# 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

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## Summary

**Background** Rapid spread of the omicron SARS-CoV-2 variant despite extensive vaccination suggests immune escape. The neutralising ability of different vaccines alone or with natural SARS-CoV-2 infection against omicron is not well-known.

**Methods** In this cross-sectional study, we tested the ability of vaccine and natural infection induced antibodies to neutralise omicron variant in a live virus neutralisation assay in four groups of individuals: (i) ChAdOx1 nCoV-19 vaccination, (ii) ChAdOx1 nCoV-19 vaccination plus prior SARS-CoV-2 infection, (iii) vaccination with inactivated virus vaccine (BBV152), and (iv) BBV152 vaccination plus prior SARS-CoV-2 infection. Primary outcome was fold-change in virus neutralisation titre against omicron compared with ancestral virus.

**Findings** We included 80 subjects. The geometric mean titre (GMT) of the 50% focus reduction neutralisation test (FRNT50) was 380·4 (95% CI: 221·1, 654·7) against the ancestral virus with BBV152 vaccination and 379·3 (95% CI: 185·6, 775·2) with ChAdOx1 nCov-19 vaccination alone. GMT for vaccination plus infection groups were 806·1 (95% CI: 478·5, 1357·8) and 1526·2 (95% CI: 853·2, 2730·0), respectively. Against omicron variant, only 5 out of 20 in both BBV152 and ChAdOx1 nCoV-19 vaccine only groups, 6 out of 20 in BBV152 plus prior SARS-CoV-2 infection group, and 9 out of 20 in ChAdOx1 nCoV-19 plus prior SARS-CoV-2 infection group exhibited neutralisation titres above the lower limit of quantification (1:20) suggesting better neutralisation with prior infection. A reduction of 26·6 and 25·7 fold in FRNT50 titres against Omicron compared to ancestral SARS-CoV-2 strain was observed for individuals without prior SARS-CoV-2 infection vaccinated with BBV152 and ChAdOx1 nCoV-19, respectively. The corresponding reduction was 57·1 and 58·1 fold, respectively, for vaccinated individuals with prior infection. The 50% neutralisation titre against omicron demonstrated moderate correlation with serum anti-RBD IgG levels [Spearman r: 0·58 (0·41, 0·71)].

**Interpretation** Significant reduction in the neutralising ability of both vaccine-induced and vaccine plus infection-induced antibodies was observed for omicron variant which might explain immune escape.

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**Keywords:** SARS-CoV-2; Omicron; Live-virus neutralisation; Covaxin; ChAdOx1 nCoV-19; Covishield; Hybrid immunity; BBV152

## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected >280 million people and claimed >5·4 million lives worldwide.[1] The most

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## Research in context

### *Evidence before this study*

Omicron variant of SARS-CoV-2 is fast becoming the dominant circulating strain world-wide. We did a literature search on PubMed between 06 January 2021 to 06 January 2022 using the terms "Omicron", "SARS-CoV-2", and "neutralisation" and found 21 results for live-virus neutralisation against omicron by vaccine/natural infection induced antibodies. We identified two published and five preprint articles relevant to our study. Preliminary reports suggest that omicron variant is significantly less susceptible to *in-vitro* neutralisation by antibodies among recipients of mRNA vaccines (BNT162b2 and mRNA-1273) and adenovirus vectored vaccine (ChAdOx1 nCoV-19), and no virus neutralisation was observed in subjects who received an inactivated virus vaccine (Coronavac). Data regarding immune escape among those with natural SARS-CoV-2 infection and vaccination are emerging from different regions of the world.

### *Added value of this study*

We report here that the proportion of neutralisers (those who demonstrated a FRNT50 titre >1:20) was significantly reduced against the omicron variant as compared to the ancestral and delta variant. The FRNT50 titres among the vaccinated individuals without a history of previous natural infection was significantly reduced against the omicron variant as compared with ancestral and delta variants. The titres among those with previous SARS-CoV-2 infection also followed a similar pattern, but the neutralising ability was better in them than in those who did not have previous infection.

### *Implications of all the available evidence*

Omicron variant of SARS-CoV-2 is capable of escaping immunity provided by currently available vaccines and even prior natural infection due to significant mutations in its spike protein. The decrease in neutralising ability against the Omicron variant might be alarming, but the real-world impact of the reduced neutralisation on major public health indices like hospitalisation rates and mortality rates have to be interpreted along with the other factors such as inherent pathogenicity of the variant, immunization uptakes and seroprevalence from natural infection in different geographical regions and the expected role of cellular immune responses to the variant. Our data may guide policy on booster vaccination to deal with an impending public health emergency as a result of surge in omicron cases worldwide.

effective strategy to contain the pandemic is vaccination. However, the emergence of variants of concern (VoC) due to mutations in the virus has led to reduced vaccine effectiveness. After the massive surge due to the delta (B·1·617·2) VoC, the emergence and rapid spread of Omicron (B·1·1·529) VoC has further caused panic around the world. The virus enters the human respiratory epithelial cells by binding the receptor-binding domain (RBD) of its spike (S) protein with the human angiotensin converting enzyme-2 (ACE2) receptors. The predominant mechanism of protection afforded by the vaccines is through generation of neutralising antibodies against the RBD that help block viral entry into host cells. The VoC harbouring mutations in the RBD may lead to a decreased neutralising ability of the vaccine-derived antibodies as has been shown against VoC such as the alpha, beta, gamma and delta variants.[2,3] Omicron has 30 amino acid substitutions, 3 deletions, and 1 insertion in its S protein (15 substitutions in RBD alone) and thus may escape vaccine induced immunity.[4]

Preliminary data derived from two recent studies from South Africa and the United Kingdom have shown markedly reduced ability of the serum from vaccinated people to neutralize omicron in live virus neutralisation assays. These data pertain to a small sample size of people vaccinated with either mRNA vaccine (BNT162b2; Pfizer-BioNTech) or adenovirus vectored vaccine (ChAdOx1 nCoV-19; Oxford-AstraZeneca).[5,6] The ability of the sera to neutralise omicron from people vaccinated with inactivated virus vaccine or those with hybrid immunity following natural SARS-CoV-2 infection plus vaccination is still unclear. This assumes significance because of the high prevalence of asymptomatic infection in many countries as detected by serosurveys.[7,8] A hybrid immunity is likely to provide better protection and thus help guide policy about vaccinating or boosting naturally infected people. India's vaccination program is driven by ChAdOx1 nCoV-19 vaccine (Covishield, Serum Institute of India, Pune, India) and an inactivated whole virus vaccine (BBV152; Covaxin, Bharat Biotech Immunologicals Limited, Hyderabad, India). India witnessed a massive surge due to the delta (B·1·617·2) VoC during April-May 2021.[9] Serosurvey following this surge showed a high prevalence (69·2 %) of serum IgG antibodies suggesting widespread infection albeit mostly asymptomatic.[7] In the present study, we tested the ability of antibodies to neutralise the omicron variant among people with vaccination alone or vaccination plus natural infection induced immunity.

## Methods

Study Participants: The participants were derived from an ongoing Department of Biotechnology (DBT) Consortium for COVID-19 Research cohort developed by Translational Health Science and Technology Institute, Faridabad, India in collaboration with hospitals in Delhi National Capital region, particularly, Employee State Insurance Corporation Medical College and Hospital, Faridabad. We randomly sampled ChAdOx1 nCoV-19 and BBV152 vaccine recipients from the cohort and

categorised them in the following four groups: (i) vaccination with two doses of ChAdOx1 nCoV-19 alone, (ii) vaccination with two doses of ChAdOx1 nCoV-19 along with an RT-PCR confirmed natural SARS-CoV-2 infection during the delta variant driven surge, (iii) vaccination with two doses of BBV152 alone, (iv) vaccination with two doses of BBV152 along with an RT-PCR confirmed natural SARS-CoV-2 infection during the delta variant driven surge. Participants with vaccination and prior SARS-CoV-2 infection were considered to have hybrid immunity. Written informed consent was obtained before enrolling the participants.

### Data collection

Specific details were obtained from the DBT Consortium cohort database and during interviews by trained research staff on the vaccination status of the participants including the name of the vaccine, number of doses, date and place of administration, and clinical manifestations of COVID-19 among those with natural infection in the past. We defined complete vaccination when the participant had completed at least 14 days after the second dose of the vaccine. The dates of immunization were documented from the certificate of immunization issued by the Government of India. Their blood samples were collected and processed for plasma and peripheral blood mononuclear cells.

*Ethics:* The studies were approved by the Institute Ethics Committees of the partnering institutions: 'Institutional Ethics Committee - Biomedical and Health Research', Translational Health Science and Technology Institute [THS 1.8.1/ (132) dated 08 Dec 2021] and 'Institutional Ethics Committee', ESIC Medical College & Hospital, Faridabad [134 X/11/13/2021-IEC/49 dated 07 Dec 2021].

### Anti-SARS-CoV-2 IgG antibody detection

Anti-RBD IgG concentrations in the serum samples were determined by enzyme-linked immunosorbent assay (ELISA) as described earlier with minor modifications required for quantitative output.[10] Internal positive control (pooled human serum from SARS-CoV-2 recovered individuals) was calibrated against the first WHO international standard for anti-SARS-CoV-2 immunoglobulin (code 20/136) as per guidelines (WHO manual for the establishment of national and other secondary standards for antibodies against infectious agents focusing on SARS-CoV-2; Draft Version 12/10/2021) leading to a calculated concentration of 718.3 binding antibody units/ml (BAU/ml) for the positive control. 96-well maxisorp polystyrene plates were coated with RBD antigen, blocked, and dry stabilized. Test sera and positive and negative controls were threefold diluted starting from 1:50 to 1:12150, and 100 µl was added to the assay wells. After the incubation, wells were washed, and the bound antibodies were detected using HRP-labelled anti-human IgG ($\gamma$-chain specific). Anti-RBD IgG concentrations (BAU/ml) in the test samples were calculated for each sample dilution by interpolation of Optical Density (OD) values on the 4-parameteric logistic (4-PL) standard curve from internal positive control using GraphPad Prism 9.3.1 software. The four parameters in 4-PL curve are the maximum value, minimum value, inflection point and the slope of the curve. Anti-RBD IgG concentrations above the assay cut-off and corresponding to the linear part of the curve were considered, and values in BAU/ml were assigned to each test sample. The lower limit of quantitation for the assay was 24 BAU/ml. Additional dilutions beyond 1:12150 were done for samples where OD values were beyond the calibration curve's linear part.

### Qualitative anti-nucleocapsid IgG ELISA

We tested for IgG antibodies against SARS-CoV-2 nucleocapsid antigen by qualitative ELISA as a measure of past infection with SARS-CoV-2. The assay procedure was similar to the process described for the RBD IgG ELISA[10] with the following modifications: (1) instead of RBD, *E.coli* expressed SARS-CoV-2 nucleocapsid antigen was coated on polystyrene wells (50 ng/50 µl); (2) sample dilution of 1:200 was used. Samples with a signal/cut-off ratio above 1 were considered positive for anti-nucleocapsid antibodies. The RBD and N protein used in ELISA were from ancestral virus.

### Live virus focus reduction neutralisation assay

Virus neutralisation assay was performed as described previously with minor changes in the incubation periods.[11] Briefly, plasma samples from the study participants were serially diluted from 1:20 to 1:640 and virus neutralization was tested in Vero E6 cells (European collection of authenticated cell cultures, Cat.no. 85020206). Cells were incubated for 24 h for ancestral (B·1) and Delta (B·1·617·2) variants and for 32 h for the omicron variant.[12] All virus stocks used in this study were propagated in Calu-3 cells (American Type Culture Collection, ATCC-HTB-55). Microplaques were quantified by AID iSPOT reader (AID GmbH, Strassberg, Germany) after staining with anti-spike rabbit polyclonal antibody (Sino Biologicals, Beijing, China; 40592-T62). 50% focus reduction neutralisation titre (FRNT50) was taken as the inverse of the plasma dilution required for 50% reduction in infection foci number. The Point-to-Point curve fit using a linear equation to fit each pair of data points was performed to calculate the FRNT50 value by SoftMax Pro (version 7.0) GxP from Molecular Devices. All virus-related experiments were performed in a biosafety level-3 lab. The FRNT50 titres are presented as geometric mean titre (GMT).

### Viral genome sequencing

The delta variant used in the study has been described earlier with GenBank accession ID MZ356566.1.[13] We have used the BA.1 sub-lineage of the Omicron variant (GISAID No. EPI_ISL_6716902).[14] Total RNA sample of the culture was processed for whole genome sequencing using an Illumina MiSeq sequencing platform to confirm the variant. Sequencing reads (>Q30) were assembled by rnaSPAdes pipeline. Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN) program version 3·1·11 with pangoLEARN 2021-09-17 were used for lineage prediction. The isolate was verified as BA.1 sub-lineage strain of omicron.

### Outcome measures

Our primary outcome was fold-change in the virus neutralisation ability of the plasma against the omicron variant as compared with ancestral virus and delta variant. A secondary outcome was correlation of the serum anti-RBD IgG titres with the FRNT50 titres.

### Statistical analysis

We report the proportion of individuals whose plasma demonstrated *in vitro* live virus neutralisation (defined as FRNT50 ≥20) against ancestral, delta and omicron variants, and compared the FRNT50 titres against different variants using analysis of variance with Tukey's multiple comparison test. For this analysis, those who demonstrated FRNT50 <20 were considered to have a titre of 10. A FRNT50 of 20 or more was taken as 50% protective neutralisation.[15] The FRNT50 of the participants was correlated with quantitative anti-RBD IgG antibody levels (BAU/ml) using Spearman's Rank correlation. The anti-RDB IgG concentrations between different groups were compared using Mann Whitney U test, with a p-value of <0·05 considered as significant.

To evaluate whether type of vaccine and past SARS-CoV-2 infection were independent predictors of neutralisation ability against the omicron variant, we constructed a multivariable logistic regression model with neutralisation ability (FRNT50 ≥20) as the dependent variable and age, sex, type of vaccine, a history of RT-PCR positive SARS-CoV-2 infection, and duration since infection or vaccination as independent variables.

### Role of the funders

Funding agencies did not have any role in the design of the study, collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the manuscript for publication.

## Results

*Participants:* We included a total of 80 participants – 20 each with ChAdOx1 nCoV-19 and BBV152 vaccination alone, and 20 each with ChAdOx1 nCoV-19 vaccine plus natural infection and BBV152 vaccine plus natural infection. Their median age was 58 years (IQR: 48, 65); 32% were females. The median duration from the second dose of vaccine was 234 days (IQR: 210, 274) and the median duration after the natural infection was 224 days (IQR: 199, 238). The clinical characteristics of the study participants are provided in Table 1.

### Serum anti-RBD IgG antibody titres

The median serum anti-RBD IgG antibody titre was 243·5 (IQR 111·3, 544·4) BAU/ml (one of the samples with a very high value of 85443·9 BAU/ml was removed from the analysis because of a history of monoclonal antibody therapy given for SARS-CoV-2 infection a month prior to sampling as elicited during a follow-up interview). The prevalence of anti-nucleocapsid

| Characteristic | ChAdOx1 nCov-19 recipients (*n* = 20) | BBV152 recipients (*n* = 20) | ChAdOx1 nCov-19 recipients plus prior SARS-CoV-2 infection (*n* = 20) | BBV152 recipients plus prior SARS-CoV-2 infection (*n* = 20) |
|---|---|---|---|---|
| **Age (years)**[$] | 61 (53, 72) | 58 (51, 62) | 62 (47, 69) | 52 (46, 60) |
| **Sex**[#] | | | | |
| **Female** | 8 (40%) | 7 (35%) | 5 (25%) | 6 (30%) |
| **Male** | 12 (60%) | 13 (65%) | 15 (75%) | 14 (70%) |
| **Duration between second dose and sampling (days)**[$] | 222 (206, 230) | 211 (200, 303) | 258 (229, 278) | 248 (218, 283) |
| **Duration between infection and sampling (days)**[$] | Not applicable | Not applicable | 212 (192, 223) | 240 (225, 250) |
| **Timing of infection with relation to vaccination** | | | | |
| **After second dose**[#] | Not applicable | Not applicable | 20 (100%) | 12 (63%) |
| **Between two doses**[#] | Not applicable | Not applicable | 0 | 8 (37%) |

***Table 1*: Clinical characteristics of the participants.**
[$] median (IQR)
[#] n (%)

antibodies was 1 of 20 ChAdOx1 nCoV-19 vaccination alone participants, 11 of 20 ChAdOx1 nCoV-19 vaccination plus infection participants, 8 of 20 BBV152 vaccination alone participants, and 18 of 20 BBV152 vaccination plus infection participants (appendix, Table S1). In addition to natural infection, anti-nucleocapsid antibodies may be induced by the whole inactivated virus BBV152 vaccine but not ChAdOx1 nCoV-19, an adenovirus-vectored recombinant coronavirus vaccine. The median anti-RBD IgG titres were higher in the vaccination plus natural infection [403·9 (IQR 164·6, 794·8) BAU/ml] and anti-nucleocapsid IgG positive group [380·7 (IQR 200·0, 665·0)] BAU/ml compared to the vaccination alone group [150·6 (IQR 81·9, 298·5) BAU/ml] (p-value <0·001) and anti-nucleocapsid IgG negative group [161·6 (IQR 86·5, 333·1) BAU/ml] (p-value 0·003, Mann Whitney U test) [appendix, Table S2].

### Live virus neutralisation ability of plasma

The neutralisation GMT was 380·4 (95% CI: 221·1, 654·7) against the ancestral virus with BBV152 vaccination alone. Similar GMT values, 379·3 (95% CI: 185·6, 775·2), were obtained with ChAdOx1 nCov-19 vaccination alone (Figure 1, Table 2). The corresponding values for hybrid immunity groups were 806·1 (95% CI: 478·5, 1357·8) and 1526·2 (95% CI: 853·2, 2730·0), respectively. The GMT showed a 2·3 to 4·3-fold reduction against the delta variant (B·1·617·2) as compared with the ancestral virus in all the

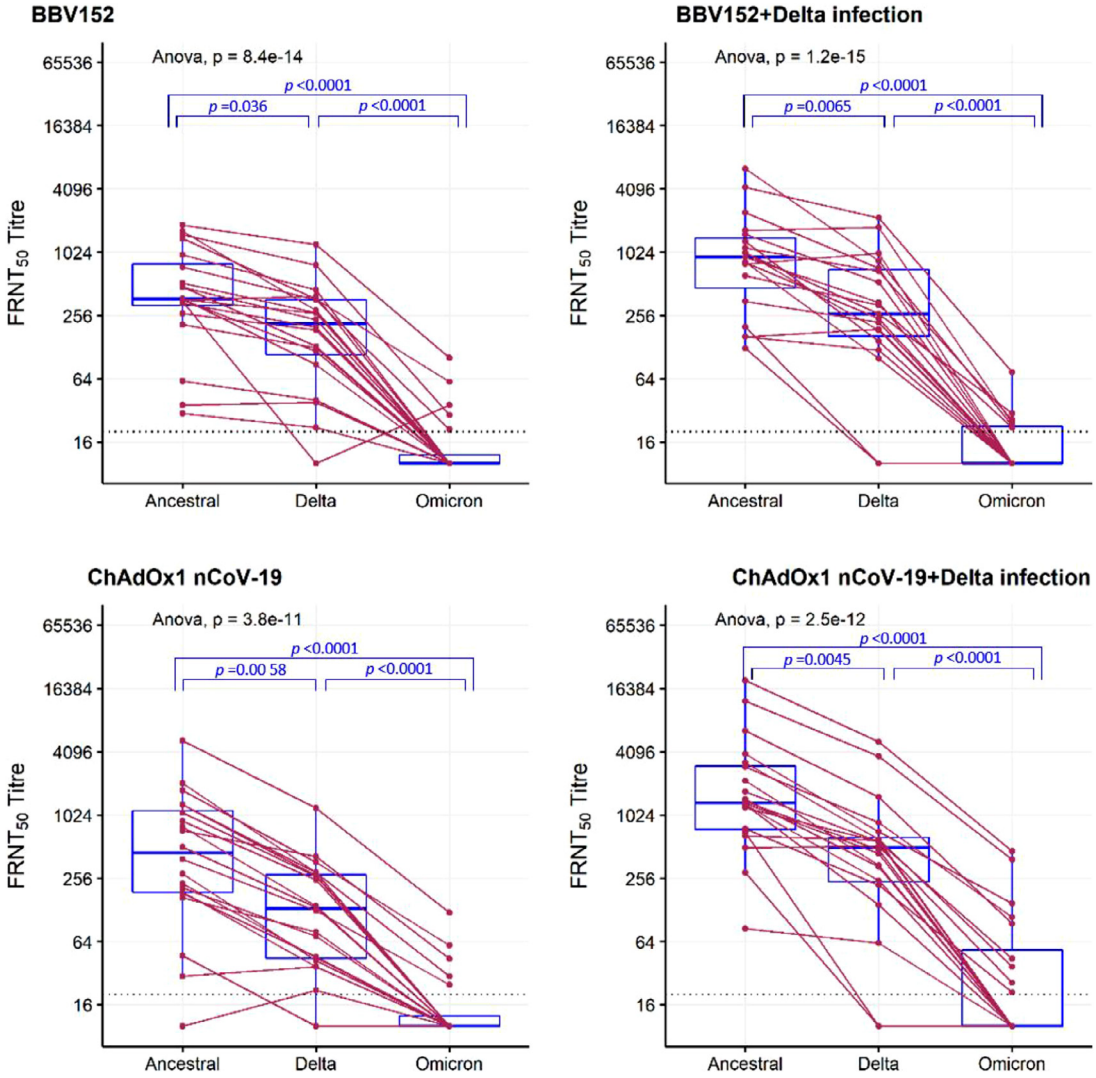


**Figure 1.** Virus neutralisation assay: Plasma samples from indicated groups were used for focus reduction neutralisation assays with indicated virus strains (*N* = 79). The dotted line represents FRNT50 of 20, which was considered as lower limit of quantification. Statistical test – Analysis of variance with Tukey's test for multiple comparisons.

| Type of participant | N[1] | GMT of FRNT50 titres (95% CI) | | | Fold Reduction Anc/Delta | Fold Reduction Anc/OMC | Fold Reduction Delta/OMC |
|---|---|---|---|---|---|---|---|
| | | Ancestral | Delta | Omicron | | | |
| **BBV152** | 20 | 380·4 (221·1, 654·7) | 164·7 (94·3, 287·8) | 14·3 (10·3, 19·9) | 2·3 | 26·6 | 11·5 |
| **BBV152 plus SARS-CoV-2 infection*** | 19[$] | 806·1 (478·5, 1357·8) | 260·2 (130·0, 520·7) | 14·12 (10·7, 18·6) | 3·1 | 57·1 | 18·4 |
| **ChAdOx1 nCov-19** | 20 | 379·3 (185·6, 775·2) | 111·9 (64·3, 194·7) | 14·7 (10·4, 20·9) | 3·4 | 25·7 | 7·6 |
| **ChAdOx1 nCov-19 plus SARS-CoV-2 Infection*** | 20 | 1526·2 (853·2, 2730·0) | 358·1 (171·5, 747·7) | 26·3 (14·2, 48·6) | 4·3 | 58·1 | 13·6 |

***Table 2*: Neutralisation geometric mean titres of participants against SARS-CoV-2 variants.**
Anc: ancestral.
[1] Number of participants whose plasma 50% virus neutralisation titres (FRNT50) were estimated by focus reduction neutralisation test against specific variantsMean value of ancestral-type was considered as reference for comparison.GMT – Geometric mean titre (Participants who demonstrated FRNT50 <20 were considered to have a titre of 10.).
* SARS-CoV2 infection during delta (B·1·617·2) driven surge.
[$] One of the 20 participants had a history of monoclonal antibody therapy and was removed from this analysis#One of the 20 participants was tested positive for anti-nucleocapsid antibody. A sensitivity analysis excluding this participant from this group showed similar neutralisation titres.

four groups (Table 2). Against the omicron variant, only 5 out of 20 in both the BBV152 and ChAdOx1 nCoV-19 vaccination only groups, 6 out of 20 in BBV152 plus prior SARS-CoV-2 infection group, and 9 out of 20 in ChAdOx1 nCoV-19 plus prior SARS-CoV-2 infection group exhibited FRNT50 of at least 20 (Figure 1, Table 2). We observed a 25 to 27-fold reduction in GMT against the omicron variant in the vaccination alone groups and 57 to 58-fold reduction in the hybrid immunity groups compared with the ancestral virus. As expected, vaccinated subjects who were infected with SARS-CoV-2 in the past showed an increase in titres against the delta variant in both BBV152 and ChAdOx1 nCoV-19 vaccinated groups. The GMT for delta variant in these cases increased from 164·7 (95% CI: 94·3, 287·8) to 260·2 (95% CI: 130·0, 520·7) in the BBV152 vaccination and from 111·9 (95% CI: 64·3, 194·7) to 358·1 (95% CI: 171·5, 747·7) in the ChAdOx1 nCoV-19 vaccination group. Interestingly, natural SARS-CoV-2 infection, most likely due to the delta variant, led to an increase in titres against the ancestral virus, from 380·4 (95% CI: 221·1, 654·7) to 806·1 (95% CI: 478·5, 1357·8) in the BBV152 group and from 379·3 (95% CI: 185·6, 775·2) to 1526·2 (95% CI: 853·2, 2730·0) in the ChAdOx1 nCoV-19 group (Figure 1, Table 2). However, the GMT declined significantly in the case of omicron. The GMT was higher among those who were positive for anti-nucleocapsid IgG as compared to those who were negative (Table 4). The FRNT50 titre against the ancestral and the omicron variant demonstrated moderate to strong correlation with serum anti-RBD IgG levels [Spearman r: 0·74 (0·62, 0·83) *p*: <0·001 and 0·58 (0·41, 0·71) *p*: <0·001, respectively] (Figure 2). Prior SARS-CoV-2 infection during the delta surge was independently (though statistically insignificant) associated with 77% increase of odds of being a neutraliser (adjusted OR: 1·77 (95% CI: 0·63, 5·14) after adjusting for age, sex and type of vaccine. The type of vaccine had no influence on the neutralisation titre against Omicron (Table 3).

## Discussion

In the present study, recipients of BBV152 and ChAdOx1 nCoV-19 vaccines demonstrated significantly reduced *in vitro* virus neutralisation against the omicron variant regardless of the past infection with SARS-CoV-2. Mutations in the RBD region of the spike protein may cause compromised binding with the neutralising IgG antibodies and thus the reduced neutralisation observed in the earlier studies as well.[5,6,16–20] There was no difference in the neutralisation between the two types of vaccines tested in the present study. However, there was a suggestion that the neutralisation titres for the omicron were better among those vaccine recipients (particularly ChAdOx1 nCoV-19 vaccine), who had a natural infection during the delta variant led surge. Nevertheless, in view of the possibility of prior asymptomatic infection in the vaccine only group, these findings may need to be corroborated by further studies. Past infection was capable of boosting the antibody titres against the ancestral virus. A recent report has shown that individuals infected with the omicron variant demonstrated an enhanced antibody response against the delta variant suggesting that there could still be shared antibody epitopes between the omicron and other variants mainly pan-Sarbecovirus neutralising antibodies binding outside the receptor binding motif region.[21,22] Though the neutralisation titres in those with infection were higher than those without an infection, the proportion of neutralisers, in general, was modest. This is probably because participants in our study were either infected or

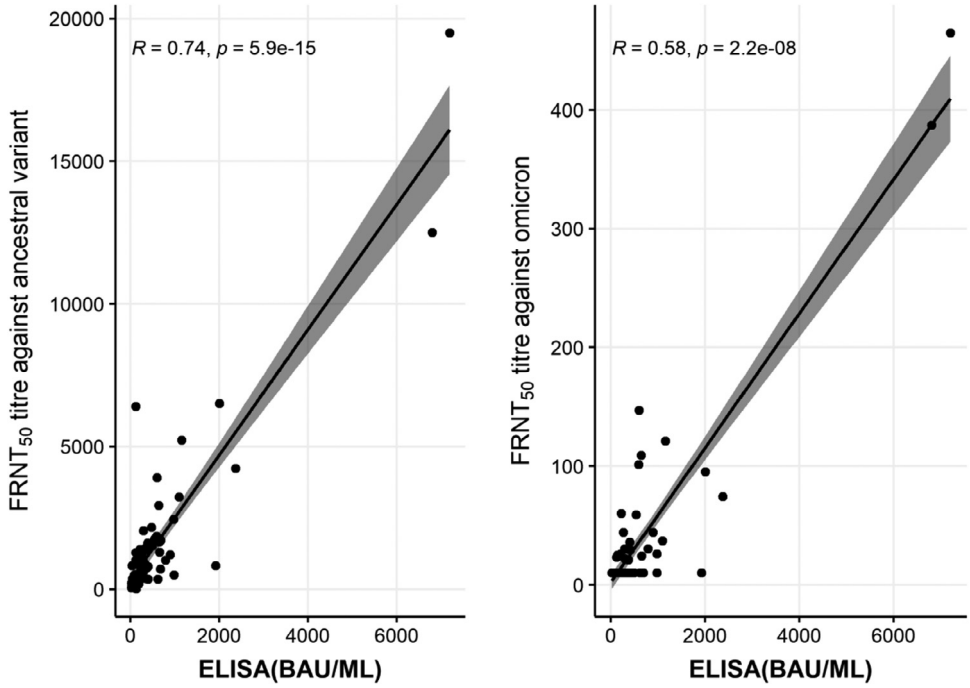


**Figure 2.** Correlation between FRNT50 titre against ancestral virus [Spearman r: 0·74 (0·62, 0·83) *p*: <0·001] and omicron variant [Spearman 0·58 (0·41, 0·71) *p*:<0·001, respectively], and anti-RBD IgG ELISA levels. The shaded region denotes the 95% confidence intervals.

vaccinated more than 6 months before the collection of samples.

It appears that anti-nucleocapsid antibodies induced by the whole inactivated virus BBV152 vaccine wane over 6 months as we could only detect these in 8 out of 20 participants in the BBV152 vaccination alone group. This is consistent with an earlier report of waning anti-nucleocapsid antibodies after natural infection.[23] Interestingly, 18 of 20 BBV152 vaccination plus infection group had anti-nucleocapsid antibodies compared to 11 of 20 ChAdOx1 nCoV-19 vaccination plus infection group suggesting boosting of anti-nucleocapsid IgG response after infection in the BBV152 vaccinated individuals. The dynamics of anti-nucleocapsid antibodies are important for population serosurvey to detect past infection.

The partial loss of neutralising ability against omicron has been demonstrated previously. Of particular interest to our population is the data from the UK that showed that just 1 out of 22 participants, whose samples were collected after 28 days from their second dose of ChAdOx1 nCoV-19 vaccine, demonstrated neutralisation against the omicron.[6] Our participants who were sampled after six months from their second dose

| | Adjusted odds ratio | 95% CI | P value |
|---|---|---|---|
| Age (years) | 1·03 | 0·99, 1·07 | 0·17 |
| Sex - male | 0·94 | 0·33, 2·79 | 0·92 |
| Sex - female | Reference | | |
| Type of vaccine - ChAdOx1 nCov-19 | 1·10 | 0·39, 3·05 | 0·86 |
| Type of vaccine – BBV152 | Reference | | |
| Prior SARS-CoV-2 infection during the delta driven surge | 1·78 | 0·63, 5·14 | 0·28 |
| No prior infection reported | Reference | | |
| Duration between infection/second-dose and sampling[$] | 0·99 | 0·98, 1·01 | 0·30 |

***Table 3*: Association between type of vaccine and prior SARS-CoV-2 infection and neutralising ability against Omicron variant (*N* = 79).**
*The odds ratios of neutralisation ability with the type of vaccine and history of infection were derived after adjusting for the age, sex, duration of sampling from vaccination/ infection of the participants.
[$] Duration was calculated from the latest event among infection and vaccination.

| Group | n | Anti-RBD IgG median (IQR) | FRNT50 titres (GMT, 95%CI) |
|---|---|---|---|
| Anti-nucleocapsid IgG positive | 37 | 380·7 (200·0, 665·0) | 380·7 (200·0, 665·0) |
| Anti-nucleocapsid IgG Negative | 42 | 161·5 (86·5, 333·1) | 161·5 (86·5, 333·1) |
| BBV152 and BBV152+Infection (Anti- nucleocapsid IgG Positive) | 25 | 301·5 (129·6, 624·3) | 301·5 (129·6, 624·3) |
| BBV152 and BBV152+Infection (Anti- nucleocapsid IgG Negative) | 14 | 105·2 (46·8, 239·7) | 105·2 (46·8, 239·7) |
| ChAdOx1 nCoV-19 and ChAdOx1 nCoV-19 + Infection (Anti- nucleocapsid IgG Positive) | 12 | 624·8 (285·4, 1,117·9) | 624·8 (285·4, 1,117·9) |
| ChAdOx1 nCoV-19 and ChAdOx1 nCoV-19 +Infection (Anti- nucleocapsid IgG Negative) | 28 | 212·0 (116·2, 338·4) | 212·0 (116·2, 338·4) |

***Table 4*: Anti-RBD IgG titres and neutralisation titres stratified by the status of anti-nucleocapsid IgG.**

demonstrated a marginally better neutralisation potential against the omicron with 5 out of 20 participants in each vaccine group showing neutralisation, possibly due to prior but undetected SARS-CoV-2 infection. The UK data showed a 29-fold reduction against the omicron in the BNT162b vaccinated sera.[6] In the present study, the fold reduction against the omicron variant was of the order of 25 to 56. A higher fold reduction in the neutralising ability of vaccinated and infected individuals as compared to those only vaccinated was presumably due to a significant boosting of immune response by infection against the ancestral virus . Since the omicron variant has a much different RBD with 15 mutations and whole spike with 30 substitutions, 1 insertion and 3 deletions, it is expected that the neutralising ability of the antibodies will be significantly compromised. However, from the point of view of vaccine/natural infection induced immunity against infection/re-infection, it is important to understand that a minimum virus neutralising ability is required for optimal protection. It has been suggested that a 1:20 neutralisation titre at which 50% virus neutralisation can be achieved is expected to provide 50% vaccine effectiveness against the infection with SARS-CoV-2.[15] Since one of the main mechanisms of action of vaccine-induced antibodies is by blocking the virus entry (and thus the reliance on virus neutralisation assay), a similar protective efficacy should be seen against omicron if antibodies can achieve similar levels of neutralisation. Therefore, we believe that it is more important to report what percentage of vaccinated/hybrid immunity people are able to neutralise the omicron variant at a minimum effective FRNT50 titre. Fold change showing very high reduction compared to the ancestral virus might be misleading. Our data should be interpreted keeping in mind that the mean age of the participants was 58 years with 47% of participants >60 years of age and the time elapsed after complete vaccination and after natural infection was around 7 months. These two factors are also likely to affect the serum binding antibody and neutralising antibody titres, leading to reduced vaccine effectiveness. However, other biological mechanisms of action such as Fc-mediated antibody functions and T-cell immune responses are likely to provide additional protection against infection and disease severity.[24] It has been shown that CD4+ and CD8+ T-cell immune responses are important for controlling SARS-CoV-2 infection.[25] This seems to be true for the omicron variant too as demonstrated by comparable and maintained CD4 and CD8 T cell response against the spike proteins of most of the variants including omicron.[26] We have recently shown that T-cell immune responses are largely preserved against the delta variant and offer protection despite decreased neutralising ability of the vaccinated plasma.[2] The final impact on the hospitalisation and mortality rates of the omicron variant-led infections will depend on viral factors such as the inherent pathogenicity of the variant and immune evasiveness, host factors such as innate and cellular immune responses, and epidemiological factors such as the proportion of individuals who might have a hybrid immunity from vaccine and past infection. Except for immune evasiveness, the other factors should favour a reduced incidence of severe COVID-19 from the omicron led surge as compared to the previous surges.[27,28]

There is a lack of data regarding the need for a booster dose in individuals who have received two doses of vaccine and had a natural infection, which by itself can act as a booster. Although a study in preprint reports a phase 2 trial of a third BBV152 dose, it doesn't give insights on the effects of this booster dose against the SARS-CoV-2 VoCs.[29] Therefore, studies on the impact of a booster (third dose) after the BBV152 and ChAdOx1 vaccination should be done to assess the response against Omicron infection.

From a policy perspective, a strong correlation between serum anti-RBD IgG titres and neutralisation titres against the omicron in the face of immune escape by this variant would lend support for an additional dose of vaccine to augment antibody response especially in those above 60 years of age who have been vaccinated in the first half of 2021. Booster dose is being implemented in a few countries including India and has the potential to offer better protection in vulnerable people after 6 months of vaccination or natural infection.

## Contributors

PKG conceptualized and designed the overall study. SB, RT and NW designed the cohort study; DM, RT, SS, MG, PK, AP, NK enrolled the participants, collected the clinical information and biospecimens. GRM, JS, AA, HS, and KP developed and conducted the FRNT experiments; SC, FM, SJD, GB developed and performed the ELISA for antibody titres; BD conducted the sequencing experiments; GRM, RT, DM and AP managed and analysed the clinical and laboratory data; PKG, SB, GRM, GB, BD, and RT reviewed and interpreted the data, and wrote the manuscript; all authors reviewed and approved the final manuscript.

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## Declaration of interests

We declare no competing interests.

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## Data sharing statement

The de-identified dataset and related codes for analysis will be made available to researchers on request after publication. Requests for data should be addressed to the corresponding author and will need to be approved by the Department of Biotechnology, Government of India (New Delhi, India).

## Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.103938.

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