

RESEARCH ARTICLE

Humoral responses after inactivated COVID-19 vaccination in individuals with and without prior SARS-CoV-2 infection: A prospective cohort study

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Abstract

We evaluated and compared humoral immune responses after inactivated coronavirus disease 2019 (COVID-19) vaccination among naïve individuals, asymptotically infected individuals, and recovered patients with varying severity. In this multicenter, prospective cohort study, blood samples from 666 participants were collected before and after 2 doses of inactivated COVID-19 vaccination. Among 392 severe acute respiratory syndrome coronavirus 2-naïve individuals, the seroconversion rate increased significantly from 51.8% (median antispikes protein pan-immunoglobulins [S-IgG] titer: 0.8 U/ml) after the first dose to 96% (median S-IgG titer: 79.5 U/ml) after the second dose. Thirty-two percent of naïve individuals had

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detectable neutralizing antibodies (NABs) against the original strain but all of them lost neutralizing activity against the Omicron variant. In 274 individuals with natural infection, humoral immunity was significantly improved after a single vaccine dose, with median S-IgG titers of 596.7, 1176, 1086.5, and 1828 U/ml for asymptomatic infections, mild cases, moderate cases, and severe/critical cases, respectively. NAB titers also improved significantly. However, the second dose did not substantially increase antibody levels. Although a booster dose is needed for those without infection, our findings indicate that recovered patients should receive only a single dose of the vaccine, regardless of the clinical severity, until there is sufficient evidence to confirm the benefits of a second dose.

KEYWORDS

humoral immunity, inactivated COVID-19 vaccine, neutralizing antibody, Omicron, pan-immunoglobulins

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). Multiple vaccines have been developed to control the ongoing COVID-19 pandemic and prevent future outbreaks. These vaccines have been shown to be effective at preventing infection, severe disease, and death.¹ As of June 20, 2022, 66.3% of the world population and 89% of mainland China's population have received at least one dose of a COVID-19 vaccine.²

Prior evaluations of humoral immunity after vaccination against SARS-CoV-2 in naïve and exposed individuals have indicated that antibody levels are higher in those with prior SARS-CoV-2 infection than in those without prior infection.^{3–5} Evaluations have also indicated that when administered to naïve individuals, the first dose of the COVID-19 vaccine can activate the immune system and the second dose can trigger a stronger protective immune response.^{6–8} However, previous studies have shown divergent results regarding whether a single dose is adequate for individuals exposed to SARS-CoV-2,^{5,8–11} especially in the case of inactivated vaccine.^{5,11} Furthermore, whether the humoral response after inactivated vaccination is positively correlated with disease severity, as in the case of the humoral response following natural infection,^{12–15} requires further evaluation.

The Omicron variant of SARS-CoV-2 (B.1.1.529), which currently dominates the pandemic, has more than 30 mutations in the spike protein (S), some of which are associated with increased transmissibility and immune evasion after natural infection and vaccination.¹⁶ The Omicron variant has shown a lower neutralizing sensitivity to immune sera elicited by vaccination and natural infection than the original strain and other variants of concern, leading to lower levels of protection in vaccinated and previously infected individuals.^{17–19} However, whether this decline in neutralizing capability varies with respect to the infection history and clinical severity remains unclear.

In this study, we employed a prospective cohort design to evaluate and compare humoral immune responses after inactivated COVID-19 vaccination in naïve individuals, asymptomatically infected individuals, and symptomatic recovered patients with varying levels of clinical severity.

2 | METHODS

2.1 | Study design and participants

This is a multicenter, prospective, ongoing cohort study. Participants were enrolled from Chongqing municipality, Hunan province, Hubei province, Sichuan province, and Zhejiang province. Permanent residents aged ≥ 18 years, who were willing to receive two doses of inactivated COVID-19 vaccine and to be followed up for 12 months were eligible. In addition to the general population, individuals with a history of natural infection were also included, regardless of whether they had experienced an asymptomatic or symptomatic infection. Key exclusion criteria for enrollment included juvenile age, inability to receive the COVID-19 vaccine, or unwillingness to be followed up.

Participants were determined to have asymptomatic infections if they had positive reverse transcription-polymerase chain reaction (RT-PCR) results or SARS-CoV-2 antibodies but never developed any signs or clinical symptoms of COVID-19. Symptomatically infected individuals were those with COVID-19-positive RT-PCR result along with related symptoms. Clinical severity was assessed by physicians according to the Chinese clinical guidance for COVID-19 pneumonia diagnosis and treatment.²⁰ Briefly, mild cases were those with mild clinical symptoms and no pneumonia on imaging. Moderate cases were those with fever, respiratory symptoms, and pneumonia detection on imaging. Severe cases were those that met any of the following criteria: respiratory distress with respiratory rate ≥ 30 beats/min; oxygen saturation $\leq 93\%$ at a resting state; arterial partial pressure of

oxygen (PaO_2)/oxygen concentration (FiO_2) ≤ 300 mmHg; lesion progression $> 50\%$ within 24–48 h on imaging. Critical cases were those that met any of the following criteria: respiratory failure requiring mechanical ventilation; occurrence of shock; failure of other organs that required monitoring and treatment in an intensive care unit.

This study was approved by the Ethical Review Board of the School of Population Medicine and Public Health, Peking Union Medical College (CAMS& PUMC-IEC-2021-021). Written informed consent was obtained from all participants before enrollment.

2.2 | Procedures

Enrollment was conducted between April 2021 and July 2021. After providing written informed consent, all participants completed a standardized questionnaire, followed by quality control by trained research staff. Demographic information and clinical information were collected from participants with a confirmed SARS-CoV-2 infection history.

After completion of the questionnaire, participants were given the first shot of inactivated COVID-19 vaccine from the Beijing Institute or Wuhan Institute of Biological Products Co., Ltd, or from Sinovac Life Science Co., Ltd, according to the local vaccine availability. Then, at the first follow-up visit, which took place ~6 weeks after baseline, participants were given a second shot of the COVID-19 vaccine.

Venous blood samples for immunogenicity testing were obtained from all participants before they were given the first and second shots of the COVID-19 vaccine. After an additional 4–6 weeks, participants were invited to a second follow-up to complete another sample collection to further evaluate their humoral immunity after the second shot (Figure 1).

2.3 | Laboratory tests

Plasma separation was performed at the local Centers for Disease Control & Prevention within 8 h of sample collection. All laboratory tests on blood samples were performed at the Christophe Mérieux Laboratory, Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China. The humoral immune response to SARS-CoV-2 infection and COVID-19 vaccination was evaluated by detecting total binding antibodies against spike (S) and nucleocapsid (N)⁷ proteins, as well as neutralizing antibodies (NAbs) against the original strain and the Omicron variant. Plasma samples were inactivated at 56°C for 30 min. Samples were tested for antispikes protein pan-immunoglobulins (S-IgS) and antinucleocapsid protein pan-immunoglobulins (N-IgS) using electrochemiluminescence immunoassay kits according to the manufacturer's instructions (Roche Diagnostics) and NAbs were tested using in-house microneutralization assays, as previously reported.¹³

A random subset of 206 participants who were positive for S-IgS was tested for NAbs against the original strain and 80 seroconverted participants were tested for NAbs against the Omicron variant. The cutoff values for S-IgS and N-IgS were 0.8 and 1.0, respectively, and the cutoff for a positive Nab titer was 1/8. Any titers below the thresholds were set to half the corresponding cutoff values.

2.4 | Statistical analysis

Median values with interquartile ranges (IQRs) were calculated for continuous variables and counts (n) with percentages were calculated for categorical variables. Multiple comparisons for antibody titers were made using the Kruskal–Wallis test, followed by posthoc Dunn's

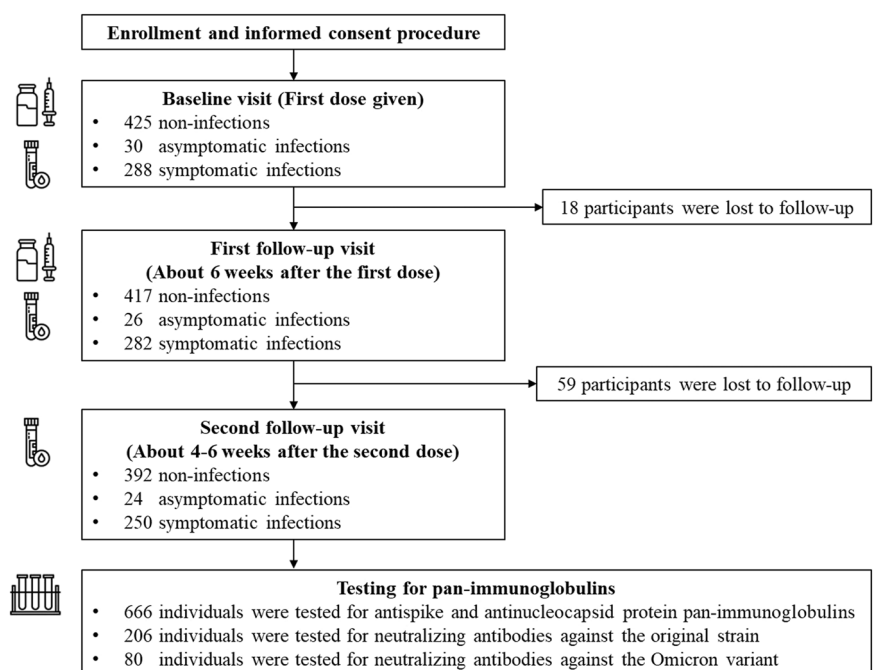


FIGURE 1 Study flowchart

correction. Correlations between NAb titers and S-IgG or N-IgG were evaluated using the Spearman rank-order correlation coefficient. A two-sided $p < 0.05$ was considered to be statistically significant. SAS software 9.4 (SAS Institute, Inc.) was used for all analyses and GraphPad Prism 9.1 (GraphPad Software) was used for illustration.

3 | RESULTS

3.1 | Study population

A total of 743 participants were enrolled at baseline and 666 (89.6%) of them completed two follow-ups. Those lost to follow-up were more likely to have an underlying disease ($p < 0.01$) and a history of SARS-CoV-2 infection ($p < 0.01$) than those who completed both visits (see Supporting Information: Table 1). Among the 666 individuals included in our analysis, almost half were male (48.7%) and most (87.8%) were younger than 65 years of age. Three hundred and ninety-two (58.9%) individuals showed no evidence of exposure to SARS-CoV-2, whereas 24 (3.6%) had asymptomatic infections, 99 (14.9%) had mild cases, 136 (20.4%) had moderate cases, and 15 (2.3%) had severe or critical cases. The median duration from symptom onset to the baseline visit was 16.7 months (IQR: 16.2–17.0) for confirmed cases. More than 93% (255/274) of individuals with SARS-CoV-2 infection were hospitalized in the acute phase and one-fourth (71/274) of them reported COVID-19-related symptoms after convalescence. Table 1 shows additional details.

3.2 | Levels of S-IgG and N-IgG

Among the naïve individuals, there were no detectable S-IgG or N-IgG before vaccination. After the first dose of the vaccine, 203 (51.8%) participants were seropositive for S-IgG and 376 (95.9%) were seropositive after the second dose (Table 2). The titers of S-IgG increased substantially after the first dose (median: 0.8 [IQR: 0.4–3.1]) and second dose (median: 79.5 [IQR: 19.2–176.2]) of the vaccine. Lower positive rates but similar trends were observed for N-IgG. Ten (2.6%) individuals were positive for N-IgG after the first dose (median: 0.5 [IQR: 0.5–0.5]) and 256 (65.5%) individuals were positive for N-IgG after the second dose (median: 2.9 [IQR: 0.5–11.9]; Figure 2).

Among asymptotically infected individuals, 22 (91.7%) and 21 (87.5%) were positive for S-IgG and N-IgG, respectively, before vaccination. All these individuals were seropositive for S-IgG and N-IgG after two doses of the vaccine. The titer of S-IgG increased significantly from 85.3 (IQR: 26.8–230) to 596.7 (IQR: 135.1–1139.8; $p < 0.0001$) after the first dose and further increased to 757.8 (IQR: 163.9–1590.0) after the second dose ($p = 0.66$). The titer of N-IgG was lower than that of S-IgG but showed similar trends (Table 2 and Figure 2).

Among people with a history of symptomatic infection, seropositivity for S-IgG and N-IgG ranged from 93% to 100% at baseline and reached almost 100% after the second dose (Table 2). Antibody titers increased significantly after the first dose (medians: 1176, 1086.5, and 1828 for

S-IgG and 143.8, 141.1, and 149.3 for N-IgG in mild, moderate, and severe/critical cases, respectively) and increased with no statistical significance after the second dose (medians: 1247, 1280, and 2367 for S-IgG and 135.4, 140.7, and 113.3 for N-IgG in mild, moderate, and severe/critical cases, respectively; Figure 2). Notably, in 115 recovered patients, the titers of S-IgG decreased to varying degrees after the second dose.

Across all visits, the concentrations of antibodies were higher in individuals with a history of natural infection than in SARS-CoV-2-naïve individuals. In particular, antibody concentrations in naïve individuals after two doses of the vaccine were still lower than those in infected individuals after only one dose ($p < 0.05$). However, no difference was observed across subgroups with different clinical severities either before or after vaccination (all $p > 0.05$).

3.3 | Levels of NAbs against original strain and omicron variant

Among the participants who were positive for S-IgG, 63 naïve individuals were tested for NAbs against the original SARS-CoV-2 strain after receiving the second dose and 143 infected individuals were tested at all 3 visits. In SARS-CoV-2-naïve individuals, 20 out of 63 (31.8%) were seroconverted after two doses of the vaccine. The seroconversion rate increased from 40.9% (9/22) to 90.91% (20/22) after the first dose in asymptotically infected individuals, from 46.3% (19/41) to 90.2% (37/41) in mild cases, from 53.5% (38/71) to 95.8% (68/71) in moderate cases, and from 77.78% (7/9) to 100% (9/9) in severe/critical cases. An additional four infected individuals seroconverted after the second dose (Table 2). In all symptomatically infected individuals, regardless of clinical severity, the titers of NAbs increased substantially after the first dose (geometric mean titer [GMT] increased from 6.9 to 39.1, from 8.5 to 30.2, and from 15.3 to 75.6 in mild, moderate, and severe/critical cases, respectively) and increased to a lesser extent after the second dose (GMT: 45.1 in mild, 37.2 in moderate, and 64 in severe/critical cases; Figure 3A). Reductions in NAbs titers were observed in 3 recovered participants after the first dose and in 49 participants after the second dose. The titer of NAbs against the original strain strongly correlated with S-IgG ($r = 0.85$, 95% confidence interval: 0.81–0.89).

All 20 SARS-CoV-2-naïve participants who were positive for NAbs against the original strain were tested for NAbs against the Omicron variant and all of them showed lost neutralizing activity against the Omicron variant. Sixty recovered individuals, who were positive for NAbs against the original strain, were tested for NAbs against the Omicron variant. Among those 44 recovered individuals, 42 (95.5%) showed loss of neutralizing activity against the Omicron variant before vaccination; the two cases of individuals who were positive for NAbs against Omicron included one moderate case and one severe case. After the first dose, 40% (2/5) of asymptomatic cases, 36.8% (7/19) of mild cases, 40% (12/30) of moderate cases, and 16.7% (1/6) of severe/critical cases did not show neutralizing activity against the Omicron variant. One asymptotically infected individual and two individuals with moderate cases were

TABLE 1 Demographic information of participants according to infection history

	All ^a (n = 666)	Individuals without past natural infection (n = 392)	Individuals with past natural infection (n = 274)	p
Sex				0.32
Male	324 (48.65)	197 (50.26)	127 (46.35)	
Female	342 (51.35)	195 (49.74)	147 (53.65)	
Age group, years				0.87
18–64	585 (87.84)	345 (88.01)	240 (87.59)	
≥65	81 (12.16)	47 (11.99)	34 (12.41)	
Education level				<0.01
Junior high school or less	281 (42.32)	157 (40.26)	124 (45.26)	
High school	155 (23.34)	77 (19.74)	78 (28.47)	
Some college or associate degree	217 (32.68)	149 (38.21)	68 (24.82)	
Bachelor's or higher degree	11 (1.66)	7 (1.79)	4 (1.46)	
Missing	2	2		
Occupation				0.03
Health worker	36 (5.41)	29 (7.40)	7 (2.55)	
Service career	131 (19.67)	69 (17.60)	62 (22.63)	
Farmer	140 (21.02)	88 (22.45)	52 (18.98)	
Retired or jobless	117 (17.57)	66 (16.84)	51 (18.61)	
Other	242 (36.34)	140 (35.71)	102 (37.23)	
Smoking status				<0.01
Never	472 (70.87)	252 (64.29)	220 (80.29)	
Ever	66 (9.91)	41 (10.46)	25 (9.12)	
Current	125 (18.77)	98 (25.00)	27 (9.85)	
Not sure	3 (0.45)	1 (0.26)	2 (0.73)	
Drinking status				0.15
Never	390 (58.56)	219 (55.87)	171 (62.41)	
Ever	137 (20.57)	90 (22.96)	47 (17.15)	
Current	138 (20.72)	83 (21.17)	55 (20.07)	
Not sure	1 (0.15)			
Underlying disease ^b				0.58
No	585 (87.84)	342 (87.24)	243 (88.69)	
Yes	81 (12.16)	50 (12.76)	31 (11.31)	
Time interval between infection and first COVID-19 vaccination (months)			16.7 (16.2, 17.0)	
Clinical severity at the acute phase				
Asymptomatic infection			24 (8.76)	
Mild cases			99 (36.13)	
Moderate cases			136 (49.64)	
Severe/critical cases			15 (5.47)	
Hospitalized at the acute phase				
No			19 (6.93)	
Yes			255 (93.07)	

(Continues)

TABLE 1 (Continued)

	All ^a (n = 666)	Individuals without past natural infection (n = 392)	Individuals with past natural infection (n = 274)	p
Had any symptoms since convalescence ^c				
Yes			71 (25.91)	
No			203 (74.09)	

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; NA, not applicable.

^aAttended three visits in total.

^bUnderlying disease included, but not limited to, coronary heart disease, diabetes, asthma, chronic obstructive pulmonary disease, bronchiectasis, and cancer.

^cCOVID-19-related symptoms included shortness of breath, weakness, headache, diarrhea, allotriomiasia, and parageusia

TABLE 2 Seroconversion rates of pan-immunoglobulins and NABs

	Not infected	Asymptomatic infection	Mild case	Moderate case	Severe/Critical case
Conservation rate of S-IgG					
No. of test	N ₁ = 392	N ₁ = 24	N ₁ = 99	N ₁ = 136	N ₁ = 15
Baseline	0 (0.00)	22 (91.67)	96 (96.97)	134 (98.53)	15 (100.00)
After the first dose	203 (51.79)	22 (91.67)	98 (98.99)	134 (98.53)	15 (100.00)
After second dose	376 (95.92)	24 (100.00)	98 (98.99)	136 (100.00)	15 (100.00)
Conservation rate of N-IgG					
No. of test	N ₁ = 392	N ₁ = 24	N ₁ = 99	N ₁ = 136	N ₁ = 15
Baseline	0 (0.00)	21 (87.50)	92 (92.93)	131 (96.32)	15 (100.00)
After the first dose	10 (2.55)	23 (95.83)	97 (97.98)	133 (98.52)	15 (100.00)
After second dose	256 (65.47)	24 (100.00)	98 (98.99)	134 (98.53)	15 (100.00)
Conservation rate of NABs against original strain					
No. of test	N ₂ = 63	N ₂ = 22	N ₂ = 41	N ₂ = 71	N ₂ = 9
Baseline	NA	9 (40.91)	19 (46.34)	38 (53.52)	7 (77.78)
After the first dose	NA	20 (90.91)	37 (90.24)	68 (95.77)	9 (100.00)
After second dose	20 (31.75)	19 (86.36)	40 (97.56)	69 (97.18)	9 (100.00)
Conservation rate of NABs against Omicron variant					
No. of test	N ₃ = 20	N ₃ = 5	N ₃ = 19	N ₃ = 30	N ₃ = 6
Baseline	NA	0 (0.00)	0 (0.00)	1 (3.33)	1 (16.67)
After the first dose	NA	3 (60.00)	12 (63.16)	18 (60.00)	5 (83.33)
After second dose	0 (0.00)	4 (80.00)	12 (63.16)	20 (66.67)	5 (83.33)

Abbreviations: NA, not applicable; NABs, neutralizing antibodies; N-IgG, anti-nucleocapsid protein pan-immunoglobulins; S-IgG, anti-spike protein pan-immunoglobulins.

seroconverted after the second dose (Table 2). NABs against Omicron increased significantly in mild and moderate cases after the first dose (GMT increased from 4.0 to 12.9 for mild cases and from 4.4 to 12.4 for moderate cases; $p < 0.05$) and remained stable after the second dose (GMT: 11.7 for mild cases and 11.8 for moderate cases; Figure 3B). The titers of NABs against the Omicron variant were lower than those against the original strain in most of the infected individuals (Figure 3C).

3.4 | Concentrations of antibodies in different subgroups

Given that no significant difference in titers of antibodies in recovered participants was observed, we grouped our participants according to infection history into two groups for further analyses.

After two doses of inactivated vaccine, female participants without natural infection showed higher titers of S-IgG and N-IgG than males

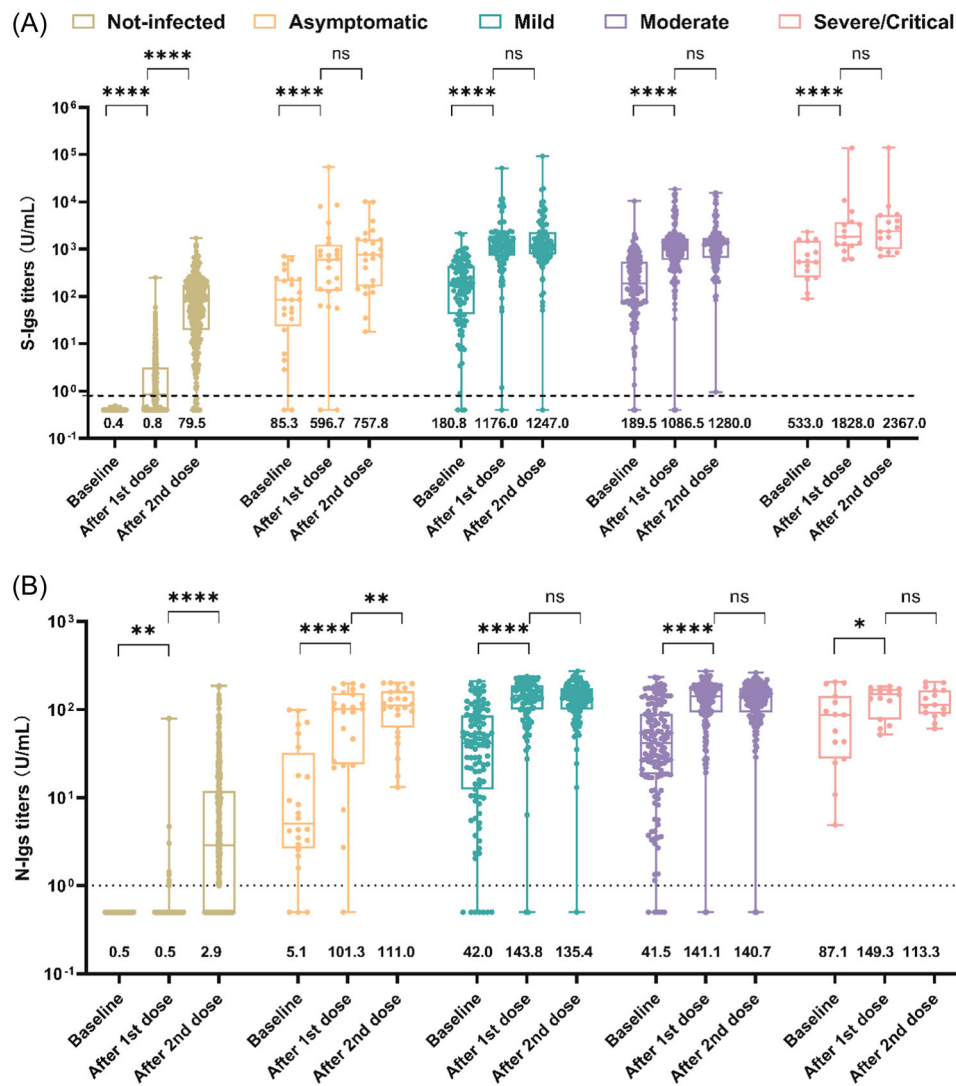


FIGURE 2 Overall distribution of pan-immunoglobulin levels before and after vaccination. (A) Antispike protein antibody titers before and after vaccination. (B) Antinucleocapsid antibody titers before and after vaccination. Dotted lines show lower limit of the assay and participants with antibody titers above the lower limit are considered seropositive. Box plots with individual data points are shown with medians (middle line), and the first and third quartiles (box). The median titers (U/ml) are shown in the figure. ns, not significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

(95.0 and 53.3 for S-IgG; 4.0 and 1.9 for N-IgG, respectively; $p < 0.01$) but the titers of NABs were comparable (GMT: 6.1 and 5.9, respectively, for NABs against the original strain). SARS-CoV-2-naïve individuals older than 65 years had higher NABs titers against the original strain than those younger than 65 years (GMT: 8.5 and 5.7, respectively; $p < 0.01$) and the titers of pan-immunoglobulins were comparable. No difference in antibody levels was observed between those with and without noncommunicable diseases (NCDs). Among individuals with a history of natural infection, antibody levels were comparable across sex, age, and NCD groups (Table 3).

4 | DISCUSSION

Our study enabled the analysis of humoral immune response to COVID-19 based on different infection histories, including variations in clinical severity. We showed that in study participants without prior

SARS-CoV-2 infection, detectable antibodies were present after the first dose of inactivated vaccine and the antibody levels improved significantly after the second dose, with 96% seroconversion for S-IgG and 32% for NABs. The development of N-IgG after vaccination paralleled the dynamics of S-IgG. In individuals with a history of natural infection, humoral immunity improved significantly after a single dose; however, the second dose of vaccine did not confer a substantial increase in antibody levels in these individuals. Overall, antibody levels were higher in recovered individuals than in SARS-CoV-2-naïve individuals and no difference was observed between recovered individuals with different clinical severities of infection. The Omicron variant showed substantial resistance to neutralization by antibodies induced by vaccination.

In SARS-CoV-2-naïve individuals, antibody levels increased significantly after the second dose. In a study on messenger RNA (mRNA) vaccination, antibody levels were comparable between those with prior infection after one dose of vaccine and those without prior

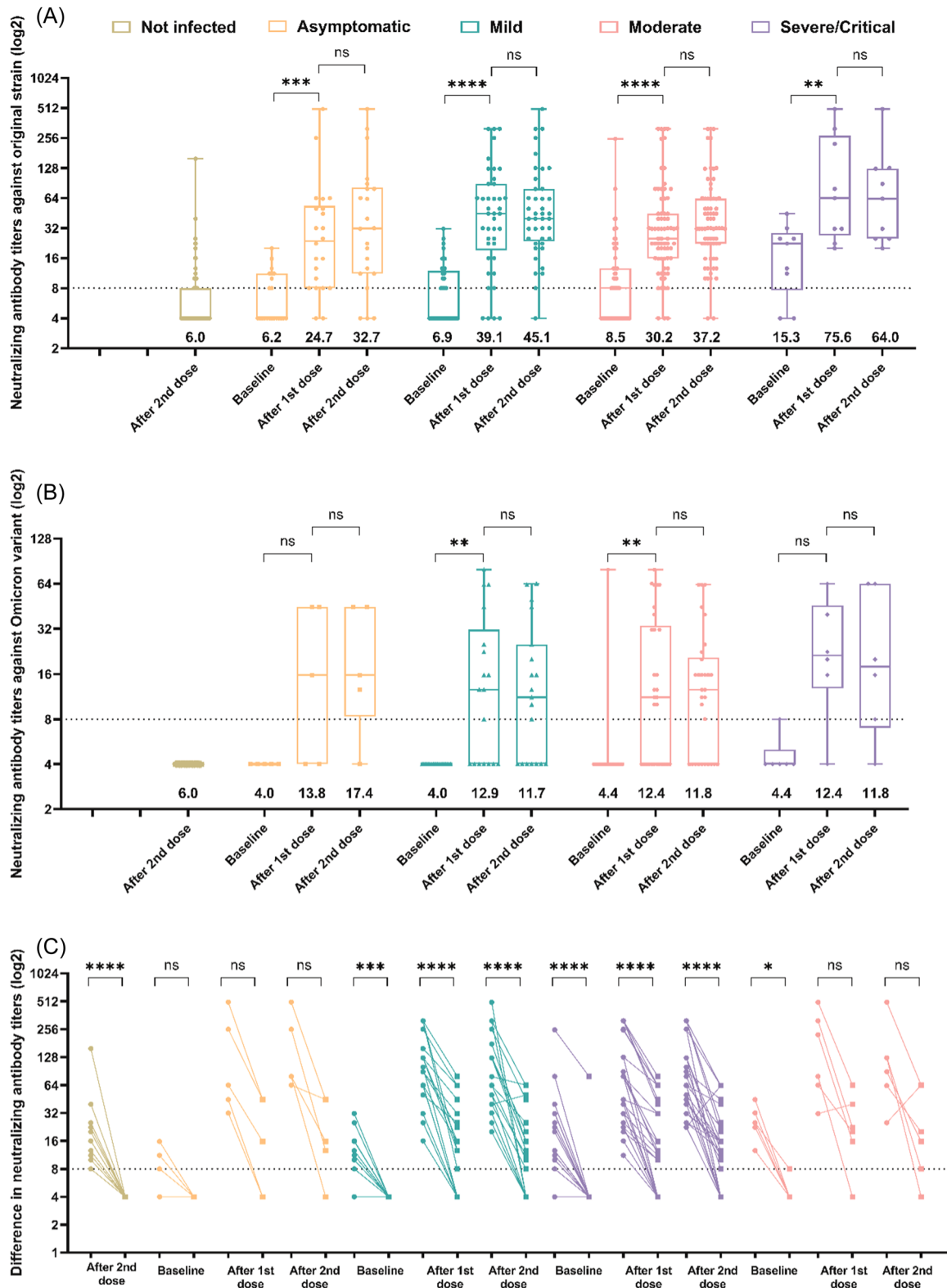


FIGURE 3 Humoral response to the original strain and Omicron variant before and after vaccination. (A) Neutralizing antibody (NAb) titers against the original strain before and after vaccination. (B) NAb titers against the Omicron variant before and after vaccination. (C) Neutralization titers against the original strain and Omicron variant before and after vaccination. Lines connect values from the same participant. Dotted lines denote the lower limit of the assay and participants with antibody titers above the lower limit are considered seropositive. Box plots with individual data points are shown with medians (middle line), and the first and third quartiles (box). Geometric mean titers are shown in the figure. ns, not significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$

TABLE 3 Concentration of pan-immunoglobulins and NABs after two doses of inactivated vaccine according to sex, age group, and NCDs

	Male	Female	p	<65 years	≥65 years	p	With NCDs	Without NCDs	p
S-IgG, median (IQR)									
Not infected	53.30 (13.08–166)	95.02 (26.03–188.9)	0.01	73.68 (18.27–174.1)	86.54 (27.94–196.8)	0.27	95.49 (15.09–182.9)	76.64 (19.25–176)	0.84
Infected	1119 (638.1–2289)	1322 (742.1–1872)	0.38	1267 (693.6–1869.5)	1298.5 (960.8–2330)	0.39	1355 (821.1–3404)	1264 (702.5–1869)	0.21
N-IgG, median (IQR)									
Not infected	1.86 (0.5–9.16)	4.00 (0.5–17.45)	<0.01	2.98 (0.5–13.88)	1.14 (0.5–9.34)	0.06	1.97 (0.5–12.89)	2.96 (0.5–11.78)	0.55
Infected	133.3 (89.42–167.4)	142 (96.14–181.3)	0.37	135 (94.77–175.1)	125.7 (99.21–169.4)	0.77	108.8 (76.65–168.2)	135.5 (100.9–175.8)	0.06
NABs against original strain, GMT (95% CI)									
Not infected	5.92 (4.72–7.42)	6.12 (4.47–8.36)	0.89	5.67 (4.63–6.94)	8.49 (5.55–13)	<0.01	7.21 (4.39–11.85)	5.85 (4.78–7.16)	0.13
Infected	37.77 (29.24–48.79)	42.17 (33.06–53.8)	0.75	39.77 (32.78–48.24)	40.65 (28.52–57.94)	0.76	51.38 (35.45–74.47)	38.61 (31.87–46.78)	0.17
NABs against Omicron variant, GMT (95% CI)									
Not infected	4 (4–4)	4 (4–4)		4 (4–4)	4 (4–4)		4 (4–4)	4 (4–4)	
Infected	12.38 (8.62–17.78)	13.08 (9.01–18.99)	0.99	12.31 (9.46–16.03)	16.19 (5.56–47.14)	0.43	22.43 (9.98–50.4)	11.65 (8.93–15.2)	0.09

Abbreviations: CI, confidence interval; GMT, geometric mean titer; IQR, interquartile range; NABs, neutralizing antibodies; NCDs, noncommunicable diseases; N-IgG, anti-nucleocapsid protein pan-immunoglobulins; S-IgG, antispikes protein pan-immunoglobulins.

infection after two doses.²¹ However, consistent with another study on inactivated vaccine,¹⁰ our study found that antibody levels were still lower in naïve participants after two doses than in recovered participants after one dose, especially for NABs. The vaccine elicited an inferior neutralizing capacity compared with natural infection, with NABs seroconverted in 32% of naïve participants, and no neutralization of Omicron was detected. Although the neutralization of Omicron was undetectable or low in those who had received a two-dose regimen of inactivated vaccine,²² an mRNA booster may lead to the development of NABs against Omicron in 80% of recipients.²³ Considering that antibody concentrations were low in SARS-CoV-2-naïve individuals, 6 months after receiving two doses of inactivated vaccine,²⁴ it may be advisable to receive a heterologous booster.²⁴⁻²⁶ However, as specific memory B cells last more than 6 months post vaccination,²⁷ the fact that antibodies wane over time does not necessarily imply loss of immune protection. Therefore, the effect of a two-dose regimen of inactivated vaccine with a booster vaccine dose needs to be explored further.

After a median of 17 months, the seropositivity rates of S-IgG remained higher than 90% in the confirmed cases in our study and almost all infected participants had a detectable antispikes antibody response. Although a previous study indicated that disease severity likely contributes to antibody responses after vaccination with CoronaVac,²⁸ our results showed that the titers of S-IgG, N-IgG, and NABs were comparable in patients with varying degrees of COVID-19 severity, both before and after vaccination. Consistent with the results of a previous study,²⁹ asymptotically infected individuals did not differ in antibody levels from participants with symptomatic disease and both groups had higher antibody levels than those observed in SARS-CoV-2-naïve individuals.

Infection-acquired immunity waned after 1 year in unvaccinated individuals but remained consistently higher than 90% in those who were subsequently vaccinated.³⁰ Consistent with existing evidence,^{3,8,9,31,32} the humoral immune response in people with natural infection was enhanced by a single dose of the COVID-19 vaccine; however, a second dose did not offer a substantial additional benefit. Some previously infected individuals even experienced a decrease in NABs titers after the second vaccine dose, which was also observed in previous studies.^{31,33} The second dose of vaccination may enhance the antibody responses to variants of concern^{34,35} in people who were exposed to SARS-CoV-2; however, only three additional individuals with natural infection had neutralizing activity against Omicron after the second dose, in our study, and the GMT of NABs even decreased after the second dose in severe and critical cases. Considering the potential risk of antibody-dependent enhancement and functional exhaustion of spike-specific lymphocytes caused by vaccination,^{36,37} it is recommended that recovered patients receive only a single dose of the vaccine until there is sufficient evidence to confirm the benefits of a second dose.

In this multicenter cohort study, we compared the antibody levels induced by vaccination in individuals with and without a history of natural infection in parallel, enabling the evaluation of the effects of infection and differences in clinical severity on the immune

response. This study relied on a serial sample before and after vaccination, allowing us to monitor the induction and maintenance of the humoral response in naïve individuals and the dynamics of reactivating pre-existing immunity with inactivated vaccines in infected individuals. Live SARS-CoV-2 assays were used to examine the presence of NABs against the original strain and Omicron variant, providing authentic data for neutralizing activity. This study had several limitations. First, we only evaluated NABs against the original SARS-CoV-2 strain and the Omicron variant even though other variants exist; further evaluation focusing on cellular immunity is ongoing. Second, we only reported data from ~1 month after the second vaccine dose and the immunity response over a longer follow-up time still needs to be characterized. We will have data with a longer follow-up time in future analyses of this ongoing cohort study.

In conclusion, our study evaluated the levels of antibodies in naïve and infected individuals before and after two doses of inactivated COVID-19 vaccination and found that a booster dose is needed for those without natural infection. However, it is recommended that recovered patients should receive only a single dose of the vaccine, regardless of the clinical severity, until there is sufficient evidence to confirm the benefits of a second dose. Our findings may help optimize vaccination strategies and maximize the overall benefit, especially in areas with a limited vaccine supply.

AUTHOR CONTRIBUTIONS

Chen Wang, Weizhong Yang, Jianwei Wang, Lili Ren, and Luzhao Feng contributed to the study design and methods. Mengmeng Jia, Li Guo, Lili Ren, Luzhao Feng, Wensheng Gong, and Qiangru Huang reviewed the literature. Wensheng Gong, Lidong Gao, Xian Liang, Enfu Chen, Wenge Tang, Guangjiong Jiang, Shanlu Zhao, Yan Feng, Li Qi, Zhiwei Leng, Yong Yue, Ju Wang, and Binshan Jiang were responsible for oversight of the study at their respective sites and contributed to the recruitment of participants. Li Guo, Lili Ren, Jingchuan Zhong, Xinming Wang, Qiao Zhang, and Tingxuan performed the laboratory analyses. Mengmeng Jia, Qiangru Huang, Luzhao Feng, Zhiwei Leng, Libing Ma, and Weizhong Yang were responsible for the data acquisition, data analysis, data interpretation, and data visualization. Mengmeng Jia, Li Guo, and Wensheng Gong wrote the original draft of the manuscript and the subsequent versions. Chen Wang, Weizhong Yang, and Jianwei Wang provided overall guidance and managed the project. All authors read and edited the manuscript. All authors approved the final version and the decision to submit the manuscript. All authors had full access to all the data and take final responsibility for the decision to submit this manuscript for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Anonymized individual-level data and data sets generated or analyzed during the current study are available to researchers who provide a methodologically sound proposal. Proposals should be directed to Dr. Chen Wang (wangchen@pumc.edu.cn), Dr. Weizhong Yang (yangweizhong@cams.cn), or Dr. Jianwei Wang (wangjw28@163.com). The data will be available beginning 3 months after publication of this article, with no end date.

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SUPPORTING INFORMATION

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

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