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Trends of humoral immune responses to heterologous antigenic exposure due to vaccination & omicron SARS-CoV-2 infection: Implications for boosting

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***Background & objectives*: Vaccination and natural infection can both augment the immune responses against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), but how omicron infection has affected the vaccine-induced and hybrid immunity is not well studied in Indian population. The present study was aimed to assess the durability and change in responses of humoral immunity with age, prior natural infection, vaccine type and duration with a minimum gap of six months post-two doses with either ChAdOx1 nCov-19 or BBV152 prior- and post-emergence of the omicron variant.**

***Methods*: A total of 1300 participants were included in this observational study between November 2021 and May 2022. Participants had completed at least six months after vaccination (2 doses) with either ChAdOx1 nCoV-19 or an inactivated whole virus vaccine BBV152. They were grouped according to their age (≤ or ≥60 yr) and prior exposure of SARS-CoV-2 infection. Five hundred and sixteen of these participants were followed up after emergence of the Omicron variant. The main outcome was durability and augmentation of the humoral immune response as determined by anti-receptor-binding domain (RBD) immunoglobulin G (IgG) concentrations, anti-nucleocapsid antibodies and anti-omicron**

RBD antibodies. Live virus neutralization assay was conducted for neutralizing antibodies against four variants – ancestral, delta and omicron and omicron sublineage BA.5.

***Results*: Before the omicron surge, serum anti-RBD IgG antibodies were detected in 87 per cent participants after a median gap of eight months from the second vaccine dose, with a median titre of 114 [interquartile range (IQR) 32, 302] BAU/ml. The levels increased to 594 (252, 1230) BAU/ml post-omicron surge (*P*<0.001) with 97 per cent participants having detectable antibodies, although only 40 had symptomatic infection during the omicron surge irrespective of vaccine type and previous history of infection. Those with prior natural infection and vaccination had higher anti-RBD IgG titre at baseline, which increased further [352 (IQR 131, 869) to 816 (IQR 383, 2001) BAU/ml] (*P*<0.001). The antibody levels remained elevated after a mean time gap of 10 months, although there was a decline of 41 per cent. The geometric mean titre was 452.54, 172.80, 83.1 and 76.99 against the ancestral, delta, omicron and omicron BA.5 variants in the live virus neutralization assay.**

***Interpretation & conclusions*: Anti-RBD IgG antibodies were detected in 85 per cent of participants after a median gap of eight months following the second vaccine dose. Omicron infection probably resulted in a substantial proportion of asymptomatic infection in the first four months in our study population and boosted the vaccine-induced humoral immune response, which declined but still remained durable over 10 months.**

Key words Anti-receptor-binding domain IgG - BBV152 - ChAdOx1 nCoV-19 - humoral immune response - immune imprinting - Omicron - SARS-CoV-2

Vaccination has proven to be an effective prevention strategy against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease. Vaccines tailored towards the original (ancestral) strain act mainly by invoking neutralizing antibodies (Nabs) [1]. Successive infection waves caused by different variants of concern (VoCs), such as alpha (B.1.1.17), delta (B.1.617.2) and omicron (B.1.1.529), have caused breakthrough infections due to mutations in the viral spike protein. Studies have demonstrated waning of antibody levels with time[1-3]. Booster and natural infection, even if asymptomatic, are likely to sustain or increase humoral immune response.

Both natural infection and vaccination might work synergistically to boost the immune response and lead to hybrid immunity[4]. The recent spike in cases primarily driven by the omicron (B1.1.529) and its sub-lineages have caused comparatively milder infections and the proportion of asymptomatic cases has increased considerably[5]. The current global surge caused by the omicron variant, which could boost the immunity (hybrid), has also triggered an urgent need for an evidence-based booster vaccination policy depending on the durability and breadth of the immune responses.

At the time of this writing, approximately 69 per cent of the world's population had been vaccinated against the SARS-CoV-2 virus. Since January 2022, India started its booster vaccination against SARS-CoV-2, first for the frontline workers and then for general public amidst omicron-led surge. While several cross-sectional assessments of the immune response exist in the literature[6,7] paired studies of the same participants, set in between two chronologically distinct periods of vaccine-acquired immunity and breakthrough natural infections, are scarce. Therefore, the aims of the present study were to measure the change in humoral immune responses among different subsets of vaccinated individuals after omicron-driven breakthrough infections and to assess the durability of humoral immune responses.

Material & Methods

This investigation followed a prospective observational study design. Participants were included through a continuing department of Biotechnology (DBT) COVID-19 Research Consortium between November 2021 and May 2022 and were followed up. This consortium primarily coordinated and managed by the Translational Health Science and Technology Institute, Faridabad, India, in partnership with the network hospitals in the Delhi National Capital Region (NCR), India, namely Employee State Insurance Corporation Medical College and Hospital, Faridabad; Maulana Azad Medical College and adjacently associated Lok Nayak Hospital (LNJP), New Delhi; and the All India Institute of Medical Sciences, New Delhi. This consortium included a group of institutions where cohorts of infected and vaccinated participants from the community were enrolled and followed up. The enrolling sites of the consortium were the major hospitals of the Delhi NCR and catered to a vast majority of the population in this region. All the participants who were vaccinated with two doses and had completed 180 days from the second dose were considered potential participants.

The study was approved by the Institute Ethics Committees at the respective institutions. All inpatients of these hospitals who provided written informed consent were enrolled and, therefore, likely to be a true representation of the population.

Participants were divided into five study groups depending on their age, vaccine type and past SARS-CoV-2 infection status. They had completed at least six months after complete past-vaccination (both doses). The first four groups had only vaccination and no past symptomatic infection.

(i) ChAdOx1 nCoV-19 (marketed as Covishield in this region) vaccination aged between 18 and 59 yr
(ii) ChAdOx1 nCoV-19 vaccination aged beyond ≥60 yr
(iii) BBV152 (marketed as Covaxin) vaccination aged between 18 and 59 yr
(iv) BBV152 vaccination aged equal to or beyond 60 yr
(v) Vaccination with either of the vaccines along with a confirmed SARS-CoV-2 infection in the past.

In addition, a control group of unvaccinated individuals was also included for comparison. A detailed clinical questionnaire was administered and blood sample was collected at the time of enrolment into the current study.

Follow up samples: To study the change in anti-receptor-binding domain (RBD) antibodies due to the omicron wave (causing communitywide infection during the third wave in India), a second follow up sample was collected after the omicron wave (March-May 2022) for all those participants who had their first sample taken before January 2022 (time when omicron variant was first reported in India). A third follow up was carried out in January-February 2023 to find out the persistence of antibody levels and per cent decline over a period of one year.

Participant interviews: The study participants were first interviewed telephonically and then subsequently in person by trained research staff for information pertaining to vaccination against SARS-CoV-2, including the type of vaccine with dosage numbers, corresponding dates, occupation, relevant medical history, *e.g.* comorbidities, previously confirmed SARS-CoV-2 infection through reverse transcription PCR test at Government of India (GoI) authorized laboratories and whether they developed influenza-like illness (ILI). Vaccination dates were cross-verified through GoI-issued vaccination certificates. Data were captured electronically based on the standard operating protocol. Five per cent of the interviews were monitored by research medical officer for quality control.

Antibody detection: Venous blood samples were collected and transported as per the biosafety protocols recommended by the GoI. A volume of 5 ml of peripheral blood was collected for antibody tests.

<u>Detection of anti-Recptor Binding Domain (RBD) IgG</u>: Plasma anti-RBD immunoglobulin G (IgG) levels (at each respective time-point) were determined by ELISA as previously described[8]. The kit was validated against two Anti-SARS-CoV-2 IgG antibody detection kits - anti-SARS-CoV-2 ELISA (IgG) (Euruimmun, Lübeck, Germany) and Covid Kavach IgG (Zydus diagnostics, Ahmedabad). In addition, based on the World Health Organization (WHO) manual for establishment of national and other secondary standards for antibodies against infectious agents, the kit was calibrated against the first WHO international standard for anti-SARS-CoV-2 immunoglobulin (code 20/136). A level of anti-RBD antibody titre of >24 BAU/ml was taken as positive. Furthermore, the detection of anti-nucleocapsid antibodies was also carried out using a qualitative ELISA[8]. The ancestral strain of the virus served as the source of the RBD and N protein used in ELISA tests. In addition, an anti-omicron RBD IgG ELISA was also developed and performed on the paired plasma samples for each participant, as described earlier[9]. The procedure followed was the same as described earlier for ancestral RBD[8].

<u>Detection of neutralizing antibodies</u>: Virus neutralization assay was carried out to determine the presence of neutralizing antibodies in the serum samples. All the samples were heat inactivated at 56°C for 1 h before testing with the SARS-CoV-2 virus. The virus was titrated in Vero-E6 cells; 1 X 102TCID50 virus (diluted in 50 µl of the serum-free media) was incubated with two-fold serially diluted serum samples for 1 h, followed by adsorption for 1 h Vero-E6 cells which were seeded 24 h before the experiment in 96 well culture plate [1 × 104 cells/well in 150 µl of Dulbecco's modified Eagle's medium (DMEM) along with 10 per cent foetal bovine serum (FBS)]. This was followed by washing the cells with 150 µl of serum free media

and 150 µl of DMEM media supplemented with two per cent FBS. The cells were incubated for 72 h at 37°C with five per cent CO_2. The presence of virus-induced cytopathic effect (CPE) was observed in cells using inverted light microscopy. The highest serum dilution at which no CPE was observed in cells, was taken as the neutralization titre of the sample. The titres were presented with a geometric mean titre.

Sample size: Previously a temporal decline was reported starting from three weeks post-vaccination with ChAdOx1 nCoV-19 until 20 wks (1025 to 342 IU/µl) in infection-naïve vaccine recipients[10]. Based on this we expected the decline to be lesser in vaccinated groups due to past infection. Data from our previous study[3] on natural infection-induced antibodies showed a decline of 22 per cent over three months (unpublished data). Therefore, we assumed the waning of anti-RBD IgG levels by 30 per cent post six months after two doses of vaccination and calculated a sample size of 296 participants in each of the five study groups with a power of 80 per cent and a significance level of five per cent after factoring in multiple comparisons using Bonferroni method. All participants, enrolled in the study, were then reached out to be followed up to assess their infection status during the omicron-driven surge.

Statistical analysis: Appropriate measures such as median [interquartile range (IQR)] and number (percentage) were used to describe the baseline characteristics. The differences in ELISA titre in the same participant over two-time points were assessed using a paired t test after log 2 transformation. ELISA titre differences among various groups were analyzed through unpaired Student's t test post log 2 transformation. Chi-square test was used to assess the categorical variables. Further, to evaluate the determinants of antibody response, we constructed a multivariate regression model with log 2 transformed anti-RBD IgG antibody titre distributed as the dependent variable and type of vaccine, number of doses of vaccine (0, 1, 2 or 3), prior infection, age in years, sex, time of blood sampling for antibody test from the last dose of vaccine received or the episode of infection, whichever was the latest in weeks. We applied log transformation on the outcome variable to meet the assumptions of parametric linear regression models. For those whose antibody titre was reported as negative, we imputed the titre as '0'. For the neutralization assay results, those who demonstrated titres <20, were considered to have a titre of 10[9]. Neutralization above 40 was deemed to provide 100 per cent protection against 100 TCID50 virus added per well in 96 well plate format. Statistical analysis was done using R 4.1.1 (R packages, R Core Team 2021). A $P<0.05$ was considered as significant.

Results

Durability of humoral response pre-omicron surge: We included 1300 vaccinated participants (683 ChAdOx1 nCoV-19 and 617 BBV152) for evaluation of a decline in humoral immune response after at least six months post-vaccination and 93 unvaccinated individuals. The median duration following the second dose till inclusion was 238 days (IQR: 218, 256). Anti-RBD IgG positivity (>24 BAU/ml) was noted in 85 per cent of the uninfected vaccinated participants and 98 per cent of those with hybrid immunity ($P<0.001$). The titres of anti-RBD IgG were significantly higher in the hybrid immunity group [352 (IQR 131, 869) BAU/ml] compared to the uninfected vaccinated group [89 (IQR 26, 229); $P<0.001$]. Higher median anti-RBD IgG titres were observed in the uninfected ChAdOx1 nCoV-19 participant groups when compared to BBV152 uninfected participant groups [147 (44, 401)] *vs*. [57 (IQR 24, 143); $P<0.001$]. Higher median titres of anti-RBD IgG were noted in the participants vaccinated with ChAdOx1 nCoV-19 group who were aged 60 and above [183 (IQR 57, 494) BAU/ml] compared to those who were below 60 [106 (IQR 39, 254) BAU/ml; $P<0.001$] (Table I).

<u>Anti-nucleocapsid antibodies</u>: Whole inactivated virus vaccines like the BBV152 or a natural infection are known to induce anti-nucleocapsid antibodies. Anti-nucleocapsid IgG was observed in 34 per cent (76 of 221) of the hybrid immunity participant group, 26 per cent (158 of 612) in the BBV152 vaccinated uninfected group and 18.2 per cent in the ChAdOx1 nCoV-19 vaccinated uninfected group. Anti-nucleocapsid positive vaccinated participants had a significantly higher median titre of anti-RBD antibodies [197 (IQR 84, 592) BAU/ml] compared to those without any anti-nucleocapsid antibodies [88 (IQR 25, 241) BAU/ml; $P<0.001$].

<u>Influence of co-variables on anti-RBD antibody titres</u>: The multivariate regression model for the first sample time point yielded a constant of 66.23 BAU/ml (47.84-91.69) with clinically small yet significant contributing factors such as age, gender, history of previous infection and type of vaccine administered ($P<0.01$).

Table I. Anti-receptor-binding domain immunoglobulin G titres (BAU/ml) among different groups of participants after six months of vaccination/infection before omicron-driven surge

Study groups	No. of study participants	Age (yr)	Female, n (%)	Comorbidity, n (%)	Proportion of ELISA positives, n (%)	Proportion of ELISA negatives, n (%)	ELISA titre (BAU/ml), median (IQR)
Vaccination alone	1079	62 (50-68)	447 (41.4)	235 (21.8)	915 (84.8)	164 (15.1)	89 (26-229)
Group 1: BBV152, <60 yr, uninfected	259	48 (40-54)	105 (40.5)	33 (12.7)	188 (72.5)	71 (27.4)	58 (25-159)
Group 2: BBV152, ≥60 yr, uninfected	353	66 (63-70)	160 (45.3)	101 (28.6)	266 (75.3)	87 (24.6)	57 (0-136)
Group 3: ChAdOx1 nCoV-19, <60 yr, uninfected	159	41 (31-52)	56 (35.2)	12 (7.5)	142 (89.3)	17 (8.8)	106 (39-254)
Group 4: ChAdOx1 nCoV-19, ≥60 yr, uninfected	308	67 (63-71)	126 (40.9)	89 (28.8)	240 (77.9)	68 (22)	183 (57-494)
Group 5: Vaccination and infection	221	57 (42-66)	65 (29.4)	49 (22.1)	218 (98.6)	3 (1.4)	352 (131-869)
Unvaccinated controls							
Total	93	34 (25-48)	30 (32.2)	0	70 (75.2)	23 (24.7)	65 (29-251)
Unvaccinated plus past infection	82	34 (25-47)	27 (32.9)	0	76 (92.6)	6 (7.3)	85 (37-270)

IQR, interquartile range; BBV, blood-borne virus

Humoral response post-omicron-driven surge: To evaluate change in humoral immune response post-omicron driven surge, 516 (349 males and 167 females) vaccinated participants (304 ChAdOx1 nCoV-19 and 215 BBV152) were included. The participants' median age was 61 yr (IQR: 48, 68). The median interval between the second dose of vaccine to enrolment was 238 (IQR 217, 267) days. A total of 142 participants had taken a homologous booster dose (106 ChAdOx1 nCoV-19, 36 BBV152) before their second sampling time point. Those who received the ChADOx1 nCoV-19 booster were enrolled at a median of 60 (42, 70) days and those who received the BBV152 booster were enrolled at a median of 64 (41, 71) days. Of the 516 participants, 40 (7.8%) had confirmed SARS-CoV-2 infection during the omicron surge. No hospitalization was required for any of these participants. In addition, 32 (6.2%) participants had ILI but had not been tested.

The baseline anti-RBD IgG median antibody for those who did not get the booster but tested positive during the omicron surge was found to be significantly lower [57 (IQR 27, 115)] compared to those who did not test positive [143, (IQR 43, 331)] (P<0.001). Post-omicron surge, anti-RBD IgG antibodies were undetectable only in 2.5 per cent of participants (13 of 516; 5 participants who received the ChAdOx1 nCoV-19 vaccine and 8 who received the BBV152 vaccine) compared to 13 per cent previously. Overall, the median serum anti-RBD IgG antibody titre increased significantly from 131 (IQR 39, 322) BAU/ml to 594 (IQR: 252, 1230) BAU/ml (P<0.001). The antibody titres increased in all the three sub-group of participants, *i.e.* those with hybrid immunity to 816 (IQR 383, 2001) BAU/ml; those vaccinated with just two doses to 528 (IQR 222, 1116); and those who received the booster to 835 (IQR (303, 1637). The difference between the three sub-groups was not statistically significant (P>0.05).

Antibody titres in the non-infected non-boosted group: Among 316 participants who neither received the booster nor tested positive or had any symptoms of COVID-19, 235 (74%) showed a rise in anti-RBD IgG titres (BAU/ml) and 67.3 per cent (128 of 190) seroconverted from negative to positive status post-omicron surge. Of these 316 participants, 247 (78.2 %) were also positive for anti-omicron RBD antibodies. In the same group (n=316), 222 (70.3%) were positive for anti-nucleocapsid antibodies, of whom 145 had seroconverted from negative to positive status (64.7%). All these results suggest substantial asymptomatic infection. The summary of antibody titre and their respective groups are mentioned in Figure 1 and Table II.

Persistence and decline in anti-RBD antibody titres over the past one year after the omicron surge: We could collect a third sample from 339 participants during January-March 2023; of these 182 had received a booster. The antibody levels declined but remained elevated (>12 times the cut-off for positivity) regardless of the booster dose. The per cent decline in the anti-RBD IgG antibodies was 34 per cent (<60 yr) and 51 per cent (≥60 yr) in those who received a booster and 28 per cent (<60 yr) and 33 per cent (≥60 yr) who did not receive a booster over a mean time period of 9.9±0.67 months (Table III).

Influence of co-variables on antibody titres: The regression model for the second sample point yielded an increase in the constant value to 142.22 BAU/ml with similar clinically small and significant contributing factors, as mentioned previously. The details are summarized in Supplementary Table I (available online).

Neutralizing antibodies: To test whether the anti-RBD antibodies were neutralizing or not, plasma from 18 paired participants was subjected to live virus neutralization assays against four major variants [ancestral, delta, omicron (B.1.1.529) and omicron BA.5]. Of these 18, 12, four, two and one participant(s) were able to neutralize ancestral, delta, omicron and omicron BA.5 variants, respectively, before the omicron-driven surge, which increased to 18, 17, 16 and 14 post-omicron surge, respectively (Fig. 2) suggesting that the induced antibodies had the neutralizing ability against the newer variants as well. The BAU/ml titres showed moderate-to-good correlation (0.62-0.82) with the neutralization titres for omicron sublineages as well, which was statistically significant (*P*<0.05). Additional information and summary for the various subgroups are mentioned in Supplementary Table II and Supplementary Figures 1 and 2.

Discussion

This study demonstrates that humoral response persisted after a median of eight months following vaccination alone and an immune boost phenomenon based on a significant rise in anti-RBD IgG antibody titres following the omicron surge, which was durable over 10 months. This was seen even in participants who neither tested positive or were symptomatic for infection nor received booster doses suggesting asymptomatic infection. These participants were found to be positive for anti-omicron RBD IgG antibodies which effectively neutralized not only the omicron but also other variants like the delta as well. However, lower neutralization titres were observed, which were directed against the omicron variant compared to the previous ones.

The emergence of omicron variant in late November 2021 led to a major surge of infections worldwide, thereby questioning the protection offered by both post-vaccination and prior COVID-19 immunity. A booster dose of vaccine, be it homologous or heterologous, was shown to be effective, at least in the short term[11-13]. Several studies have highlighted the more infectious but milder pathogenic nature of the omicron variant leading to widespread mildly symptomatic or asymptomatic infections[5,14-16]. Preliminary studies demonstrated an overall immune-boosting effect caused by the omicron infection.

An important observation in the present study was that the neutralizing antibodies were most effective against the ancestral virus after the omicron infection. Omicron with significant mutations in the spike protein would constitute a heterologous antigen exposure, and thus, this observation suggests the phenomenon of immune imprinting, as has also been shown by a few other studies as well[17,18]. Kaku *et al*[19] highlighted that post-vaccination breakthrough infections seem to be majorly factored by the recall of vaccine-induced B memory cells and their capacity to recognize the omicron variant. Furthermore, the study also highlighted the fact that antibodies expressed by these B memory cells after infection by the omicron variant had higher affinity to the ancestral variant of the SARS-CoV-2 virus, indicating that these were vaccine-induced cells. These results are supported by the higher neutralization titres observed against the ancestral virus in the present study as well. Similar sub-optimal neutralization titres against the omicron variant were also observed by other studies, including one authored by some of co-authors of this manuscript[9,20].

In the present study, there was nearly a 4-fold increase in antibody titres seen in the participants following the omicron surge, implying that a sizable population had been exposed to the omicron variant with the background complete vaccination rate of >80 per cent. In addition, of the participants, who neither received the booster nor tested positive or had any symptoms, 74 per cent showed a rise in

Table II. Antibody levels in various sub-groups following omicron surge

Participant groups	Median anti-RBD IgG BAU/ml (IQR)	
	1st time point (November-December 2021)	2nd time point (March-May 2022)
All paired samples (n=516)	131 (39-322)	594*** (252-1230)
Participants who received the booster dose		
Received booster dose (n=142)	161 (25-421)	848*** (289-1684)
Those who received booster dose, not tested positive nor symptomatic (n=128)	176 (32-427)	835*** (309-1684)
ChAdOx1 booster overall (n=106)	230 (41-541)	1011*** (530-2190)
ChAdOx1 booster, not tested positive nor symptomatic (n=96)	253 (80-555)	983*** (532-2161)
BBV152 booster overall (n=36)	42 (0-81)	251*** (119-886)
BBV 152 booster, not tested positive nor symptomatic (n=32)	37 (0-83)	250*** (114-906)
Participants who did not receive the booster dose		
Total (n=374)	127 (42-287)	554*** (229-1120)
Tested positive for SARS-CoV-2 (n=37)	57 (27-115)	648*** (338-1138)
Symptomatic but not tested (n=21)	66 (30-193)	549*** (180-1076)
Not tested positive nor symptomatic (n=316)	143 (47-331)	528*** (222-1116)

P ***<0.001. RBD IgG, receptor-binding domain immunoglobulin G

Table III. Anti-receptor-binding domain immunoglobulin G antibody levels in various sub-groups one year after the omicron surge

Participants	Median BAU/ml (IQR)		Per cent decline	Mean interval between 2 samples (days)
	Post-omicron 1st sample (March-May 2022)	Post-omicron 2nd sample (January-March 2023)		
Total participants (n=339)	573 (239-1185)	339 (197-596)	41	299±21
<60 yr of age, with booster (n=64)	477 (231-1009)	314 (207-487)	34	306±21
<60 yr of age, no booster (n=92)	418 (163-917)	302 (134-437)	28	299±22
≥60 yr of age, with booster (n=118)	796 (288-2047)	384 (233-715)	51	299±20
≥60 yr of age, no booster (n=65)	580 (236-1345)	387 (168-927)	33	295±19

antibody titres. Our data are important as most regions globally have been afflicted by at least three waves of differing SARS-CoV-2 variants, omicron being the most recent. The boosting of neutralizing antibodies, including those against the recent BA.5 variant, seems to demonstrate the protective ability of the omicron natural infection. One could infer that the natural infections may be equated to at least one vaccine booster, bolstering the protection conferred by a 2-dose vaccination regimen. There is also a possibility that other protective mechanisms, such as cellular immune responses stimulated by natural infection, are superior to those induced by vaccines alone. It has been documented that clinically, naturally infected people demonstrate better protection than those who received only vaccination[14,21-23]. Further, previous studies demonstrated that antibody titres in those with hybrid immunity are higher than those with either natural infection or vaccination[7,23,24]. Based on the results, there was no overt contribution of the type of vaccine, pre-existing antibody titres and past infection history for risk reduction to contracting asymptomatic omicron infections, and the variability in the humoral immune response observed in the study could be attributed to individual host response.

The inclusion of a strategically timed paired sample (just before and after the emergence of the omicron variant), enrolment of different age groups (people aged at 60 or above are considered to be a vulnerable group), anti-nucleocapsid antibody profiling and anti-RBD

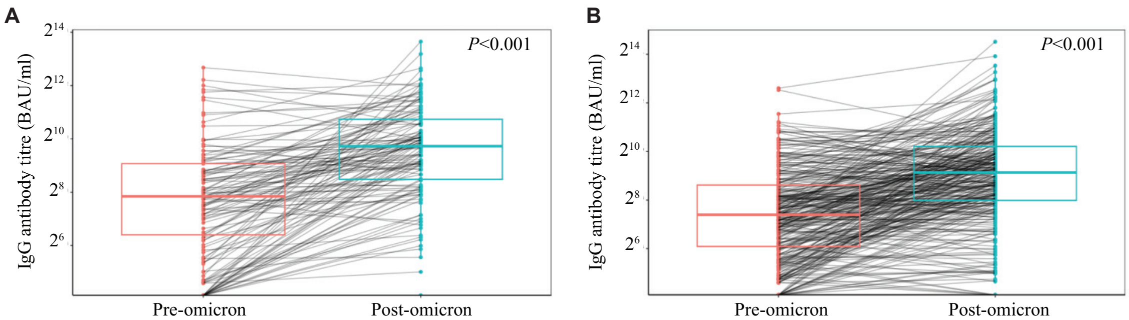

Fig. 1. Plots highlighting the change in the BAU levels from pre - to post - omicron surge in different sub-sets of the study population (**A**) Participants who received the booster dose and did not test positive for SARS-CoV-2 with no symptoms reported; (**B**) Participants who did not receive the booster dose and did not test positive for SARS-CoV-2 with no symptoms reported. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

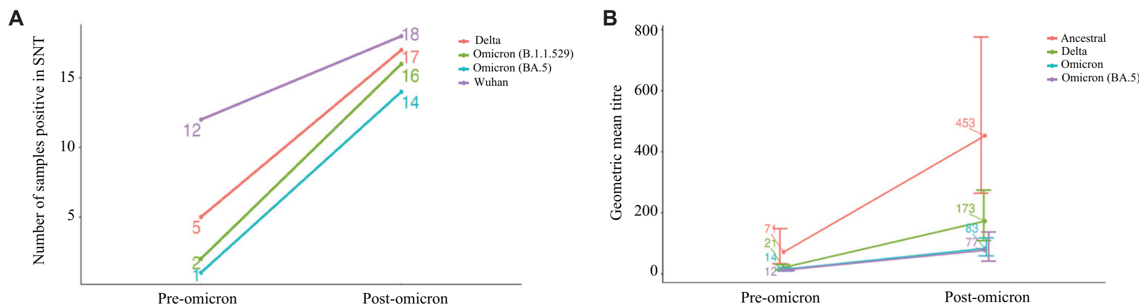

Fig. 2. (**A**) Rising trend in neutralization positivity (≥1:40) and (**B**) geometric mean titres from pre-omicron to post-omicron against different variants in paired plasma samples (n=18).

(Omicron) antibody positivity to account for silent infections are the major strengths of the present study. We chose six months post-vaccination to understand the durability of the immune response beyond six months, by which time the humoral response was likely to wane. This was based on a longitudinal cohort study where it was observed that antibody titre declined after six-months[3].

There were certain limitations in this study. One is that we do not know how long the immunity induced by omicron infection would last. To overcome this, the antibody levels were measured in a subset of participants and showed that there was an average of 41 per cent decline in antibodies over the past one year, but still, the antibody levels were adequate even in those who had not received any booster. Second, the sample size fell short of the estimated sample size in some groups. This was because we could not include participants after the emergence of the omicron variant in January 2022 to obtain a chronological baseline sample from the adequate number of participants before the emergence of the omicron variant.

Our findings are important in the face of challenges in implementing universal booster rounds of immunization, particularly in those settings where the availability and acceptability of vaccines are suboptimal. Moreover, how frequently one needs to boost is another question. A targeted booster vaccination to the most vulnerable and those who do not have protective antibodies seems a prudent way forward. Another pertinent question that would require further insight is the need to tweak the existing vaccine for VoCs (current and emerging). It has been shown that while existing vaccine boosters seem to augment an individual's existing immunity by engaging the vaccine-imprinted B memory cells, enhancement of immunity against variants would require the engagement of naïve B cells, which may not be straightforward based on the 'original antigenic sin' phenomenon documented very thoroughly from studies on influenza vaccinations[25,26]. The results of this study also clinically validate this phenomenon, as even those with natural infection possessed higher antibodies against the ancestral strain rather than the disease-causing strain itself.

In summary, anti-RBD IgG antibodies were detected in 85 per cent of the participants after a median time of eight months after the second vaccine dose.

Omicron infection resulted in a substantial proportion of asymptomatic infection in the first four months in our study population and the infection boosted the vaccine-induced humoral immune response, which although declined, remained durable over 10 months.

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Conflicts of Interest**:** None.

References

1. Li D, Luan N, Li J, Zhao H, Zhang Y, Long R, *et al.* Waning antibodies from inactivated SARS-CoV-2 vaccination offer protection against infection without antibody-enhanced immunopathology in rhesus macaque pneumonia models. *Emerg Microbes Infect* 2021; *10* : 2194-8.
2. Seow J, Graham C, Merrick B, Acors S, Pickering S, Steel KJA, *et al.* Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbiol* 2020; *5* : 1598-607.
3. Thiruvengadam R, Chattopadhyay S, Mehdi F, Desiraju BK, Chaudhuri S, Singh S, *et al.* Longitudinal serology of SARS-CoV-2-infected individuals in India: A prospective cohort study. *Am J Trop Med Hyg* 2021; *105* : 66-72.
4. Reynolds CJ, Gibbons JM, Pade C, Lin KM, Sandoval DM, Pieper F, *et al.* Heterologous infection and vaccination shapes immunity against SARS-CoV-2 variants. *Science* 2022; *375* : 183-92.
5. Garrett N, Tapley A, Andriesen J, Seocharan I, Fisher LH, Bunts L, *et al.* High rate of asymptomatic carriage associated with variant strain omicron. *medRxiv* 2022. Doi: 10.1101/2021.12.20.21268130.
6. Barouch DH, Stephenson KE, Sadoff J, Yu J, Chang A, Gebre M, *et al.* Durable humoral and cellular immune responses 8 months after Ad26.COV2.S Vaccination. *N Engl J Med* 2021; *385* : 951-3.
7. Havervall S, Jernbom Falk A, Klingström J, Ng H, Greilert-Norin N, Gabrielsson L, *et al.* SARS-CoV-2 induces a durable and antigen specific humoral immunity after asymptomatic to mild COVID-19 infection. *PLoS One* 2022; *17* : e0262169.
8. Mehdi F, Chattopadhyay S, Thiruvengadam R, Yadav S, Kumar M, Sinha SK, *et al.* Development of a fast SARS-CoV-2 IgG ELISA, based on receptor-binding domain, and its comparative evaluation using temporally segregated samples from RT-PCR positive individuals. *Front Microbiol* 2020; *11* : 618097.
9. Medigeshi GR, Batra G, Murugesan DR, Thiruvengadam R, Chattopadhyay S, Das B, *et al.* Sub-optimal neutralisation of omicron (B.1.1.529) variant by antibodies induced by vaccine alone or SARS-CoV-2 Infection plus vaccine (hybrid immunity) post 6-months. *EBioMedicine* 2022; *78* : 103938.
10. Aldridge RW, Yavlinsky A, Nguyen V, Eyre MT, Shrotri M, Navaratnam AMD, *et al.* Waning of SARS-CoV-2 antibodies targeting the Spike protein in individuals post second dose of ChAdOx1 and BNT162b2 COVID-19 vaccines and risk of breakthrough infections: Analysis of the virus watch community cohort. *medRxiv* 2021. Doi: *http://medrxiv.org/lookup/doi/10.1101/2021.11.05.21265968*.
11. Abu-Raddad LJ, Chemaitelly H, Ayoub HH, AlMukdad S, Yassine HM, Al-Khatib HA, *et al.* Effect of mRNA vaccine boosters against SARS-CoV-2 omicron infection in Qatar. *N Engl J Med* 2022; *386* : 1804-16.
12. Accorsi EK, Britton A, Shang N, Fleming-Dutra KE, Link-Gelles R, Smith ZR, *et al.* Effectiveness of homologous and heterologous COVID-19 boosters against omicron. *N Engl J Med* 2022; *386* : 2433-5.
13. Brockman MA, Mwimanzi F, Lapointe HR, Sang Y, Agafitei O, Cheung PK, *et al.* Reduced magnitude and durability of humoral immune responses to COVID-19 mRNA vaccines among older adults. *J Infect Dis* 2022; *225* : 1129-40.
14. Sheikh A, Kerr S, Woolhouse M, McMenamin J, Robertson C, EAVE II Collaborators. Severity of omicron variant of concern and effectiveness of vaccine boosters against symptomatic disease in Scotland (EAVE II): A national cohort study with nested test-negative design. *Lancet Infect Dis* 2022; *22* : 959-66.
15. Modes ME, Directo MP, Melgar M, Johnson LR, Yang H, Chaudhary P, *et al.* Clinical characteristics and outcomes among adults hospitalized with laboratory-confirmed SARS-CoV-2 infection during periods of B.1.617.2 (Delta) and B.1.1.529 (Omicron) variant predominance – One hospital, California, July 15-September 23, 2021, and December 21, 2021-January 27, 2022. *MMWR Morb Mortal Wkly Rep* 2022; *71* : 217-23.
16. Maslo C, Friedland R, Toubkin M, Laubscher A, Akaloo T, Kama B. Characteristics and outcomes of hospitalized patients in South Africa during the COVID-19 omicron wave compared with previous waves. *JAMA* 2022; *327* : 583-4.
17. Reynolds CJ, Pade C, Gibbons JM, Otter AD, Lin KM, Muñoz Sandoval D, *et al.* Immune boosting by B.1.1.529 (Omicron) depends on previous SARS-CoV-2 exposure. *Science* 2022; *377* : eabq1841.
18. Wheatley AK, Fox A, Tan HX, Juno JA, Davenport MP, Subbarao K, *et al.* Immune imprinting and SARS-CoV-2 vaccine design. *Trends Immunol* 2021; *42* : 956-9.
19. Kaku CI, Bergeron AJ, Ahlm C, Normark J, Sakharkar M, Forsell MNE, *et al.* Recall of preexisting cross-reactive B cell memory after omicron BA.1 breakthrough infection. *Sci Immunol* 2022; *7* : eabq3511.
20. Chauvin C, Levillayer L, Roumier M, Nielly H, Roth C, Karnam A, *et al.* Tocilizumab-treated convalescent COVID-19 patients retain the cross-neutralization potential against SARS-CoV-2 variants. *iScience* 2023; *26* : 106124.
21. Lea CS, Simeonsson K, Kipp AM, McNeill C, Wilcox L,

Irish W, *et al.* Waning of SARS-CoV-2 seropositivity among healthy young adults over seven months. *Vaccines (Basel)* 2022; *10* : 1532.

22. Brlić PK, Pavletić M, Lerga M, Krstanović F, Matešić MP, Miklić K, *et al.* SARS-CoV-2 spike and nucleocapsid antibody response in vaccinated Croatian healthcare workers and infected hospitalized patients: A single center cohort study. *Viruses* 2022; *14* : 1966.
23. Altarawneh HN, Chemaitelly H, Ayoub HH, Tang P, Hasan MR, Yassine HM, *et al.* Effects of previous infection and vaccination on symptomatic omicron infections. *N Engl J Med* 2022; *387* : 21-34.
24. Blom K, Marking U, Havervall S, Norin NG, Gordon M, García M, *et al.* Immune responses after omicron infection in triple-vaccinated health-care workers with and without previous SARS-CoV-2 infection. *Lancet Infect Dis* 2022; *22* : 943-5.
25. van Zelm MC. Immune memory to SARS-CoV-2 Omicron BA.1 breakthrough infections: To change the vaccine or not? *Sci Immunol* 2022; *7* : eabq5901.
26. Monto AS, Malosh RE, Petrie JG, Martin ET. The doctrine of original antigenic sin: Separating good from evil. *J Infect Dis* 2017; *215* : 1782-8.

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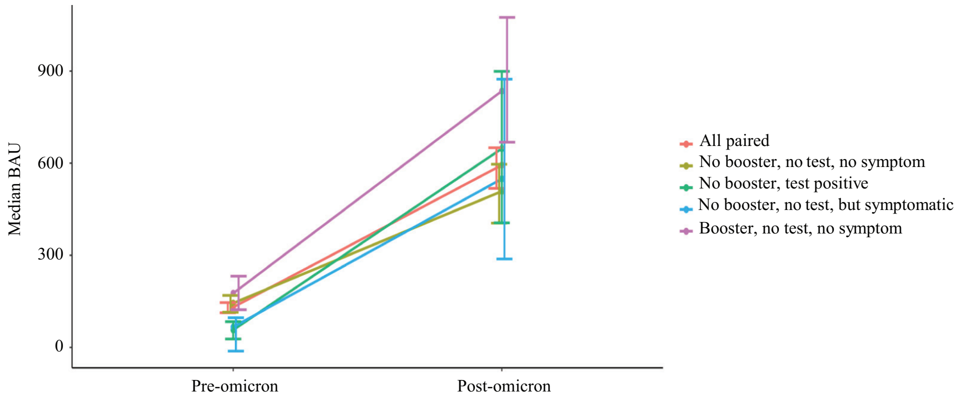
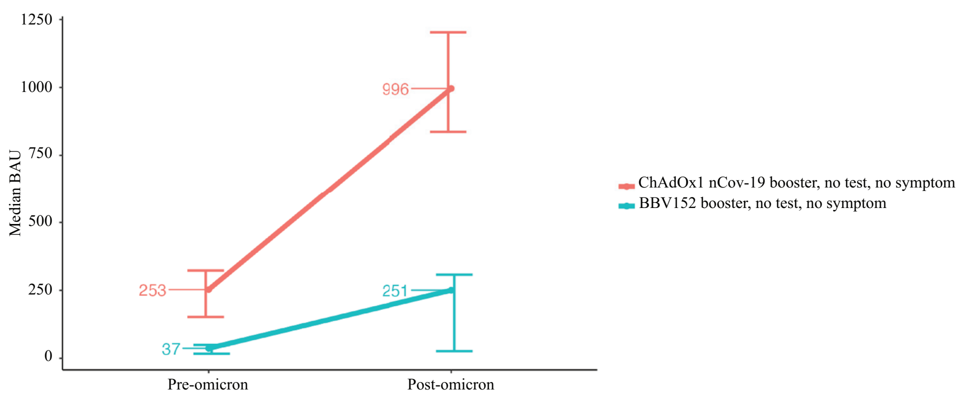


Supplementary Fig. 1. Graphical depiction of the change in antibody titres from pre- to post-omicron surge in the paired samples with their respective sub-groups.



Supplementary Fig. 2. Graphical depiction of the change in antibody titres from pre- to post-omicron surge in the paired samples who received a homologous booster dose.

Supplementary Table I. Multivariate regression model for assessing determinants of humoral immune response (antibodies) before and after the emergence of the omicron variant

Pre-omicron sample	Beta coefficient	2.5%	97.5%	SE	*P*	Post-omicron sample	Beta coefficient	2.5%	97.5%	SE	*P*
Intercept	66.2373	47.8475	91.695	0.1659	0	Intercept	142.2269	52.2962	386.8064	0.5105	0
Age	1.0067	1.0026	1.0108	0.0021	0.0013	Age	1.0156	1.0079	1.0234	0.0039	1E-04
Gender (male)	1.2032	1.057	1.3696	0.0661	0.0052	Gender (male)	1.1852	0.9386	1.4967	0.119	0.154
Type of vaccine (ChAdOx1 nCoV-19)	1.7768	1.5642	2.0183	0.065	0	Booster dose (yes)	1.1033	0.8528	1.4275	0.1314	0.4548
Previous history of COVID-19	2.2426	1.8694	2.6902	0.0929	0	Previous history of COVID-19	1.2394	0.8237	1.8649	0.2085	0.3037
Duration from infection/2nd dose to sample collection (weeks)	1.0014	0.9956	1.0073	0.003	0.6339	Type of vaccine (ChAdOx1 nCoV-19)	1.5789	1.2565	1.9841	0.1166	1E-04
						Duration from infection/2nd dose/booster to sample collection (weeks)	1.0022	0.9864	1.0182	0.0081	0.7879

SE, standard error

Supplementary Table II. Additional sub-group results comparing the pre- and post-omicron surge paired samples with respect to anti-N and anti-omicron receptor-binding domain seroconversion and per cent rise in antibody titrs

Participant groups	Anti-N seroconversion (negative-positive)	Anti-omicron RBD seroconversion (negative-positive)	Rise in antibody titre (BAU/ml) per cent samples
All paired (n=516)	61.4% (234 out of 381)	72.8% (236 out of 324)	80% (412 out of 516)
No booster, no test, no symptom (n=316)	64.7% (145 out of 224)	67.3% (128 out of 190)	74% (235 out of 316)
No booster, test positive (n=37)	63.3% (19 out of 30)	90% (27 out of 30)	92% (34 out of 37)
No booster, no test, but symptomatic (n=21)	76.4% (13 out of 17)	75% (12 out of 16)	90% (19 out of 21)
Received homologous booster, no test, no symptom (n=128)	51.5% (51 out of 99)	80.5% (62 out of 77)	86% (110 out of 128)
Received ChAdOx1 nCoV-19 booster, no test, no symptom (n=96)	53% (40 out of 75)	89% (42 out of 47)	84%* (81 out of 96)
BBV152 booster, no test, no symptom (n=32)	46% (11 out of 24)	67% (20 out of 30)	90% (29 out of 32)
No booster, no test, no symptom (history ChAdOx1 nCoV-19) (n=158)	63% (78 out of 124)	67% (56 out of 84)	72% (113 out of 158)
No booster, no test, no symptom (history BBV152) (n=158)	67% (67 out of 100)	68% (72 out of 106)	77% (122 out of 158)
No booster, no test, no symptom (history BBV152 uninfected) (n=141)	67% (62 out of 93)	68% (68 out of 100)	79% (112 out of 141)
No booster, no test, no symptom (history BBV152 infected) (n=17)	71% (5 out of 7)	67% (4 out of 6)	59% (10 out of 17)

BBV, blood-borne virus; RBD, receptor binding domain