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Longitudinal Follow-Up of the Specific Antibody Response to SARS-CoV-2 Vaccination in Colombia

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ABSTRACT

A total of 5011 adult volunteers attending vaccination centers in different regions of Colombia were enrolled in a 1-year prospective observational cohort study to evaluate the immunogenicity and effectiveness of SARS-CoV-2-based vaccines as part of a National Vaccine Program established to contain the COVID-19 pandemic. Following informed consent, 5,011 participants underwent a sociodemographic survey and PCR testing to assess SARS-CoV-2 infection. Blood samples were collected, and serum fractions were obtained from a participant subsample ($n = 3441$) at six-time points to assess virus-specific IgG responses to the Spike protein, its Receptor Binding Domain, and the Nucleoprotein by ELISA. Additionally, antibody-neutralizing activity was evaluated using a cPass SARS-CoV-2 neutralization kit. Most participants (95.8%; $n = 4802$) received between one Ad26. COV2.S (Janssen vaccine) and four vaccine doses of BNT162b2 (Pfizer/BioNTech), AZD1222 (AstraZeneca), mRNA-1273 (Moderna), CoronaVac (Sinovac), with some receiving vaccine combinations; a small group, 4.2% ($n = 209$), remained unvaccinated. Throughout the study, only 8.76% ($n = 439$) of the participants tested positive for SARS-CoV-2 by PCR. Notably, all participants seroconverted for IgG antibodies, with high seropositivity rates for S (99.8%; $n = 4795$), RBD (99.7%; $n = 1691$), and N (92.7%; $n = 3072$) proteins. Moreover, significant (92%–97%) neutralizing activity was observed for all four SARS-CoV-2 circulating variants. This study highlights the importance of assessing the duration of the IgG response to SARS-CoV-2 elicited by vaccination and infection, and the antibody neutralizing activity as a potential surrogate marker of protection. These findings provide important insight for further strengthening the vaccination strategies to control COVID-19.

1 | Introduction

More than 4 years have passed since the emergence of the severe acute respiratory syndrome (SARS) outbreak in China in 2019, and despite the monumental efforts of a global vaccination campaign, coronavirus-2 (CoV-2) continues to impact populations globally [1]. The World Health Organization (WHO) has documented over 774.6 million confirmed cumulative cases and nearly 7 million deaths worldwide [2], including approximately 193.2 million cases in the Americas region [3]. The first clinical COVID-19 case in Latin America was reported in Brazil in February 2020 [4], followed by Colombia a month later [5], where spread rapidly led to 1.8 million cases by the end of 2020 [6].

After completing phase I and II studies [7, 8], mass vaccination campaigns were initiated in several countries, including China, the USA, and Europe. The Sinovac (CoronaVac) and BNT162b2 (Pfizer/BioNTech) vaccines were rapidly distributed and widely administered in January 2021 [9]. Gradually, vaccination expanded to encompass other regions starting in the spring of 2021 [10]. Colombia launched a National Vaccine Program (NVP) on February 17, 2021 [11], using various vaccines, including BNT162b2 (Pfizer/BioNTech), AZD1222 (AstraZeneca), mRNA-1273 (Moderna), CoronaVac (Sinovac), and Ad26.COV2.S (Janssen), some acquired under the multilateral COVID-19 Vaccines Global Access (COVAX) initiative. Initially, the recommended vaccination regimen consisted of two homologous vaccine doses at 3–12-week intervals [12]; however, as the pandemic evolved, the vaccine strategy underwent modifications, allowing the combination of vaccine types [13, 14] to ensure effective protection against new transmission peaks and virus variants. Nevertheless, all vaccines were designed to target the SARS CoV-2 spike (S) protein, whose receptor binding domain (RBD) fragment is involved in virus attachment to the ACE receptor and the subsequent host cell viral invasion [15]. Consequently, numerous publications have measured seroprevalence and neutralizing antibodies (Ab) in severe and non-severe COVID-19 cases, focusing on the anti-S antibody responses, particularly those to the RBD fragment [15] and their association with protection induced by infection or vaccination [16–19].

However, there is a scarcity of reported longitudinal studies in Latin America and Colombia [20, 21], where a significant reduction in COVID-19 incidence and associated complications was observed in response to vaccination. To support future COVID-19 management policies, this study presents a 1-year follow-up of the evolution of the specific IgG antibody responses postvaccination (six-time points) and disease in representative regions of Colombia as well as the antibody-neutralizing activity against the four circulating SARS- CoV-2 variants (Wuham, Mu, Omicron BA.1 and BA.1).

2 | Methods

2.1 | Study Design and Ethical Aspects

An observational prospective cohort study was conducted on 5011 adult participants across seven cities in Colombia (Figure 1). Adult subjects were recruited at 12 Official Vaccination Posts (OVP), where they were voluntarily seeking for vaccination. OVPs were primarily located within Hospitals/Clinics authorized by the Ministry of Health (MoH), as part of the COVID-19 NVP.

The study protocol, including the case report forms (CRF) containing socio-demographic (SDS) and clinical information, and the informed consent (IC) documents were reviewed and approved by the Ethical Committees affiliated with Centro Internacional de Vacunas in Cali (CECIV code No. 2103), Fundación Santa Fe de Bogotá (code No. CCEEI-13717-2021) and all remaining participating institutions to ensure compliance with the International Conference on Harmonization guidelines. After written IC, all participants underwent comprehensive assessments, including SDS, comorbidities and medication consumption. Immunoreactivity evaluations were conducted at predefined intervals to monitor the kinetics of the immune response postvaccination and disease, including assessment of virus-specific IgG levels and sera-neutralizing activity, together with the evaluation of vaccines' long-term effectiveness (manuscript in preparation). Participants were also followed up weekly by telephone for 1 year. Any self-reported symptoms, consistent with a clinical COVID-19 case prompted PCR testing to confirm potential infections.

Study data were collected and managed using REDCap electronic data capture tools, a secure web application for building and managing online surveys and databases [22]. This ensured the accuracy, completeness, and integrity of the datasets and facilitated robust data collection and analysis. Confidentiality

Location of study sites	No. participants (%)
Barranquilla:	559 (11.1)
Bogotá:	621 (12.4)
Bucaramanga:	383 (7.6)
Cali:	511 (10.2)
Cúcuta:	902 (18.0)
Manizales:	752 (15.0)
Pasto:	1,287 (25.7)

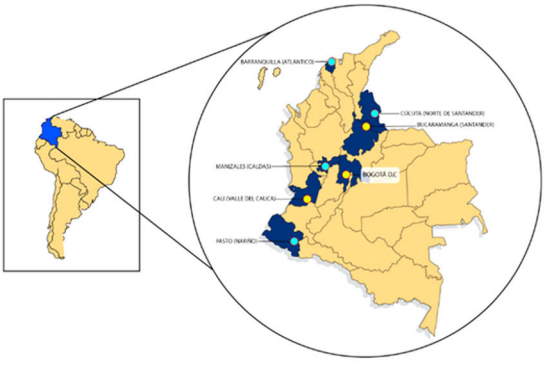

FIGURE 1 | Site of the study and distribution of study participants.

and data protection measures were rigorously maintained throughout the study duration to safeguard the privacy and rights of participants.

2.2 | Study Sites and Laboratory Assays

Volunteers were recruited in 12 OVPs across seven designated cities in Colombia: Barranquilla, Bogotá, Bucaramanga, Cali, Cucuta, Manizales, and Pasto, strategically selected based on their accessibility and robust health infrastructure, also ensuring representation of the country's significant ethnic and socioeconomic diversity. Moreover, these cities served as reference sites for areas accounting for ~26% of the total Colombian population [23]. Physicians and technical staff trained in COVID-19 management and good laboratory practices actively participated in the recruitment and sample collection processes at each OVP (Figure 1).

2.3 | Enrollment and Follow-Up

The study cohort of 5011 individuals was stratified into three categories: unvaccinated, fully vaccinated, or receiving one or two booster vaccine doses. Participants were provided with comprehensive oral information about the study before providing written IC. Booster doses refer to vaccinations after the complete two-dose or one-dose vaccine protocols recommended by WHO and pharmaceutical companies [24]. At enrollment, participants were requested a nasopharyngeal swab for SARS-CoV-2 qPCR molecular testing and an arm venipuncture for collecting whole blood (7–15 mL).

Subsequently, a subsample of 3441 subjects accepted to participate in the evaluation of long-term specific IgG antibody response at designated time points: 0, 1-, 2-, 5-, 8-, and 11-months post-enrollment. Blood samples were collected into Vacutainer clot activation gel tubes and separated by centrifugation into sera and cell fractions. Sera fractions maintained frozen at −20°C until analysis were periodically transferred to three designated reference laboratories in Bogotá, Bucaramanga, and Cali, selected to ensure consistency and reliability in sample handling and analysis. The coordination of sample preparation and transportation to these laboratories was carried out by trained staff in adherence to the WHO Guidelines for Infectious Substances 2021–2022 [2], with the assistance of specialized biological cargo companies.

2.4 | ELISA Studies

2.4.1 | Reference Research Laboratories

Three collaborative research institutions with extensive experience, advanced facilities, and strategic location were selected to perform the ELISA test: BioMedical Science Laboratory, Universidad de Los Andes (UniAndes, Bogotá), Laboratory of Molecular Genetics of Infectious Diseases and Cancer (GEMEICA), Universidad Industrial de Santander (UIS, Bucaramanga), and Serology Laboratory at Centro Internacional de Vacunas (CIV, Cali). An in-house-developed binding antibody assay protocol, previously standardized at CIV, was transferred to these research institutions along with negative and positive sera control pools [25, 26].

2.4.2 | Recombinant SARS CoV-2 Antigens

The antibody response was determined by ELISA, using as antigens the virS protein, corresponding to a recombinant trimer protein expressed in Chinese Hamster Ovary (CHO) cells and the recombinant RBD domain (Wuham-Hu-1, Arg 319-Phen 541, code 002) expressed in Human Embryonic Kidney 293 (HEK-293) cells [27]. Both antigens were produced by Excellgene SA (Montey, Switzerland). The recombinant N (His Tag) protein produced in *E. coli* was acquired from GenScript Inc (860 Centennial Av, Piscataway NJ 08854, Catalog No: Z03480-1).

2.5 | ELISA Test

Samples seroreactivity was evaluated by ELISA using 96-well plates (Nunc-Immuno Plate, Maxisorp, Roskilde, Denmark) coated with S, RBD, and N recombinant proteins at a concentration of 1 μg/mL, incubated overnight in PBS 1×, pH 7.4 at 4°C [26]. After plates were blocked with 5% skim milk solution (PBS 1×, 0.05% Tween 20, [PBS-T]) for 2 h, sera samples were added at 1:100 dilution in 1.5% skim milk in PBS-T and were incubated for 2 h. Then, plates were washed and incubated with alkaline phosphatase-conjugated anti-Human IgG antibody (Sigma Chemical Co., St Louis, MO) at a 1:5000 dilution for 1 h. Reactions were revealed with para-nitrophenyl phosphate substrate (p-NPP) (Sigma Aldrich) and read at 405 nm wavelength (Dynex Technologies Inc. MRX Chantilly, VA). ELISA cut-off points were calculated as three standard deviations (SD) above the mean absorbance value at 405 nm of sera from a pool of naïve pre-pandemic donors ($n = 50$) from Cali kept at the CIV cryo-bank, used as a negative control [28]. The results were expressed as reactivity index (RI), defined as OD values of tested samples divided by the cut-off value. $p < 0.05$ were considered significant.

2.6 | Neutralization Antibody Test

The serological test to detect neutralizing antibodies to the SARS-CoV-2 binding to the RBD domain (cPass SARS-CoV-2 Neutralization Antibody Detection Kit, GenScript, Diagnostics Technology Co. Ltd.) was kindly donated by Dr. J.F. Drexler from the Institute of Virology, Charité, Berlin, Germany. It detects neutralizing antibodies able to block the interaction between the angiotensin-converting enzyme 2 (ACE2) receptor protein and the RBD domain of four SARS-CoV-2 variants (Wuhan, Omicron BA.1 and BA.2, and Mu) [29]. Briefly, Nunc Maxisorp ELISA plates (Nunc-Immuno Plate, Maxisorp, Roskilde, Denmark) were previously precoated with 1 μg/mL hACE2. All reagents were equilibrated to room temperature (20°–25°C) before use. The RBD conjugated with HRP was then diluted with HRP dilution buffer at a 1:1000, and the wash solution was also diluted with deionized or distilled water at a 1:20 volume ratio. Controls (positive and negative) and samples were diluted with sample dilution buffer at a 1:10. The diluted

controls and samples were mixed with the diluted HRP-RBD solution in a 1:1 and incubated at 37°C for 30 min. Then, 100 μL of each mixture (controls and samples) was added to each well. The plate was covered with a plate sealer and incubated at 37°C for 15 min, after which it was washed four times with 260 μL of 1× wash solution. Then, 100 μL of TMB solution was added to each well, and the plate was incubated in the dark at 25°C for 15 min, followed by the addition of 50 μL of stop solution to each well. Absorbance was read at 450 nm wavelength (Dynex Technologies Inc. MRX Chantilly, VA). Positive and negative controls were included in the cPass SARS-CoV-2 neutralization kit. OD of negative control had to be > 1.0 and OD of positive control < 0.3. The inhibition was estimated as (1-Sample OD/Negative control OD) *100. Therefore, cutoff values were positive ≥ 30% for neutralizing antibodies and ≤ 30% negative.

2.7 | Statistical Analysis

Data from the coded CRF were directly entered into REDCap, a metadata-driven methodology and workflow process for providing translational research informatics support during data and sample collection [22]. Each participant was assigned a number code known only to the investigators. The laboratory information was directly entered into Excel, and ELISA data were imported into Excel and associated with the participants' sociodemographic and epidemiological data registered in REDCap.

The purpose of this study was to evaluate the prevalence of antibody responses against the SARS-CoV-2 S protein in all participants, and to RBD and N proteins in a volunteer subsample. A descriptive analysis was conducted to evaluate trends in humoral responses in the corresponding study group (S, RBD and N antigens), and each group's antibody kinetics were depicted. The Friedman test was used to compare antibody titers for each protein at each time point and between vaccine groups, followed by Conovert's test for multiple comparisons for differences between means results. All statistical tests were performed using Prism 6 (GraphPad Software) or R (version 3.5.3, R Foundation for Statistical Computing), and $p < 0.05$ were considered significant.

3 | Results

The study involved 5011 adult volunteers enrolled over a period of 19 months between May 20, 2022, and December 19, 2023. A subsample of 3441 was followed for an 11-month period to assess virus-specific IgG responses to S, RBD, and N by ELISA. From this subsample, 635 participants were further evaluated for neutralizing antibodies. The participants' ages ranged from 18 to 95 years, with a median age of 38 years.

Demographic characteristics revealed that the majority of participants (61.4%, $n = 3079$) were female. The largest age group comprised individuals 19–35 years (44.8%), followed by 36–45 years (19.4%), 46–55 years (28.1%), 56–65 years (12.9%), and the smallest proportion (7.7%) were those older than 65 years (Table 1). There was a 49% compliance rate in adherence to the study follow-up timeline, facilitating the serological evaluations.

TABLE 1 | Sociodemographic characteristics of participants.

Characteristics	*n*	%
Age group mean (SD)	40.67 (15.83)	
19–35	2245	44.8
36–45	974	19.4
46–55	760	15.2
56–65	647	12.9
> 65	385	7.7
Gender		
Female	3079	61.4
Male	1932	38.6
Ethnicity		
Mestizo	4290	85.6
Afro-colombian	703	14.0
Indigenous	11	0.2
Raizal	3	0.1
Rom	3	0.1
Palenquero	1	0.0
City		
Pasto	1287	25.7
Cucuta	902	18.0
Manizales	752	15.0
Bogotá	619	12.4
Barranquilla	557	11.1
Cali	511	10.2
Vaccinated		
Yes	4764	95.1
No	209	4.2
No reported	38	0.8

3.1 | COVID-19 Status of Study Participants

As shown in Figure 2, the study protocol was formulated and approved at the beginning of the Colombian NVP implementation on 20 May 2022, aligning with the prevailing international consensus recommending a two-dose vaccination schedule to prevent or minimize the severe COVID-19 clinical manifestations and death [30]. It had been determined that despite the high protective efficacy of vaccination, the lifespan of elicited antibodies was shorter than expected, and more than two vaccine doses were considered necessary [31]. As the pandemic progressed, Colombia faced at least five transmission peaks [6]. The NVP vaccination activities were initiated in February 2021; therefore, the cohort represented a population with diverse COVID-19 experience. We found that 1239 out of the 5011 participants (24.7%) reported previous COVID-19 infection at enrollment; this information was confirmed in the

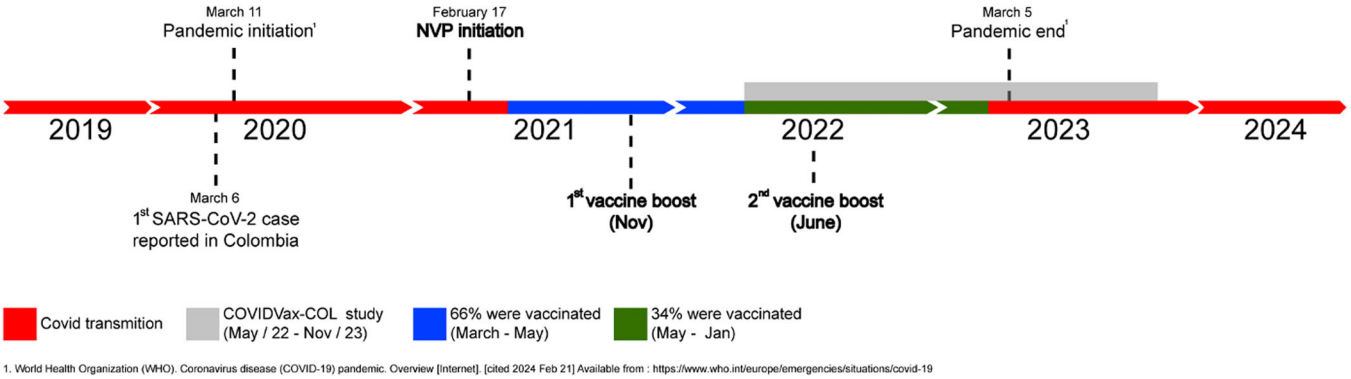

FIGURE 2 | Timeline of events during the COVID-19 pandemic and its relation to the study period. 1 Word Health Organization (WHO). Coronavirus disease (COVID-19) pandemic. Overview [Internet]. [cited 2024 Feb 21] Avialable from: https://www.who.int/europe/emergencies/situations/covid-19.

National Public Health Surveillance System (SIVIGILA) database. Additionally, in this visit, 167 (3.33%) who reported no COVID-19 symptoms, were confirmed to be infected, and 272 (5.43%) additional participants were confirmed by RT-PCR during the follow-up period.

In addition, volunteers were immunized with different COVID-19 vaccine schemes.

From the total cohort of 5011 participants, 4802 (96%) were vaccinated with at least one dose and 209 (4%) participants received no vaccination. From the vaccinated group, 4481 (93%) completed the recommended regime of either one dose (Jansen) or two doses (all others), and 321 (7%) received an incomplete scheme, one dose but not Janssen vaccine. Moreover, 3059 (68%) participants received booster doses using any available vaccine during the study period. The cohort group with complete vaccination scheme was further divided into a homologous scheme with 4258 (95%) volunteers, 626 (15%) receiving one dose of Jansen and 3632 (85%) receiving two doses of other vaccines (1684 participants (46.4%) received Pfizer, 622 (17.1%) Moderna, 554 (15.3%) AstraZeneca, and 772 (21.3%) Sinovac vaccine). In contrast, a heterologous scheme group was composed of 223 (5%) volunteers with two doses of any vaccine type (Figure S1).

3.2 | Seroprevalence of Antibodies Against S, Receptor-Binding Domain, and Nucleocapsid

While 4802 (95.83%) of the total 5011 volunteers underwent vaccination, the remaining 209 did not. The serological analyses indicated that virtually all of the vaccinated, as well as the nonvaccinated, were seropositive to the S protein 99.85% and 99.04%, respectively, as well as to RBD, and N proteins (Table 2). For anti-RBD antibodies, 1691 (99.7%) out of 1696 vaccinated subjects were positive, and only 5 (0.30%) were negative, whereas from the 48 nonvaccinated subjects, 47 were also positive. A significance of $p < 0.0001$ with a correlation ranged 0.40 and 0.48 (T2–T5) by Spearman test) was found between individuals positive for anti-S and anti-RBD antibodies. Out of 3469 subjects 3313 were vaccinated, of whom 3072 (92.73%) were positive and 241 (7.27%) negatives for anti-N antibodies, whereas 153 (98.07%) out of 156 nonvaccinated individuals were positive (Table 2).

The seroprevalence of samples was arbitrarily categorized based on the reactivity index (RI) into low (RI 1–5), moderate (6–10), and high (> 11). From the 4795 positive individuals, 94.9% developed moderate to high reactivity (Table 3). Most of the volunteers, 3044 (63.48%), were allocated to the highest reactivity category (> 11), 1507 (31.43%) to moderate, and 244 (5.09%) to the lowest category. Volunteers vaccinated with the Pfizer (BNT162b2) vaccine developed the highest reactivity, with 76% of the vaccinees corresponding to moderate and high (38% each).

3.3 | Kinetics of Anti-Spike Antibodies

Overall, IgG anti-Spike antibodies levels showed a significant decrease (21%–33%) ($p < 0.05$) between the first (T0) and the last sampling (T5) in all vaccine types when volunteers were subjected to the two-dose or one-dose (Janssen– Ad26.COV2S) recommended regime only, without booster doses (Figure 3A). However, in a group of individuals who received one or two booster doses, regardless of the vaccine type, the antibody levels remained high (Figure 3B); at the initial sampling (T0), a statistically significant difference in the average immune response ($p = 0.0077$) was observed. No difference ($p < 0.05$) was found in the RI at T1 ($p = 0.8497$), T2 ($p = 0.2176$), and T3 ($p = 0.1842$), however, at T4 ($p = 0.001$) and T5 ($p = 0.0023$), significant differences were noted between the means RI of individuals vaccinated with three and four doses. Despite the statistical disparity in the T4 and T5 measurements, the group with four doses was only 8.3% higher than the group that received three doses. Inferential statistics for this analysis are summarized in Table S2.

No statistical difference was observed between RI at time T0 and T3 (month 5) (RI = 13.00 and 11.83) ($p > 0.05$) in the AstraZeneca (AZD1222) IgG kinetics; however, there was a significant decrease (23.7%) between T0 and T5 (month 11) ($p = 0.036$, Table S1). Likewise, for the Janssen vaccine, no statistical differences were observed in the mean values between T0 and T3 (month 5) ($p > 0.05$), but a significant decrease was evident when comparing T0 and T5 (RI = 21.5%) ($p = 0.0304$).

In the case of the Moderna (mRNA-1273) vaccine, no statistical difference between the averages of T0 and T2 (month 2) was

TABLE 2 | Prevalence of antibodies against SARS-CoV2 antigens.

Status	Total	Anti-spike (%) $n = 5011$		Total	Anti-RBD $n = 1744$		Total	Anti-nucleopcapside $n = 3469$	
		Positive	Negative		Positive	Negative		Positive	Negative
Vaccinated	($n = 4802$)	4795 (99.85)	7 (0.15)	($n = 1696$)	1691 (99.7)	5 (0.30)	($n = 3313$)	3072 (92.73)	241 (7.27)
Nonvaccinated	($n = 209$)	207 (99.04)	2 (0.96)	($n = 48$)	47 (97.93)	1 (2.07)	(n = 156)	153 (98.07)	3 (1.93)

observed, however, a significant RI decrease (29.3%) was evident between T0 and T5 ($p < 0.0001$, see Figure 3A). Individuals who received the Pfizer vaccine displayed a constant decrease in reactivity between T0 and T4, with a decrease of 23.5% ($p < 0.0001$). However, stable reactivity was observed between T4 and T5 with a non-statistically significant difference. Sinovac's reactivity displayed the greatest reduction (32.3%) between the initial T0 and the T5 dose ($p < 0.05$).

Regarding differences in long-term anti-Spike antibody kinetics induced by different vaccines, a significant overall difference between vaccines was observed ($p = 0.0007$) except for Janssen vaccine. The Pfizer vaccine showed the highest mean ranks (Supporting Information S1: Table S1, Supplement).

3.4 | Age-Dependent Reactivity in the Three SARS-CoV2 Antigens

When the sera reactivity to each one of the SARS-CoV-2 proteins was evaluated by age group, at T0, significant differences to S and RBD were observed between most groups, although, no specific trend was observed (Figure 4). No statistical differences were observed in the seroreactivity to the N protein, among the different age groups. No significance differences were observed among the different age group related to the number of doses ($p < 0.07$).

3.5 | Prevalence of Neutralizing Antibodies

A sera subsample ($n = 608$) was evaluated to determine the neutralizing activity against the different circulating SARS-CoV-2 variants. All sera were selected based on those previously confirmed to display ELISA IgG antibody response to RBD (Table 2). As shown in Figure 5, a broad dispersion of the neutralizing antibodies (AcN) activity was observed. A greater number of sera without activity (< 30%) against the Omicron BA-1 variant can be observed; however, the same sera displayed significantly greater neutralizing activity on the other variants (Wuhan, Omicron BA-2, and Mu) and maintained positive neutralization activity throughout the study.

4 | Discussion

The Colombian government sponsored this prospective observational cohort study to determine the antibody lifespan and functional effectiveness as determined by their capacity to neutralize viral invasion and replication in the host cells and, therefore, prevent COVID-19 death and severe disease. The MoH acquired a variety of vaccines, including BNT162b2 (Pfizer/BioNTech), AZD1222 (AstraZeneca), mRNA-1273 (Moderna), CoronaVac (Sinovac), and Ad26. COOV2.S (Janssen) [32], all with valuable data on the safety, tolerability, and, more importantly, high efficacy profiles demonstrated in previous trials [33–35].

Results indicate that the different vaccines were highly immunogenic, as confirmed by the almost complete seroreactivity

TABLE 3 | Seroprevalence of IgG anti-Spike antibodies and the type of vaccine.

Type of vaccine	Low RI 1–5 (%)*	Moderate RI 6–10 (%)	High RI > 11 (%)
AstraZenaca	43 (17.7)	240 (15.9)	476 (15.6)
Janssen	44 (18.0)	199 (13.3)	382 (12.6)
Moderna	23 (9.4)	240 (15.9)	515 (16.9)
Pfizer	62 (25.4)	573 (38.0)	1156 (38.0)
Sinovac	72 (29.5)	255 (16.9)	515 (16.9)
Total	**244 (5.09)**	**1507 (31.43)**	**3044 (63.48)**

*RI: Reactivity Index: OD sample/OD negative control.

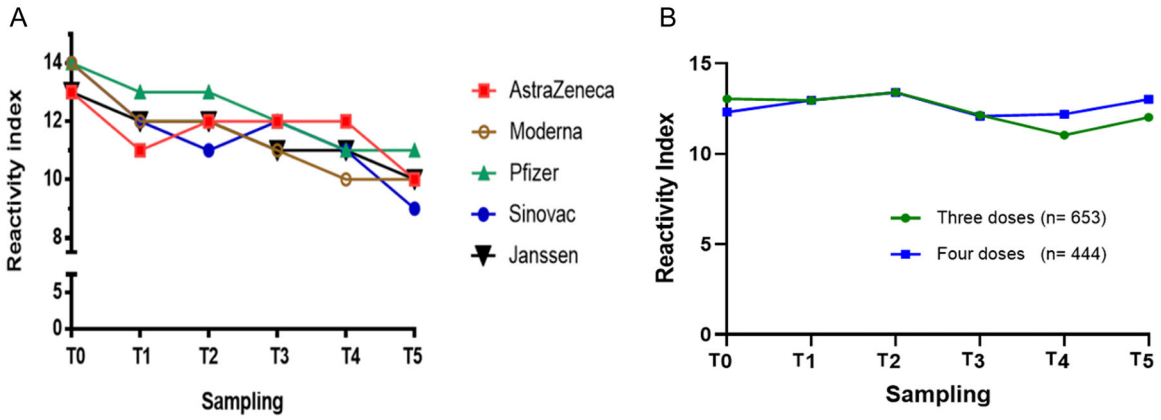

FIGURE 3 | Antibody kinetics are shown for subjects that received the complete schedule of the indicated vaccine (two-dose or one-dose (Janssen) recommended regime, without booster doses) (A); and for individuals with three of four doses regardless of the vaccine type (B).

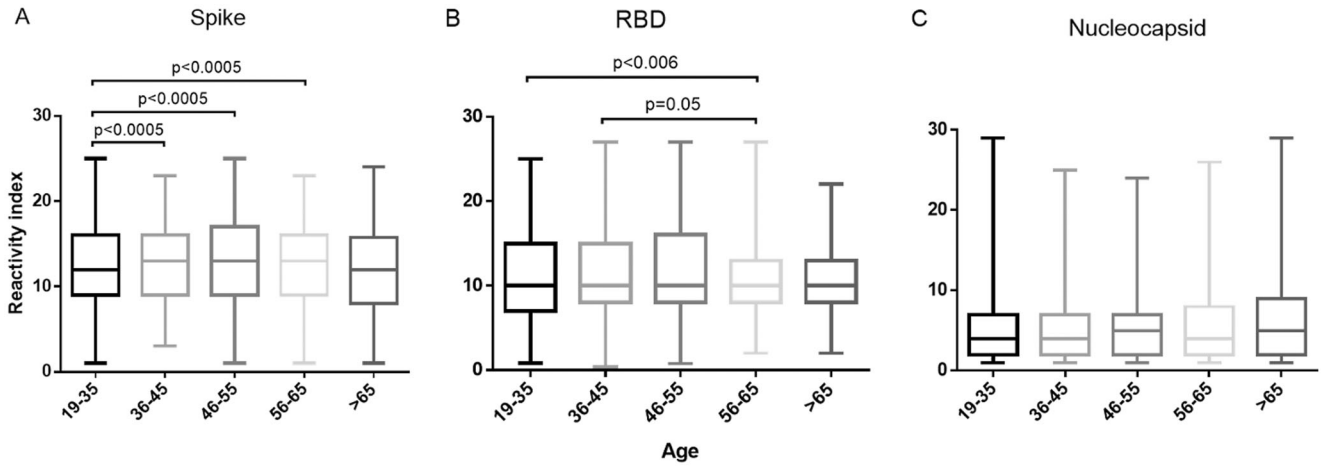

FIGURE 4 | Age-dependent reactivity is shown to SARS CoV2 proteins Spike (A), RBD (B), and N (C). Error bars correspond to mean values plus 2 SD. Statistical significance was observed between each age group in the different proteins ($p < 0.0005$).

after exposure to the vaccine-recommended protocol, either one dose (Janssen) or two doses (all others). Interestingly, more than 97% of vaccinated participants were reactive to the three selected viral proteins (S, RBD, and N), indicating the previous contact with the virus. As mentioned above, a significant number of clinical and presumably asymptomatic COVID-19 cases were already circulating in the country when the study started. This is confirmed by the fact that, 99% of the unvaccinated participants ($n = 209$) were also immunoreactive to S and N proteins at baseline time (T0) ($p < 0.0001$), suggesting their previous contact with the virus. In addition, except in individuals immunized with CoronaVac (16.5%; $n = 509/3079$), a lower positive antibody rate to the N protein than to the S and RBD proteins was observed, which has been already reported by others [36, 37] suggesting either a lower immunogenicity of the N protein or less access of this protein to the immune system due to its internal location within the virus, or both. Because most participants, had been immunized at

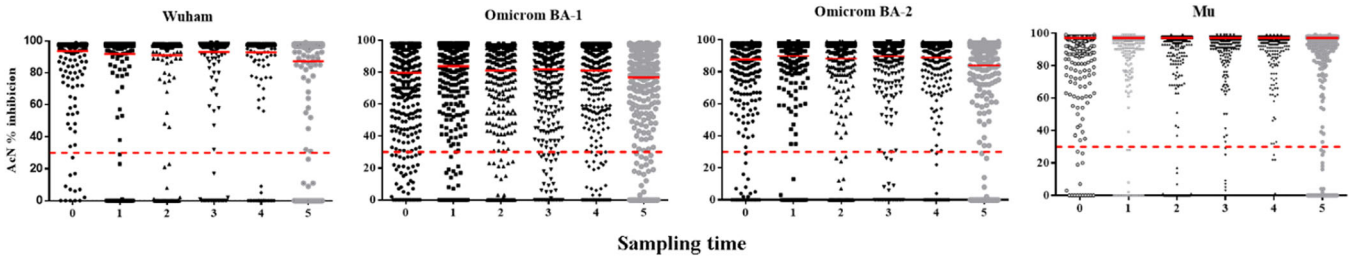

FIGURE 5 | Positive sera for IgG antibodies to the RBD protein were tested for their binding to the ACE2 receptor using the cPass neutralization antibody detection with the four virus variants. Only sera with neutralization activity above 30% were considered positive; the red line indicates the cut-off value.

enrollment, it is uncertain how many of them had also suffered symptomatic and asymptomatic infection, which will influence the frequency and intensity of the specific antibody response.

The negative response to the N protein in 241 (7%) participants suggest neither previous natural exposure to the virus nor exposure to the virus-attenuated vaccine (CoronaVac).

As described before, despite the high immunogenicity of all vaccine types, the two-dose schedule is not sufficient to provide long-term protection. As shown in Figure 3, there is an overall decline in reactivity of between 21% and 33% from T0 to T5 ($p < 0.05$). However, a group of individuals who received 1–2 booster doses, regardless of the vaccine type, maintained high antibody levels for the 11-month follow-up. This also occurred with the single dose recommended for Janssen´s vaccine, which required a booster dose to maintain anti-spike antibody levels high enough during the same period. Although not yet conclusive, it appears that a booster dose is sufficient to maintain protective antibody levels, probably for more than a year. This is a very encouraging observation, as a reduction in the number of vaccine doses and a longer interval between doses may maintain similar or even better vaccine effectiveness [38]. A longer interval between boosters improving the responsiveness of the immune system as a consequence of the recovery of the immune system has been reported, however, it has also been shown in other microorganisms (i.e. influenza, malaria) that repeated vaccinations are associated with reduced antibody-affinity maturation by the mechanisms of vaccine exhaustion, which may lower vaccine effectiveness [39–42]. In addition, repeated vaccination has been shown to raise the risk of inducing or potentiating adverse events in some cases [39].

Although significant differences were observed in response to the different viral proteins between most age groups, no specific trend was observed. Some studies have shown a reduced humoral response to the COVID-19 vaccine in individuals over 65 years of age due to immune-senescence; however, this was not significant [43].

It is interesting to note that the antibody response to the RBD occurs early after immune system priming. As shown in Figure 5, most sera were neutralizing immediately after the first vaccine doses and there was significant parallelism between the overall antibody titers and the sera-neutralization activity throughout the study. This observation further supports the convenience of spacing vaccine doses. In addition, the Omicron variant has had a significant impact on the development of AcN due to its immune evasion capabilities. It has been demonstrated lower AcN when compared to previous variants like Alpha, Beta, Gamma, and Delta, making it more challenging to control with pre-existing immunity, whether from infection or vaccines [44, 45].

It is important to highlight that in this study, the majority of volunteers produced antibodies against the wild-type strain and the viral variants Mu and Omicron BA.1, BA.2. Previous studies have shown that immunization with the COVID-19 vaccine hinders the production of neutralizing antibodies against the Omicron [44, 46] strains. Nonetheless, vaccinated individuals who have had a postvaccination infection generate higher titers of specific antibodies against Wuhan and Omicron strains [47] This is probably because during natural infection, the immune system is exposed to a more diverse set of antigens, which confers greater breadth and potency to the neutralization response. However, the protective effect conferred by vaccination is compromised by the appearance of viral variants with immune escape mutations such as Omicron [48–50]. Nonetheless, boosters, hybrid immunity, and the cellular immune response help maintain protection against severe outcomes, even as neutralization capacity against Omicron diminishes [44].

When we assessed the vaccination and infection history of the study volunteers, we identified that 94.5% (154/158) of the volunteers with neutralizing activity against the Omicron variants had hybrid immunity resulting from a combination of natural infection and vaccination.

Although neutralizing antibodies against the SARS-CoV-2 receptor-binding domain (RBD) represent a significant proportion of the total neutralizing antibodies targeting the spike (S) protein [44] other conformations of the S protein, such as the full spike trimer, would contribute to a more comprehensive view of the neutralization mechanisms [51]. The N-terminal domain, for example, is a key region where neutralizing antibodies can prevent conformational changes in the S protein structure, thus inhibiting viral invasion [51, 52]. As we evaluated AbN targeting the RBD domain of the S protein, the neutralization activity may be even higher than the described here considering the potential contribution of other neutralizing antibodies [51], this might be a limitation means that our study may overlook the broader spectrum of neutralizing antibodies involved in the immune response.

Although our study design did not allow us to determine the minimal levels of neutralization needed for functional protection, it is notable that despite the significant decrease (~30%) in neutralizing antibody levels by the end of the follow-up period (T4–T5), this reduction may still support herd immunity [53]. Further studies are warranted to determine the minimal antibody and neutralization levels necessary for protection and the frequency of vaccination required to achieve such levels.

The results of this study suggest that the high levels of functional antibodies attained shortly after the initial vaccination and sustained over an extended period, along with the minimal difference in antibody titers between recipients of three and four doses, emphasize the potential benefits of reevaluating the current vaccination schedule. Such a reassessment could pave the way for a more cost-effective and efficient COVID-19 vaccination strategy.

Author Contributions

Conceived and designed the experiments: Myriam Arévalo-Herrera, Sócrates Herrera- Valencia, Sonia Marcela Herrera-Arévalo, Juliana Quintero-Espinosa, Darío Londoño-Trujillo, David Suarez-Zamora. Patient recruitment coordination: David Suarez-Zamora, María A. Nieto-Rojas, Juliana Quintero-Espinosa, Paula Andrea Serna-Ortega. Performed the experiments: John Mario González-Escobar, Brandon Rosero-López, Sebastián Quiceno-García, Nicolas Palacio-Muñoz, Elena Carrasquilla-Agudelo, Ivette Freyle-Roman, Brayan Mendoza-Landinez, Juan Carlos Santos-Barbosa, Natalia Bolaños-Cristancho and Francisco Bohórquez-Martínez. Analyzed the data: Sonia Marcela Herrera-Arévalo, Myriam Arévalo-Herrera, Sócrates Herrera-Valencia, Brandon Rosero-López, Bladimiro Rincón-Orozco. Contributed reagents/materials/analysis tools: Myriam Arévalo-Herrera, Elena Carrasquilla-Agudelo, Paula Andrea Serna-Ortega, Juan Carlos Santos-Barbosa, Natalia Bolaños-Cristancho, and Sócrates Herrera- Valencia. Wrote the paper: Myriam Arévalo-Herrera, Sonia Marcela Herrera-Arévalo, Sócrates Herrera- Valencia, Juliana Quintero-Espinosa, Bladimiro Rincón-Orozco, John Mario González-Escobar. All authors contributed to the manuscript and approved the submitted version.

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Ethics Statement

All research involving human participants conducted for this study were reviewed and approved by Institutional Review Board (IRB) at the Centro Internacional de Vacunas (CEICIV, Cali-Colombia) (code CEICIV No. 2103) Ethics Committee of the Fundación Santa Fe de Bogota (Code No. CCEEI-13717-2021). Additionally, ethical approvals were obtained from all other participating institutions. Informed consent was obtained from all participants involved in the study, ensuring that they were provided with comprehensive information regarding the nature, purpose, and potential risk of the research. Participants were assured of their voluntary participation and their right to withdraw from the study at any time without penalty.

Conflicts of Interest

The authors declare that they have no competing interests related with this publication.

Data Availability Statement

Data collected for the study, including individual deidentified participant data and a data dictionary defining each field in the set, will be made available. Study protocols and IC data will be available upon request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.