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Long-term dynamics of SARS-CoV-2 immunity in a university hospital in Colombia: A cohort study

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ABSTRACT

Objectives: This prospective cohort study aimed to estimate the natural, vaccine-induced, and hybrid immunity to SARS-CoV-2, alongside the immunogenicity of the messenger RNA (mRNA)-1273 booster after the BNT162b2 primary series in health care workers in Colombia.
Methods: Immunoglobulin (Ig) G, IgA, and neutralizing antibodies were measured in 110 individuals with SARS-CoV-2 infection or a BNT162b2 primary series. Humoral responses and related factors were explored in a subgroup (n = 36) that received a BNT162b2 primary series, followed by a mRNA-1273 booster (2BNT162b2 + 1mRNA-1273), and T-cell responses were evaluated in a subgroup of them (n = 16).
Results: For natural immunity, IgG and IgA peaked within 3 months, declining gradually but remaining detectable up to 283 days post-infection. Neutralizing antibody inhibition post-infection was below positive range (≥35%) but exceeded 97% in vaccine-induced and hybrid immunity groups. After 2BNT162b2 + 1mRNA-1273, IgG peaked 3-4 months post-booster, gradually declining but remaining positive over 10 months, with IgA and neutralizing antibodies stable. Age and blood group were related to IgG response, whereas obesity and blood type were related to IgA response post-booster. Autoimmunity and blood type B were associated with lower neutralizing antibody inhibition. There were no differences in T-cell responses according to previous infection.
Conclusions: These findings provide long-term insights into the immunity against SARS-CoV-2 and the immunogenicity of mRNA vaccines.

Introduction

The COVID-19 pandemic has posed significant threats to public health and health systems worldwide [1]. Various strategies have been adopted worldwide to mitigate the disease burden. These included prevention measures such as social distancing, travel restrictions, and lockdowns [2], as well as the accelerated spread of safe and effective vaccines and the strengthening of health systems to facilitate the prevention, detection, and treatment of COVID-19 [3].

SARS-CoV-2 infections generate an immune response capable of reducing the risk of re-infection [4]. However, this response is influenced by various factors, including virus-dependent mechanisms, such as the evolution of antigenically distinct viral variants. For instance, protection from natural immunity against re-infection remains strong for pre-Omicron variants but weakens faster for Omicron and its sublineages [4]. Although past infections can trigger a robust immune response, vaccination has played a vital role in mitigating the spread of SARS-CoV-2 [5].

The development and implementation of COVID-19 vaccines have led to a progressive decline in morbidity and mortality [6]. Multiple vaccines using different platforms have been made available to the public, and some are still under development [7]. After the application of primary schedules, the emergence of viral variants with higher transmissibility and virulence, and the waning of vaccine effectiveness and immunogenicity over time, leading to the implementation of booster doses [8]. Vaccine boosters were administered with the same vaccine as

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the primary schedule (homologous booster) or a different vaccine (heterologous booster) [9]. The combination of COVID-19 vaccines is a safe and reassuring alternative, particularly, useful in the context of vaccine scarcity, such as in low- and middle-income countries [9].

Previous studies addressing the immunogenicity of heterologous boosters showed a significant increase in binding and neutralizing antibody titers that correlated with greater protection against viral variants of concern and a higher T-cell response and interferon (IFN)-γ secretion than homologous boosters [10]. Although there is some evidence regarding the combination of a two-dose primary series with BNT162b2 and a messenger RNA (mRNA)-1273 booster, most studies originate from high-income countries [10,11]. As the body of evidence continues to grow, further replication in diverse settings and populations is necessary. This is particularly relevant because this vaccine combination was frequently used among health care workers (HCWs) in middle-income settings, such as Colombia.

A broader understanding of the long-term protection against SARS-CoV-2 conferred by infection, vaccines, and a combination of both is necessary to aid policymaking and prepare for future emergent diseases, especially, considering the potential applications of mRNA vaccines to prevent severe outcomes of infectious diseases, offering a versatile and rapid response strategy that will likely be effective for various emerging pathogens. Thus, we aimed to (i) estimate the natural, vaccine-induced, and hybrid humoral immunity against SARS-CoV-2 in HCWs and (ii) assess the humoral and cellular responses elicited by the mRNA-1273 booster in HCWs previously vaccinated with two doses of BNT162b2.

Materials and methods

Setting

The study was conducted at the University Hospital Fundación Santa Fe de Bogotá, a tertiary care hospital in Bogotá, Colombia. In February 2021, Colombia gradually started the COVID-19 vaccination process in two phases. The first phase sought to reduce mortality and incidence of severe disease and protect HCWs, whereas the second sought to reduce infectivity to reach herd immunity [12]. HCWs were among the first to complete their primary vaccination schedule. Thereafter, by November 2021, the government approved a booster dose for adults that was administered at least 6 months after completing the initial schedule [13]. The vaccines available in the country included BNT162b2 (Pfizer), mRNA-1273 (Moderna), Ad26.COV2.S (Janssen), AZD1222 (AstraZeneca), and CoronaVac (Sinovac) [12]. For the booster dose, HCWs could access a homologous booster or a heterologous booster (using an mRNA or a viral vector-based vaccine) according to their preferences and vaccine availability [13].

Study design and participants

In 2020, the University Hospital Fundación Santa Fe de Bogotá, in collaboration with Universidad de los Andes, conducted the CoVIDA-FSFB study [14]. The prospective cohort study enrolled a cohort of 420 voluntary adult hospital workers recruited between June 25 and October 30, 2020, who underwent routine SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) and serological testing over 6 months until April 30, 2021 (Figure 1). During the follow-up by March 2021, a subgroup of participants received a two-dose BNT162b2 schedule. Collected serum samples were stored at −70°C until further analysis. To estimate the infection-induced, vaccine-induced, and hybrid humoral immunity against SARS-CoV-2, this analysis focused on a subgroup of the CoVIDA-FSFB participants who met any of the following inclusion criteria (n = 110): (i) RT-PCR–confirmed SARS-CoV-2 infection before study recruitment (n = 29), (ii) RT-PCR–confirmed SARS-CoV-2 infection during the study follow-up (n = 57), and (iii) received a primary vaccination schedule with BNT162b2 (n = 24). Subsequently, for the analysis, individuals were divided into subgroups: 86 with natural immunity and 24 with vaccine-induced immunity. Of these, 11 participants developed hybrid immunity. For this analysis, participants with re-infections, contraindications for phlebotomy, and characteristics that hindered follow-up were excluded (e.g. change of residence, planned long-term travel outside the city). Stored samples of eligible participants were subsequently sent to the Center for Autoimmune Diseases Research analysis.

Subsequently, the participants received the first vaccine booster between November 26, 2021 and January 4, 2022. Those who received a booster dose of mRNA-1273 after a two-dose primary schedule of BNT162b2 (2BNT162b2 + 1mRNA-1273 schedule) (n = 36) were invited to participate in an ancillary component to study the humoral immunogenicity of this vaccine combination (Figure 1). The cellular immune response was evaluated in a subset without underlying comorbidities, acute infections, and chronic or acute use of medication (n = 16). Individuals were scheduled a new visit between March 24 and April 11, 2022 to assess eligibility for this study component and collect additional information and blood samples to assess humoral and cellular immunogenicity in those who were eligible. Subsequently, they were followed up for 6 additional months, which concluded on October 25, 2022. During this period, blood samples for humoral immunity assessment were collected at 6 and 9 months after the booster, and participants were contacted monthly to identify laboratory-confirmed COVID-19 cases (Figure 1).

Outcomes

Measurement of immunoglobulin G, immunoglobulin A, and neutralizing antibodies

The EUROIMMUN anti–SARS-CoV-2 enzyme-linked immunosorbent assay (EUROIMMUN, Luebeck, Germany) was used for serological detection of human immunoglobulin (Ig) G and IgA antibodies against the SARS-CoV-2 wild-type spike (S) 1 structural protein, following the manufacturer's instructions, as previously described. To evaluate results, a ratio of the optical density of the patient sample over the optical density of the calibrator was calculated. Ratios <0.8 were deemed negative, ≥0.8 to <1.1 were considered borderline, and ≥1.1 were classified as positive. Antibody positivity was determined using a 1:100 dilution.

The anti-S SARS-CoV-2 IgG II Quant assay (S IgG) (Abbott, Sligo, Ireland) was used to assess the IgG response to the 2BNT162b2 + 1mRNA-1273 schedule. The assay was conducted on the Abbott ARCHITECT i2000SR system, according to the manufacturer's instructions. The assay allows qualitative and quantitative determination of IgG antibodies against the SARS-CoV-2 glycoprotein receptor binding domain (RBD) in human serum and plasma. The positivity threshold for this assay is 50.0 arbitrary units per milliliter (AU/ml). The units of the quantitative Abbott anti-S assay (AU/ml) were converted to the World Health Organization units, binding antibody units per milliliter (BAU/ml), by multiplying by a factor of 0.142, according to the manufacturer's instructions.

To evaluate the neutralizing capacity of anti-SARS-CoV-2 antibodies, the semi-quantitative assay NeutraLISA kits (EUROIMMUN, Lübeck, Germany) was used. This kit detects IgG, IgM, and IgA Ig classes capable of neutralizing the S1 subunit, where RBD of the SARS-CoV-2 S protein is located. Results were reported as percent inhibition (%Inhibition), following the manufacturer's instructions. Samples were classified as negative (<20% inhibition), positive (≥35% inhibition), or inconclusive (20-34% inhibition). This assay was performed only in participants with positive IgG antibodies. For all EUROIMMUN assays, the color intensity of the enzyme-linked immunosorbent assay was measured at a wavelength of 450 nm, with a reference of 655 nm using an iMark microplate reader (Bio-Rad).

Peripheral blood mononuclear cell isolation and cryopreservation

Blood collected in tubes coated with ethylenediaminetetraacetic acid was used to isolate peripheral blood mononuclear cells (PBMCs) through

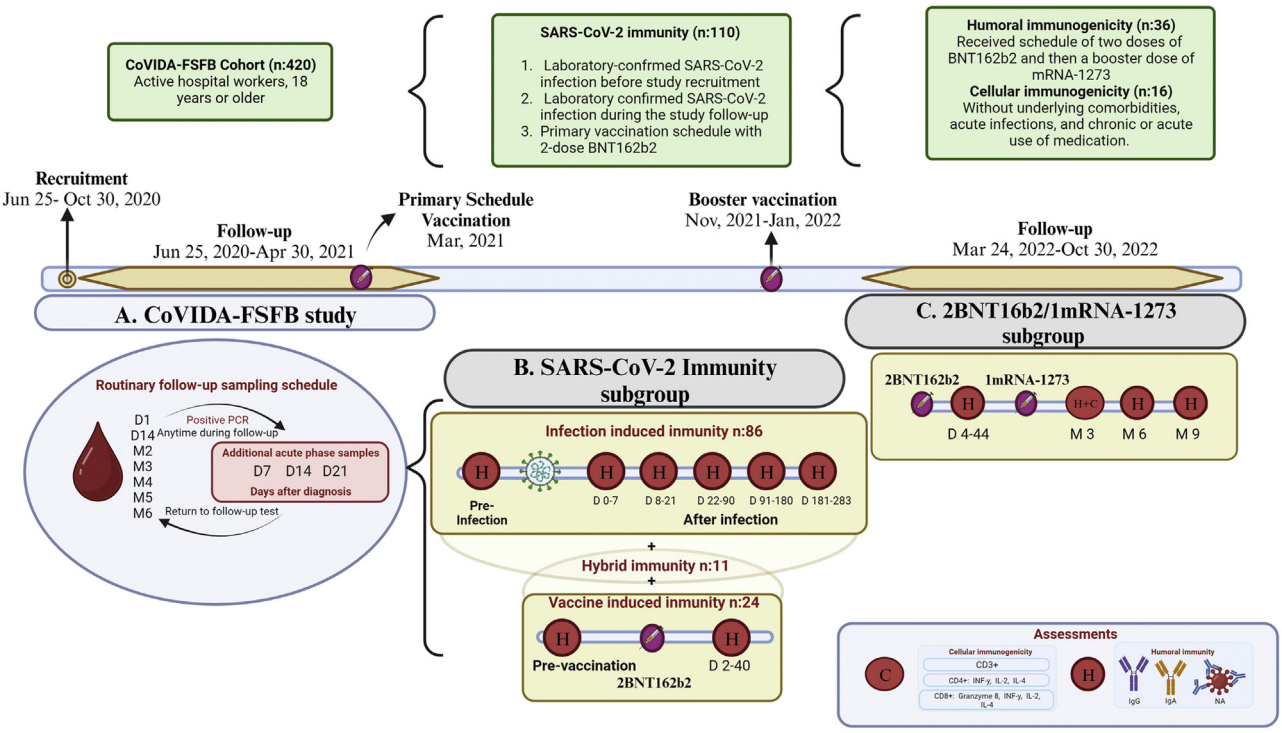

Figure 1. Study design. The CoVIDA-FSFB cohort included a group of 420 adult hospital workers. (a) During the CoVIDA-FSFB project, periodic blood samples were collected over 6 months. Additional samples were collected from participants with reverse transcription-polymerase chain reaction–confirmed SARS-CoV-2 infection during the acute phase. (b) The samples stored from a subset of 110 participants of the CoVIDA-FSFB cohort were analyzed, including those who had a SARS-CoV-2 infection before recruitment or during the cohort follow-up time or who had received a two-dose BNT162b2 schedule. The humoral response to SARS-CoV-2 was analyzed in three subgroups: those with natural immunity (with any confirmed infection before or during the study, n = 86) and those with vaccine-induced immunity (n = 24). Of the 86 individuals with natural immunity, 11 participants also received vaccination and were, thus, categorized as having hybrid immunity. The immune response was assessed by measuring IgG, IgA, and neutralizing antibodies. Samples were analyzed according to the time after infection or vaccination. (c) A subgroup of 36 participants who received a two-dose BNT162b2 primary schedule and an mRNA-1273 booster continued to be followed up. In this group, a blood sample was collected 4-44 days after the two-dose BNT162b2 schedule and 3, 6, and 9 months after the mRNA-1273 booster to assess the humoral immunogenicity (IgG, IgA, and neutralizing antibodies). On month 3 after the booster, cellular immunogenicity was analyzed in a subgroup of 16 of them, without underlying comorbidities, acute infections, and chronic or acute use of medication. Figure was created using BioRender (BioRender.com).
Ig, immunoglobulin; mRNA, messenger RNA.

a density gradient centrifugation method using Ficoll-Histopaque 1077 (Sigma-Aldrich, St Louis, MO, USA), following the manufacturer's instructions. For cryopreservation, the isolated PBMCs were washed twice with complete RPMI-1640 media (Gibco, NY, USA) and then frozen and stored in fetal bovine serum (BioWest, Riverside, USA) containing 10% dimethyl sulfoxide (Sigma-Aldrich, St Louis, USA). Cryovials containing the PBMCs were initially stored at −70°C to allow a gradual temperature decrease. After 24 hours, these cryovials were transferred to a liquid nitrogen tank, where they were stored until further use.

Measurement of SARS-CoV-2–specific T-cell response

To explore the SARS-CoV2–specific T-cell response to the 2BNT162b2 + 1mRNA-1273 schedule, three different peptide pools of SARS-CoV-2 wild type (Mabtech AB, Nacka Strand, Sweden) (Supplementary File S1) were used:

1. SARS-CoV-2 S1 scanning pool, which contains 166 peptides from the human SARS-CoV-2 virus; the peptides are 15-mers overlapping with 11 amino acids, covering the S1 domain of the S protein (amino acid 13-685).
2. SARS-CoV-2 SNMO defined peptide pool that contains 47 synthetic peptides from the human SARS-CoV-2 virus; the peptides are derived from the S, nucleoprotein, membrane protein, ORF3a and ORF7a.
3. S2 N defined peptide pool, which contains 41 peptides derived from the S and nucleoprotein of SARS-CoV-2.

According to the manufacturer's instructions, each pool of peptides was individually resuspended in 40 µl of 100% dimethyl sulfoxide and 85 µl of sterile 1X PBS, resulting in a final stock concentration of 200 µg/ml. Then, cryopreserved PBMCs were thawed in a 37°C water bath, washed twice with RPMI-1640 media pre-warmed to 37°C, and centrifuged at 350 g for 10 minutes. Afterward, cells were analyzed for viability using trypan blue and seeded at a density of 1 × 106 cells per well in a 96-well plate in RPMI-1640, supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine (Gibco, NY, USA). After, cells were rested for 2 hours and then stimulated with each SARS-CoV-2 peptide pool independently at a final concentration of 2 µg/ml overnight (~18 hours) at 37°C and 5% CO_2. As positive control, cells were stimulated with 5 ug/ml of phytohemagglutinin (Sigma-Aldrich, St Louis, USA), and, as negative control, cells were left unstimulated. All conditions were seeded with Brefeldin A at 10 µg/ml (Sigma-Aldrich, St Louis, USA) to inhibit protein transport. The percentage of SARS-CoV-2–specific IFN-γ, interleukin (IL)-2, IL-4, and granzyme B-producing cells were evaluated by flow cytometry. After stimulation with the SARS-CoV-2 peptide pools, cells were harvested and stained with 7AAD-PERCP, anti-clusters of differentiation (CD)3-APCH7, anti-CD4-V500, and anti-CD8-APC antibodies (BD Biosciences, CA, USA) at room temperature for 30 minutes. For intracellular cytokine staining, cells were fixed and permeabilized with BD Cytofix/Cytoperm (BD Biosciences, CA, USA), followed by staining with anti-IFN-γ-FITC, anti-IL-2-V450, anti-IL-4-PECy7, and anti-Granzyme B-PE antibodies (BD Biosciences, CA, USA) for 30 minutes at 4°C in the

darkness. The semi-automatic compensation was performed using BD CompBeads. These beads were mixed with specific conjugated antibodies and analyzed on a FACS Canto II flow cytometer (BD Biosciences). Data were analyzed with FlowJo software version 9 (BD Biosciences). Compensation matrixes were generated to correct for spectral overlap between fluorochromes. The analysis strategy adjustments are detailed in Supplementary Figure S1.

Data sources

During the cohort's first visit, participants were asked about their sociodemographic information and medical history. This included information regarding comorbidities, flu vaccination, and previous viral infections (e.g. dengue, chickenpox, zika, chikungunya, influenza, measles, or hepatitis). These infections were self-reported by the participants, and no specific diagnostic tests were conducted to confirm them.

For the 2BNT162b + 1mRNA-1273 immunogenicity subgroup, additional data were collected through an electronic questionnaire implemented in REDCap (Supplementary File S2 File in Spanish, Supplementary File S3 for the English translation). This included data on sociodemographic characteristics (e.g. age, sex, socioeconomic status, city in which they live, and profession), clinical characteristics (e.g. height, weight, body mass index [BMI], blood type, comorbidities, medications, and previous laboratory-confirmed COVID-19 infection by either polymerase chain reaction or antigen tests), and habits (e.g. physical activity, alcohol, and smoking cigarettes). To avoid inter-interviewer bias, the questionnaire was administered by the same investigator. Information regarding COVID-19 vaccination was obtained from the participant's vaccination card, including the vaccine batch, laboratory, dosage, administration dates, and the health provider institution that administered the vaccine. During the follow-up period, the administration of a second booster dose was approved in Colombia. Participants were asked about receiving this dose, and verification was conducted through the vaccination certificate by the end of the follow-up.

Statistical analysis

For the descriptive analysis, qualitative variables were presented as frequencies and proportions, and quantitative variables were presented as means or medians with SDs or interquartile ranges (IQRs), depending on their distribution, according to the Shapiro–Wilk test. Sociodemographic and clinical characteristics of patients in the natural and vaccine-induced immunogenicity groups were compared using the Mann–Whitney U test and Fisher exact test to assess statistical significance. There were no missing data on the independent variables. Missing data on the humoral immunogenicity outcome corresponded to 4.86%, which were not included in the analysis.

IgG and IgA titers were compared before and after a two-dose BNT162b2 primary schedule using the Wilcoxon signed-rank test. To compare IgG, IgA, and neutralizing antibodies after the mRNA-1273 booster, the Skillings–Mack test was used. Differences in IgG, IgA, and neutralizing antibodies according to sociodemographic, clinical variables, and habits were graphically explored, and the Mann–Whitney U test was used to assess statistical significance.

The chemiluminescent microparticle immunoassay kit used to measure anti-S IgG, for 2BNT162b2 + 1mRNA-1273 immunogenicity assessment, provides values up to 5680 BAU/ml. Values above that threshold were set as equal to the threshold; thus, the data for this variable were right-censored. To address this and given the longitudinal nature of our data, a random-effects Tobit model was used to determine factors related to anti-S-RBD IgG antibodies. In this model, all observations made for each participant were included because each person was observed at three points in time after the vaccine booster. Regression models were constructed using anti-S-RBD IgG post-booster as the dependent variable. All clinically relevant variables with biological plausibility previously identified through literature search were included as independent variables. Two models were constructed, a bivariate model and a multivariate reduced model with the minimum number of independent variables that best suited the data, using a 0.2 significance level for variable removal from the model. The multivariate model was used to adjust for confounders and detect effect modifiers. All possible interactions between the variables of interest were explored; however, these were not included in the final model because they were not statistically significant. Multicollinearity was assessed using the variance inflation factor, with a 5.0 cut-off point. Bootstrapping was used to provide more reliable standard errors. The random-effects model used account for autocorrelation that may arise from within cluster dependencies. The quadrature approximation used in the random-effect estimators was checked, with no relative differences in the coefficients larger than 0.01%. The normal distribution of raw residuals was confirmed.

T-cell responses in participants with 2BNT162b2 + 1mRNA-1273 were compared according to whether they previously had COVID-19. Specifically, CD4+ cells producing IFN-γ, IL-2, and IL-4 and CD8+ cells expressing granzyme B, IFN-γ, IL-2, and IL-4. Responses were evaluated post-stimulation with three distinct peptide pools (S1, SNMO, and S2 N), and the Mann–Whitney U test was used to assess statistical significance. A P <0.05 was considered to indicate statistical significance for all statistical tests. Analysis was performed using Stata SE 17.0 [15] and visualized in GraphPad Prism version 9 [16].

Results

Immunity to SARS-CoV-2

This study included 110 participants: 86 with natural immunity and 24 with vaccine-induced immunity; in addition, 11 participants had hybrid immunity (Figure 1). The median age of participants was 40 years (IQR 33-44 years, range 25-64 years), and most participants were female (81.8%) and from a middle socioeconomic background (66.4%) (Table 1). The majority were professional nurses (32.7%), followed by medical doctors and students (15.5%), and more than half had a health care position (67.6%). The median BMI was 24.8 (IQR 22.6-26.9 kg/m^2), and 23% had some sort of comorbidity.

We assessed the humoral natural immunity up to 283 days post-infection (Figure 2a) (see Supplementary Table S1 for sampling timing details). IgA antibodies peaked earlier, reaching a median ratio of 8.079 on days 8-21 after infection, then they began to descend, reaching a median ratio of 3.273 by the end of the follow-up (191-283 after infection). On the other hand, IgG antibodies peaked on days 22-90, reaching a median ratio of 5.111, and then descended, with a median ratio of 3.352 by days 191-283 after infection. We measured neutralization only in participants with positive IgG anti–SARS-CoV-2 antibodies (ratio ≥1.1). On days 8-21, the mean percentage of inhibition was −17.85%, followed by 14.86% on days 22-90, 12.68% on days 91-180, and 22.06% on days 181-283 after the infection (Figure 2b).

In the group with vaccine-induced immunity, for IgG, the median ratio increased from 0.205 before vaccination to 8.797 after vaccination (P <0.0001) (Figure 2c). For IgA, the median ratio increased from 0.392 to 9.788 after vaccination (P <0.0001). The median percentage of inhibition of neutralizing antibodies after vaccination was 97.00% (IQR 94.70-97.80%). In the group with hybrid immunity, the median ratio for IgG was 9.676 and 8.010 for IgA (Figure 2d). Regarding the neutralizing antibodies, the median percentage of inhibition was 97.61% (IQR 97.25-98.20%).

Humoral immunogenicity of 2BNT162b2 + 1mRNA-1273

A subgroup of 36 participants was included for this analysis. Their mean age was 42 ± 8 years (range 27-60 years), and most were female (78%) (Table 2). Professional nurses accounted for the largest proportion of roles (39%), followed by nursing assistants (22%), and medical

Table 1
Sociodemographic and clinical characteristics of cohort's participants (n = 110).

Characteristic		Total N = 110	Vaccine-induced immunity N = 24	Natural immunity N = 86	*P*-value
Age	Median (interquartile range)	40 (33-44)	41 (37-43)	39 (33-44)	0.49
Sex	Male	20 (18.2%)	5 (20.8%)	15 (17.4%)	0.77
	Female	90 (81.8%)	19 (79.2%)	71 (82.6%)	
Socioeconomic status	Low (1-2)	21 (19.1%)	4 (16.7%)	17 (19.8%)	0.58
	Mid (3-4)	73 (66.4%)	15 (62.5%)	58 (67.4%)	
	High (5-6)	16 (14.5%)	5 (20.8%)	11 (12.8%)	
Occupation	Nurse	36 (32.7%)	11 (45.8%)	25 (29.1%)	0.090
	Nurse assistant	14 (12.7%)	2 (8.3%)	12 (14.0%)	
	Medical doctors and students	17 (15.5%)	5 (20.8%)	12 (14.0%)	
	Laboratory workers	13 (11.8%)	5 (20.8%)	8 (9.3%)	
	Administrative assistants	12 (10.9%)	1 (4.2%)	11 (12.8%)	
	Therapists	9 (8.2%)	0 (0.0%)	9 (10.5%)	
	Other	9 (8.2%)	0 (0.0%)	9 (10.5%)	
Type of position	Administrative	23 (21.9%)	0 (0.0%)	23 (28.0%)	0.006
	Blended	11 (10.5%)	3 (13.0%)	8 (9.8%)	
	Health care	71 (67.6%)	20 (87.0%)	51 (62.2%)	
Comorbidities	No	85 (77.3%)	20 (83.3%)	65 (75.6%)	0.58
	Yes	25 (22.7%)	4 (16.7%)	21 (24.4%)	
Active smoking	Nonsmoker	82 (74.5%)	21 (87.5%)	61 (70.9%)	0.26
	Previous smoker	23 (20.9%)	3 (12.5%)	20 (23.3%)	
	Current smoker	5 (4.5%)	0 (0.0%)	5 (5.8%)	
Passive smoking	No	78 (72.2%)	15 (62.5%)	63 (75.0%)	0.30
	Yes	30 (27.8%)	9 (37.5%)	21 (25.0%)	
Previous viral infections[a]	No	72 (65.5%)	7 (29.2%)	65 (75.6%)	<0.001
	Yes	38 (34.5%)	17 (70.8%)	21 (24.4%)	
Influenza vaccine	No	18 (16.4%)	3 (12.5%)	15 (17.4%)	0.76
	Yes	92 (83.6%)	21 (87.5%)	71 (82.6%)	
Body mass index	Median (interquartile range)	24.8 (22.6-26.9)	24.4(21.4-26.3)	25.0 (23.0-27.1)	0.28
	Underweight	1 (0.9%)	0 (0.0%)	1 (1.2%)	0.74
	Healthy weight	58 (52.7%)	15 (62.5%)	43 (50.0%)	
	Overweight	39 (35.5%)	7 (29.2%)	32 (37.2%)	
	Obesity	12 (10.9%)	2 (8.3%)	10 (11.6%)	
SARS-CoV-2 infection during follow-up	No	52 (47.3%)	24 (100.0%)	28 (32.6%)	<0.001
	Yes	58 (52.7%)	0 (0.0%)	58 (67.4%)	
Previous SARS-CoV-2 infection	No	81 (73.6%)	24 (100.0%)	57 (66.3%)	<0.001
	Yes	29 (26.4%)	0 (0.0%)	29 (33.7%)	

[a] Any of the following: dengue, chickenpox, Zika, chikungunya, influenza, measles, and hepatitis.

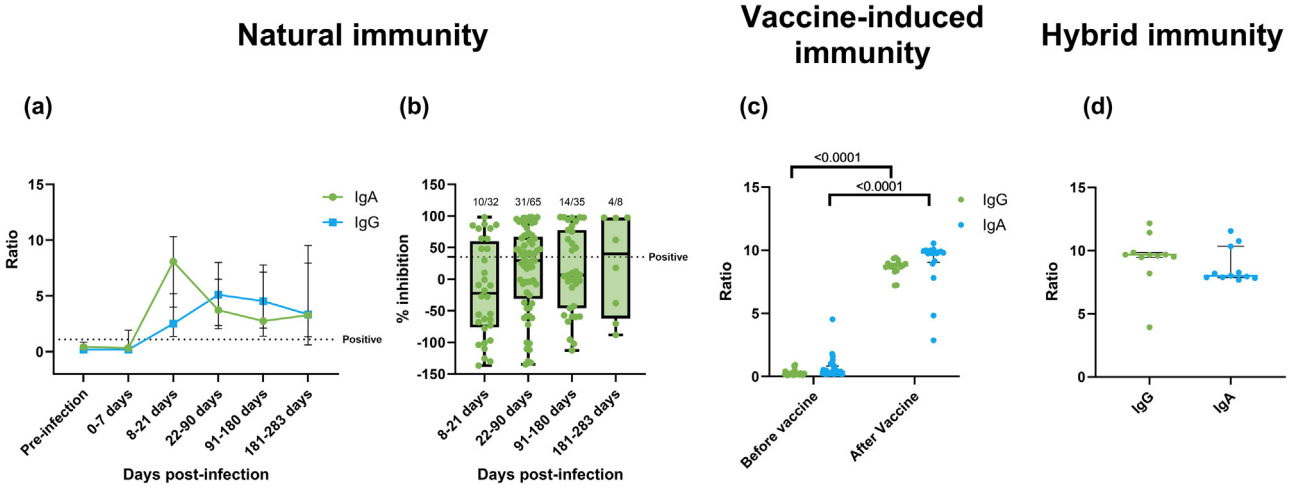

Figure 2. Dynamic of SARS-CoV-2 immunity. Levels of anti-SARS-CoV-2 IgA, IgG, and neutralizing antibodies over time. (a and b) Show the dynamics of humoral immune response in individuals after infection. (a) Circles and squares show median IgA and IgG antibodies, respectively. Horizontal lines represent interquartile ranges. Ratios equal to or above 1.1 were considered positive. Antibodies were measured before infection and up to 283 days after infection. The number of individuals tested varied according to the time point evaluated. (b) Neutralizing antibodies were measured only in individuals with positive IgG antibodies over time. Values above 35% of inhibition were considered positive. The proportion of positive samples over the total samples measured at each time point is displayed above each box. (c) IgG and IgA antibodies in naïve individuals before and after vaccination with a two-dose schedule of BNT162b2. (d) IgG and IgA antibodies in vaccinated in individuals with a two-dose schedule of BNT162b2 who then had a laboratory-confirmed SARS-CoV-2 infection. Statistical significance was measured using a Wilcoxon signed rank test at a significance level of 5%.
Ig, immunoglobulin; mRNA, messenger RNA.

Table 2
Demographics, clinical characteristics, and habits of the subgroup of participants for the assessment of the humoral immunogenicity of 2BNT162b2 + 1messenger RNA-1273 (n = 36).

Characteristic		Total N = 36 N (%)
Sex	Female	28 (78)
City	Bogotá	31 (86)
	Outside the city	5 (14)
Occupation	Professional nurse	14 (39)
	Nursing Assistant	8 (22)
	Medical doctor	5 (14)
	Administrative position	4 (11)
	Microbiologist or bacteriologist	4 (11)
	Nutritionist	1 (3)
Socioeconomic status	Low (1-2)	6 (17)
	Mid (3)	16 (44)
	High (4-6)	14 (39)
Previous SARS-CoV-2 infection	Yes	18 (50)
Number of previous SARS-CoV-2 infections	0	18 (50)
	1	13 (36)
	2	5 (14)
Comorbidities	No	17 (47)
	Yes	19 (53)
Autoimmunity	No	34 (94)
	Yes	2 (6)
Use of chronic medications	No	34 (94)
	Yes	2 (6)
Blood group	A	14 (39)
	B	2 (6)
	O	20 (56)
Tobacco smoking	Never	33 (92)
	Past	2 (6)
	Current	1 (3)
Moderate alcohol consumption[a]	No	13 (36)
	Yes	23 (64)
Physical activity[b]	No	20 (56)
	Yes	16 (44)
Second vaccine booster	No	33 (92)
	Yes	3 (8)

[a] ≤2 drinks/day for men and ≤1 drink/day for women.
[b] ≥150 minutes/week of moderate-intensity physical activity or ≥75 minutes/week of high-intensity physical activity during the free time.

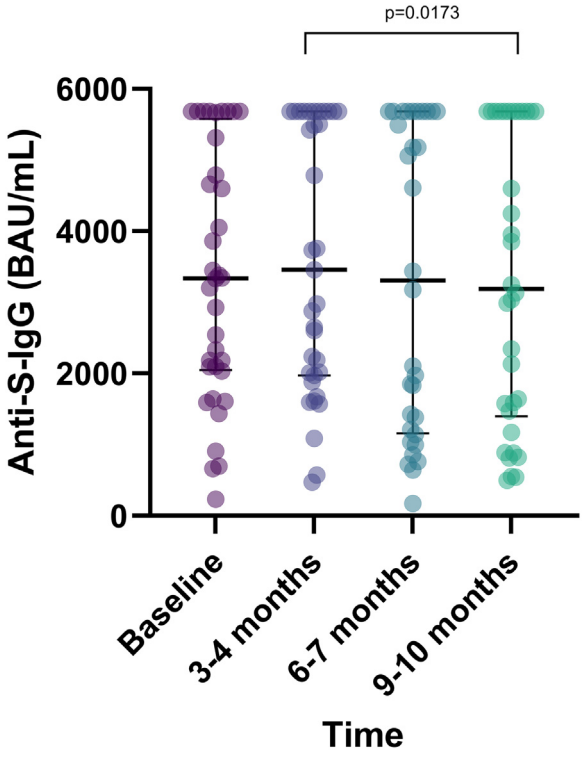

Figure 3. Anti-spike SARS-CoV-2 IgG levels after the BNT162b2 primary schedule and the messenger RNA-1273 booster. IgG antibodies were measured at baseline on days 4-44 after the two-dose primary schedule of BNT162b2. There were no statistically significant differences between antibodies before the booster and 3-4 months after the booster. After the vaccine booster, antibodies decreased over time. Statistical significance was measured using the Skilling–Mack test at a significance level of 5%.
Ig, immunoglobulin.

doctors (14%). A total of 53% percent of participants had any type of comorbidities, including one participant who had rheumatoid arthritis that was treated with methotrexate and one who had ulcerative colitis that was treated with azathioprine. Other reported comorbidities included gastritis, migraine, allergies, acne, alopecia, and hypothyroidism. Before baseline, half of the participants had COVID-19, and 14% had been re-infected. During the follow-up period, four laboratory-confirmed SARS-CoV-2 infections occurred. In addition, three participants received a second booster during the study follow-up.

Participants' mean BMI was 25 ± 3 kg/m^2, and the majority had type O (56%) or A blood type (39%) (Table 2). The majority had never smoked tobacco (93%) and had a moderate alcohol consumption (two or less drinks/day for men and one or less drink/day for women [17]) (64%), with a median consumption of two portions (IQR one to three). More than half of participants (58%) engaged in physical activity regularly, and 44% complied with World Health Organization recommendations for physical activity (≥150 minutes/week of moderate-intensity physical activity or ≥75 minutes/week of high-intensity physical activity [18]). The median time for physical activity was 95 minutes (IQR 0-210) per week.

At baseline, the median SARS-CoV-2 anti-S IgG was 3337 BAU/ml (IQR 2060-5489) (Figure 3). On days 4-9, after the second dose of BNT162b2, the median anti-S IgG was 3384 BAU/ml (IQR 2090-5666), and on days 29-44 days, after the second dose, it was 2540 BAU/ml (IQR 1642-3341) (Supplementary Figure S2).

Participants received the booster dose on average 267 days after completing the two-dose BNT162b2 schedule. The median SARS-CoV-2 anti-S IgG 3-4 months after receiving the mRNA-1273 booster was 3459 BAU/ml (IQR 988-5680). The difference in medians before the booster and 3-4 months after the booster was not statistically significant (P = 0.6257) (Figure 3). During the follow-up period, SARS-CoV-2 anti-S IgG decreased, with a median of 3306 BAU/ml (IQR 1177-5680) by 6-8 months after the booster and 3188 BAU/ml (IQR 1471-5680) 9-10 months after the booster (P = 0.0173) (Figure 3). None of the participants had negative SARS-CoV-2 anti-S IgG at baseline or during follow-up. At baseline, four (11%) participants had SARS-CoV-2 anti-S IgG levels in the lowest 10th percentile, ranging from 233 to 908 BAU/ml. Three of them had a history of COVID-19, and two had comorbidities, specifically, hypothyroidism. In all cases, IgG levels increased significantly after the booster, accompanied by high IgA levels and strong neutralization activity (Supplementary Figure S3).

The median IgA antibodies ratio 3-4 months after the booster was 8.550 (IQR 7.918-8.763). This remained stable over the course of the follow-up, with a median ratio of 8.630 (IQR 7.973-8.803) 6-7 months and 8.610 (IQR 7.840-8.785) 9-10 months after the booster. There were no statistically significant differences across these measurements (P = 0.7036) (Figure 4a). Throughout the observation time, none of the participants had an IgA ratio below the positivity threshold. Regarding neutralizing antibodies, the median percentage of inhibition in months 3-4 after the booster was 98.28% (IQR 97.81-98.69%). There was an increase that remained stable over the rest of the follow-up (98.57%, IQR 98.21-98.85%, on months 6-7; 98.57%, IQR 98.34-98.71%, on months 9-10 after the booster) (P = 0.0340) (Figure 4b). Neutralization was

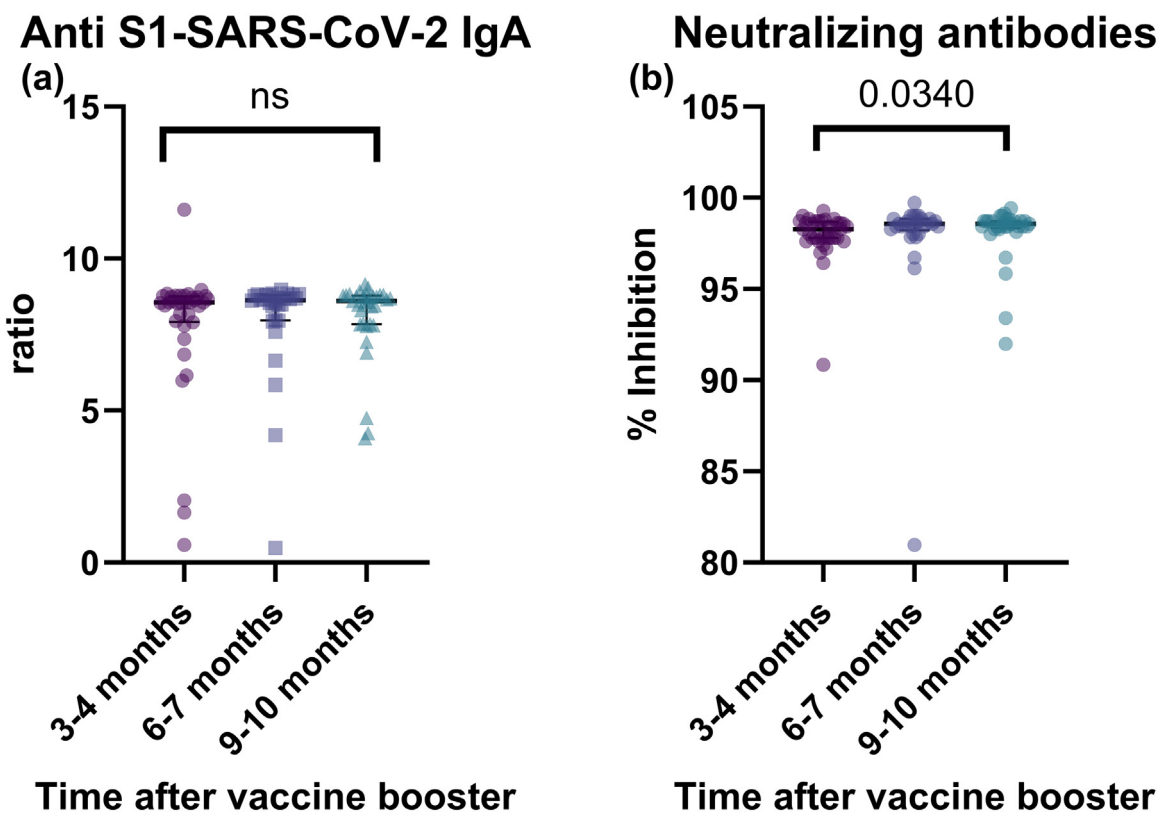

Figure 4. Anti-spike SARS-CoV-2 IgA and neutralizing antibodies after the messenger RNA-1273 booster in participants with the BNT162b2 primary schedule. IgA and neutralizing antibodies were measured after the booster. Statistical significance was measured using the Skilling–Mack test at a significance level of 5%. Ig, immunoglobulin.

above the positivity threshold for all participants during the observation period.

The multivariate regression model of factors related to anti-S IgG after the vaccine booster was adjusted for anti-S IgG levels after the second vaccine dose, age, BMI, blood group, SARS-CoV-2 infection, and a history of receiving a second booster. In this model, for each additional year of age over time, post-booster SARS-CoV-2 anti-S IgG increased, on average, 165 BAU/ml (95% confidence interval 60-269, $P = 0.002$) (Table 3). Individuals with B blood type had on average 5881 BAU/ml less IgG antibodies post-booster compared to people with group A (95% confidence interval −10037 to −1726, $P = 0.006$).

In addition, those with obesity and group B blood type had fewer IgA antibodies ($P = 0.0278$ and 0.0331, respectively) (Figure 5). Similarly, participants with autoimmunity and those with group B blood type had fewer neutralizing antibodies ($P = 0.0158$ and 0.0064, respectively) (Figure 5).

Cellular immunogenicity

For the cellular immunogenicity analysis, a subgroup of 16 participants without acute or chronic diseases or use of medications were included. Their median age was 45 years (IQR 38.5-48.5). The majority were women (75%), 62.5% previously had COVID-19 infection, and their median BMI was 23.98 kg/m^2 (IQR 22.48-27.73 kg/m^2). There were no statistically significant differences in T-cell responses based on previous SARS-CoV-2 infection (Supplementary Figure S4).

Discussion

Our study provides insights into the long-term humoral immune response to SARS-CoV-2 infection and the humoral and cellular immunogenicity of the mRNA-1273 booster in HCWs previously vaccinated with two doses of BNT162b2. In HCWs with natural immunity, IgG and IgA responses peaked within the initial 3 months after infection, remaining positive through follow-up, up to 283 days after infection. However, inhibition by neutralizing antibodies was below the positive range (≥35%) throughout the follow-up period. Conversely, vaccine-induced and hybrid immunity resulted in a higher percentage of inhibition by neutralizing antibodies, exceeding 97%. After receiving a 2BNT162b2 + 1mRNA-1273 schedule, IgG titers decreased over time but remained positive for up to 10 months post-booster. IgA and neutralizing antibodies remained stable for the same duration. We identified factors related to humoral response, including age, BMI, autoimmunity, and blood type.

Although detectable IgG and IgA responses were identified in patients with natural immunity in our study, these were accompanied by a low percentage of inhibition by neutralizing antibodies. This is likely associated with participants exhibiting a milder disease presentation. Previous studies have evaluated neutralization capacity in individuals exposed to SARS-CoV-2 based on disease severity and found low levels of neutralizing activity in those with asymptomatic and mild infections [19].

After booster administration, an initial increase in antibody levels is expected. A clinical trial conducted in the United States evaluated the humoral immunogenicity of homologous and heterologous schedules up to a month after booster administration [10]. In participants vaccinated with a 2BNT162b2 + 1mRNA-1273 schedule, an increase in antibody levels was evident, peaking by day 15 post-booster [10]. However, prospective studies with longer follow-up periods have shown that humoral responses decrease over time. For instance, a study conducted in Spain evaluating humoral and cellular responses to a 2BNT162b2 + 1mRNA-1273 schedule identified that circulating humoral responses due to the booster declined after 6 months [11]. Yet,

Table 3
Factors related to anti-Spike SARS-CoV-2 IgG antibody levels over time after receiving the messenger RNA-1273 vaccine booster.

Variables	Bivariate analysis				Multivariate reduced model[a]			
	Coeff	*P*-value	95% CI		Coeff	*P*-value	95% CI	
IgG pre-booster	−0.149	0.510	−0.594	0.295	−0.321	0.185	−0.796	0.153
Sampling time								
3-4 months post-booster	Ref							
6-7 months post-booster	−506.045	0.176	−985.711	312.376				
9-10 months post-booster	−422.297	0.429	−1468.064	623.4687				
Age	145.727	**0.001**	62.656	228.798	164.564	**0.002**	60.312	268.815
Sex								
Male	Ref							
Female	92.634	0.992	−1751.331	1936.600				
Socioeconomic status								
Low (1-2)	Ref							
Mid (3)	−1223.235	0.395	−4039.221	1592.752				
High (4-6)	−966.225	0.525	−3432.769	1941.230				
Body mass index								
Normal weight	Ref				Ref			
Overweight	723.9461	0.414	−1011.420	2459.312	1237.538	0.186	−596.299	3071.375
Obesity	−2133.121	**<0.001**	−3195.610	−1070.632	−2008.773	0.099	−4395.815	378.2678
Blood group								
A	Ref				Ref			
B	−2143.335	**0.013**	−3835.438	−451.232	−5881.276	**0.006**	−10,036.960	−1725.593
O	518.621	0.553	−1193.917	2231.160	−510.676	0.574	−2289.469	1268.117
Autoimmunity								
No	Ref							
Yes	−287.732	0.799	−2498.100	1922.635				
Tobacco								
No	Ref							
Current or previous	−1921.504	**<0.001**	−2911.658	−931.350				
Alcohol consumption								
No	Ref							
Yes	−241.064	0.786	−1981.401	1499.273				
SARS-CoV-2 infection								
No	Ref				Ref			
Previous infection	−751.919	0.388	−2457.954	954.116	−96.340	0.922	−2029.309	1836.629
Infection during follow-up	−1799.949	0.233	−4760.037	1160.139	835.445	0.590	−2199.680	3870.570
Second booster								
No	Ref				Ref			
Yes	2011.721	0.600	−5505.627	9529.070	3663.525	0.350	−1036.802	8363.853

n_participants: 36, n_observations:101.
[a] Wald test P <0.0001.

despite the decline in IgG titers over time, antibody functions, including neutralizing capacity and Fc-dependent effector functions, remain highly relevant for protection against COVID-19 [20]. In our study, antibody neutralization was sustained throughout the observation period, with the percentage of inhibition remaining above 98% up to 10 months post-booster.

In our study, obesity was associated with lower IgA titers after booster administration. Obesity is known to impair immune responses through multiple mechanisms involving alterations in leukocyte development, phenotypes, and activity [21,22]. In addition, obesity is a risk factor for chronic diseases and often accompanied by multimorbidity [23]. It has been consistently associated with worse COVID-19 outcomes, including increased risk of hospitalization and mortality [24]. A systematic review showed that obesity was significantly associated with lower antibody titers after COVID-19 vaccination [25]. In addition, previous research suggests that the waning of vaccine-induced humoral immunity is accelerated in individuals with severe obesity [26].

We identified that blood type B was associated with lower IgG, IgA, and neutralization responses. There is scarce and controversial evidence regarding the association between blood type and humoral response. Blood group antigens are important receptors or coreceptors for microorganisms and may influence responses to other vaccines, such as polio [27]. Regarding COVID-19, blood group B was reported to be associated with a higher susceptibility to infection in non-vaccinated individuals [28]. Studies with larger sample sizes are required to confirm whether ABO type influences humoral responses after COVID-19 vaccination.

We also identified that older age was associated with higher IgG responses post-booster, likely because older individuals had greater deficits after the primary vaccination series. This aligns with previous research indicating that the response after the vaccine booster was enhanced in older people without previous infection who exhibited lower baseline levels [29]. Booster vaccination may overcome the effects of frailty and age on antibody responses in the elderly population [30,31]. In older individuals lower humoral and IFN-γ responses after primary vaccine series have been reported; however, the booster dose significantly increases humoral responses, reducing the discrepancies between age groups [32]. Given the higher risk of severe COVID-19 in older adults, these findings highlight the importance of booster doses in maintaining adequate protection. Our study is focused on HCWs and not representative of the elderly population.

Monitoring antibody levels over time helps determine how long a response may last. However, immune correlates of protection have not been established; thus, it is not yet clear whether higher levels of antibodies correlate with better outcomes. A randomized clinical trial analyzed the association between antibody levels and SARS-CoV-2 infection, showing that higher levels of all immune markers were correlated with a reduced risk of symptomatic SARS-CoV-2 infection [33]. Nevertheless, infection can still occur in the presence of high levels of antibodies [34]. Correlates of protection after COVID-19 vaccination are

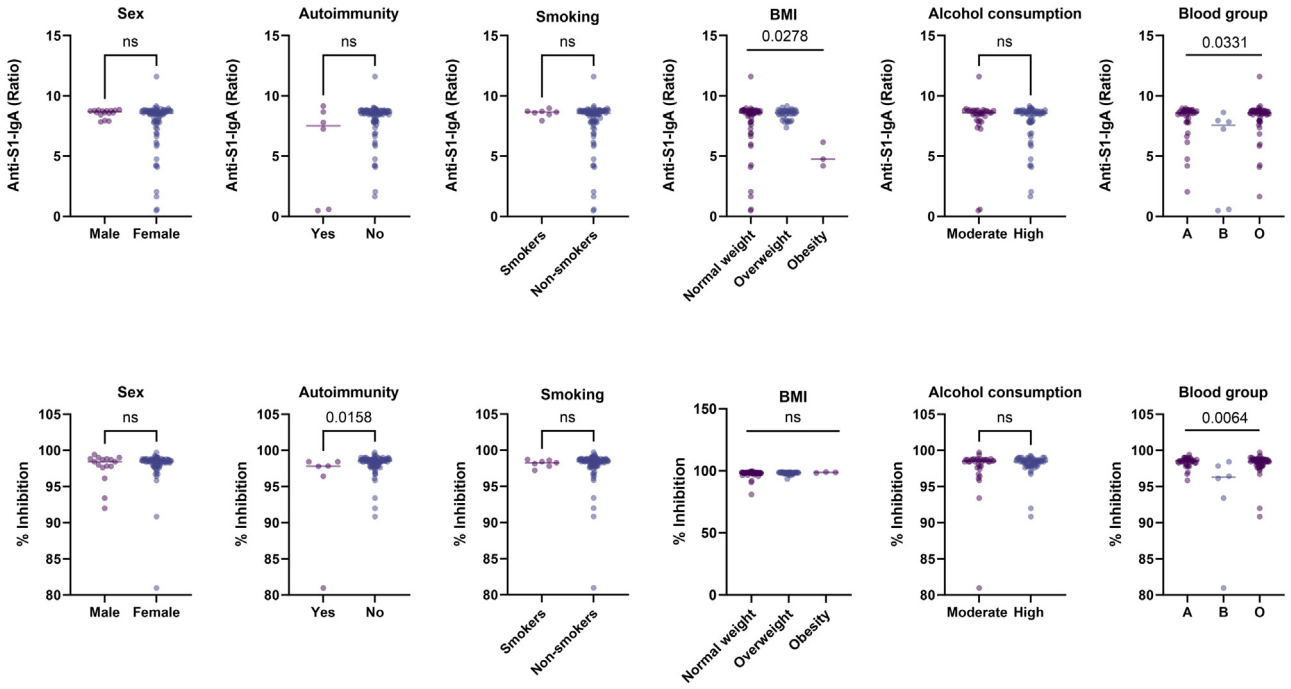

Figure 5. Anti-spike SARS-CoV-2 IgA and neutralizing antibodies after the mRNA-1273 booster according to participants characteristics. IgA and neutralizing antibodies were measured according to participants' sex, autoimmunity, smoking, BMI, and alcohol consumption. Statistical significance was measured using the Mann–Whitney test at a significance level of 5%. For IgA antibodies, ratios above 1.1 were considered positive. For neutralizing antibodies, values above 35% of inhibition were considered positive.
BMI, body mass index; Ig, immunoglobulin.

probably relative, meaning that most infections are prevented at a particular level of response, but some will occur above that level, likely because of host-dependent factors [34].

Previous findings have demonstrated that vaccination combined with natural infection is better than vaccination alone [35]. In our analysis, we did not find differences among T-cell responses to peptides pools; this is likely due to the small sample size for this analysis, which limits our ability to draw definite conclusions. However, other studies have shown higher IFN-γ and IL-2 responses to the Spike, Membrane, and Nucleocapsid proteins in previously infected and vaccinated individuals than those in uninfected participants after mRNA vaccines [36].

Although our study did not evaluate the specific SARS-CoV-2 variants causing the infections; multiple variants of concern, including Mu, Delta, and Omicron, were circulating in the country during the study period. The 21H (Mu) variant was predominant during the first half of 2021, followed by the 21J (Delta) variant, which predominated in the second half of 2021 [37]. Subsequently, the 21K and 21L (Omicron) variants became predominant during 2022 [37]. These circulating variants could impact the immune responses. A previous study conducted in Colombia assessed SARS-CoV-2–specific antibody and T-cell responses against the Mu, Gamma, and Delta variants. The authors found detectable binding antibody cross-recognition for the Gamma, Mu, and Delta variants, but the antibodies poorly neutralized the Mu variant [38].

This study has some limitations. We consecutively included participants without using probabilistic sampling methods; thus, our study results may only be extrapolated to populations that share similar characteristics. In addition, because this is an observational study conducted in a university hospital setting, the participants' demographic characteristics reflect real-world trends. The strengths of the present study include the longitudinal design with quantitative repeated measures of antibodies over time and the consideration of factors related to humoral response. We analyzed multiple components of the humoral response, including IgG, IgA, and neutralizing antibodies. We prospectively followed up participants up to 9.4 months after infection and 10 months after receiving the vaccine booster, providing insights into the kinetics of the humoral response in the long term. To address the longitudinal and censored nature of the chemiluminescent microparticle immunoassay kit data, we constructed a random-effects Tobit model. By considering the within-subject variation in antibody responses over time, the random-effects model accounts for fluctuations in responses that may be because of factors other than the vaccine booster.

In conclusion, our results provide insights into the long-term immune response against SARS-CoV-2. The 2BNT162b2 + 1mRNA-1273 schedule generated a humoral response in HCWs for up to 10 months. Further exploration of factors related to immune responses to vaccines is relevant to tailor efficient vaccination strategies.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethics statement

The study protocol was approved by the Fundación Santa Fe de Bogotá Ethics Committee (CCEI-12183-2020, and CCEI-13882-2022). This study was conducted in compliance with Act 008430-1993 of the Ministry of Health of Colombia and is classified as minimal-risk research. All patients provided their written informed consent and were informed about the Colombian data protection law (1581 of 2012). All research was performed under relevant guidelines and regulations and in accordance with the Declaration of Helsinki.

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Author contributions

Nohemi Caballero (conceptualization, methodology, validation, formal analysis, investigation, data curation, writing original draft, visualization, project administration, funding acquisition), Diana M. Monsalve (methodology, validation, investigation, data curation, writing review and editing), Yeny Acosta-Ampudia (methodology, validation, investigation, data curation, writing review and editing), Natalia Fajardo (methodology, investigation, writing - original draft, visualization), Sergio Moreno (conceptualization, methodology, formal analysis, writing review and editing), Oscar Martínez Moreno (conceptualization, writing review and editing), Catalina González-Uribe (conceptualization, writing review and editing), Carolina Ramírez-Santana (conceptualization, methodology, writing original draft, project administration, funding acquisition, supervision), and Juliana Quintero (conceptualization, methodology, writing review and editing, project administration, funding acquisition, supervision).

Data availability

Data collected from the study, including individual participant data that underlie the results reported in this article (after de-identification), and other related study documents (questionnaires) are available in the supplementary materials. The study data set is available in Supplementary File S4.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2025.100641.

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