SARS-CoV-2, the original Omicron variant (BA.1), and its subvariants FE.1.2 and BQ.1.1. In addition, immunoinformatics analyses permitted the quantification and location of highly conserved NAb epitopes in the WT SARS-CoV-2 and Omicron subvariants.

2 | METHODS

2.1 | Study design and ethics

In this cross-sectional study, we investigated the antibody neutralization response against different variants of SARS-CoV-2 using serum samples from 93 healthy volunteers (31 males and 62 females), aged between 16 and 84 years, from Barreiras, BA, Brazil. They received three or four doses of COVID-19 monovalent vaccines ($n = 61$) or a booster shot with a bivalent vaccine ($n = 32$). The vaccination recordings of volunteers who received only monovalent vaccines varied in immunizations with homologous or heterologous regimens of Sinovac-CoronaVac (based on a purified inactivated virus),[8] Oxford/Astrazeneca (AZD1222 or ChAdOx1-S), which is based on an adenovirus vector encoding the spike (S) protein of SARS-CoV-2,[9,10] and Pfizer (BNT126b2, RNA-based vaccine).[11,12] The bivalent vaccine (COMINARTY Original/Omicron BA.4-5 COVID-19 vaccine) used as a booster shot was mainly a fifth dose after monovalent-based vaccine regimens. The group that received only monovalent vaccines consisted of 43 females with average age of 46 ± 14.92 years, and 18 males with average age of 47 ± 15.34 years. The group the received the bivalent vaccine was composed of 19 females with average age of 39 ± 14.24 years, and 13 males with average age of 39 ± 14.39 years (Supplementary Material S1). The group that received only monovalent vaccines had an average time interval of 6.72 ± 2.5 months between the last two doses, and a 2.11 ± 1.57 months' interval between the last dose and sampling. The group that received the bivalent vaccine had an average time interval of 7.92 ± 1.12 months between the last two doses, and a 0.4 ± 0.16 months' interval between the last dose and sampling. In addition, none of the participants of this study were infected with

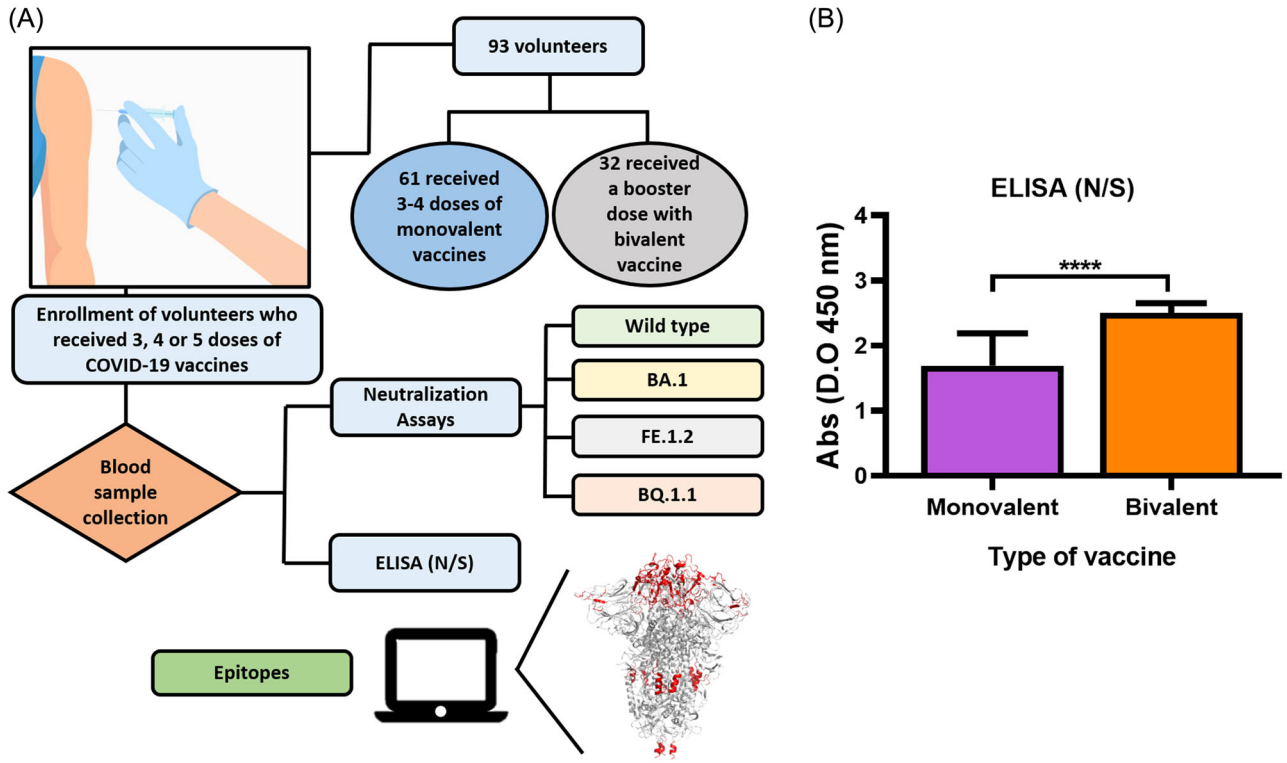

FIGURE 1 The antibody neutralization response against different variants of SARS-CoV-2 using serum samples from 93 healthy volunteers was investigated. (A) study design. Volunteers from Barreiras, BA, Brazil (31 males and 62 females) aged between 16 and 84 years that received three or four doses of COVID-19 monovalent vaccines ($n = 61$) or a booster shot with a bivalent vaccine ($n = 32$) were enrolled. The group that received only monovalent vaccines consisted of 43 females with an average age of 46 ± 14.92 years and 18 males with an average age of 47 ± 15.34 years. The group that received the bivalent vaccine was composed of 19 females with an average age of 39 ± 14.24 years and 13 males with an average age of 39 ± 14.39 years. Demographic information and serum samples were collected. An enzyme-linked immunosorbent assay (ELISA) was carried out to measure the serum levels of antibodies capable of recognizing the spike (S) and the nucleocapsid (N) proteins. Neutralization assays using the wild-type SARS-CoV-2, the original Omicron variant (BA.1), and its subvariants FE.1.2 or BQ.1.1 were carried out. Additionally, an immunoinformatic analysis was conducted to highlight unique and shared conserved neutralizing epitopes (NAb) between SARS-CoV-2 strains (wild type, BA.1, BA.4, BA.5, and BQ.1.1). (B) ELISA was carried out using the EIE COVID-19 IgG N/S Kit (Bio-Manguinhos, Fiocruz) to verify differences between antiviral antibody levels between samples from volunteers immunized only with monovalent vaccines and those who received a booster shot with the bivalent vaccine. Statistical significance was set as $p \leq 0.05$. ****$p \leq 0.0001$. DO, optical density; NAb, neutralizing antibody; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2 for at least 1 year before the study period, as determined by the lack of registered evidence of illness (COVID-19), confirmed by RT-qPCR and presentation of symptoms, as previously described.[13,14] Demographic information (Supplementary Material S1) as well as serum samples were collected. An enzyme linked immunosorbent assay (ELISA) was carried out to measure the serum levels of antibodies capable of recognizing the S and the nucleocapsid (N) proteins, as previously described.[13,15] Neutralization assays using the WT SARS-CoV-2, the original Omicron variant (BA.1), and its subvariants FE.1.2 or BQ.1.1 were carried out, as previously described.[13] Additionally, we conducted immunoinformatic analysis to highlight unique and shared conserved NAb epitopes between SARS-CoV-2 variants (WT, BA.1, BA.4, BA.5, and BQ.1.1), as previously described.[16,17] All the research complied with all relevant ethical and biosafety guidelines. Ethical approval was obtained from the Institutional Ethics Committee of the Federal University of Western Bahia (CAAE 40779420.6.0000.8060). All the procedures and possible risks were explained to the volunteers. Informed consent was obtained from all study participants.

2.2 | Serological assays

2.2.1 | ELISA assay

The EIE COVID-19 IgG N/S Kit (Bio-Manguinhos, Fiocruz) was used to analyze the serum samples, according to the manufacturer's instructions, as previously described.[15] Titers of IgG antibodies specific to the SARS-CoV-2 structural proteins S and nucleoprotein (N) were defined according to the optical density values. Briefly, an ELISA with solid-phase bound N and S recombinant antigens was performed using serum samples from volunteers. Kit controls and samples were added to the wells after dilution (1:101) with the kit diluent. After incubation for 30 min at 37°C, plates were washed five

times with kit washing buffer. Subsequently, the diluted (1:100) conjugate provided in the kit was added to each well and the plates were further incubated for 30 min at 37°C. The plates were then washed five times again and the reaction was initiated by addition of the developing solution to the wells. After incubation at room temperature for 10 min, the reaction was terminated with 2 M H_2SO_4 and the absorbance was measured at 450 nm.

2.2.2 | Cell culture and SARS-CoV-2 propagation

All the experiments using SARS-CoV-2 were performed in a Biological Safety Level 3 (BSL3) laboratory, in accordance with the WHO recommendations. African Green monkey kidney cells Vero E6 (ATCC® CRL-1586™) and Vero CCL-81 (ATCC® CCL-81) were maintained according to the recommendations of ATCC®. Vero E6 cell monolayer was infected with SARS-CoV-2 variants to propagate a viral stock. The following SARS-CoV-2 strains were used in the present study: (i) WT SARS-CoV-2 (Wuhan strain—WT) (GISAID: EPI_ISL_2499748), a kind gift from Dr. José Luiz Proença-Módena (University of Campinas—UNICAMP, Campinas, SP, Brazil); (ii) Omicron BA.1 subvariant (GISAID: EPI_ISL_6794907); (iii) Omicron FE.1.2 subvariant (GISAID: EPI_ISL_ 18277186); (iv) Omicron BQ.1.1 subvariant (GISAID: EPI_ISL_18277185). The SARS-CoV-2 viral stocks were subjected to titration (in tissue culture infectious dose [TCID] 50/mL), as described previously,[13] and were used for viral neutralization tests.

2.2.3 | Cytopathic effect-based virus neutralization test (CPE-VNT) for SARS-CoV-2 WT and omicron subvariants

The CPE-VNT assay was performed in a BSL3 laboratory, in accordance with the WHO recommendations. NAb titers against SARS-CoV-2 variants were measured as described previously.[13,14,18] Briefly, cell monolayers (5×10^4 Vero CCL-81 cells/well) in 96-well culture plates were exposed to 1×10^3 TCID50/mL of SARS-CoV-2 Wuhan strain—WT or Omicron subvariants (BA.1, FE.1.2, BQ.1.1) that were previously incubated with 1:20–1:1280 twofold diluted, heat-inactivated human serum samples, in a final volume of 150 µL. After 72 h of incubation, the plates were evaluated microscopically for the presence of characteristic SARS-CoV-2 cytopathic effects (CPEs). The absence of CPEs in the 1:20 diluted sample was considered as a positive result for the presence of NAbs against SARS-CoV-2.

2.2.4 | Statistical analyses

Data generated in ELISA, and neutralization assays were subjected to the Mann–Whitney U test. For comparison between multiple (>2) groups, the Kruskal–Wallis test followed by Dunn's multiple comparisons was used. In all cases, statistical significance was set at $p \leq 0.05$. Statistical power was set to be of at least 80%.

2.3 | Immunoinformatics

2.3.1 | Datasets of SARS-CoV-2 S protein amino acid sequences and epitopes

We built datasets with S protein amino acid sequences for each SARS-CoV-2 strain: WT ($n = 30$), BA.1 ($n = 30$), BA.4/BA.5 ($n = 30$), and BQ.1.1 ($n = 30$) (Supplementary Material S2). The amino acid sequences were retrieved from the National Center for Biotechnology Information (NCBI virus) (https://www.ncbi.nlm.nih.gov/labs/virus) from May to June 2023. The criteria for selecting the S proteins sequences were as follows: (i) complete sequences; and (ii) absence of unidentified amino acids. In addition, other data set consisting of NAb epitopes (epitope amino acid sequences) was built. Sequences were retrieved from the Immune Epitope Database (IEDB) (https://www.iedb.org/). This last data set consisted of 415 epitopes validated by virus neutralization assays (Supplementary Material S3). Replicates of the same epitope sequences were removed from analyses. NAb epitopes with more than one chain were also removed from analyses.

2.3.2 | Epitope conservation analysis

As previously described,[16,17] the IEDB conservation analysis tool (http://tools.iedb.org/conservancy) was used to determine epitope conservation among the SARS-CoV-2 S protein sequences contained in our data sets. In the present study, only fully conserved B cell epitopes which are target for NAbs were considered (100% conserved in the S protein amino acid sequences of the datasets used). The epitope data set was used for conservation analysis of each variant separately.

2.3.3 | Structural biology analysis

A S protein three-dimensional (3D) model (https://doi.org/10.2210/pdb7DDD/pdb) retrieved from the Protein Data Bank (PDB, https://www.rcsb.org) was used to localize fully conserved epitopes for NAbs using PyMol (https://pymol.org/2/).

3 | RESULTS

As expected, ELISA results clearly demonstrated that anti-SARS-CoV-2 serum antibodies levels were increased in samples from volunteers who received a booster shot with the bivalent vaccine in comparison to those who received three or four doses of monovalent vaccines (Figure 1B). Surprisingly, despite having monovalent vaccination with

three or four doses, we observed a stronger statistical support when the data was analyzed comparing the samples following monovalent or bivalent immunization protocols and not individual monovalent doses, which led us to compare both groups as a whole during the study. Equivalent results were seen when neutralizing titers of serum samples were measured with the WT SARS-CoV-2 (Figure 2A), the original Omicron variant (BA.1) (Figure 2B), and its subvariants FE.1.2 (Figure 2C) and BQ.1.1 (Figure 2D). The bivalent vaccine boosted the NAb responses against all viruses tested in significant higher levels in comparison to those induced by three or four doses with monovalent vaccines. Moreover, we observed that, for both monovalent-only (Figure 2E) and bivalent groups (Figure 2F), the WT SARS-CoV-2 was neutralized in higher rates than Omicron BA.1, which was also significantly more neutralized than FE.1.2 and BQ.1.1 Omicron subvariants. The only difference between the groups was that significantly differences were not detected regarding neutralization titers for the WT SARS-CoV-2 and Omicron BA.1 when serum samples from those immunized with the bivalent vaccine were tested. Collectively, these results indicate that the booster shot with the bivalent vaccine, although capable to induce important immune responses against the Omicron variant and its subvariants, have boosted mainly the serum levels of NAb to the WT virus.

To better understand the results presented above, we performed a quantification analysis representing viruses used for the neutralization assays to detect highly conserved NAb epitopes that are shared among some viral strains or unique in each of them (Table 1). As expected, the WT SARS-CoV-2 showed higher numbers of highly conserved/shared, as well as unique NAb epitopes in comparison to Omicron BA.1, BA.4, BA.5, and BQ.1.1. However, the fluctuations of conserved/shared and unique NAb epitopes did not explain the observed boosted responses against the WT virus and the priming for the Omicron viruses. Then, we used a S protein 3D model to highlight the amino acids that compose NAb epitopes in each of the tested viruses. As shown in Figure 3A–D, the amount of NAb epitopes conserved in receptor-binding domain (RBD) visibly diminished from the WT SARS-CoV-2 (Figure 3A) to the Omicron BA.1 variant

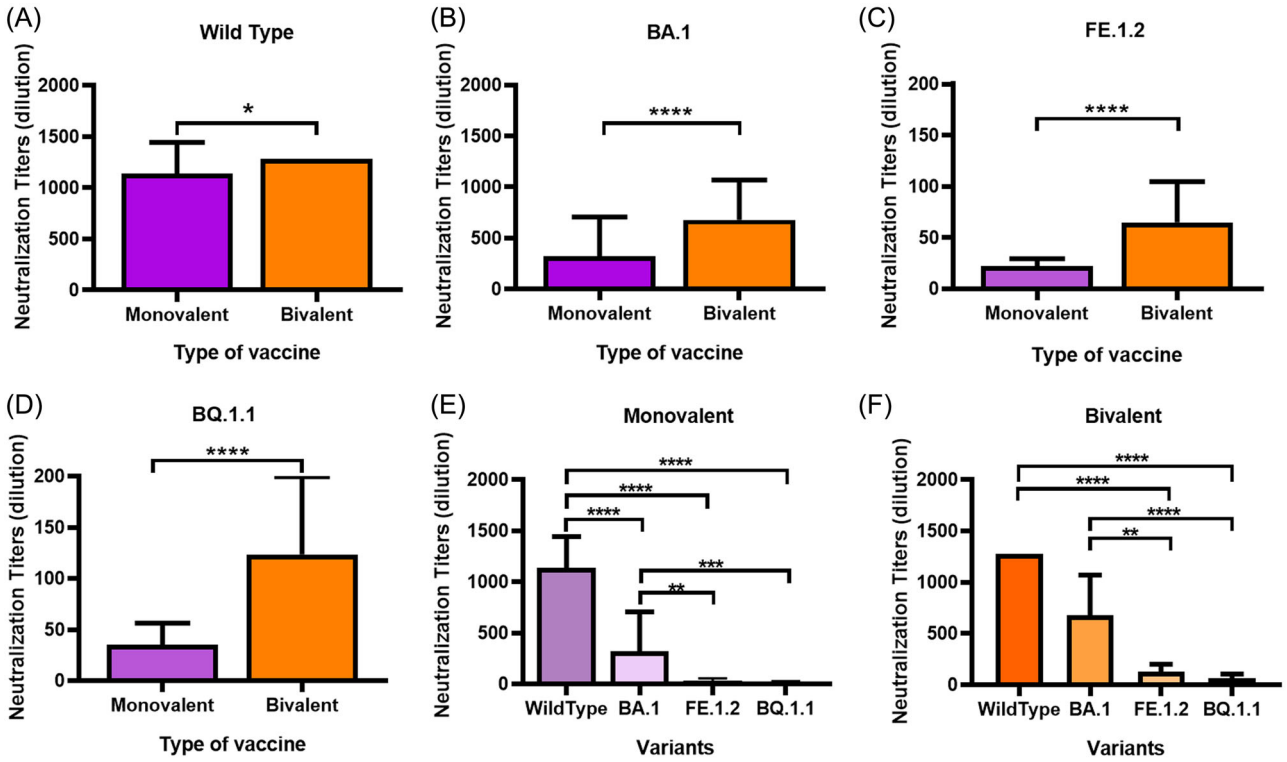

FIGURE 2 Neutralization titer according to SARS-CoV-2 strain. Neutralization assays using the wild-type SARS-CoV-2, the original Omicron variant (BA.1), and its subvariants FE.1.2 or BQ.1.1 were carried out. In brief, cell monolayers (5×10^4 Vero CCL-81 cells/well) in 96-well culture plates were exposed to 1×10^3 TCID50/mL of SARS-CoV-2 Wuhan strain—wild type or Omicron subvariants (BA.1, FE.1.2, BQ.1.1) that were previously incubated with 1:20–1:1280 twofold diluted, heat-inactivated human serum samples, in a final volume of 150 µL. After 72 h of incubation, the plates were evaluated microscopically for the presence of characteristic SARS-CoV-2 CPEs. The absence of CPEs in the 1:20 diluted sample was considered a positive result for the presence of neutralizing antibodies against SARS-CoV-2. Comparisons between neutralization titers measured in samples from individuals immunized only with monovalent vaccines and those who received a booster shot with the bivalent vaccine are shown with regard to the wild-type SARS-CoV-2 (A), Omicron BA.1 (B) and its subvariants FE.1.2 (C), and BQ.1.1 (D). In addition, neutralization titers with regard to each SARS-CoV-2 strain were compared considering values of samples from volunteers immunized only with monovalent vaccines (E) or those who received a booster shot with the bivalent vaccine (F). Statistical significance was set as $p \leq 0.05$. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$. CPEs, cytopathic effects; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCID, tissue culture infectious dose.

TABLE 1 Epitope conservation analyses for each of the SARS-CoV-2 variants were carried out.

Conserved epitopes[a]					Unique epitopes[b]			
Region	Wild type	BA.1	BA.4/BA.5	BQ.1.1	Wild type	BA.1	BA.4/BA.5	BQ.1.1
RBD	107	31	30	24	79	05	01	0
NTD	09	01	20	06	03	0	14	0
S2	08	08	05	08	0	0	0	0
Total	124	40	55	38	82	05	15	0

Note: Epitopes highly conserved in each variant, and that can alsobe shared with other variants, as well as epitopes which are unique for each viral variant are presented. The amounts of conserved/unique epitopes are presented according to each variant and according to domains and subunits of the spike protein.

Abbreviations: NTD, N-terminus domain; RBD, receptor-binding domain; S2, subunit 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Epitopes 100% conserved in the specific variants, and that can also be shared with other variants.

[b]Epitopes 100% conserved, and unique in the specific variants.

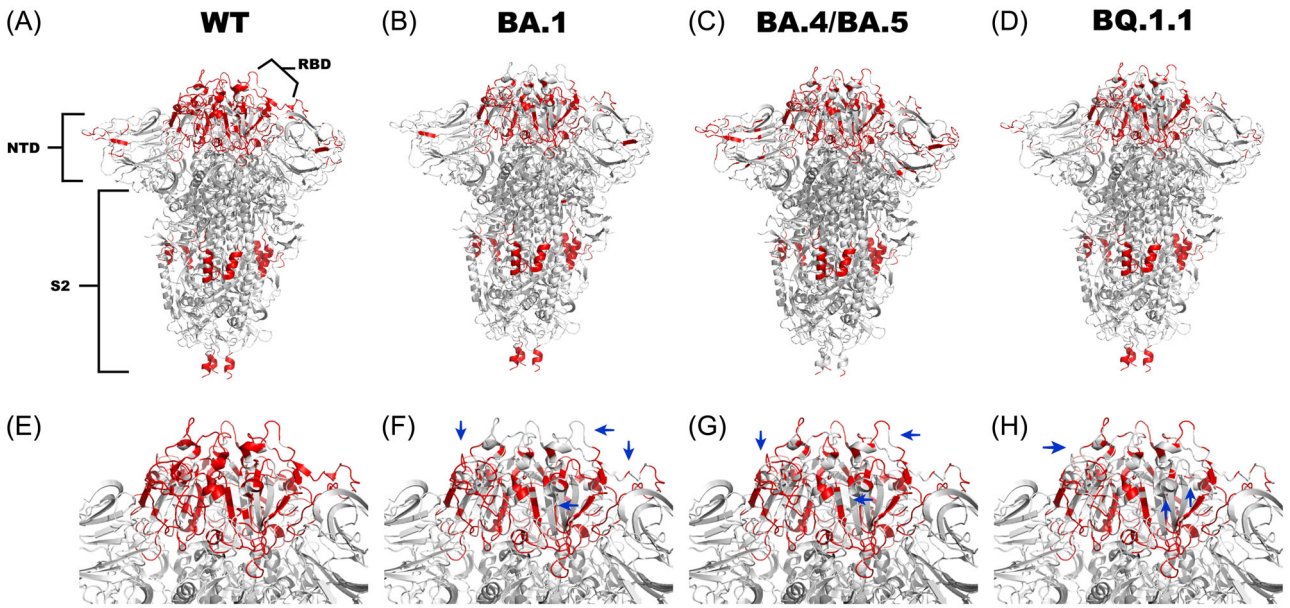

FIGURE 3 Arrangement of highly conserved epitopes targeted by neutralizing antibodies in the spike protein of each SARS-CoV-2 strain. The arrangement of epitopes in the whole S protein (RBD, NTD, and S2) is shown for the WT (A), Omicron BA.1 (B), BA.4/BA.5 (C), and BQ.1.1 (D) viruses. The positions of NAb epitopes are also shown in the RBD for WT (E), Omicron BA.1 (F), BA.4/BA.5 (G), and BQ.1.1 (H) viral strains. Blue arrows indicate key changes in loops and beta sheets. NTD, N-terminus domain; RBD, receptor-binding domain; S2, subunit 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WT, wild type.

(Figure 3B). Moreover, slight fluctuations in the amounts of epitopes can be observed from Omicron BA.1 (Figure 3B) to BA.4/BA.5 (Figure 3C) and BQ.1.1 (Figure 3D) variants. Similarly, the epitope occupancy of the N-terminus domain (NTD) visibly diminished from the WT virus (Figure 3A) to the Omicron BA.1 variant (Figure 3B), and fluctuated between BA.4/BA.5 (Figure 3C) and BQ.1.1 (Figure 3D). In the subunit 2, the only difference observed was a decrease in the epitope occupancy in the BA.4/BA.5 variants (Figure 3C). When special attention was given to the RBD (Figure 3E,F), it was possible to confirm a higher concentration of conserved NAb epitopes in the WT protein in comparison to the other variants. The occupancy of surface loops of the RBD by amino acids which compose epitopes in the Omicron BA.1 visibly diminished (Figure 3F). In addition, some beta sheets fluctuated in occupancy by conserved epitopes in BA.1 (Figure 3F), BA.4/BA.5 (Figure 3G), and BQ.1.1 (Figure 3H). Such fluctuations may help to explain, at least in part, the results seen in the serological assays.

4 | DISCUSSION

In the present investigation about the impact of immunizing with the COVID-19 bivalent vaccine on the NAb response, we found that although it has induced important antibody responses against the

Omicron variant and its subvariants, it boosted mainly the serum levels of NAb to the WT virus. It is important to remember that the bivalent vaccine contains the original virus in its composition. It seems that the nature of such a vaccine formulation has only primed the immune response against Omicron viruses, especially the newest ones, even though the priming was still enough to reach statistically significant differentiation. Furthermore, it is important to highlight that, in this study, even the individuals who received the bivalent vaccine were previously immunized with at least three doses of monovalent vaccines. As such, the observed neutralization effects were higher for the WT virus and reduced according to the distance of the variants to the WT strain. The higher amount of conserved epitopes on the WT SARS-CoV-2 S protein with regard to those of the original Omicron (BA.1) and some of its newest subvariants (BA.4, BA.5, and BQ.1.1) explains, at least in part, the results observed after the serological analyses (Figure 3). It is important to highlight that antigens derived from Omicron BA.4, and BA.5 are contained in the bivalent vaccine that was administered to part of the study population. Thus, this result represents the immunological context of this study.

A boosted NAb response against all viruses tested in this study, including Omicron, was observed when the bivalent vaccine was administered. This result is in line with previous studies that show that the bivalent vaccine increases neutralization and protection against the original Omicron, and its subvariants.[19–21] A specific study showed an interpretation that the use of the bivalent vaccine produces low neutralization of Omicron subvariants.[22] Our findings and concomitant data interpretation show that both observations make sense. On the one hand, a booster effect on the NAb response is observed after administration of the bivalent vaccine. On the other hand, the levels of NAb targeting the WT virus are higher than those targeting Omicron and two of its newest subvariants. Our structural modeling analyses of the whole S protein regarding concentration of conserved NAb epitopes showed that RBD is the main target. However, the number of conserved epitopes was dramatically diminished from the WT SARS-CoV-2 to Omicron and its subvariants, including those which are part of the vaccine formulation: BA.4 and BA.5. Furthermore, the fluctuations of numbers and positions of conserved NAb epitopes among Omicron subvariants have a lesser extension. This finding is in line with those observed after our neutralization assays because Omicron subvariants tested in this study (FE.1.2 and BQ.1.1) were neutralized in indistinguishable levels. Collectively, our results indicate that COVID-19 vaccines should be updated to based only on antigens from viruses that are currently circulating in human populations, not those which were epidemiologically replaced. This is particularly important to boost immune responses against circulating viruses and thus, contribute to control their community transmission.

4.1 | Limitations

This study has strengths and limitations. A strength is that serum samples were collected from a diverse multiracial Brazilian population with a well-known vaccine recording. Another strength is that serum samples were tested with viral strains that represent important stages of COVID-19 and its vaccine history, including two of the newest Omicron subvariants which circulate nowadays, in contrast to the WT SARS-CoV-2, and the original Omicron BA.1. In this way, BQ.1.1 was reported to have mutations in its RBD that confer a relevant neutralization escape capacity,[23,24] which seems to be in line with our results. FE.1.2[25] is one of the newest Omicron subvariants that is becoming epidemiologically important in the United States and in Brazil. An important limitation is that we used a convenience population sample due to difficulties in enrolling volunteers at the time of sample collection, which happened in the early beginning of administration of the bivalent vaccine in our study area (Barreiras, Brazil). In addition, instead of using plaque or focus reduction neutralization test (PRNT or FRNT, respectively) to access the antibody neutralization titers, we used CPE-VNT, a technique that has been successfully applied to measure serum NAbs both in humans and in other mammalian species. Although PRNT, and FRNT are considered golden standard, the CPE-VNT has shown to be particularly useful for studying larger sets of samples.[18,26,27] The same technique has been used in our previous studies[13,14] and we preferred not to change the methodology in the present study. Nevertheless, we present robust statistical analysis that support our main statements and conclusions.

5 | CONCLUSION

Our results indicate that the NAb response after immunization with the COVID-19 bivalent vaccine was mainly boosted against the WT SARS-CoV-2, while was primed against the more recent Omicron subvariants. As so, our conclusions highlight that the update of future COVID-19 vaccines should consider to use only circulating viruses, and not viruses which were epidemiologically replaced and no longer circulate in the human population. This is particularly important considering the need to control community transmission of new viral variants.

AUTHOR CONTRIBUTIONS

Milena Silva Souza, and Jéssica Pires Farias collected samples, and volunteer's data, carried out enzyme-linked immunosorbent assay (ELISA), analyzed the data, prepared figures and tables, and wrote the paper. Robert Andreata-Santos carried out cell and virus culture, serum neutralization assays, analyzed the data, and wrote the paper. Marianne Pereira Silva collected samples, organized samples storage, and analyzed the data. Ruth Dálety da Silva Brito carried out ELISA, and analyzed the data. Marcia Duarte Barbosa da Silva and Cristina Mendes Peter carried out neutralization assays. Marcus Vinícius de França Cirilo, Wilson Barros Luiz, Alexander Birbrair, and Paloma Oliveira Vidal collected samples, gave scientific support, and wrote the paper. Maria Fernanda de Castro-Amarante, Erika Donizetti Candido, Aldilene Silva Munhoz, Fernanda de Mello Malta, Erik Gustavo Dorlass, and Rafael Rahal Guaragna Machado

carried out virus propagation and titration, and gave scientific support. João Renato Rebello Pinho, Danielle Bruna Leal Oliveira, Edison Luiz Durigon, Juliana Terzi Maricato, Carla Torres Braconi, Luís Carlos de Souza Ferreira, and Luiz Mário Ramos Janini gave scientific support, provided funding, and wrote the paper. Jaime Henrique Amorim conceived the study, collected samples, analyzed the data, provided funding, and wrote the paper. All authors critically revised the manuscript and approved the final version for submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Datasets used in this study are available as Supporting Information: Data. Results of analyses are shown in the main text, tables, and figures.

ETHICS STATEMENT

All the research complied with all relevant ethical and biosafety guidelines. Ethical approval was obtained from the Institutional Ethics Committee of the Federal University of Western Bahia (CAAE 40779420.6.0000.8060). All the procedures and possible risks were explained to the volunteers. Informed consent was obtained from all study participants.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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