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Neutralizing antibody test supports booster strategy for young individuals after SARS-CoV-2 Omicron breakthrough

Yichuan Yao[1,2†], Yunru Yang[1,2†], Qiqin Wu[1,2], Mengyao Liu[1,2], Wei Bao[3], Qiutong Wang[4], Meijun Cheng[5], Yunuo Chen[1,2], Yiting Yu[6], Yuan Cai[1], Mei Zhang[1,2,5], Jingxue Yao[1,2], Hongliang He[1], Changjiang Jin[4], Changcheng Zheng[1,7*], Tengchuan Jin[1,2,7*] and Dali Tong[1,7*]

Abstract

Background The SARS-CoV-2 Omicron variant, since its initial detection, has rapidly spread across the globe, becoming the dominant strain. It is important to study the immune response of SARS-CoV-2 Omicron variant due to its remarkable ability to escape the majority of existing SARS-CoV-2 neutralizing antibodies. The surge in SARS-CoV-2 Omicron infections among most Chinese residents by the end of 2022 provides a unique opportunity to understand immune system's response to Omicron in populations with limited exposure to prior SARS-CoV-2 variants.

Methods We tested the levels of IgG, IgA, and IgM specific to the prototype SARS-CoV-2 RBD (receptor-binding domain) in blood samples from 636 individuals by chemical luminescence assay, ELISA and pseudovirus-based neutralization assay.

Results Inoculation with inactivated prototype SARS-CoV-2 vaccines or recombinant protein vaccines showed higher IgG levels after infection than the unvaccinated individuals. Moreover, the age resulted in different IgG levels after the Omicron infection as IgG level of the patients aged > 60 years was lower than that of patients aged < 60 years. This indicates that the IgG induced by SARS-CoV-2 Omicron breakthrough infection was different between old and young individuals. We found that a booster dose of the prototype SARS-CoV-2 vaccine led to a significant increase in the neutralizing immune response against the prototype SARS-CoV-2 and helped induce neutralizing antibodies against BA.5 and BF.7 variants after an Omicron breakthrough infection in young individuals, which is different from a previous report on older people.

Conclusions These data suggest that the prototype SARS-CoV-2 booster vaccination helps induce high levels of neutralizing antibodies against Omicron BA.5 and BF.7 variants after Omicron breakthrough infection in young individuals.

Trial registration: This study is a purely observational study.

Keywords Survey, SARS-CoV-2 variants, Neutralizing antibody, Vaccine, Booster

†Yichuan Yao and Yunru Yang have contributed equally.

*Correspondence:
Changcheng Zheng
zhengchch1123@ustc.edu.cn
Tengchuan Jin
jint@ustc.edu.cn
Dali Tong
tdl@ustc.edu.cn
Full list of author information is available at the end of the article

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Background

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread rapidly worldwide, threatening global public health. Since its emergence, SARS-CoV-2 has developed into many variants, such as Alpha, Beta, Delta, and Omicron. Currently, the Omicron variant, including the Omicron offshoots BA.5, BF.7, and XBB, is the major circulating viral strain. Since its initial detection in November 2021, the Omicron variant has rapidly spread across the globe, becoming the dominant strain. Its significantly enhanced infectivity and transmission speed surpass those of previous variants like Alpha, Beta, Gamma, and Delta. According to the classification by the World Health Organization (WHO), Omicron has been designated as a Variant of Concern (VOC), suggested that it became a significant threat to global public health. The SARS-CoV-2 spike (S) protein interacts with the human angiotensin-converting enzyme 2 (ACE2) receptor (respiratory system, enterocytes, brain, eye and so on), enabling its entry into target cells [1–5]. The Omicron variant has more than 30 mutations in the spike protein and 15 mutations in the RBD of the S protein (G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y and Y505H). These mutations leading to the escape of neutralization activity by most of the identified anti-SARS-CoV-2 neutralization antibodies [6, 7]. For example, K417N and N501Y contribute to immune escape and higher infectivity [8, 9]. G446S, E484A and Q493R could also lead to the escape of neutralization [6].

Based on the pathogenic mechanism of SARS-CoV-2, some drugs, including Paxlovid, have been developed for the treatment of COVID-19 [10–12]. Furthermore, many vaccines have been developed to control the infection and spread of SARS-CoV-2, including those based on messenger RNA (mRNA) [13, 14], viral vectors [15, 16], recombinant proteins [17, 18], inactivated SARS-CoV-2 [19, 20], and bivalent vaccine or boost strategy [21–23]. Immunization with these vaccines has reduced infection rates and post-infection mortality rates. A booster dose of inactivated SARS-CoV-2 vaccine led to a significant increase in the neutralizing immune response against the prototype SARS-CoV-2 and the Omicron variant, despite incomplete escape [24]. In contrast, repeated vaccination with an inactivated SARS-CoV-2 vaccine has been reported to dampen the neutralizing antibody (nAb) response against new Omicron variants in breakthrough infections due to a stronger immune imprint on the ancestral strain [25]. Extensive research has reported immune imprinting, a phenomenon involving the creation of epitope-specific B cell memory following initial exposure to an antigen. This memory subsequently influences future B cell and antibody responses to variant epitopes, particularly in the context of SARS-CoV-2 infection and vaccination [26–29]. This suggests that the current herd immunity may not efficiently prevent infections with highly mutagenic Omicron variants. However, 84.4% of the patients were > 50 years of age in the investigation [25], and it remains unclear how breakthrough infection affects the immune responses of young people who received a 3-dose or 4-dose compared to those who received a 2-dose vaccination or were unvaccinated. The objective of this study is to explore whether booster vaccination can enhance the induction of higher neutralizing antibodies (nAbs) following breakthrough infection, particularly among young individuals.

From mid-December 2022 to early January 2023, the majority of Chinese residents experienced a surge in SARS-CoV-2 Omicron breakthrough infection wave. According to data released by the Chinese Center for Disease Control and Prevention, the SARS-CoV-2 that caused the spread of this current epidemic was mainly Omicron BA.5 and BF.7 variants. The surge in SARS-CoV-2 Omicron infection among most Chinese residents by the end of 2022 provides a unique opportunity to understand how the immune system responds to Omicron infection in populations with limited contact with prior SARS-CoV-2 variants. Therefore, we surveyed 750 individuals with SARS-CoV-2 infection and collected fingertip blood samples from 636 individuals (the remaining volunteers voluntarily withdrew from the blood tests for personal reasons) without immunodeficiency disorders in Hefei, Anhui Province, China in January 2023. Among the 636 people, 441 were infected with SARS-CoV-2 for the first time. We tested the nAb titer in the plasma of venous blood against the prototype, Omicrons BA.5, BF.7, and XBB. 1.5 variants by the pseudovirus-based neutralization assay. The result suggested that the prototype SARS-CoV-2 booster vaccination helps induce high levels of neutralizing antibodies against Omicron BA.5 and BF.7 variants after Omicron breakthrough infection in young individuals.

Methods

Survey and human blood samples

This study was reviewed and approved by the Medical Ethical Committee of the First Affiliated Hospital of the USTC (the medical ethical approval numbers: 2023-ky-001). The first surveys were collected from 750 people during the Omicron Variants pandemic from December 2022 to January 2023 in Hefei, Anhui Province, China. Blood samples were collected from 636 individuals at USTC Hospital. None of the patients diagnosed with severe or critically ill COVID-19 at the hospital were included in the survey. A history of vaccination was

recorded, and the patients were categorized as unvaccinated, 2-dose vaccine, 3-dose vaccine, and 4-dose vaccine. The second survey was conducted on 222 people approximately 4 months after the breakthrough infection wave. All participants were fully informed about the study and provided written informed consent. As specific comorbidities (diabetes, hypertension and so on) can either intensify these pathological mechanisms or diminish the patient's resilience to organ damage [30–32], among the volunteers who participated in the venous blood collection, none of them had any specific comorbidities, including metabolic diseases like diabetes or respiratory diseases.

We used fingertip blood collection and negative pressure venous blood collection in the study. All blood collection procedures were completed by professional medical staff at the USTC Hospital. When collecting fingertip blood, we used sterile 28G blood collection needles and sterile pipettes to collect about 20 μL of blood from the ring finger fingertip and transfer it to the chemical luminescence kits reagent for subsequent testing. When collecting negative pressure venous blood, we used negative pressure EDTA-2K blood collection tubes to collect about 2 mL of venous blood. Centrifugation at 3000 rpm for 10 min, the upper plasma was collected in the biosafety cabinet. The collected plasma was stored in a − 80 ℃ refrigerator. The following pseudovirus-based neutralization assay involving the plasma were conducted within the biosafety cabinet to prevent contamination.

Pseudovirus-based neutralization assay

The SARS-CoV-2-Fluc pseudovirus (Vazyme, prototype: DD1702, BA.5: DD1176, BF.7: DD1789 and XBB. 1.5: DD1797) was used in the pseudovirus-based neutralization assay. The viral system employed utilizes HIV-1, which carries a luciferase reporter gene as its structural backbone, and expresses the SARS-CoV-2 Spike protein on its viral coat. These pseudotyped virus particles are capable of infecting exogenous cell lines that exhibit high levels of ACE2 expression, mimicking the invasion process of the SARS-CoV-2 virus into target cells via the Spike–ACE2 interaction. The level of infection in target cells by these pseudotyped virus particles is directly proportional to the luciferase-based luminescence intensity and inversely proportional to the neutralizing activity of the antibodies present.

The undiluted plasma samples were inactivated in a water bath at 56 °C for 30 min. Serially diluted plasma (three times diluted with the first dilution of 1:20 by DMEM (Gibco, 11965-092) with 10% FBS (Gibco, 16000-044)) was added to the plates with 200 $TCID_{50}$ pseudovirus (total: 100 μL), which were then incubated at 37 °C for 1 h. 2×10^4 cells/well HEK293–ACE2 cells (100 μL) was added to the plates and incubated at 37 °C (5% CO_2) in DMEM medium with 10% FBS for 48 h. After remove the medium and wash with 200 μL PBS, 100 μL Enhanced Firefly Luciferase Reporter Gene Assay Cell Lysis Buffer (Beyotime, RG127) was added to the plates and then incubated at room temperature for 10 min. The plates were then centrifuged at 300 rpm for 5 min. The supernatant was transferred to a 96-well plate. Add 100 μL of the Bio-Lumi Firefly Luciferase Reporter Gene Assay Kit (Beyotime, RG042). Incubated at room temperature for 2 min. The full spectrum of luminescence is measured using a microplate reader.

The 50% pseudovirus neutralizing titers ($pVNT_{50}$) of each sample in the prototype and the Omicron variant pseudovirus assays were determined. We plotted the nAb titers of the samples and calculated the geometric mean titers (GMTs) for each vaccine dose group. Results below the detection threshold ($pVNT_{50}=40$) were set to 0.5 times of the detection threshold ($pVNT_{50}=20$).

Protein expression and purification

The methods for purifying the SARS-CoV-2 RBD [amino acid (AA) 321–591], SARS-CoV-2 RBD variants, and human ACE2 extracellular domain (AA 19–615) were based on previous research [33]. Briefly, target genes were inserted into the pTT5 vector, which contained an IFNA1 signal peptide at the N-terminus and a tobacco etch virus (TEV) protease site connected to the human IgG1 Fc at the C-terminus. The expression vectors were transiently transfected into HEK-293F cells using polyethyleneimine (PolyScience). After 3 days, the supernatant was collected by centrifugation at 5000×*g* for 15 min at 4 °C. Approximately ¼ volume of 1×DPBS (138 mM Sodium chloride, 2.67 mM Potassium chloride, 8.1 mM Disodium hydrogen phosphate, 1.47 mM Potassium dihydrogen phosphate) was added to adjust the pH of the supernatant. The supernatant was then loaded onto the protein A column and the target protein was eluted with 0.1 M acetic acid on ÄKTA pure (GE Healthcare). 150 mM Tris 7.5 was added to the collection protein to adjust the pH. To get the target protein without Fc tag, 1 mM edetate disodium (EDTA), 5 mM dithiothreitol (DTT), and a tenfold molar ratio of 6×His-tagged TEV protease were added to the protein, and the Fc tag were cleaved at 4 °C overnight. Furthermore, the digested protein was dialyzed with 1×DPBS at 4 °C. Then, a 5 mL Protein A column was used to remove Fc and undigested protein, and a 5 mL Ni–NTA column was used to remove 6×His-TEV. The target protein was collected from the flow-through. Amicon Ultra-15 centrifugal filter with 10 kDa (Millipore, UFC9010) was used to concentrate the target proteins and SDS–PAGE was used to check purity.

ELISA

The ELISA assay was based on previous research [15]. Nunc MaxiSorp plates were coated with 100 µL of 3 µg/mL of recombinant RBD, BA.5 RBD, BF.7 RBD, or XBB.1.5 RBD at room temperature for 2 h. After washing four times with PBS (3 min each), the plates were blocked with 5% non-fat milk in PBS at room temperature for 2 h. Serially diluted plasma (the serial dilution is performed with threefold dilutions, starting with a 1:100 dilution in 5% non-fat milk in PBST (PBS with 0.1% Tween-20) was added to the plates, which were then incubated at room temperature for 1 h. After washing thrice with PBST (3 min each), horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Sangon Biotech, D110050, 1:10,000 diluted with 5% non-fat milk in PBST) was added, followed by incubation at room temperature for 1 h. After washing three times with PBST (3 min each time), 100 µL of TMB substrate was added and incubated in the dark for 8 min and the reaction was stopped with 50 µL of 1 M H_2SO_4. Absorbance was measured at 450 nm using a microplate reader. The antibody titer was calculated as the dilution of plasma that induced an A450 value twice that of the A450 value of the negative control.

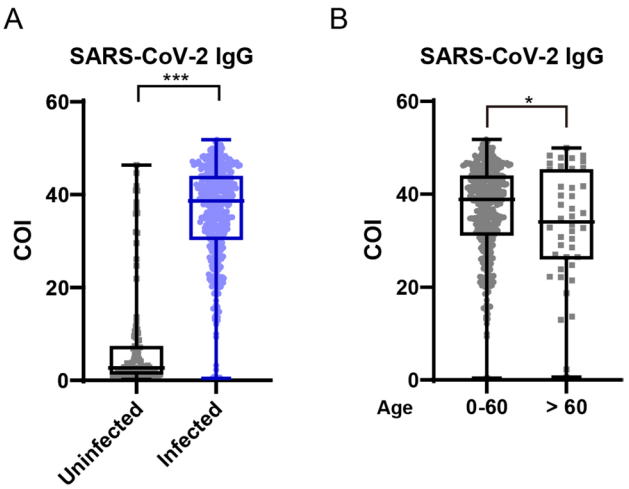

Fig. 1 IgG level of the patients aged > 60 years was lower than that of patients aged < 60 years. **A** IgG levels against the prototype SARS-CoV-2 in infected and uninfected individuals were measured using chemical luminescence kits. ($n = 108$ in the uninfected group; $n = 441$ in the infected group; COI, cutoff index). B IgG levels in infected young and old patients ($n = 396$ in the 0–60 group; $n = 45$ in the > 60 year group). Values are expressed as medians (range). Mann–Whitney test analyzed using GraphPad Prism v8, $^{*}P < 0.05$; $^{***}P < 0.001$

Chemical luminescence assay

Previous studies demonstrated that the serum level of IgG that specifically binds to the RBD highly correlates with that of neutralizing antibody activity in blocking infection of SARS-CoV-2 or ACE2 targeting pseudoviruses [34, 35]. Coated the purified RBD viral antigens onto magnetic particles for catching SARS-CoV-2-specific IgA, IgM and IgG in sera. The second antibody recognizes IgA, IgM or IgG conjugated with acridinium was used for detecting the IgA, IgM or IgG caught by antigen, respectively. 20 µL fingertip blood was used to measure levels of IgG, IgA, and IgM specific to the prototype SARS-CoV-2 spike RBD using the chemical luminescence kits and an automatic chemical luminescent immune-analyzer, Kaeser 1000 (Kangrun Biotech, Suangzhou, China). Virus-inactivated serum samples were then diluted 40 times with dilution buffer and subjected to testing at room temperature [36, 37].

Statistical analyses

All data are presented as the median (quartile, minimum to maximum) or geometric mean + geometric standard deviation for antibody titers. Since the antibody samples we tested did not follow a Normal Distribution, we chose the Mann–Whitney test for difference analysis between the two groups of samples (Fig. 1, Figures S1A, B, D, G, H, S3A), ANOVA was used for related multiple sample analysis, and the Kruskal–Wallis multiple comparison test for multiple sample adjustments was used for multiple comparisons (Figs. 2, 3, Figures S1C, E, F, I, J, S2, S3B–F). Pearson Correlation Analysis was used for correlation analysis between the two factors (Fig. 4). The hypothesis was tested to be that there was no significant difference between the two samples. When $P < 0.05$, we rejected the hypothesis. Quantification graphs were analyzed using GraphPad Prism v8 (GraphPad Software). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Results

Characterization of the study participants

Among the 750 people participating in the survey, only 636 individuals completed blood collection. Table 1 summarizes the demographic and epidemiological characteristics of these 636 individuals in January 2023. Regarding the people surveyed, the median age was 32 years (interquartile range: 27 and 49 years; range: 2–69 years). Twenty-two people were younger than 15 years, and 57 were older than 60 years. Of them, 308 are women. Among the 636 people, 441 were infected, as confirmed by antigen or nucleic acid testing, 142 were negative for both tests, and the remaining 53 were not tested at the time of blood collection (excluded from the antibody analysis as we could not know they were infected or not). The ratio of positive test results was similar between men and women. The percentage of infected patients increased with age (Spearman Correlation Analysis, $r = 1$, $P = 0.0167$). A total of 611 people (96.07%) were

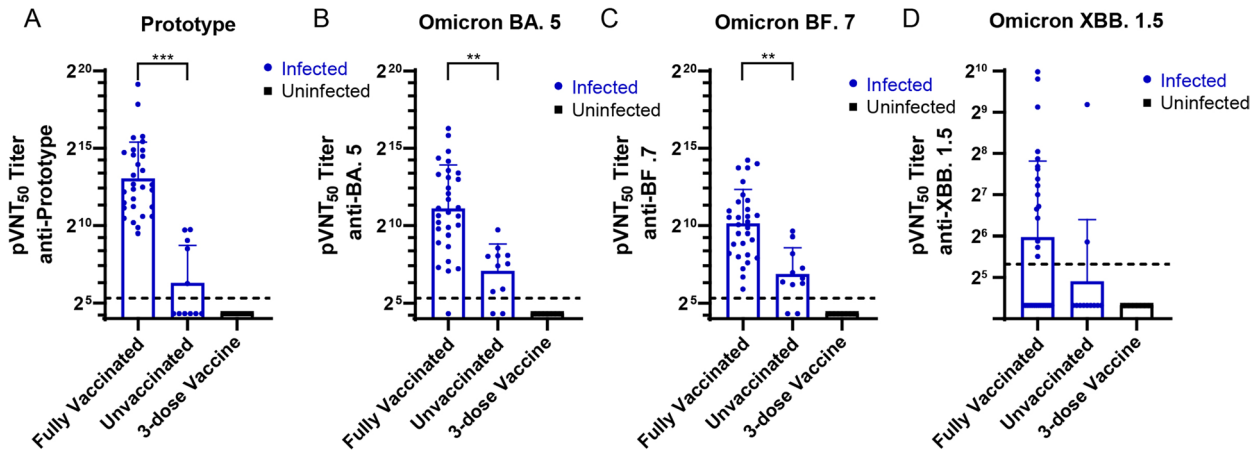

Fig. 2 Vaccination of prototype vaccines could help patients produce higher nAbs against the Omicron variants. **A** Quantitative analysis of the $pVNT_{50}$ titer against the prototype SARS-CoV-2 was calculated using a pseudovirus-based neutralization assay. **A**–**D** The detection threshold ($pVNT_{50}$ = 40) is shown as a dotted line; n = 30 in the fully vaccinated infected group which received 2-dose or more IV vaccines; n = 11 in the unvaccinated infected group; n = 8 in the 3-dose uninfected group that received three doses of the IV vaccine). **B** Quantitative analysis of the $pVNT_{50}$ titer against the Omicron BA.5 variant calculated using a pseudovirus-based neutralization assay. **C** Quantitative analysis of the $pVNT_{50}$ titer against the Omicron BF.7 variant calculated using a pseudovirus-based neutralization assay. **D** Quantitative analysis of $pVNT_{50}$ titers against Omicron XBB. 1.5 variant were calculated using a pseudovirus-based neutralization assay. Values represent the geometric mean + geometric standard deviation of the $pVNT_{50}$ titer. Kruskal–Wallis multiple comparison test analyzed using GraphPad Prism v8; $^{**}P < 0.01$; $^{***}P < 0.001$

fully vaccinated, including 2-dose inactivated SARS-CoV-2 vaccine (10.53%); 3-dose recombinant protein vaccines (4.09%); 2-dose inactivated SARS-CoV-2 vaccine with 1-dose inactivated SARS-CoV-2 vaccine booster (57.55%); 2-dose inactivated SARS-CoV-2 vaccine with 1-dose recombinant protein vaccine booster (12.42%); 2-dose inactivated SARS-CoV-2 vaccine with 1-dose Adv vaccine booster (1.26%); 2-dose mRNA vaccines (0.63%); 3-dose mRNA vaccines (0.16%) and 4-dose vaccines (8.49%). Nineteen people (2.99%) were unvaccinated, four (0.63%) were vaccinated with 1-dose inactivated SARS-CoV-2 vaccine, and two (0.32%) were vaccinated with 1-dose recombinant protein SARS-CoV-2 vaccine. Of the 611 fully vaccinated individuals, 5 (0.86%) received the latest vaccine for less than 7 days, 11 (1.80%) received the latest vaccine between 7 and 13 days, 49 (8.02%) received the latest vaccine between 14 and 30 days, 6 (0.98%) received the latest vaccine between 1 and 6 months, and the others (540, 88.38%) received the latest vaccine for more than 6 months. The infection rate was 76% in the group that received the last dose of the vaccine after > 6 months.

A total of 222 individuals (from the 636 volunteers who participated in the initial round of testing) participated in the second study visit, and blood samples were collected in April 2023 for the second time. All 222 participants who participated in the second test did not experience reinfection with SARS-CoV-2 during 4 months, as confirmed by their self-administered antigen tests. The median individual age was 34 years (interquartile range: 27–46 years; range: 20–68 years). A total of 156 people were infected, as confirmed by antigen or nucleic acid testing; 50 people were negative for both tests, and the other 16 people were not tested during the breakthrough infection wave (from mid-December 2022 to early January 2023). No SARS-CoV-2 reinfection was reported in the 222 individuals after breakthrough infection. The vaccination strategies for the 222 individuals are summarized in Table S1.

Clinical features of the study participants

The patients of SARS-CoV-2 showed symptoms of viral pneumonia and other system [38–41]. The clinical characteristics of the 441 individuals who tested positive for antigens or nucleic acids are summarized in Table 2. Only four patients (0.91%) reported no special symptoms, and the most common symptoms were fever (86.62%), cough (84.6%), weakness (67.35%), sputum production (65.99%), headache (50.79%), myalgia (50.79%), sore throat (49.43%), runny nose (35.37%), loss of taste and smell (30.39%) and conjunctivitis (3.17%). The mean symptom duration was 5 days (range, 1–20 days). Among the patients, 24.26% exhibited mild symptoms, 63% displayed moderate symptoms, and 12.70% self-reported severe symptoms, although none of the cases were officially classified as severe infections by the hospital. A total of 355 patients (80.50%) used medication to alleviate their symptoms in accordance with the guidelines provided by the Chinese Center for Disease Control and Prevention. The drugs used were ibuprofen (93.80%), paracetamol

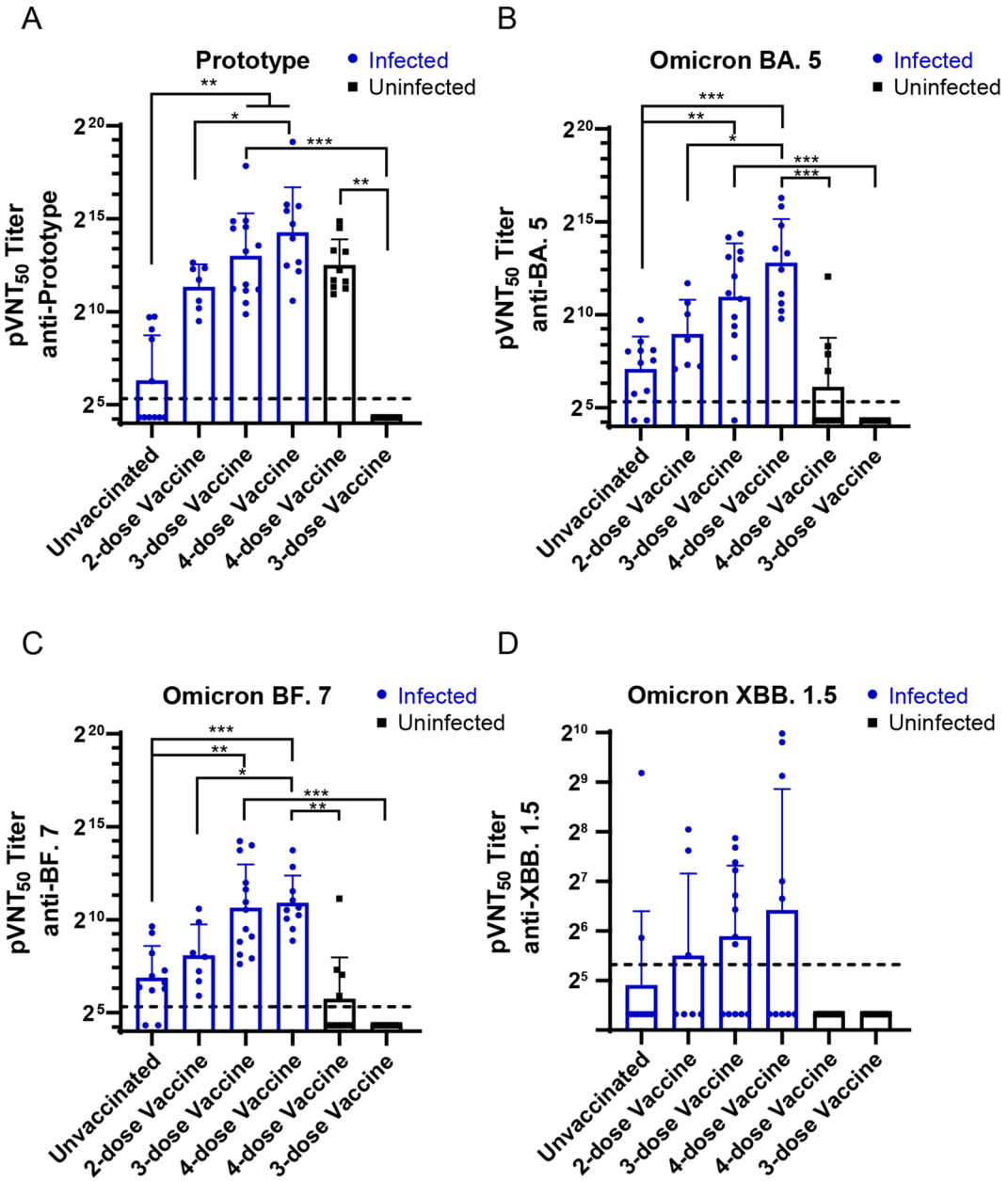

Fig. 3 Booster vaccination of prototype vaccines could help patients produce higher nAbs against the Omicron variants. **A** Quantitative analysis of the $pVNT_{50}$ titer against the prototype SARS-CoV-2 was calculated using a pseudovirus-based neutralization assay. **A**–**D** The detection threshold ($pVNT_{50}$ = 40) is shown as a dotted line, n = 11 in the unvaccinated infected group; n = 7 in the 2-dose infected group (2-dose inactivated vaccine); n = 13 in the 3-dose infected group (3-dose inactivated vaccine); n = 10 in the 4-dose infected group (3-dose IV and 1-dose RP vaccine); n = 10 in the 4-dose uninfected group (3-dose IV and 1-dose RP vaccine); n = 8 in the 3-dose uninfected group (3-dose IV vaccine)). **B** Quantitative analysis of the $pVNT_{50}$ titer against the Omicron BA.5 variant calculated using a pseudovirus-based neutralization assay. **C** Quantitative analysis of the $pVNT_{50}$ titer against the Omicron BF.7 variant calculated using a pseudovirus-based neutralization assay. **D** Quantitative analysis of $pVNT_{50}$ titers against Omicron XBB. 1.5 variant were calculated using a pseudovirus-based neutralization assay. Values represent the geometric mean + geometric standard deviation of the $pVNT_{50}$ titer. Kruskal–Wallis multiple comparison test analyzed using GraphPad Prism v8, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

(44.79%), Chinese medicine (44.79%), antibiotics (2.82%), and paxlovid (0.56%).

Serological results of the study participants

IgG level of the patients aged > 60 years was lower than that of patients aged < 60 years

Using a chemical luminescence assay, we found that the

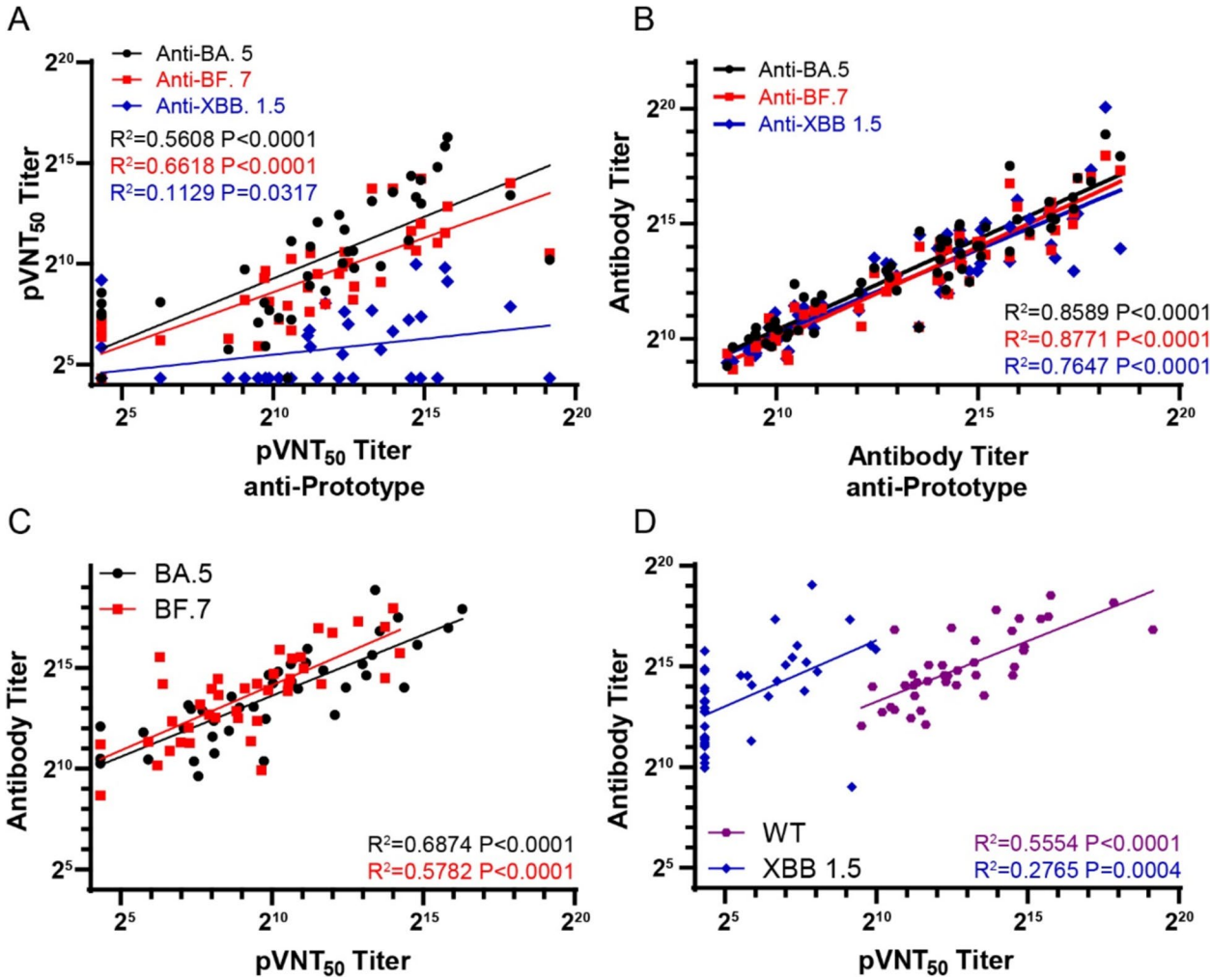

Fig. 4 Correlation analysis of $pVNT_{50}$ titer and antibody titer. **A** Pearson correlation coefficient analysis of $pVNT_{50}$ titers against prototype SARS-CoV-2 and Omicron variants in infected individuals ($n=41$). **B** Pearson correlation coefficient analysis of antibody titers against the prototype SARS-CoV-2 and Omicron variants. ($n=56$). **C** Pearson correlation coefficient analysis of $pVNT_{50}$ titer and antibody titer against Omicron BA.5 and BF.7 variants in infected individuals ($n=41$). **D** Pearson correlation coefficient analysis of $pVNT_{50}$ titer and antibody titers against prototype SARS-CoV-2 and Omicron XBB. 1.5 variants in infected individuals ($n=41$). Pearson correlation analysis analyzed using GraphPad Prism v8

IgG levels in infected patients were higher than those in uninfected individuals (Fig. 1A). IgA and IgM levels were low in both infected and uninfected individuals and were lower than the cutoff value of the positive control (Figure S1A, B) [36]. Therefore, we focused on the IgG levels. There was no significant change in the IgG levels of the patients in terms of sex or body mass index (BMI, Figure S1C–E), whereas the IgG level of the patients aged > 60 years was lower than to that of patients aged < 60 years. This indicates that the IgG induced by SARS-CoV-2 Omicron breakthrough infection was different between old and young individuals (Fig. 1B). The IgG levels in patients with severe symptoms were higher than those in patients with mild symptoms (Figure S1F). Similarly, the IgG levels of patients taking medicines were higher than those of patients receiving non-drug treatment (Figure S1G), which may be due to the worse symptoms of patients taking medicines (Figure S1H). No significant changes in IgG levels were found in the groups treated with the different types of medicines (Figure S1I).

Vaccination is one of the most effective methods for controlling the spread of SARS-CoV-2. To investigate the impact of immunization strategies on IgG production, we tested whether the vaccination strategy resulted in different IgG levels after Omicron infection, with the last vaccination more than 6 months. Omicron infection failed to induce measurable levels of prototype SARS-CoV-2 IgG in unvaccinated

Table 1 Demographics and baseline characteristics of the uninfected people and patients infected with SARS-CoV-2 Omicron variants

Characteristics	No. (%) of people[a]
Age groups, years	
0–15	22 (3.46%)
16–30	206 (32.39%)
31–45	203 (31.92%)
46–60	148 (23.27%)
> 60	57 (8.96%)
Sex	
Male	328 (51.57%)
Female	308 (48.43%)
Confirmed with SARS-CoV-2 infection[b]	
Uninfected	142 (22.33%)
Infected	441 (69.34%)
Not sure	53 (8.33%)
Infection of male	
Uninfected	74 (22.56%)
Infected	224 (68.29%)
Not sure	30 (9.15%)
Infection of female	
Uninfected	68 (22.08%)
Infected	217 (70.45%)
Not sure	23 (7.47%)
Incidence rate of SARS-CoV-2 with different ages	
0–15	8 (36.36%)
16–30	137 (66.50%)
31–45	139 (68.47%)
46–60	112 (75.68%)
> 60	45 (78.95%)
SARS-CoV-2 vaccination strategy[c]	
Unvaccinated	19 (2.99%)
1-Dose IV	6 (0.94%)
2-Dose IV	67 (10.53%)
3-Dose RP	26 (4.09%)
2-Dose IV and 1-dose IV (homologous)	366 (57.55%)
2-Dose IV and 1-dose RP (heterologous)	79 (12.42%)
2-Dose mRNA	4 (0.63%)
3-Dose mRNA	1 (0.16%)
2-Dose IV and 1-dose Adv (heterologous)	8 (1.26%)
4-Dose vaccine[d]	54 (8.49%)
Infected rate (interval days of latest SARS-CoV-2 vaccination and diagnosis day)	
0–6 days	0/5 (0%)
7–13 days	0/11 (0%)
14–30 days	27 (55%)
1–6 months	6 (100%)
> 6 months	408 (76%)

[a] Data are shown as number (%) or number/total number (%) in the 0–6 days and 7–13 days of the interval between the latest SARS-CoV-2 vaccination and diagnosis day

Table 1 (continued)

[b] Infected: antigen or nucleic acid testing positive; uninfected: antigen or nucleic acid testing negative; Not sure: no antigen or nucleic acid test

[c] IV: inactivated SARS-CoV-2 vaccine (BBIBP-CorV (BBIBP-CorV, Sinopharm, Beijing, China) or CoronaVac (Sinovac Life Sciences, Beijing, China)); RP: Recombinant Protein vaccine (Recombinant Novel Coronavirus Vaccine (CHO Cell, Zhifei Longcom Biopharmaceutical, Anhui, China)); mRNA: mRNA vaccine; Adv: Adv-based vaccine

[d] All the individuals received 4-dose vaccines were under the heterologous prime-boost strategy

Table 2 Clinical features of the 441 patients infected with SARS-CoV-2 Omicron variants

Characteristics	No. (%) of patients[a]
Initial presenting symptoms	
Fever	382 (86.62%)
Cough	373 (84.6%)
Weakness	297 (67.35%)
Sputum production	291 (65.99%)
Headache	224 (50.79%)
Myalgia	224 (50.79%)
Sore throat	218 (49.43%)
Runny nose	156 (35.37%)
Loss of taste and smell	134 (30.39%)
Conjunctivitis	14 (3.17%)
Symptom score[b]	
0–3	107 (24.26%)
4–7	278 (63.04%)
8–10	56 (12.7%)
Medicine-use	
Medicine	355 (80.50%)
Non-medicine	86 (19.50%)
Medicine type[c]	
Ibuprofen	333 (93.80%)
Paracetamol	159 (44.79%)
Chinese medicine	159 (44.79%)
Antibiotic	10 (2.82%)
Paxlovid	2 (0.56%)

[a] Data are shown as number (%)

[b] The symptom Score was provided by the patients: 0, no special symptoms. 10: Feels the most severe symptoms. No patients diagnosed with severe or critically ill COVID-19 at the hospital were included in the survey

[c] The patients who took this type of medicine are summarized in the table below, and they may have taken other types of medicine at the same time

individuals. All vaccination strategies using vaccines targeting the prototype SARS-CoV-2 could induce antibodies against the prototype SARS-CoV-2 after infection with Omicron; however, there was no significant difference in the prototype SARS-CoV-2 IgG levels produced by the different vaccination strategies (Figure S1J).

Booster immunization could induce higher nAbs of SARS-CoV-2 after Omicron breakthrough

A previous report suggested that repeated vaccination with an inactivated SARS-CoV-2 vaccine dampens the nAb response against the new Omicron variants in breakthrough infections owing to a stronger immune imprint on the ancestral strain [25]. To test the nAbs against different variants of SARS-CoV-2 in young individuals, we collected the plasma of 41 infected patients (11 unvaccinated patients, 7 with 2-dose inactivated SARS-CoV-2 vaccine, 13 with 3-dose inactivated SARS-CoV-2, and 10 with 3-dose inactivated SARS-CoV-2 and 1-dose recombinant protein vaccine (between 14 and 30 days) as the 4-dose inactivated SARS-CoV-2 vaccination strategy is discouraged in China) and 18 uninfected people, including 10 people (3-dose inactivated SARS-CoV-2 and 1-dose recombinant protein vaccine uninfected group) who received the latest boost (4th dose) between 14 and 30 days and 8 people (3-dose uninfected group) who received the latest boost of more than 6 months. All participants were aged < 60 years (range, 7–52 years; median age, 26 years). We then tested the nAb titer in the plasma of venous blood against the prototype Omicrons BA.5, BF.7, and XBB. 1.5 variants in the pseudovirus-based neutralization assay. GMTs of nAbs against the prototype, Omicron BA.5, BF.7, and XBB. 1.5 variants were not detected in the 3-dose uninfected group (Fig. 2). SARS-CoV-2 Omicron infection can induce nAbs against Omicron BA.5, and BF.7; however, there are limited nAbs against the prototype and Omicron XBB. 1.5 variant in the unvaccinated group (Fig. 2). Compared with the unvaccinated infection group, the basic fully vaccinated group (2-dose) did not show significant higher induction of nAbs against the prototype, Omicron BA.5, and BF.7 variants (Fig. 3A–C). However, booster immunization by receiving the booster shot (the 3rd and 4th doses) could induce higher nAbs against the prototype, Omicron BA.5, and BF.7 variants than the unvaccinated infection group after the Omicron Breakthrough (Fig. 3A–C). In addition, the 4-dose vaccinated infected group showed higher nAb levels against the prototype, Omicron BA.5, and BF.7 variants than the 2-dose vaccinated infected group (Fig. 3A–C). However, the nAbs against XBB.1.5 (that were not found in China on the plasma sampling date) in each infected group were low and showed no significant difference after Omicron infection (Fig. 3D). Conversely, we found that the 3-dose uninfected group, who received their last vaccine more than 6 months ago, showed undetectable neutralizing antibodies against either the prototype strain or any Omicron strains. In addition, the 4-dose vaccine booster (administered between 14 and 30 days prior) only induced neutralizing antibodies against the prototype strain in uninfected individuals, but not any Omicron strains (Fig. 3). This suggests that the booster inactivated vaccine against the prototype SARS-CoV-2 only elicits immune responses specific to the prototype strain but not to Omicron strains in uninfected individuals. Moreover, we found that the GMTs of nAbs against Omicron BA.5, and BF.7 had a high positive correlation with antibody levels against the prototype SARS-CoV-2 (Fig. 4A).

GMTs of nAbs had a high positive correlation with the antibody level

In addition, we tested the antibody titer (IgG) in the plasma of the venous blood against the prototype, Omicron BA.5, BF.7, and XBB. 1.5 variants using by ELISA [15]. Antibodies against Omicron BA.5, BF.7, and XBB. 1.5 of the individuals had a high positive correlation with antibody levels against the prototype SARS-CoV-2 (Fig. 4B). Interestingly, antibodies against the prototype, Omicron BA.5, BF.7, and XBB. 1.5 variants induced by Omicron infection in the fully vaccinated group was higher than that in the unvaccinated groups (Figure S2A–D). We also found that the 4-dose vaccinated infected group showed higher antibody levels against the prototype, Omicron BA.5, BF.7 and XBB. 1.5 variants than the 2-dose vaccinated infected group after Omicron infection (Figure S2E–H). Furthermore, the $pVNT_{50}$ of nAbs against the prototype, Omicron BA.5, and BF.7 variants of the infected individuals had a high positive correlation with antibody levels (Fig. 4C, D). It is noteworthy that we observed that neither infection nor vaccination was able to induce a high level of neutralizing antibodies against Omicron XBB.1.5 variants (Figs. 2D, 3D).

Booster immunization could induce higher antibody level of SARS-CoV-2 Tested 4 months after Omicron breakthrough infection wave

To detect the dynamics of antibodies after breakthrough infection, we also analyzed the serological results of the study participants 4 months after the breakthrough infection wave using chemical luminescence kits and Elisa Test (Figure S3, none of the 222 individuals received the booster vaccine during the 4 months). The antibody level against the prototype, Omicron BA.5, BF.7 and XBB. 1.5 variants of infected group decreased 4 months after the Omicron breakthrough infection wave (Figures S2E–H, S3C–F). However, booster immunization by receiving a booster shot (3rd and 4th doses) induced higher antibody levels against the prototype, Omicron BA.5, BF.7, and XBB. 1.5 variants than the unvaccinated infection group at 4 months after the Omicron breakthrough infection wave (Figure S3C–F). Antibody titers against prototypes BA, 5, and BF. 7 and XBB. 1.5 variants in individuals who

received the 4th vaccine were higher than those who received only 2-dose vaccines.

Discussion

In this study, blood samples were collected from 636 people in Hefei, Anhui Province, China, in mid-January 2023, ~ 3 weeks after the quick pandemic infection. The IgG levels in patients with severe symptoms were higher than those in patients with mild symptoms. Moreover, the age resulted in different IgG levels after the Omicron infection as IgG level of the patients aged > 60 years was lower than that of patients aged < 60 years. Omicron infection did not induce prototype SARS-CoV-2 IgG levels in unvaccinated patients. All vaccination strategies using vaccines targeting the prototype SARS-CoV-2 could induce antibodies against the prototype SARS-CoV-2 after infection with Omicron; however, there was no significant difference in the prototype SARS-CoV-2 IgG levels produced by the different vaccination strategies (Figure S1J).

A previous report suggested that repeated vaccination with an inactivated prototype SARS-CoV-2 vaccine dampens the nAb response against the new Omicron variants in breakthrough infections due to a stronger immune imprint on the ancestral strain in old individuals. Unfortunately, we did not find evidence that young people do not exhibit immune imprinting following vaccination with prototype vaccines when exposed to an Omicron infection. Our results suggest that, although young individuals who received booster shots showed high levels of nAbs against the prototype SARS-CoV-2 after Omicron infection, they also generated higher levels of nAbs against Omicron BA.5 and BF.7 variants than unvaccinated individuals. However, the nAbs against XBB.1.5 of different vaccination strategy were low and showed no significant difference after Omicron infection. This result revealed an Omicron XBB. 1.5 variant escape from the immune protection elicited by SARS-CoV-2 infection and vaccination with the prototype COVID-19 vaccine. We also conducted a pseudovirus assay to assess antibody responses in both infected and uninfected individuals against both the prototype and Omicron strains. The results indicated that SARS-CoV-2 Omicron breakthrough infections were capable of inducing neutralizing antibodies against the prototype strain and the Omicron BA.5 and BF.7 variants, but not against the XBB.1.5 variant (3-dose infected group vs. 3-dose uninfected group). Moreover, infection was the primary factor in inducing neutralizing antibodies against the Omicron BA.5 and BF.7 variants. The 4-dose booster vaccine against the prototype SARS-CoV-2 could elicit neutralizing antibodies against the prototype strain but not against Omicron variants (4-dose infected group vs. 4-dose uninfected group). Though the 4th vaccine did not immediately induce the production of nAbs against BA.5, BF.7 variants without infection, it helped young individuals produce higher nAbs against the Omicron BA.5 and BA.7 variants after Omicron breakthrough infection (Fig. 3).

There are still some limitations in this study. The interval between the last immunization time and the blood collection time in the 4-dose groups was between 14 and 30 days; therefore, we could not distinguish the apparent effectiveness of a 4th dose of vaccine from the effectiveness of a more recent vaccination. In addition, we were unable to conduct a follow-up survey with all volunteers and invite them for the second round of testing 4 months later. Consequently, in the blood samples taken 4 months later, we observed a reduction in data from some groups, which is likely to have a certain impact on the accuracy of the experimental results. Another limitation of this study is that we did not measure the nAbs of the booster vaccination against more novel SARS-CoV-2 variants. In our study, we found that the vaccination of booster shots could only produce limited immunity against Omicron XBB. 1.5 neutralizing antibody which might due to the multiple mutations. Therefore, further research is needed on other new SARS-CoV-2 variants especially these with variant has multiple or significant mutations.

The COVID-19 infection–fatality ratio was different by age, time, and geography [42, 43]. This might due to the variation of different population. For example, the single nucleotide variants or insertion/deletion polymorphism of ACE2 [44–47]. The sample population collected in this study was only the Han population in China, so further research is needed to determine whether the result is applicable to other populations.

Despite the fact that we gathered fingertip blood samples from 636 individuals without immunodeficiency disorders and ensured that all volunteers participating in the venous blood collection did not have any specific comorbidities, including metabolic conditions like diabetes or respiratory diseases, comorbidities continue to be a significant consideration that may potentially impact our conclusions.

Conclusion

Our data suggest that booster vaccination with prototype vaccines could help young individuals produce higher nAbs against the Omicron BA.5 and BA.7 variants, even though it recalls a strong immune response to target the prototype strain and contributes to establishing a booster vaccination strategy against COVID-19 in young individuals. On the other hand, the strong immune escape ability of new SARS-CoV-2 variants such as XBB.1.5 highlights the necessity of developing broad-spectrum coronavirus vaccines based on

conserved regions. This result suggested that the booster vaccination of young individuals with the inactivated vaccine against the prototype SARS-CoV-2 could help to produce nAbs against the new variants of the SARS-CoV-2. In clinical prevention work, vaccinating suitable people with booster shots could help preventing the infection and spread of the SARS-CoV-2 due to these young individuals could produce nAbs against the new SARS-CoV-2 variants by booster shot. However, it is also worth noting that if the SARS-CoV-2 variant has multiple or significant mutations, it may reduce the ability of the booster shot to produce nAbs against the variant.

Abbreviations

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus disease 2019
RBD	Receptor-binding domain
ELISA	Enzyme-linked immunosorbent assay
WHO	World Health Organization
VOC	Variant of Concern
mRNA	Messenger RNA
nAb	Neutralizing antibody
BMI	Body Mass Index
COI	Cutoff Index
$pVNT_{50}$	50% Pseudovirus neutralization titer

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-024-02240-5.

Supplementary Material 1.

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Author contributions
D.T., T.J., C.Z., C.J., and H.H. conceived of the project and designed the experiments. D.T., Y. Yao. and Y. Yang. designed and analyzed the questionnaire, tested the antibody titer, and wrote the manuscript. Q.Wu. and M. L. used chemical luminescence kits and W. B. analyzed the questionnaire. Q. Wang Collected blood samples from people. M. C., Y. Chen, Y. Yu, Y. Cai, M. Z., J. Y., H. H., and C. J. worked on the data collection, analysis, and discussion. All authors edited and proofread the manuscript.

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Data availability
All data needed to evaluate the conclusions of the paper can be found in the manuscript or Supplementary Material. Additional data related to this paper may be requested from the lead contact Dr. Dali Tong.

Declarations

Ethics approval and consent to participate
The study conformed to the Helsinki Declaration of 1975 (revised in 2008) concerning Human and Animal Rights. This study was reviewed and approved by the Medical Ethical Committee of the First Affiliated Hospital of USTC with the approval number: 2023-ky-001, dated: 2023-01-05. All participants or their proxies/legal guardians provided written informed consent for the publication of their anonymised case details and images. Assent was obtained from minor under the age of 18 years in addition to consent from the parents/legal guardians. For one participant (Age: 2 years), assent was obtained from the parents.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Ophthalmology, The First Affiliated Hospital of USTC, School of Life Sciences, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230026, China. [2] School of Life Sciences, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230026, China. [3]Institute of Public Health Sciences, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230026, China. [4]The Hospital of USTC, University of Science and Technology of China, Hefei 230026, China. [5]Hefei National Research Center for Physical Sciences at the Microscale, Neurodegenerative Disorder Research Center, CAS Key Laboratory of Brain Function and Disease, CAS Key Laboratory of Innate Immunity and Chronic Disease, Biomedical Sciences and Health Laboratory of Anhui Province, University of Science and Technology of China, Hefei 230026, China. [6]Institute of Advanced Technology, University of Science and Technology of China, Hefei 230031, China. [7]School of Life Science, West Campus University of Science and Technology of China, Room 718, No.443 Huangshan Road, Hefei 230022, Anhui, China.

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