

ORIGINAL ARTICLE

EVALUATION OF THE HUMORAL RESPONSE INDUCED BY BBIBP-CORV VACCINE BY DETERMINING NEUTRALIZING ANTIBODIES IN PERUVIAN HEALTHCARE PERSONNEL

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ABSTRACT

Objective. To determine the titer of antibodies against the receptor binding domain (RBD) of the spike protein (S) in health personnel between the 4th and 12th week after receiving the BBIBP-CorV vaccine (Sinopharm). **Materials and methods.** We included a total of 168 healthcare workers from two hospitals in the region, who complied with the complete Sinopharm vaccine schedule; serum antibodies were measured using the Elecsys® Anti-SARS-CoV-2 test. **Results.** All participants developed antibodies to the RBD domain. The lowest antibody titer level was 1.78 U/mL. Levels equal to or above 250 were found in 70 (41.7%) participants. The geometric mean was 82.6 (95% CI: 67.8-100.6). Women had higher antibody levels. Participants whose antibodies were measured between 4- and 7-weeks post-vaccination showed significantly higher antibody levels than patients whose antibody levels were measured between 10- and 12-weeks post-vaccination. Among patients with a history of COVID-19, antibody levels were found to be at or above 250 U/mL in 88% of cases, compared to 6% among those without a history of COVID-19, ($p < 0.001$). **Conclusion.** All participants immunized with BBIBP-CorV vaccine were positive for antibodies against the SARS-CoV-2 spike protein RBD. The correlation between the titer level and protection against COVID-19, as well as the length of the protection provided by the vaccine, needs to be evaluated.

Keywords: Neutralizing Antibodies; Vaccination; Immunity; COVID-19; SARS-CoV-2 (source: MeSH NLM).

INTRODUCTION

Since the SARS-CoV-2 pandemic began, several research teams worldwide started the development of vaccines against COVID-19, mainly inactivated virus, recombinant protein, vectored and RNA vaccines, especially aimed at producing antibodies against SARS-CoV-2 spike (S) proteins⁽¹⁻⁶⁾. The efficacy of these vaccines has been demonstrated to be in the range of 70 to 95%⁽⁷⁻¹⁰⁾, showing immunogenicity comparable to that developed by convalescent patients^(1,3,6,11,12).

The various antibody tests are a useful tool for identifying subjects who have had prior exposure to COVID-19⁽¹³⁾, these antibody titers vary depending on several factors (age, sex, COVID-19 severity, and days since infection)⁽¹⁴⁾ and may decline substantially over time⁽¹⁵⁻¹⁷⁾. Reports by Manisty *et al.* and Long *et al.* have associated clinical severity of the infection with the magnitude of initial antibody responses and the duration of circulating antibody titers^(14,18), which would explain the sustained antibody levels in hospitalized patients for 3 to 6 months⁽¹⁹⁻²¹⁾.

Neutralizing antibodies are considered to be a good marker for measuring humoral responses⁽²²⁾. The plaque reduction method is the gold standard for measuring antibody levels⁽²³⁾. However, this methodology is expensive and requires infrastructure and biosafety

Cites as: Soto A, Charca-Rodríguez FdM, Pareja-Medina M, Fernández- Navarro M, Altamirano-Cáceres K, Sierra Chávez E, *et al.* Evaluation of the humoral response induced by BBIBP-CoRV vaccine by determining neutralizing antibodies in Peruvian healthcare personnel. Rev Peru Med Exp Salud Publica. 2021;38(4):493-500. doi: <https://doi.org/10.17843/rpmesp.2021.384.9244>

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Received: 09/08/2021
Approved: 15/12/2021
Online: 22/12/2021

conditions that are inaccessible for most health facilities. The measurement of antibodies against the receptor binding domain (RBD) of the virion spike (S) protein is a useful methodology to quantify neutralizing antibodies. This antibody determination has shown good correlation with the plaque reduction method, which is currently considered to be the gold standard ⁽²⁴⁻²⁶⁾.

High mortality rates among health personnel were reported during the pandemic in Peru, mainly due to the lack of biosafety materials and overcrowding ⁽²⁷⁾. By July 14, 502 physicians had died from COVID-19 complications ⁽²⁸⁾, therefore this population group was considered in the first phase of the national vaccination program, which began in February. For this vaccination phase, the inactivated virus vaccine BBIBP-CorV from the Sinopharm laboratory was used, a vaccine with a reported efficacy of 86% ⁽¹⁰⁾. By June 30, more than 85% of physicians had received the second dose ⁽³⁰⁾.

Although published phase II clinical trials on the BBIB-CorV vaccine show the production of neutralizing antibodies in 99% of cases ⁽⁵⁾, there are doubts regarding the immunogenicity in the Peruvian population, which motivated the Peruvian health authorities to modify the vaccination scheme against COVID-19, implementing a booster dose of the vaccine produced by the Pfizer/BioNTech laboratory, in order to achieve a better immune response.

The aim of this study was to evaluate the humoral response determined by the titer of antibodies against the receptor binding domain (RBD) of the spike (S) protein in health personnel between the 4th and 12th week after receiving the BBIBP-CorV vaccine (Sinopharm) as an indicator of the acquired immune response following vaccination.

MATERIALS AND METHODS

Study type

Descriptive, prospective observational study.

Study population and sample

We included health personnel from the Hipólito Unanue and Carlos Lanfranco La Hoz national hospitals, who received the second dose of the COVID-19 vaccine, with and without a history of previous diagnosis of COVID-19. A sample size of 97 participants was calculated based on a 95% confidence level and a 50+/-10% neutralizing antibody positivity ratio. Healthcare workers who received the second dose of BBIBP-CorV vaccine (Sinopharm) against SARS CoV-2, who agreed to be part of the study and signed the informed

KEY MESSAGES

Motivation for the study: One of the priorities during the current pandemic is to ensure the efficacy of the preventive measures used to reduce contagion, so we must understand the immunological responses obtained after vaccination, which is important for adequate decision making. This study is a step towards understanding the complex relationship between the virus and the immune system, which should be subsequently studied in greater depth.

Main findings: Vaccination with the BBIBP-CorV vaccine (Sinopharm) provides adequate immunogenicity.

Implications: Further studies should be carried out in order to better understand the behavior of the immune system in response to vaccination.

consent form were included. Participants diagnosed with COVID-19 after vaccination and prior to antibody dosing, as well as those pregnant and those diagnosed with HIV/AIDS, cancer, autoimmune diseases or diseases associated with immunosuppression were excluded.

Procedures

This study was approved by the Institutional Ethics Committee of the Hospital Nacional Hipólito Unanue and by the Hospital Nacional Carlos Lanfranco La Hoz. Informed consent was obtained from all participants before enrollment. Health workers who received the second dose of the BBIBP-CorV vaccine (Sinopharm) between the 4th and 12th post-vaccination week were selected for enrollment. Between the 26th and 30th of May, health workers from different areas of the Hospital Nacional Hipólito Unanue were enrolled as participants; on April 21, health workers from the Hospital Nacional Carlos Lanfranco La Hoz were enrolled. A study form was filled out including information on age, sex, profession, date of vaccination and history of COVID-19.

Venous blood collection

Blood was obtained from a peripheral vein, in 5 mL sample tubes with coagulation activator to obtain serum. Once the sera were obtained by centrifugation, they were stored at 20 °C until processing. Sera that were not processed on the same day were stored at +5 °C (+/- 3 °C) within the first two hours until processing within 14 days of sample collection, as recommended by the reagent manufacturer.

Method verification

Prior to sample analysis, we verified the precision of the measurement procedure, following the recommendations of the guideline for the verification of quantitative analysis procedures of the Instituto Nacional de Calidad (INACAL); the laboratory obtained a variation coefficient (VC) lower than the VC of the reagent manufacturer, so the result was accepted.

Analysis

Samples that met the acceptance criteria were analyzed using the Elecsys® Anti-SARS-CoV-2 S assay, an immunoassay for the quantitative *in vitro* detection of antibodies to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein (S) in human serum and plasma. The cobas® e601 automated analyzer, which uses the electrochemiluminescence method, was used for measurement. The assay employs a recombinant protein representing the RBD of the S antigen in a dual-antigen sandwich assay format, which detects high-affinity antibodies to SARS-CoV-2. The detected antibody titers showed good correlation with neutralizing antibodies in neutralization assays⁽²⁴⁻²⁶⁾; however, during the execution of this article the electrochemiluminescence method for *in vitro* quantitative detection of antibodies against the receptor binding domain (RBD) of the SARS-CoV-2 spicule (S) protein had only emergency use approval from the U.S. Food and Drug Administration (FDA). The linear range is 0.4 to 250 U/mL. A result above 0.8 U/mL is interpreted as reactive^(31,32); however, suitable cut-off points could not be determined because values above 250 U/mL could not be considered for statistical analysis. Internal quality control was carried out with Preci Control Anti-SARS-CoV-2 S.

Tests were conducted in the laboratory of the Hospital Nacional Hipólito Unanue for the participants from that facility and in the Instituto Nacional de Salud for the participants from the Hospital Carlos Lanfranco La Hoz.

Statistical analysis

The data were entered into a database created in Microsoft Excel and subsequently exported to a dta file. Data processing and analysis were conducted in the statistical program Stata v16.0 (Stata Corporation, College Station, Texas, USA). Numerical variables are presented with the appropriate measures of central tendency and dispersion, according to their distribution. Normality was assessed using histograms and formal tests. Categorical variables are presented as frequencies and percentages. Antibody titers were evaluated by presenting their measures of central tendency and

dispersion according to their distribution. The geometric mean concentration is used for antibody titers because titer data generally do not fit a linear scale. When data are skewed and not normally distributed, it is recommended to calculate the geometric mean rather than the arithmetic mean, which may not provide a good representation of the results⁽³³⁾. A 95% confidence interval and a p-value of less than 0.05 were considered significant. Spearman's correlation was used for comparison of antibody titers against numerical variables, and the Mann Whitney or Kruskal Wallis test was used for the comparison with categorical variables. Due to the different times of vaccination and sample collection techniques in the two hospitals, one group of participants underwent antibody dosing between 4- and 6-weeks post-vaccination while another group of participants underwent dosing between 8- and 12-weeks post-vaccination. The difference in vaccination times was considered as an additional variable. A multivariate analysis was carried out using the robust linear regression method including age, sex, history of COVID-19 and time of vaccination as independent variables. It was not possible to collect pre-vaccination sera in order to compare the increase in pre-vaccination sera in the studied individuals.

Ethical considerations

Our study respected the ethical principles in research according to the Helsinki Principles: autonomy, nonmaleficence, justice and beneficence. The study was authorized by the ethics committees of the Hipólito Unanue National Hospital (038-2021-CIEI- HNHU) and by the Carlos Lanfranco La Hoz Hospital. To participate in the study, patients voluntarily signed an informed consent form. The study protocol has been submitted to the Registry of Health Research Projects (PRISA) under code: EI00000001792.

RESULTS

A total of 168 participants were included, 108 from the Hospital Nacional Hipólito Unanue and 60 from the Hospital Lanfranco La Hoz. Most of the participants (60.1%) were female. The predominant occupation was physician (Table 1). None of the participants developed COVID-19 during the follow-up of up to 3 months after antibody dosing.

Antibody titers against the receptor binding domain (RBD) of the SARS CoV-2 S protein

All participants developed antibodies against the RBD domain. The median was 137.05 with an interquartile range between 40.04 and 250. The minimum value was 1.78 U/mL. Seventeen

participants (10.1%) had values below 10 U/mL and 70 (41.7%) had values equal to or above 250, the maximum antibody level determined by the test. The geometric mean was 82.6 (95% CI: 67.8-100.6). In patients with a history of COVID-19 the geometric mean was 219.51 (95% CI 195.04-247.06). All patients with a history of hospitalization for COVID-19 had values greater than or equal to 250 U/mL. The overall and by sex distribution of antibodies can be seen in Figure 1 and 2.

Bivariate analysis

Females had higher antibody levels ($p=0.04$) (geometric mean of 100.97; 95% CI: 81.11-125.70) than males (geometric mean of 60.54; 95% CI: 41.90-87.48). The antibody levels of patients in whom antibodies were measured between weeks 4 and 6 post-vaccination were significantly higher (geometric mean 129 U/mL; 95% CI: 100.93-166.78) than those in whom antibodies were determined between 8- and 12-weeks post-vaccination (geometric mean 62.23 U/mL; 95% CI: 47.46-81.59). Patients with a history of COVID had significantly higher levels (geometric mean 219.51; 95% CI: 195.03-247.06) than those without a history of COVID (geometric mean 38.97; 95% CI: 30.39-49.96). Among patients with COVID history, antibody levels were found at or above 250 U/mL in 88% of cases compared to 6% in those without a history of COVID ($p<0.001$). No association was found between age and antibody titers (Spearman correlation coefficient -0.04 ; $p=0.56$). Fourteen participants older than 60 years were included; the geometric mean of this group of participants was 53.44 (95%CI 21.29-134.16), compared to 86.11 (95%CI 70.28- 105.49) with no statistically significant difference ($p=0.28$). No association was found between working in COVID-19 areas developing post-vaccination symptoms with the antibody titer. Comparison details are shown in Table 2.

Multivariate analysis

In the multivariate model (Table 3), history of COVID-19 had a significant association with a higher antibody titer (beta coefficient=213.87). This can be interpreted as the difference (adjusted for sex and time since the second dose) in antibody titer between those participants with history of COVID-19 compared to those without such a history. Male sex was associated with lower antibody titer (beta coefficient=-8.37). Likewise, the time between the second dose and the measurement of antibody titers was also associated with lower antibody titers (beta coefficient=-6.67).

DISCUSSION

Our study demonstrated the presence of antibody responses in all participants. This corroborates the data from other studies and confirms an adequate immunogenicity in the humoral response. Our data are concordant with the clinical trial published by Xia *et al.* who found adequate immunologic responses in a population of 143 vaccinees, where 100% of participants had increased neutralizing antibody titers compared to pre-immunization baseline⁽⁵⁾. Likewise, a recent study carried out by the Instituto Nacional de Salud provides information quite similar to our findings; in this study 95 persons with no history of infection and 34 persons with a history of SARS-CoV-2 infection were evaluated. In the first group, 21 days after the first immunization, 31% of the participants produced IgG antibodies for the B.1.1 lineage, and 15% produced IgG antibodies against the Gamma variant (P.1); but 21 days after the second dose with BBIBP-Cor-V vaccine, these percentages increased to 99 and 96%, respectively for each lineage. In the group with history of SARS-CoV-2 infection, 21 days after the first dose, 82% of the participants produced IgG antibodies for the B.1 .1 lineage and 77% produced IgG antibodies against

Table 1. Participants' employment and post-vaccination symptomatology.

Employment	n	%	Symptoms after vaccination							
			Headache 31 (18.5%)	Fatigue 22 (13.1%)	Arm pain 9 (5.7%)	Increased sleep 6 (3.6%)	Dizziness 4 (2.4%)	Nausea 6 (3.6%)	Fever 2 (1.2%)	Other symptoms 6 (3.7%)
Physician	67	39.8	13 (19.4)	10 (14.9)	5 (7.5)	2 (3.0)	3 (4.5)	1 (1.5)	0 (0.0)	1 (1.5)
Administrative	33	19.6	5 (15.2)	4 (12.1)	1 (3.0)	2 (6.1)	0 (0.0)	2 (6.1)	0 (0.0)	0 (0.0)
Nursing Tech.	28	16.7	7 (25.0)	3 (10.7)	0 (0.0)	1 (3.6)	0 (0.0)	1 (3.6)	1 (3.6)	3 (10.7)
Technologist	17	10.1	2 (11.8)	1 (5.9)	2 (11.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.9)	1 (5.9)
Nurse	13	7.7	2 (15.4)	3 (23.1)	0 (0.0)	1 (7.7)	1 (7.7)	2 (15.4)	0 (0.0)	1 (7.7)
Obstetrician	8	4.7	2 (25.0)	1 (12.5)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pharmacy Tech.	2	1.2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

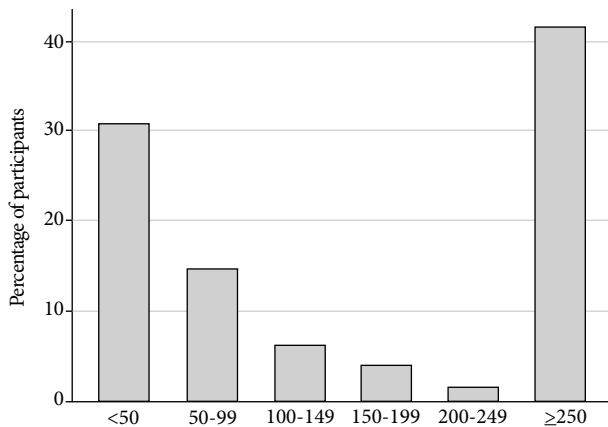


Figure 1. Distribution of antibody titers against the receptor binding domain of S protein in U/L in health personnel from two general hospitals. Lima, Peru.

the Gamma variant (P.1), and 21 days after the second dose 100% produced IgG antibodies against the B.1.1 lineage and the Gamma variant ⁽³⁴⁾.

Among the factors associated with a higher antibody titer, history of COVID-19 clearly stands out. This is predictable and to some extent limiting, since it is not possible to distinguish the immune responses to vaccination from those originated by the SARS CoV-2 infection itself. In any case, our data are consistent with a higher production of antibodies in those persons with history of COVID-19. This has also been evidenced by a study published by Xiangyu Chen *et al.* in which they observed that the neutralizing antibody titers showed a positive relationship with history

of the disease and its severity, being higher in those patients with severe disease ⁽²²⁾.

The T-cell-mediated immunogenic response appears to be more intense in women, while the levels of several chemokines and innate immune cytokines appear to be higher in male patients. The severity of COVID-19 is known to be lower in women ⁽³⁵⁾, and these differences in the immune response between women and men could explain the differences in disease severity.

Although the sample is small, immunogenicity does not appear to decrease with age. However, studies with a larger population are required to determine whether there are significant differences in this regard. In this regard, it is important to note that data from persons over 60 years of age is scarce and often misinterpreted as vaccine ineffectiveness. However, published studies have found 100% seroconversion rates in people older than 60 years, depending on vaccine doses ⁽³⁴⁾.

On the other hand, history of COVID-19 is associated with increased immune responses. It has been suggested that only one vaccine dose may be necessary in patients with history of COVID-19 and an adequate immune system ⁽³⁶⁾. However, there is a significant number of patients who do not achieve high antibody levels, so we could not make a recommendation in this regard. The correlation between titers and the ability to protect against SARS-CoV-2 infection remains uncertain.

A limitation for our study is the fact that we did not determine the presence of neutralizing antibodies directly, so

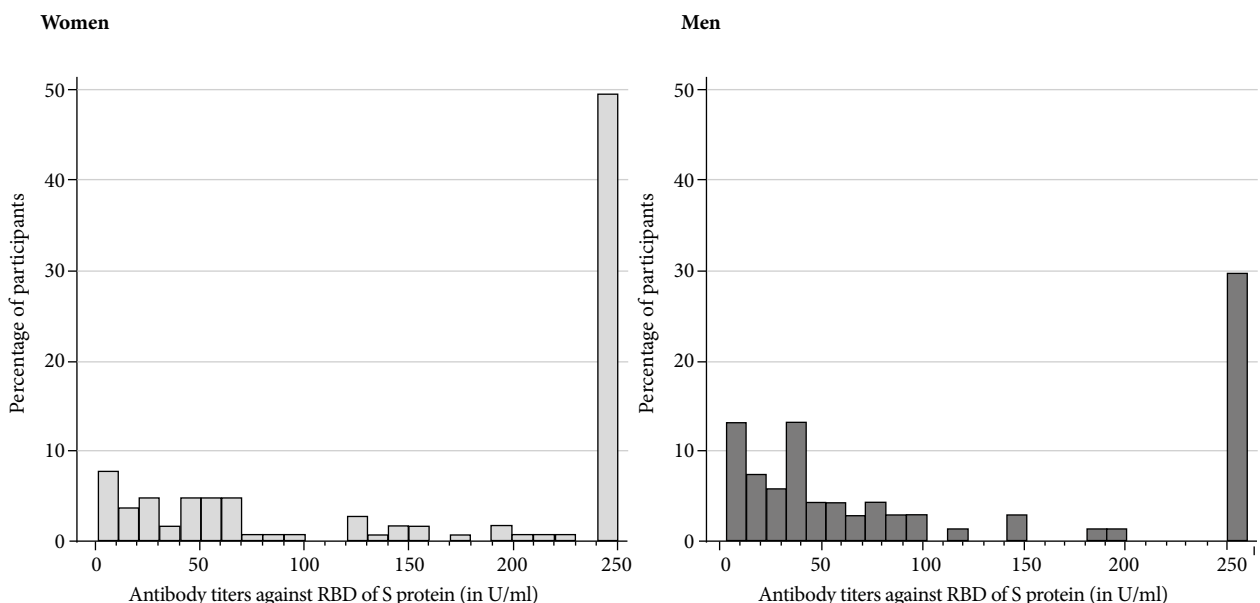


Figure 2. Comparison of antibody titers against the receptor binding domain in women and men. The maximum detection limit of the test is 250 U/L..

Table 2. Medians and geometric means of antibody levels against the S protein receptor binding domain in U/L in health personnel from two general hospitals. Lima, Peru.

	n = 168	%	Geometric mean (95% CI)	Median (IQR)	p-value ^a
Sex					
Women	102	60.7	100.97 (81.11-125.70)	150.30 (51.06 - 250)	0.04
Men	66	39.3	60.54 (41.90-87.48)	77.22 (22.74 -250)	
Diabetes mellitus					
No	162	96.4	82.11 (67.50-99.88)	122.95 (39.88-250)	0.27
Yes	6	3.6	96.80 (9.31-1007.00)	250 (206.50-250)	
Arterial hypertension					
No	158	94.0	79.83 (65.03- 98.00)	121.40 (39.88-250)	0.18
Yes	10	6.0	141.35 (63.46- 314.83)	250 (206.50-250)	
Asthma					
No	160	95.2	84.00 (68.68- 102.72)	143.75 (40.04-250)	0.43
Yes	8	4.8	58.96 (16.21-214.44)	83.91 (24.66-233.5)	
Works in COVID-19 area					
No	92	54.8	89.75 (68.28-117.97)	195.90 (45.08-250)	0.23
Yes	76	45.2	74.68 (55.82-99.92)	104.39 (31.22-150)	
COVID-19 history					
No	95	56.5	38.97 (30.39-49.96)	47.45(20.52-90.39)	<0.001
Yes	73	43.5	219.51 (195.04-247.06)	250 (250-250)	
Time between second dose and antibody measurement					
4 to 7 weeks	62	36.9	129.74 (100.93-166.78)	250 (62.30-250)	<0.001
10 to 12 weeks	106	63.1	62.23 (47.46-81.59)	72.38 (24.09-250)	
Symptoms after second vaccine dose					
No	90	53.6	88.36 (68.56-113.88)	143.75 (41.98-250)	0.77
Yes	78	46.4	76.40 (55.76-104.69)	122.90 (34.83-250)	

^a Mann Whitney U test to evaluate median difference
IQR: interquartile range; 95% CI: 95% confidence intervals.

we cannot necessarily infer that the evidenced immunological responses necessarily imply protection from the clinical point of view; likewise, the test used for the detection of antibodies against the receptor binding domain (RBD) of the spike protein (S) has only been approved for emergency use.

However, a good correlation with the neutralization tests by plaque reduction has been observed. Another limitation for our study was having too wide cut-off points, not being able to compare values above 250 U/mL; however, the study seeks to measure seroconversion and found that all participants

Table 3. Robust regression model for antibody levels against the S protein receptor binding domain in U/L in health personnel from two general hospitals. Lima, Peru.

Variable	Beta coefficient	95% CI	p-value
COVID-19 history	213.87	208.24; 219.49	<0.001
Men	-8.37	-14.09; -2.65	0.004
Dosage 10 to 12 weeks post-vaccination ^a	-6.67	-12.51; -0.82	0.026

^a Compared with those vaccinated at 4 to 7 weeks.
95% CI: 95% confidence intervals.

achieved seroconversion regardless of the titers found. Another limitation was not having pre-vaccination sera to compare the increased effect provided by the vaccine; however, for the purposes of this study this limitation did not interfere with the conclusions. On the other hand, response against the new variants may be different, as has been evidenced by the lower neutralizing capacity of the BBIBP-CorV vaccine against variant B.1.351⁽³⁷⁾.

Considering that the vaccine we used is based on an inactivated virus, it is reasonable to hypothesize that protection may last for a limited time, given that natural immunity in the case of COVID-19 seems to be diluted after a few months, as suggested by experiences in Manaus where, despite having obtained a prevalence of more than 70% (and thus postulated herd immunity), a second wave of significant proportions emerged. Therefore, it may be reasonable to consider a booster dose after a few months for health personnel, particularly in front-line workers, in accordance with the recommendations issued by regulatory institutions such as the Center for Disease Control and Prevention and the World Health Organization, always

prioritizing collective health. However, we consider it is a mistake to state that the chosen vaccine has no utility, which may motivate reluctance to vaccination; such as the refusal of the teachers' union in rural areas who have been offered vaccination with BBIBP-CorV.

In conclusion, our data showed adequate immunogenicity of the BBIBP-CorV vaccine (Sinopharm) as assessed by antibodies against RBD. However, it is necessary to evaluate the correlation between the magnitude of titers and protection against COVID-19 and the time of protection conferred by the vaccine.

Authors' contributions: All authors participated in the conception, recruitment of participants, critical evaluation of the manuscript, and approval of the final version. AST and JRV participated in the statistical analysis and prepared the draft version of the manuscript. All authors are responsible for the content of the article.

Funding: self-funded. The supplies for antibody dosing at the Hospital Hipólito Unanue were donated by the ROCHE laboratory, which had no role in this research.

Conflicts of interest: the authors declare that they have no conflicts of interest.

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