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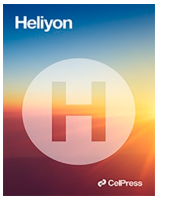

Research article

Serum SARS-CoV-2 antibodies in HIV-1-infected patients after inactivated vaccination and SARS-CoV-2 infection

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ABSTRACT

Objective: To monitor post-vaccination antibody production, neutralizing activity, and their dynamics over time in people living with HIV (PLWH).
Methods: We collected sera from 147 PLWH and 94 healthy controls after vaccination at different time points and examined changes in antibody levels and neutralizing activity using enzyme-linked immunosorbent assay (ELISA) and pseudovirus neutralization assay.
Results: IgG levels were substantially increased in both PLWH and healthy controls after the booster injection. Antibody levels decreased significantly in both PLWH and controls five months after the booster injection. However, the rate of decrease was not significantly different between the two groups. The generated antibodies demonstrated protective efficacy against the wild-type SARS-CoV-2 strain, but very low protection against the mutant strains. Furthermore, the protection decreased over time. The vaccine was less effective in PLWH with <200/μl CD4 T cells. During the SARS-CoV-2 recovery period, participants had substantially increased serum antibody levels and protective efficacy compared with those who received the booster.
Conclusion: Both PLWH and controls demonstrated comparable antibody production ability. Vaccines and booster development against SARS-CoV-2 mutant strains should be prioritized in PLWH, especially in those with low CD4 counts.

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic has persisted for 3 years and has caused numerous deaths and substantial economic losses worldwide. Following the successful development and application of vaccines, an increasing number of people have been protected against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. In vaccinated populations, people living with HIV (PLWH) have received widespread attention owing to their differing immune levels, antibody production levels post vaccination, and neutralizing antibody protection levels compared to those of people without HIV. In a small cohort of PLWH who received mRNA vaccines, the vaccine was less immunogenic in those with low $CD4^+$ T cell counts compared to those with $CD4^+$ T cell

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counts >200 cells/μl [2,3]. A study on BNT162b2 vaccine safety and immunogenicity in 143 PLWH and 261 controls reported a good safety profile and similar immunogenicity in both groups [4]. Similarly, studies from South Africa and England have reported no significant differences in vaccine immunogenicity between PLWH and healthy controls [5]. However, only a relatively few studies have assessed the immunogenicity of inactivated vaccines in PLWH.

Following the continuous mutation of the COVID-19 SARS-CoV-2 virus, Beta, Delta, Omicron BA.2, Omicron BA4/5, and Omicron BF.7 have been the dominant mutant strains [6]. A previous study reported that serum protection efficacy against the Delta and Omicron strains 8 weeks after vaccination decreases 1.47- and 11.65-fold, respectively [7]. Owing to the decrease in protective efficacy and vaccination policy modifications, there have been post-COVID-19 vaccine breakthrough infections, and antibody production in PLWH with post-COVID-19 vaccine infections should be monitored in a timely manner. A study from Germany reported that participants developed serum antibodies after two doses of the BNT162b2 mRNA vaccine: HIV-1-infected participants had significantly lower serum neutralization and anti-spike immunoglobulin (Ig)A levels than non-HIV-1-infected individuals [8]. A study from the UK demonstrated that among well-treated PLWH, COVID-19-infected PLWH had post-ChAdOx1 vaccination antibody levels comparable to those without HIV [9]. A study from the US also demonstrated that antibody levels in breakthrough infections in PLWH correlated with disease severity and $CD4^+$ T cell count [10,11]. These results suggest that the ability of PLWH to produce antibodies after a breakthrough infection is influenced by various factors and exhibits unique characteristics. Therefore, monitoring the ability of PLWH to maintain humoral protection after COVID-19 is critical for understanding the reinfection risk and the need for additional vaccine boosters in the future [12].

In view of the above findings, we collected sera from 147 PLWH and 94 healthy people who had been immunized with the inactivated vaccine and followed them up by testing the above participants' sera two months after the breakthrough COVID-19 infection. We assessed the immunogenicity and dynamics of the inactivated vaccine in PLWH and examined the complex body fluid environment stemming from HIV-1 and SARS-CoV-2 coinfection to present recommendations for subsequent pandemic prevention strategies.

2. Materials and methods

2.1. Serum samples

This study enrolled 22 unvaccinated PLWH，147 PLWH and 94 healthy control volunteers from the Qingchun Hospital of Zhejiang Province and the First Affiliated Hospital of Zhejiang University from September 2018 to June 2022. This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang University (approval no. IIT20200060A). Informed consent was obtained from all the volunteers. Blood samples were collected 4 months after the second dose of the COVID-19 vaccine, 1 and 5 months after the third dose, and 2 months after COVID-19 infection. Serum was obtained by 10-min centrifugation at 3000 rpm and inactivated at 56 °C for 1 h.

2.2. Enzyme-linked immunosorbent assay (ELISA)

S, receptor-binding domain (RBD), and nucleocapsid (N) protein-specific binding IgG antibodies were measured using commercial kits from Vazyme Biotech Co., Ltd. according to the manufacturer's protocols. All plasma samples were heat-inactivated at 56 °C for 30 min before use and were diluted in 5 % bovine serum albumin, added to high-affinity binding ELISA plates pre-coated with S, RBD, or N antigen, and incubated for 1 h at 37 °C. The wells were then washed three times with phosphate-buffered saline [PBS] containing 0.05 % Tween-20, and secondary antibodies were added and incubated for 30 min at 37 °C. After another three washes with PBS-T, the tetramethylbenzidine substrate was added at 37 °C in the dark. After 15 min, the reaction was stopped with 2 M H_2SO_4 solution, and the absorbance was measured at 450 and 630 nm.

2.3. Pseudotype neutralization assays

The pseudotyped vesicular stomatitis virus incorporated into the S proteins of the SARS-CoV-2 variants was obtained from Vazyme Biotech Co., Ltd. Plasma samples were heat-inactivated at 56 °C for 30 min to remove complement activity. Samples were serially diluted three-fold with Dulbecco's modified Eagle's medium (DMEM) from 1:15 to 1:3465 in white, flat-bottomed 96-well plates (Thermo Fisher Scientific) in a total volume of 50 μl. Subsequently, 1.3×10^4 median tissue culture infective dose (TCID50) SARS-CoV-2 pseudotyped particles in 50 μl medium were added to each well and incubated at 37 °C for 1 h. ACE-2-293T cells (4×10^5) in 100 μl complete medium were added to each well and incubated for 24 h at 37 °C and 5 % CO_2. Firefly luciferase activity (luminescence) was measured using the Bright-Lite Luciferase Assay System (Vazyme Biotech Co., Ltd.) and GloMax Multi Detection System (Promega) according to the manufacturer's protocols. Neutralizing antibody titers were calculated as the median inhibitory dose (ID50), which was expressed as the plasma dilution that resulted in a 50 % reduction in luciferase luminescence compared to the virus control in a single-round pseudovirus infection assay.

2.4. Statistical analysis

GraphPad Prism 8 (GraphPad Inc., La Jolla, CA, USA) was used for the statistical analysis. Data are presented as the mean ± SD. Statistical differences between groups were analyzed with the Mann–Whitney *U* test. Paired samples were compared using a paired *t*-

test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Cohort baseline characteristics

From September 2018 to June 2022, we recruited 22 unvaccinated PLWH, 147 PLWH, and 94 healthy control volunteers who were vaccinated with Sinovac (Beijing, China). Age, sex, viral load, and CD4 count are shown in Table 1. Vaccination candidates should be over 18 years old, and those with contraindications, such as uncontrolled epilepsy, should be excluded. The entire immunization process consisted of three injections, with an interval of more than 3 weeks and less than 8 weeks between the first and second doses. A third booster dose was administered 6 months after completing the second dose. All volunteers completed their first dose in April 2021, their second dose in May, and received a booster shot in January 2022.

The median age of the 147 vaccinated PLWH was 39 years (range, 21–49 years), and 87.8 % of them were males. Fifty-eight patients had viral load >20 copies/ml and the median $CD4^+$ T cell count was 513/μl (range, 78–1198/μl). Classification of the $CD4^+$ T cell counts was as follows: 10 PLWH, <200/μl (6.8 %); 59 PLWH (39.5 %), 200–500/μl; and 78 PLWH (53.1 %), >500/μl. The median age of the 94 healthy controls was 41 years (range, 22–60 years), and 68.2 % were males.

Ninety-four PLWH and 37 healthy controls were followed up until December 2022. The median age of the 94 PLWH was 38.5 years (range, 26–56 years); 88.3 % were male, and 78.7 % of the viral load was <20 copies/ml. The median $CD4^+$ T cell count was 517/μl (range, 78–1087) in 94 patients. The $CD4^+$ T cell counts were as follows: two PLWH (2.1 %), <200/μl; 40 PLWH (42.6 %), 200–500/μl;

Table 1
Participants' characteristics.

	PLWH	People without HIV
COVID-19-vaccinated		
n	147	94
Age (years), median (range)	39 (21–49)	41(22–60)
Gender		
Male, n (%)	129 (87.8 %)	64(68.1 %)
Female, n (%)	18 (12.2 %)	30(31.9 %)
Plasma HIV viral load		
>20 copies/ml, n (%)	58(39.5 %)	/
<20 copies/ml, n (%)	89(60.5 %)	/
Median $CD4^+$ T cell count (range)	513 (78–1198)	/
CD4 count (cells/μl)		
<200 , n (%)	10 (6.8 %)	/
200-500 , n (%)	59 (39.5 %)	/
>500 , n (%)	78 (53.1 %)	/
COVID-19-infected		
n	94	37
Age (years), median (range)	38.5 (26–56)	41 (22–57)
Gender		
Male, n (%)	83 (88.3 %)	28 (75.7 %)
Female, n (%)	11 (11.7 %)	9 (24.3 %)
Plasma HIV viral load		
>20 copies/ml, n (%)	20 (21.3 %)	/
<20 copies/ml, n (%)	74 (78.7 %)	/
Median $CD4^+$ T cell count (range)	517 (78–1087)	/
CD4 count (cells/μl)		
<200 , n (%)	2 (2.1 %)	/
200-500 , n (%)	40 (42.6 %)	/
>500 , n (%)	52 (55.3 %)	/
COVID-19-unvaccinated		
n		
Age (years), median (range)	38(21–54)	/
Gender		
Male, n (%)	13(59.1 %)	/
Female, n (%)	9(40.9 %)	/
Plasma HIV viral load		/
>20 copies/ml, n (%)	4(18.2 %)	/
<20 copies/ml, n (%)	18(81.8 %)	/
Median $CD4^+$ T cell count (range)	749(85–1132)	/
CD4 count (cells/μl)		
<200 , n (%)	3(13.6 %)	/
200-500 , n (%)	5(22.7 %)	/
>500 , n (%)	14(63.6 %)	/

Abbreviations: PLWH, People living with HIV; COVID-19, coronavirus disease 2019.

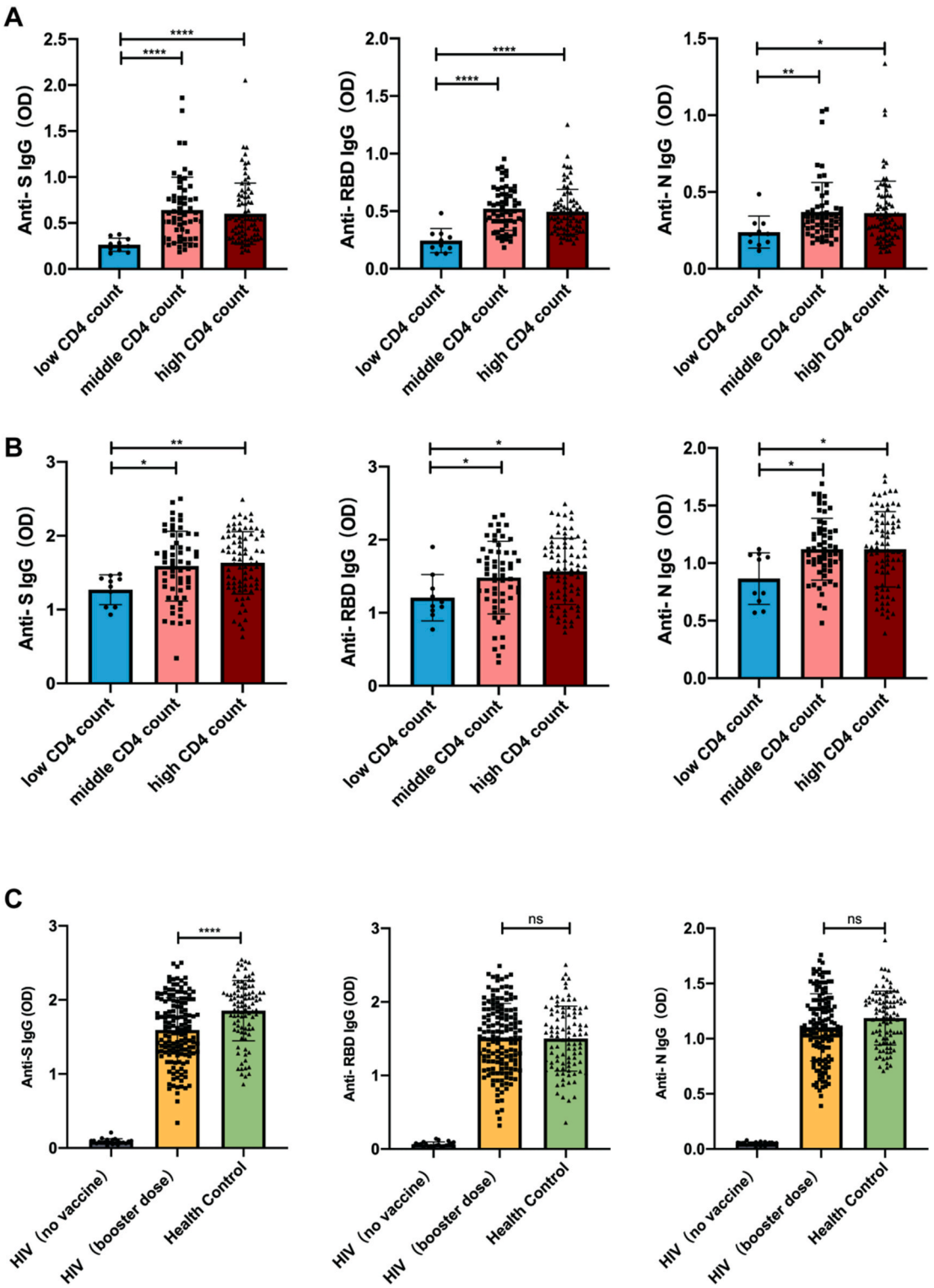

Fig. 1. Comparison of anti-S, -RBD, and –N IgG antibodies of PLWH and healthy controls after second and third doses of SARS-CoV-2 inactivated vaccine. (A) IgG levels of PLWH after the second vaccine dose (after 4 months) (B) IgG levels of PLWH after the booster (after 1 month). (C) Overall anti-S, -RBD, and –N IgG antibody levels in PLWH vs. controls after booster injection (after 1 month). Statistical significance was determined using the 2-sided Mann–Whitney *U*test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Abbreviations: PLWH, People living with HIV; Ig, immunoglobulin; N, nucleocapsid protein; RBD, receptor-binding domain; S, spike; OD, optical density.

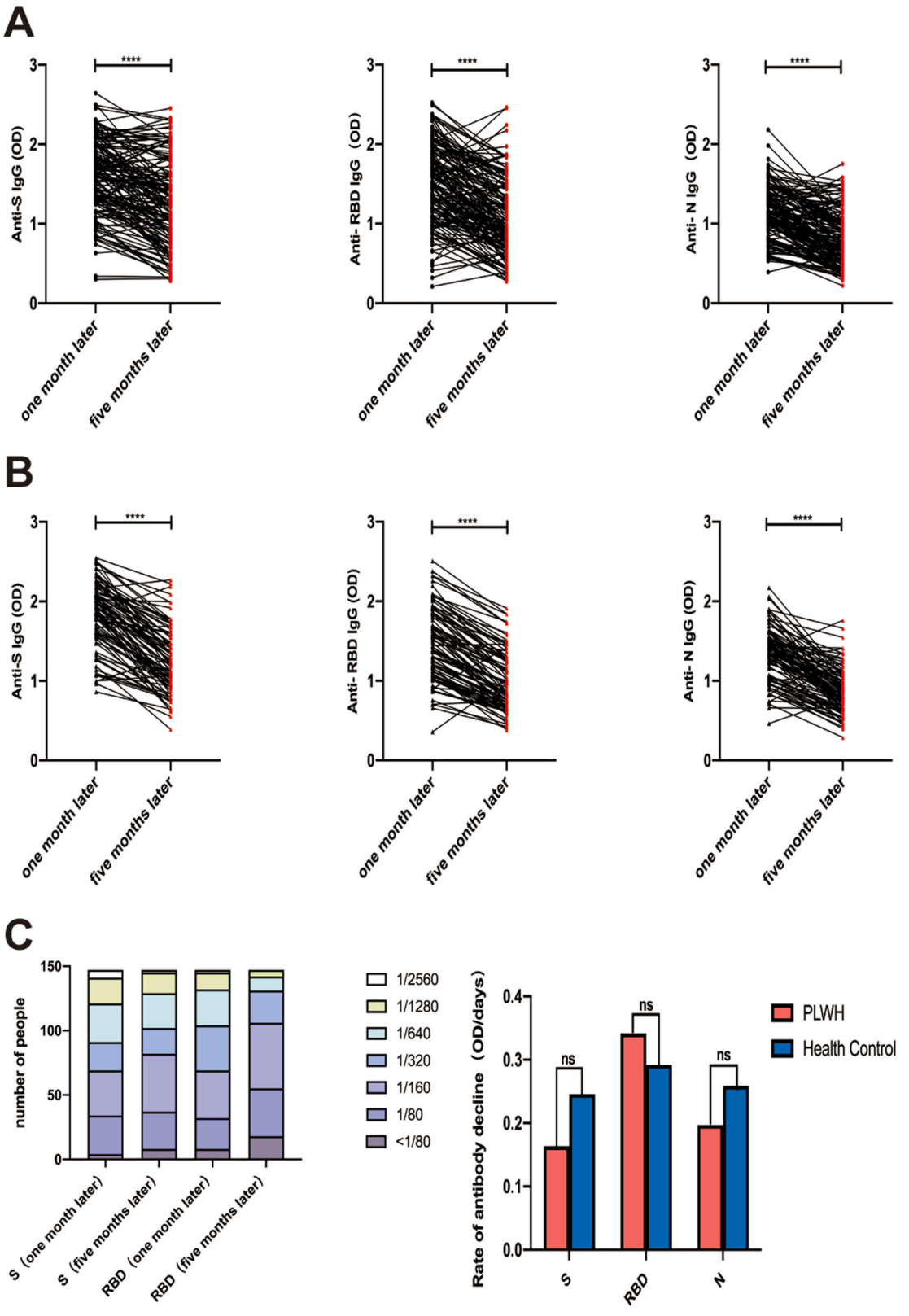

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Fig. 2. Dynamics of anti-S, -RBD, and –N IgG antibodies after booster injections in PLWH and healthy controls. (A) Antibody levels in PLWH at 1 and 5 months after booster. (B) Antibody levels in controls at 1 and 5 months after booster. (C) Distribution of the number of volunteers with the highest antibody titers at different booster injection times (left). Comparison of the antibody change rate of PLWH and healthy controls (right). Statistical significance was determined using the paired *t*-test. ****$P < 0.0001$.
Abbreviations: PLWH, People living with HIV; HC, healthy controls; Ig, immunoglobulin; N, nucleocapsid protein; RBD, receptor-binding domain; S, spike; OD, optical density.

and 52 PLWH (55.3 %), >500/μl. The median age of the 37 healthy controls was 41 years (range, 22–57 years), and 75.7 % were male. Twenty-two PLWH who did not receive an inactivated vaccine contributed to the laboratory-retained 2018 plasma. Their median age was 38 years (range, 21–54 years), and 59.1 % were male. Of these 22 PLWH, four had viral loads >20 copies/ml and the median $CD4^+$ T cell count was 749/μl (range, 85–1132/μl). The $CD4^+$ T cell counts were classified as follows: three PLWH (13.6 %), <200/μl; five PLWH (22.7 %), 200–500/μl, and 14 PLWH (63.6 %), >500/μl.

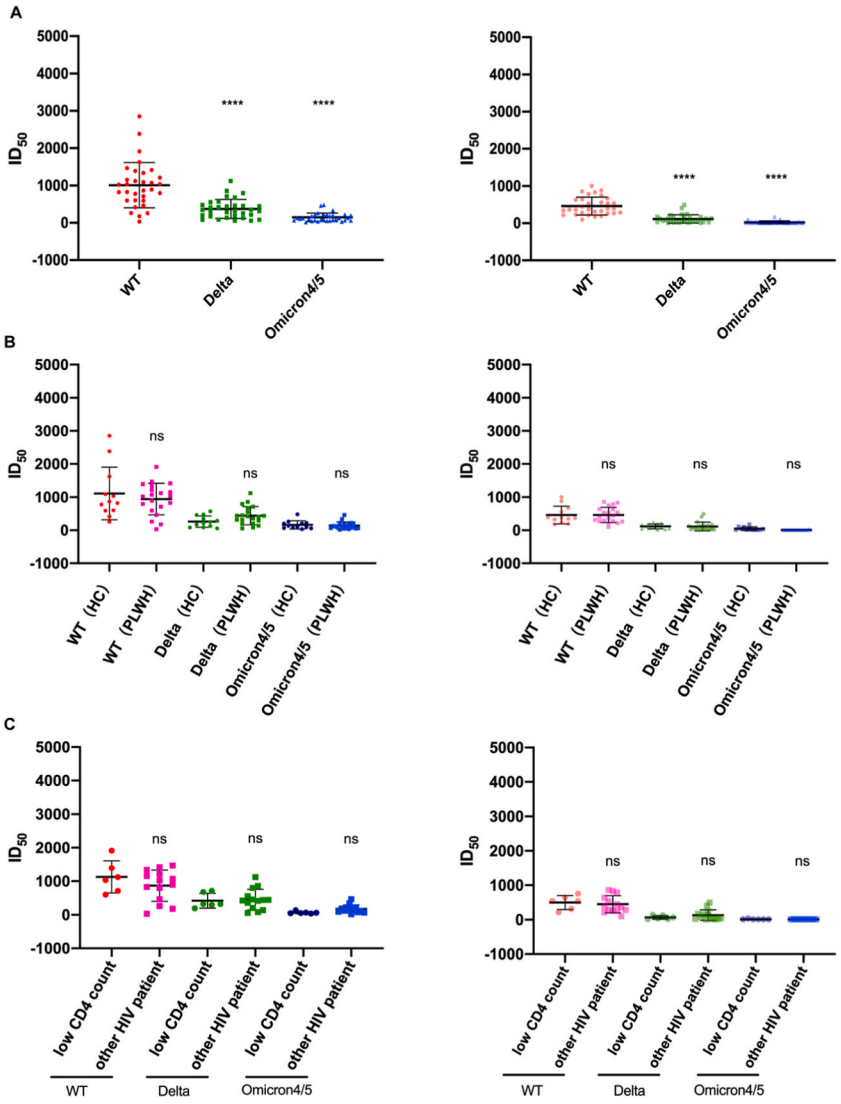

Fig. 3. Dynamic changes in the protective efficacy of serum neutralizing antibodies in PLWH and healthy controls (HC) after receiving inactivated vaccine. (A) Neutralizing antibody titers (ID50) against the WT, Delta, and Omicron BA4/5 strains 1 month (left) and 5 months (right) after the booster. (B) PLWH and control neutralizing antibody titers against the WT, Delta, and Omicron BA4/5 strains at 1 month (left) and 5 months (right) after the booster. (C) PLWH neutralizing antibody titers at 1 month (left) and 5 months (right) after the booster. Statistical significance was determined using a 2-sided Mann–Whitney *U* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Specimens with ID50 < 20 were assigned a value of 10.
Abbreviations: PLWH, People living with HIV; HC, healthy controls; ID50, median inhibitory concentration; WT, wild-type.

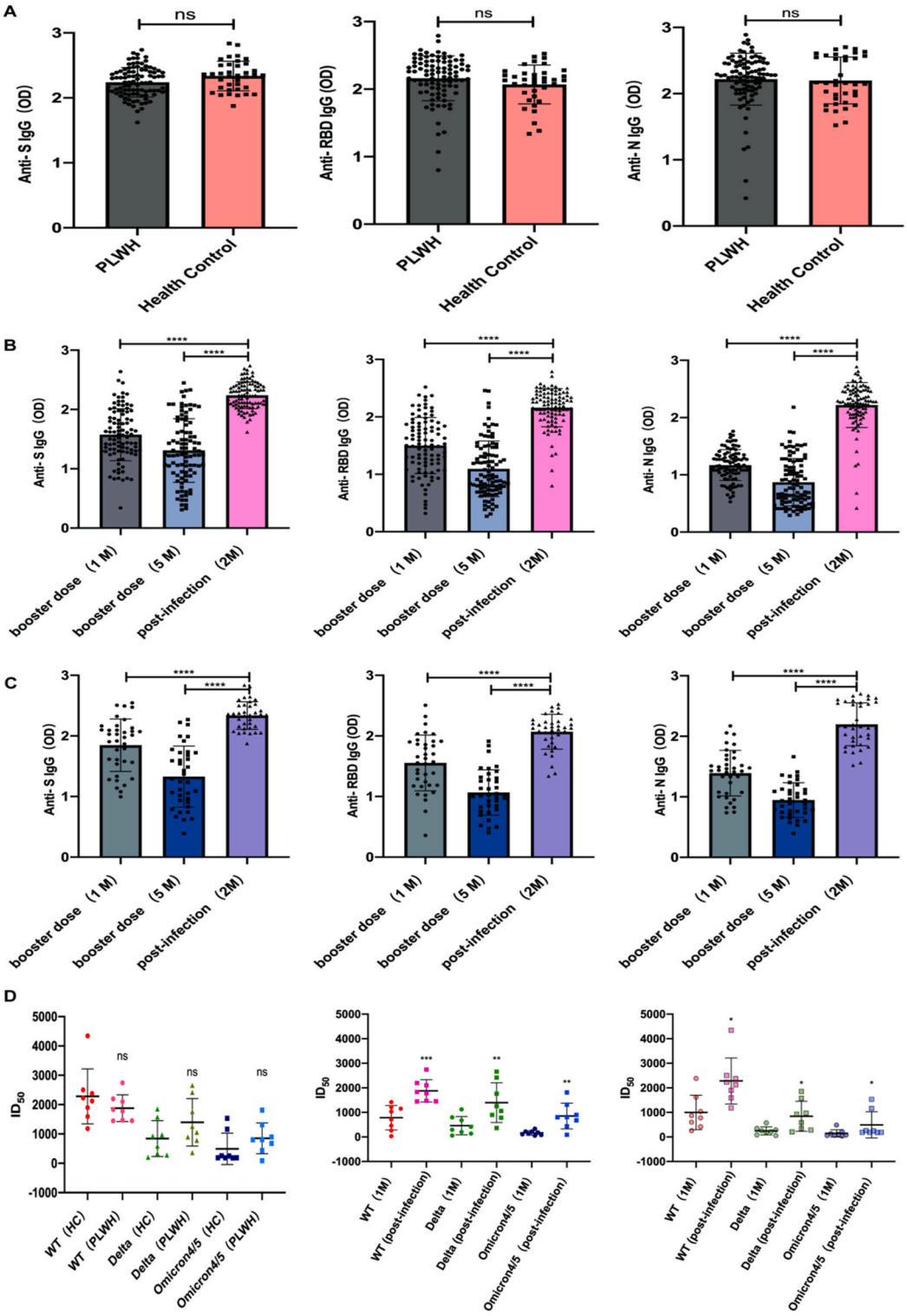

Fig. 4. The antibody production and neutralizing activity of convalescent serum from PLWH and healthy controls. (A) Anti-S, -RBD, and –N IgG production in the convalescent serum of PLWH vs. controls. (B) Comparison of anti-S, -RBD, and N IgG antibody production in PLWH convalescent serum sera and after booster injections. (C) Comparison of anti-S, -RBD, and –N IgG antibody production in control convalescent serum sera and after booster injections. (D) Serum neutralization viability dynamics in PLWH vs. controls post-infection (left). Serum neutralization viability dynamics in PLWH post-infection and 1 month after booster (middle). Serum neutralization viability dynamics in controls post-infection and 1 month after booster (right). Statistical significance was determined using the 2-sided Mann–Whitney *U* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P <$

0.0001.
Abbreviations: PLWH, People living with HIV; HC, healthy controls; 1 M: 1 month after booster dose; 5 M: 5 months after booster dose; Ig, immunoglobulin; N, nucleocapsid protein; RBD, receptor-binding domain; S, spike.

3.2. Assessment of SARS-CoV-2 antibodies after booster vaccination

IgG levels against the S glycoprotein, viral RBD, and N protein of the 147 PLWH were measured using ELISA. Based on the $CD4^+$ T cell counts, the PLWH were grouped into low- (<200/μl), medium- (200–500/μl), and high-count (>500/μl) groups. The low-count group had lower IgG antibody production than the other two groups (Fig. 1A). Although anti-S, anti-RBD, and anti-N IgG antibody levels increased in all PLWH after the booster injection, the antibody levels in the low-count group remained low (Fig. 1B). As antibody production increased significantly in PLWH after the third vaccine dose, we compared the degree of antibody production with that of 94 healthy controls who had received a booster shot (Fig. 1C). Compared with unvaccinated PLWH, the anti-S, -RBD, and anti-N IgG antibodies were significantly enhanced in PLWH and healthy controls after the booster injection, and the PLWH had significantly lower anti-S IgG production than the controls. Nevertheless, anti-RBD and –N IgG production was not significantly different between PLWH and controls (Fig. 1C). This may be because the N protein is inside the viral particle and the RBD is in the S protein [13,14].

3.3. Dynamic changes in antibody production after inactivated vaccine booster

The participants' antibody persistence ability was measured and their serum was collected 5 months after the third injection. After 5 months, the anti-S, anti-RBD, and anti-N IgG antibodies in the PLWH decreased, but their antibody levels remained high (Fig. 2A). Titration of the S and RBD antibody titers of the 147 PLWH demonstrated significantly reduced numbers of PLWH with antibody titers >1:640 after 5 months (Fig. 2C). In the control group, the target IgG antibodies decreased significantly after 5 months (Fig. 2B). Detection of the decline rates of IgG antibodies in PLWH and controls revealed no significant difference between the two groups, indicating good persistence of the domestic inactivated vaccine on PLWH immunogenicity (Fig. 2C).

3.4. Neutralizing effect of serum on wild-type (WT) COVID-19 strains and variants 1 month and 5 months after booster

To test the neutralizing efficiency of serum against circulating COVID-19 variants, we analyzed the ID50 against the WT and mutant Delta and Omicron variants using SARS-CoV-2 pseudotype neutralization assays. Plasma samples from 20 PLWH and 12 healthy controls were randomly selected for the analysis. The results demonstrated that the protective efficacy of the serum was mainly against the WT strain and was significantly lower against the Delta strain, especially against Omicron BA4/5. Five months after the booster injection, the serum antibodies demonstrated significantly weaker protective efficacy against the WT and mutant strains (Fig. 3A). In comparison with controls, the protective efficacy of serum antibodies between strains was not significantly different in PLWH at 1 or 5 months after the booster (Fig. 3B). CD4+T cell counts were conducted on 20 PLWH individuals, among which 6 had counts below 200/μl, classified as the low count group, while 14 had counts above 200/μl, categorized into another group. Serum testing was performed on these groups to assess their protective efficacy against wild-type and variant strains. The results indicated that the differences between the low-count group and other groups were not significant (Fig. 3C).

3.5. Antibody production and neutralizing activity against SARS-CoV-2 after infection

As SARS-CoV-2 continued to mutate, participants' breakthrough infections were counted. Convalescent sera were obtained from 94 PLWH and 37 healthy controls 2 months after the onset of COVID-19 breakthrough infection. All COVID-19-infected participants were identified using commercial antigen kits or nucleic acid tests. ELISA testing of anti-S, anti-RBD, and anti-N IgG antibodies during the recovery period revealed that the antibody levels in PLWH with breakthrough infection were not significantly different from those in the controls (Fig. 4A). This is consistent with the results of a previous study on breakthrough infections in PLWH after BNT162b2 mRNA vaccination [15]. This indicates that PLWH demonstrated a good humoral immune response to SARS-CoV-2 and that HIV-1 infection status exerted little effect on the organism's immune response to the novel coronavirus. Nevertheless, comparison with the antibody levels 1 month post-booster revealed that serum antibody levels were significantly higher during the recovery period than after vaccination, indicating a stronger immune response after breakthrough infection (Fig. 4B and C). The neutralizing activity of convalescent sera was examined, and the convalescent sera of 16 of the 32 samples examined earlier were analyzed. Among these 16 individuals, 8 were PLWH, and 8 were healthy controls.

The serum neutralizing ability of antibodies against the major prevalent COVID-19 strains was examined using a pseudotyped viral neutralization assay, which determined that the serum neutralizing potency against SARS-CoV-2 was substantially increased after breakthrough infection, and the neutralizing activity in PLWH was not different from that of the controls (Fig. 4D). This illustrates the increased ability of viruses to escape after mutation and the need to develop vaccines against prevalent mutant strains.

4. Discussion

We compared the antibody production and neutralizing activity of PLWH and healthy controls with those of the WT and major prevalent mutant SARS-CoV-2 strains after inactivated vaccination against COVID-19. Serum antibody levels and neutralizing activity

during the recovery period after breakthrough infection were also compared. The results demonstrated that serum anti-S, anti-RBD, and anti-N IgG antibodies after two or three inactivated vaccine doses were not well produced in COVID-19-infected PLWH with <200 CD4 T cells/μl. This was similar to the results of a study of the same type that reported similar results for an mRNA vaccine [16]. This might be due to immunosuppression or treatment failure and impaired immune levels in PLWH, such that their post-vaccination immune response was poor. For example, some studies reported significantly lower B cell counts and RBD-specific memory B cell subpopulations in this population than in PLWH with >200 CD4 T cells/μl after vaccination, and weaker antibody neutralizing activity [17]. Following the booster, the anti-S, anti-RBD, and anti-N IgG antibody levels increased substantially, although the PLWH had lower levels than the healthy participants [11,18]. This was consistent with the antibody level changes in the participants after receiving the BNT162b2 booster vaccine [19]. The anti-S, -RBD, and anti-N IgG antibodies increased slightly after booster injections, perhaps due to the viral particle structure or the sequence of viral protein-induced antibody production [20,21,22]. Some studies have reported that anti-N and anti-S IgM peaked in some non-intensive care unit patients 2 weeks after infection or vaccination, while anti-N and anti-S IgG continued to increase 3 weeks after infection or vaccination [23].

We also examined the persistence of anti-S, anti-RBD, and anti-N IgG antibodies in PLWH and healthy controls post-vaccination and determined that the levels of all three antibodies decreased in PLWH and controls after ~150 days but remained high. Determination of the antibody decline rate in different populations indicated no significant differences. This indicates that the SARS-CoV-2 inactivated vaccine has good immunogenicity for PLWH and can trigger a durable humoral immune response. Similarly, several cohort vaccination studies of PLWH demonstrated that high antibody levels persisted in such patients 4 months after booster injection [12,24].

We also evaluated the virus-neutralizing ability of the antibodies produced after booster vaccination. The WT strain and the Delta and Omicron BA4/5 variants were selected based on the continuous viral mutation and the current epidemiological situation. Overall, the antibodies demonstrated strong neutralizing activity against the WT strain and decreased neutralizing activity against the delta strain 1 month after booster vaccination. Notably, the antibodies had little protective efficacy against the currently prevalent strain Omicron BA4/5, which is consistent with previous studies [25,26]. Furthermore, immune escape by the Omicron BA4/5 variant occurs not only in vaccine breakthrough infections but also in some antibody drugs such as the LY233CoV016/LY-CoV555 cocktail and REGN-10933/REGN-109876 cocktail [27].

Given the weakening antibody-protective efficacy and vaccination policy changes, a large breakthrough infection is expected to occur in China in late 2022. For participants with follow-up, serum samples were collected from 94 PLWH and 37 healthy controls. The results demonstrated substantially elevated IgG antibodies against the S, RBD, and N proteins 2 months post-infection compared to antibody levels 1 month after the vaccine booster. Furthermore, serum antibody levels were comparable between PLWH and controls during the recovery period after breakthrough infection, indicating that PLWH were able to mount a strong immune response to SARS-CoV-2. The convalescent serum demonstrated a significantly elevated neutralization ability against the WT and prevalent mutant strains, and the neutralization viability of the two populations was comparable. These results have important implications for the protective potential of patients in the recovery period after infection and are similar to those of previous studies [28].

However, health issues arising from the production of serum autoantibodies during vaccine administration cannot be ignored. Some studies have reported a significant increase in autoantibody levels after SARS-CoV-2 infection. Pascolini et al. [29]. analyzed the presence and function of autoantibodies in patients with COVID-19-related pneumonia. The team conducted a prospective study on 33 COVID-19 patients, all of whom underwent testing for antinuclear antibodies (ANA), antiphospholipid antibodies, and anti-neutrophil cytoplasmic antibodies (ANCA). The results showed that 45 % of the patients tested positive for at least one type of autoantibody, including 11 cases of positive ANA (33 %), 8 cases of positive anti-phospholipid antibodies (IgG and/or IgM) (24 %), and 3 cases of positive anti-β2 glycoprotein antibodies (IgG and/or IgM; 9 %). No antineutrophil cytoplasmic antibody (ANCA) reactivity was observed. These antibodies, particularly the antinuclear antibodies, are significantly associated with severe lung damage [30]. Cases related to Autoimmune diseases have also been reported. Pascolini et al. reported three cases of immune-mediated thrombocytopenia occurring during SARS-COV-2 infection. All three patients tested positive for anti-APA antibodies [31]. Seohyun et al. explored the adverse events following immunization (AEFI) related to autoimmune diseases and the COVID-19 vaccine using the World Health Organization's global database, VigiBase. Four potential safety signals related to autoimmune diseases after COVID-19 vaccination were detected, including ankylosing spondylitis, psoriatic arthritis, inflammatory bowel disease, polymyositis, and thyroiditis [32]. Therefore, it is important to actively monitor patients during the vaccination process, such as monitoring the production of self-antibodies after vaccination completion and monitoring the development and changes in self-antibody levels after breakthrough infections or reinfections.

This study has some limitations. In the post-vaccination monitoring of antibody levels, sample detection after the first injection was missing because of vaccination prevention measures. Furthermore, the convalescent sera of PLWH and controls relied mainly on self-assessment using symptom attenuation or antigen kits. Consequently, the exact recovery times might have been biased. In the future, we will strive to avoid such issues and give them more timely and comprehensive attention when it comes to monitoring reinfections among such special groups and immune responses to different variants.

Despite these shortcomings, our findings provide an objective evaluation of the neutralizing ability of the virus and its mutant strains by examining antibody production and dynamic changes in PLWH and healthy controls after COVID-19 vaccination and breakthrough infection. Moreover, the immune response to the COVID-19 inactivated vaccine and SARS-CoV-2 in PLWH were characterized.

5. Conclusion

We verified the humoral immunity levels of PLWH to the inactivated vaccine and SARS-CoV-2 infection after breakthrough

infection by examining the changes in antibody levels and neutralizing ability of PLWH and healthy controls. As the novel coronavirus continues to mutate, with the emergence of variants, such as XBB1.9, XBB1.16, and JN.1, vaccines are constantly being updated. Vaccines targeting the newly emerged variants have also been developed. In PLWH patients with $CD4^+$ T cell counts below 200/μl, although their ability to produce antibodies is weaker, they still provide some level of protection. Considering the prevalence of variant strains, it is advisable to recommend booster vaccine shots that target these variants in such populations.

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Ethical approval statement

Studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (ethical approval no. IIT20200060A). Informed consent was obtained from all the volunteers. Details that might disclose the identities of the study participants have been omitted.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Xiaodi Zhang: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Dating Han:** Validation. **Nanping Wu:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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