COVID-19 Vaccine-induced antibody response to BNT162b2 mRNA in frontline healthcare workers

Respuesta de anticuerpos COVID-19 inducida por la vacuna ARNm BNT162b2 en trabajadores de atención médica de primera línea

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Abstract

The emergence of SARS-CoV-2, the virus causing COVID-19, has resulted in a pandemic that has disrupted all sectors of society. Less than a year after the sequencing of the virus's genome, emergency use authorization for the BNT162b2 vaccine was requested. The aim of this study was to evaluate the response to the BNT162b2-mRNA COVID-19 vaccine in frontline workers from two hospitals in Colombia. Nasopharyngeal swabs were collected for the molecular detection of SARS-CoV-2 using real-time PCR, and blood samples were taken to assess seroconversion using qualitative and quantitative IgA, IgG, and IgM test kits. The study was conducted at a high-complexity healthcare institution in Bucaramanga, Colombia. 245 people were included in the first round and 129 in the second. SARS-CoV-2 molecular tests were conducted by RT-qPCR, and peripheral blood samples were collected to measure IgG, IgM, and IgA. The main outcome was to establish natural infection and the antibody response induced by the vaccine. The entire population was tested at two fixed times with RT-PCR tests and antibody level measurements approximately 4 and 8 months after receiving the second vaccine dose. 62 (25.3%) and 35 (14.3%) participants had a history of positive PCR in the first and second rounds, respectively. All positive cases showed elevated levels of all immunoglobulins, especially IgG. The average concentrations of IgA, IgM, and IgG at 90 days were 1149.5 U/mL (95% CI 828.2-1470.9); 320.3 U/mL (95% CI 218.4-422.3); and 9277.3 U/mL (95% CI 8989.2-9565.3). Frontline healthcare workers showed an adequate response to the BNT162b2 mRNA vaccine.

Keywords: Seroepidemiologic studies; Coronavirus infections; Vaccine; Occupational health; Immunoglobulins.

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Resumen

La aparición del SARS-CoV-2, causante de la COVID-19, resultó en una pandemia que afectó a todos los sectores de la sociedad. Menos de un año después de la secuenciación del genoma del virus, se solicitó el uso de emergencia para la vacuna BNT162b2. El objetivo de este trabajo fue evaluar la respuesta a la vacuna BNT162b2-mRNA COVID-19 en trabajadores de primera línea de dos hospitales en Colombia. Se tomaron muestras nasofaríngeas para la detección del SARS-CoV-2 mediante PCR en tiempo real y muestras de sangre para evaluar la seroconversión con kits de prueba IgA, IgG e IgM. El estudio se realizó en una institución de salud de alta complejidad en Bucaramanga, Colombia. Se incluyeron 245 personas en la primera ronda y 129 en la segunda. Las pruebas moleculares de SARS-CoV-2 se realizaron por RT-qPCR y se obtuvieron muestras de sangre periférica para medir IgG, IgM e IgA. La medida principal fue establecer la infección natural y la respuesta de anticuerpos inducida por la vacuna. Toda la población fue testada en dos momentos: aproximadamente a los 4 y 8 meses después de la segunda dosis de la vacuna. Se encontraron 62 (25,3 %) y 35 (14,3 %) participantes con antecedentes de PCR positiva en las dos rondas respectivamente. Todos los casos positivos presentaron niveles elevados de todas las inmunoglobulinas, especialmente IgG. Las concentraciones promedio de IgA, IgM e IgG a los 90 días fueron: 1149,5 U/mL (IC 95 % 828,2-1470,9); 320,3 U/mL (IC 95 % 218,4-422,3); 9277,3 U/mL (IC 95 % 8989,2-9565,3). Los trabajadores de salud de primera línea mostraron una adecuada respuesta a la vacuna de ARNm BNT162b2.

Palabras clave: Estudios seroepidemiológicos; Infecciones por coronavirus; Vacuna; Salud ocupacional; Inmunoglobulinas.

Introduction

The COVID-19 pandemic has caused significant morbidity and mortality worldwide, as well as major social, educational, and economic disruption. There is an urgent global need for effective and safe vaccines, and to make them available on a large scale and equitably in all countries. The two-dose messenger RNA (mRNA) vaccine BNT162b2 was administered at an interval of 21 days, and after follow-up with a median duration of 2 months, conferred 95% protection seven days after the second dose (95% CI: 90.3%; 97.6%) against symptomatic SARS-CoV-2 infection in people older than 16 years[1].

The immune response of the BNT162b2 vaccine is directed against the SARS-CoV-2 S1 spike protein, and the antibody titers are associated with the functional neutralization of the virus[1]. Once the safety and efficacy endorsement of the BNT162b2 mRNA vaccine was granted by different international and national regulatory agencies, vaccination focused on health workers as a strategy to guarantee their protection and safety at work and to ensure the sustainability of the system sanitary[2,3]. In Colombia, the vaccination process was divided into 5 stages according to the risk of exposure and age group[4]. The first stage began on February 17th, 2021, including people over 80 years (Sinovac-CoronaVac) and the workforce (Pfizer-BioNTech) in charge of COVID-19 patients' healthcare at the first line of care.

Although research has already been carried out on the population of this study, which has reported the seroprevalence and incidence of SARS-CoV-2 infection in health workers on the front line of care for COVID-19[5,6], it is necessary to know the antibody response to vaccines for greater epidemiological surveillance. Therefore, the objective of this article was to assess the response to the BNT162b2 mRNA COVID-19 vaccine in a sample of frontline workers from two high-complexity hospitals in Bucaramanga, Santander, Colombia.

Methodology

Design and population

A cross-sectional observational study was carried out in the Metropolitan Area of Bucaramanga (Santander, Colombia), between June and July 2021 (round 1) and November 2021 (round 2). Health workers or front-line workers (hospital staff) from two (2) health institutions with the vaccination schedule for COVID-19 (two doses with BNT162b2 Pfizer/BioNTech [Messenger RNA]).

Sampling and sample.

Convenience sampling was carried out. Recruitment took place between June 21th and July 16th, 2021 (round 1) and between November 9th and 27th, 2021 (round 2) among the same participants.

Data collection and variables.

All participants self-completed an online survey on socio-demographic data (age, sex, socioeconomic level, and health institution), working area, cigarette smoking status, medical conditions, and possible contact with people with suspected or confirmed COVID-19 infection. The samples from the first round were taken before vaccination. Study data were collected and managed using REDCap electronic data capture tools hosted at Fundación Cardiovascular de Colombia[7,8].

Electronic informed consent was obtained from all subjects involved in the study. This consent was available to be downloaded and saved by each participant.

To establish natural infection in both rounds, we performed either polymerase chain reaction (PCR) or IgM/IgG quantification as follows:

PCR assessment

SARS CoV-2 molecular detection tests were performed by RT-qPCR. The MGIEasy Nucleic Acid Extraction kits were used, with which the automated extraction of viral RNA was started from respiratory samples obtained from nasopharyngeal swabs. Then, the genetic material of the virus was amplified by the reverse transcription PCR technique (RT-qPCR) using RNA as a template to synthesize complementary DNA (cDNA), with which the corresponding PCR was subsequently performed. The test was performed using commercial eDiagnosis® molecular diagnostic kits and with the CFX96TM BioRad® and Gentier96® detection system using real-time PCR. After the end of the amplification reaction, the results were determined by analyzing the cycle threshold (Ct) of each channel. In addition, the RNaseP gene, designed as an internal control, was included to monitor sampling, extraction, annealing, amplification, and other processes to effectively prevent the appearance of false positives and false negatives.

Participants with a positive PCR test were immediately informed through the email address they provided in the virtual survey and were reported to the Health and Safety at Work department in their institution to assess the need to confirm a possible infection with PCR.

To establish the antibody response induced by the vaccine, the following parameters were determined:

IgG, IgM, and IgA measurement

A 5 cc peripheral blood sample was obtained from each participant in a yellow cap tube with a coagulant activator and gel, they were centrifuged at 3,500 RPM in a Thermo ScientificTM Megafuge ™ 16 centrifuges, then the components were separated and the sera were stored in cryovials at -80°C. Samples that did not meet the manufacturer's quality requirements were excluded: icteric, lipemic, hemolytic, or with bacterial contamination.

The selected sera were prepared at room temperature for processing. For the detection of IgG/IgM/IgA antibodies against the SARS-CoV-2 virus, the qualitative and quantitative test kits AESKULISA® SARS-CoV-2 S1 NP IgA, IgG, and IgM were used. This kit detects the S1 domain of the glycosylated Spike protein of SARS-CoV-2t. AESKULISA® immunoassays have a sensitivity of > 95% and a specificity of > 99%.

The SkanIt Software version 2.4.3 and the Varioskean Flash microplate reader (Thermo Fisher) were used for the analysis. The immunoassay was calibrated with internal reference sera. A standard curve was generated with the application of optical measurement signals (optical density, OD) of the calibrators for their antibody activity in IU/ml or U/ml. The limit ranges were those established by the supplier in the quality control certificate: detection limit range 8-12 U/ml and measurement range 3-100 U/ml. The evaluation of a sample below the limit range was established as negative and above as positive. The qualitative evaluation was carried out by comparing the OD of the sample with the mean optical density of calibrator B applied twice (cut-off calibrator CAL B). If the OD of the sample was within a range of +/- 20% of the average OD of the CAL B cut-off calibrator, this was considered as the cutoff. When the OD was higher, it was considered positive, and lower it was considered negative.

Results are reported according to previous positive PCR or negative history of COVID-19 infection and a complete vaccination scheme.

Time delta days post vaccine

This variable was calculated to establish the time (days) between the second vaccination dose and the date of blood sampling in round 1 and round 2. Time

delta is reported as a day range and two range deltas are reported (Delta 1=31 and 137 days; Delta 2=185 and 272 days).

Statistical methods

Variables are reported as means with a 95% Confidence Interval (CI) and absolute and relative frequencies. Quantitative variables are presented with mean and standard deviation. The p-values to establish the difference of increase or decrease were quantified with paired t-test. Statistical analysis was done in Stata 15.

Ethical considerations

The Research Ethics Committee (CEI) of Fundación Cardiovascular de Colombia reviewed and approved this study. Written informed consent was obtained directly by all participants.

Patient and Public Involvement statement: It was not appropriate or possible to involve patients or the public in the design, conduct, reporting, or dissemination plans of our research

Results

General characteristics

A total of 245 participants were included in round 1 and 129 participants in round 2. In both rounds, the majority of the population were female. The majority socioeconomic level of the participants was distributed between the medium-low and medium levels. The working area with the highest participation in both rounds was non-COVID-19 health workers. Most of the study population had no associated risk factors (smoking/medical conditions) and reported no contact with a suspected or confirmed COVID-19 patient, however, this contact was more frequent for those with a previous positive PCR ($p<0.0001$) (Table 1).

On the other hand, in round 1 and round 2 a total of 62 (25.3%) and 35 (14.3%) participants, respectively, with a history of positive PCR, were found (Table 1).

Table 1. Sociodemographic, clinical, and SARS-CoV-2 exposure variables according to the history of previous COVID-19 infection and post-vaccination time.

	Time delta 31 days and 137 days				Time delta 185 days and 272 days			
Variable	**All**	**No prior infection**	**Prior PCR (+)**	**p-values**	**All**	**No prior infection**	**Prior PCR (+)**	**p-values**
n	245	183	62		129	94	35	
Age, (years)*	35.1 (9.5)	35.5 (9.9)	33.8 (8.4)	0.3628	36.1 (9.6)	36.3 (10.0)	35.4 (8.7)	.66
Sex								
Women	185 (75.5)	137 (74.9)	48 (77.4)	0.686	101 (78.9)	76 (80.8)	25 (73.5)	.37
Men	60 (24.5)	46 (25.1)	14 (22.6)		28 (21.1)	18 (19.1)	10 (26.5)	
Socioeconomic status								
1 (lowest)	11 (4.5)	8 (4.4)	3 (5.1)	0.292	7 (5.4)	5 (5.3)	2 (5.88)	.90
2	46 (18.8)	35 (19.1)	11 (18.6)		22 (17.1)	16 (17.0)	6 (17.6)	
3	73 (29.8)	55 (30.6)	18 (29.0)		32 (25.0)	25 (26.6)	7 (20.5)	
4	67 (27.3)	53 (29.0)	14 (22.6)		41 (32.0)	31 (32.9)	10 (29.4)	
5	30 (12.2)	21 (11.5)	9 (15.2)		16 (12.5)	11 (11.7)	5 (14.7)	
6 (higher)	16 (6.5)	11 (6.0)	5 (8.5)		11 (7.8)	6 (6.3)	5 (11.7)	

		Time delta 31 days and 137 days			Time delta 185 days and 272 days			
Unknown	2 (0.8)	0 (0.0)	2 (3.4)		0 (0.0)	0 (0.0)	0 (0.0)	
Health institution								
Institution 1 (no COVID-19 attention)	122 (49.8)	91 (49.7)	31 (50.0)	0.970	70 (54.7)	51 (54.3)	19 (55.8)	.87
Institution 2 (COVID-19 attention)	123 (50.2)	92 (50.3)	31 (50.0)		59 (45.3)	43 (45.7)	16 (44.1)	
Working area								
Administrative Staff	39 (15.9)	29 (15.9)	10 (16.1)	0.689	18 (14.1)	11 (11.7)	7 (20.6)	.58
COVID-19 healthcare	58 (23.7)	45 (24.6)	13 (21.0)		29 (22.6)	22 (23.4)	7 (20.6)	
Healthcare	145 (59.9)	106 (57.9)	39 (62.9)		81 (62.5)	60 (63.8)	21 (58.8)	
Cleaning staff	3 (1.2)	3 (1.6)	0 (0.0)		1 (0.7)	1 (1.0)	0 (0.0)	
Smoking								
Yes (currently)	6 (2.4)	5 (2.7)	1 (1.6)	0.867	2 (1.6)	1 (1.0)	1 (2.9)	.48
Yes (past)	41 (16.7)	32 (17.5)	9 (14.5)		24 (18.7)	17 (18.1)	7 (20.5)	
Yes (passive)	13 (5.3)	9 (4.9)	4 (6.4)		5 (3.9)	5 (3.9)	0 (0.0)	
No	185 (75.5)	137 (74.9)	48 (77.4)		98 (75.7)	71 (75.5)	27 (76.4)	
Medical conditions								
No	197 (80.4)	146 (79.8)	51 (82.7)	0.671	101 (78.9)	73 (77.6)	28 (82.3)	.56
Yes	48 (19.6)	37 (20.2)	11 (17.7)		28 (21.1)	21 (22.3)	7 (17.6)	
Contact with people with suspected or confirmed COVID-19								
No	156 (63.7)	130 (71.0)	26 (41.9)	<0.0001	79 (61.7)	70 (74.5)	9 (26.5)	<.0001
Yes	81 (33.7)	47 (25.7)	34 (54.8)		45 (35.2)	22 (23.4)	23 (67.6)	
Do not know	8 (3.3)	6 (3.3)	2 (3.2)		5 (3.1)	2 (2.1)	3 (5.8)	

Abbreviations: PCR, polymerase chain reaction

*It represents a continuous variable where the mean and standard deviation.

Post-vaccination concentration of IgA, IgM, and IgG (Time delta 1, range 31 days and 137 days)

Two participants (3.22%) with previous positive PCR infection reached the maximum threshold of IgA concentration and 2 individuals (1.09%) without previous infection. Regarding IgM, most of those who reached values above 1000 U/mL were individuals with a previous infection for COVID-19: 6 participants (9.67%) and 2 participants (1.09%) without previous infection. For IgG, 53 participants (85.48%) with positive PCR reached the maximum threshold, and 139 people (75.95%) without previous infection (**Figure 1**).

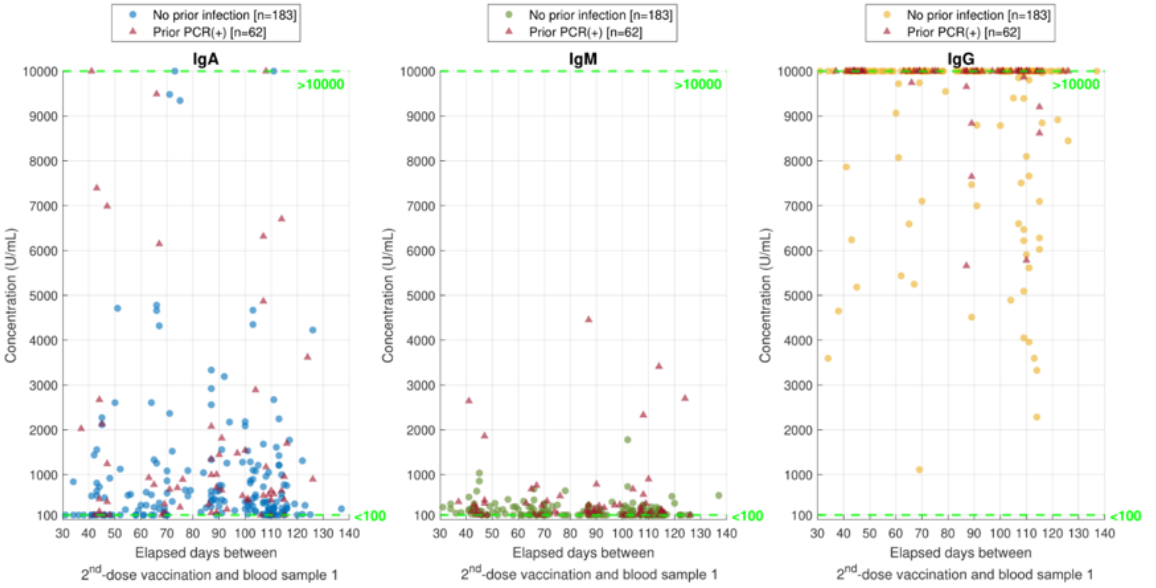

Figure 1. Concentration of IgA, IgM, and IgG with post-vaccination time delta 31 days and 137 days. Abbreviations: PCR, polymerase chain reaction; U/mL, units per milliliter. The green dotted line indicates the minimum (100 U/mL) and maximum (10,000 U/mL) cut-off values for the detection of each antibody isotype.

For IgA concentration, only one participant (2.85%) with a previous SARS-CoV-2 infection reached the maximum concentration threshold vs no person in the uninfected group (0%). No participant reached maximum IgM thresholds, but about 12 people (34.28%) with the previous infection for SARS-CoV-2 achieved concentration values above 100 U/mL vs 9 people (9.57%) without previous infection. A total of 17 participants (48.57%) with the previous infection for SARS-CoV-2 exceeded the maximum threshold of IgG immunoglobulin concentration at 180 days, vs 17 people (18.08%) without previous infection (**Figure 2**).

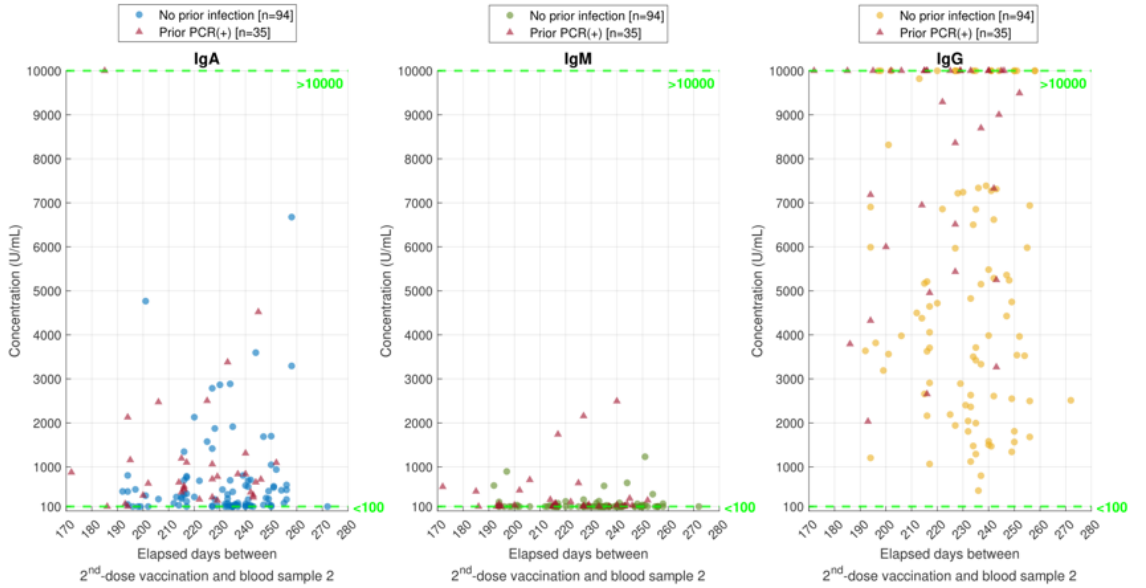

Figure 2. The average concentration of IgA, IgM and IgG at 90 days was respectively: 1149.5 U/mL (95% CI 828.2-1470.9); 320.3 U/mL (95% CI 218.4-422.3); 9277.3 U/mL (95% CI 8989.2 – 9565.3). On the other hand, the average concentration of IgA, IgM and IgG at 180 days was respectively: 840.4 U/mL (95% CI 610.0 – 1070.7); 209.9 U/mL (95% CI 150.0 – 269.7); 5854.7 U/mL (95% CI 5300.6 – 6408.7).

The difference in immunoglobulin concentration between delta 1 and delta 2 was calculated through the percentage of change, with a decrease in the concentration of IgA, IgM, and IgG of 36.78% (P 0.0266), 52.63% (P 0.0003), and 58.45% (P < 0.0000) respectively over time, statistically significant decrease (Table 2).

Discussion

The results of our study establish that the population is homogeneous in both rounds, with the only statistically significant difference being the possible exposure to infection through contact with individuals that had confirmed or suspected of COVID-19 infection for participants with and without prior COVID-19 infection. For participants who were recruited during the first days of round 1, the immunoglobulins increased in concentration of immunoglobulins and at specific moments of the round Some individuals with a history of positive PCR for SARS-CoV-2 presented a greater immunological response compared to uninfected individuals; this finding could be interpreted because of the reinforcement of antibodies with a vaccine after a natural infection. Regarding immunoglobulin G, the antibody that lasts the longest as supported by some studies,[9,10] a similar pattern was observed in most individuals, except in some participants who did not have a previous infection for SARS-CoV-2, in which IgG concentration decreased rapidly between the range of 31 days and 137 days (time delta 1).

In round 2 of the study, corresponding to the time delta 2 (range 185 days and 272 days), a decrease in the concentration of all immunoglobulins was observed, due to the time between the moment of vaccination and assessment of immunological response. However, considering that IgA levels remain for 2.5 months from natural infection[11], the result of one participant was novel, as it maintained elevated levels at the threshold of immunoglobulin concentration. This demonstrates a stronger immune response concerning other individuals, particular characteristics that could be used for future research.

These findings are supported by quantitative data, observing a decrease in the amount of each antibody over time (for example, IgA concentration at 3 months of 1149.5 vs 840.4 at 6 months), with a percentage of change as the indicator that would confirm the previous findings, accompanied by a decrease in IgG from the first round of the study to the second of 58.45%, which had a value of p-value less than .005 according to the relationship in the last table (Table 2).

Table 2. Concentration of antibodies (IgA, IgM, and IgG) of first-line personnel with complete vaccination schedule after 30 days and 170 days.

Variables	Time delta 1 (31 days and 137) days (a)		Time delta 2 (185 days and 272) days (b)		Difference (a – b)	95% CI	% Change	p-values
	Mean	95% CI	Mean	95% CI				
IgA (n=129)	1149.5	828.2 - 1470.9	840.4	610.0 - 1070.7	309.1	36.3 - 581.9	-36.78	0.02
IgM (n=129)	320.3	218.4 - 422.3	209.9	150.0 - 269.7	110.4	51.1 - 169.7	-52.63	0.0003
IgG (n=129)	9277.3	8989.2 - 9565.3	5854.7	5300.6 - 6408.7	3422.5	2913.4 - 3931.7	-58.45	< 0.0000

Abbreviations: CI, Confidence Interval.
[a]Represents Time delta 1.
[b]Represents Time delta 2.

According to the published literature about the results of this article, we observed that something similar occurred in 2 studies carried out with health workers in Italy, in which those individuals who had a history of the previous infection for SARS-CoV-2 or positive serology at the beginning of the study, generated, after the second dose of the BioNTech & Pfizer vaccine, higher concentrations of immunoglobulins than the participants without the previous characteristics[11,12]. In our study, around 85.48% of participants with previous SARS-CoV-2 infection had IgG concentrations above the maximum cut-off for antibody detection (> 10,000 U/mL), in the first 90 days after application of the second dose. These results differ from those found in a study carried out by Kelsen et al.[13] who discovered that health workers with a previous infection had a faster response after the first dose of the vaccine, but the IgG level was attenuated or nil after the second dose, after 56 days of the initial vaccination. However, in our study, it was observed that 48.57% of the participants with a previous infection for SARS-CoV-2 exceeded the maximum threshold of IgG immunoglobulin concentration at 180 days, which would mean a notable decrease in the response to the second dose of the BioNTech & Pfizer vaccine. The decrease in the concentration of antibodies influenced by time elapsed after the application of the second dose was seen in other similar studies[14,15], also corroborated in a systematic review, in which it was even appreciated that individuals with a previous infection for SARS-CoV-2 began a decline in antibody concentration much earlier than seronegative individuals[16]. It is important to mention that, despite the decrease in antibody concentration over time, most of the 2 study groups had IgG concentrations above the minimum threshold (100 U/mL) at 180 days, probably as Memory B cell response to SARS-CoV-2 antigens[17]. The percentage of change applied to our results (**Table 2**), which to date has not been reported in the scientific literature regarding COVID-19, made it possible to observe the difference in antibody concentrations in individuals during the 2 phases, finding a drop below 60% in peak IgG concentration at 180 days; this data would indicate the need for a booster dose between the third and sixth month after the complete vaccination schedule.

An advantage of this study was the possibility of performing real-time PCR tests and informing health professionals that they were seropositive and probably unaware of their immunological status. One limitation of the study was the decrease in participants in the second round, which could have generated some type of bias and limited further stratification. However, the objective of the study was achieved despite the attrition in the sample size.

Conclusions

The vaccination policy for SARS-CoV-2 has allowed the reduction of cases in the population, especially among front-line health workers, which demonstrates the efficacy of the BNT162b2 mRNA vaccine. Continuing research on the antibody response to vaccination, especially in the long term, is necessary to elucidate whether it is essential to periodically carry out booster immunizations for COVID-19, fundamentally in those individuals with natural immunity due to the previous infection.

Implications for policy & practice

The research findings provide evidence of the effectiveness of vaccination in frontline health workers, reinforcing the validity of the immunization policy for Sars-CoV-2 implemented globally.

The study findings also made possible to identify the antibody response to vaccination, being considerably higher in those people who had a previous infection for Sars-CoV-2. The previous contributes to governments' decision-making, encouraging the continuation and implementation of health policies that allow the administration of booster doses against COVID-19 in the population to maintain optimal collective immunization.

Authors contribution

NSD and DCQL are the principal investigators who designed the study and led the idea for this paper. DCQL and CCM performed data analysis, interpretation, and writing of the draft manuscript, PBN, AS, DPS, and ISA supported data analysis and results interpretation. LM and OLSR coordinated and performed sample analysis for immunoglobulin A, G, and M assessment. NSD and DCQL led human resources assignments and project administration. All authors have made substantial contributions to the content of this paper and have read and approved the final version and submission of the manuscript. The manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language.

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Ethical considerations

The study was conducted following the standards issued by the World Medical Association's Declaration of Helsinki guidance and the Research Ethics Committee (CEI) of Fundación Cardiovascular de Colombia approved this study (protocol code CEI-2020-01485, September 17, 2020).

Written informed consent was obtained directly by all participants and health institutions.

The datasets generated and analyzed for this study can be requesting the authors of the article since a request must be made to the research ethics committee to be able to share the database that is in an institutional repository.

Conflict of Interest

The authors declare no conflict of interest.

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AI Technological support

The authors report that they did not use artificial intelligence, language models, machine learning or similar technologies to create or assist with the elaboration or editing of any of the contents of this document

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