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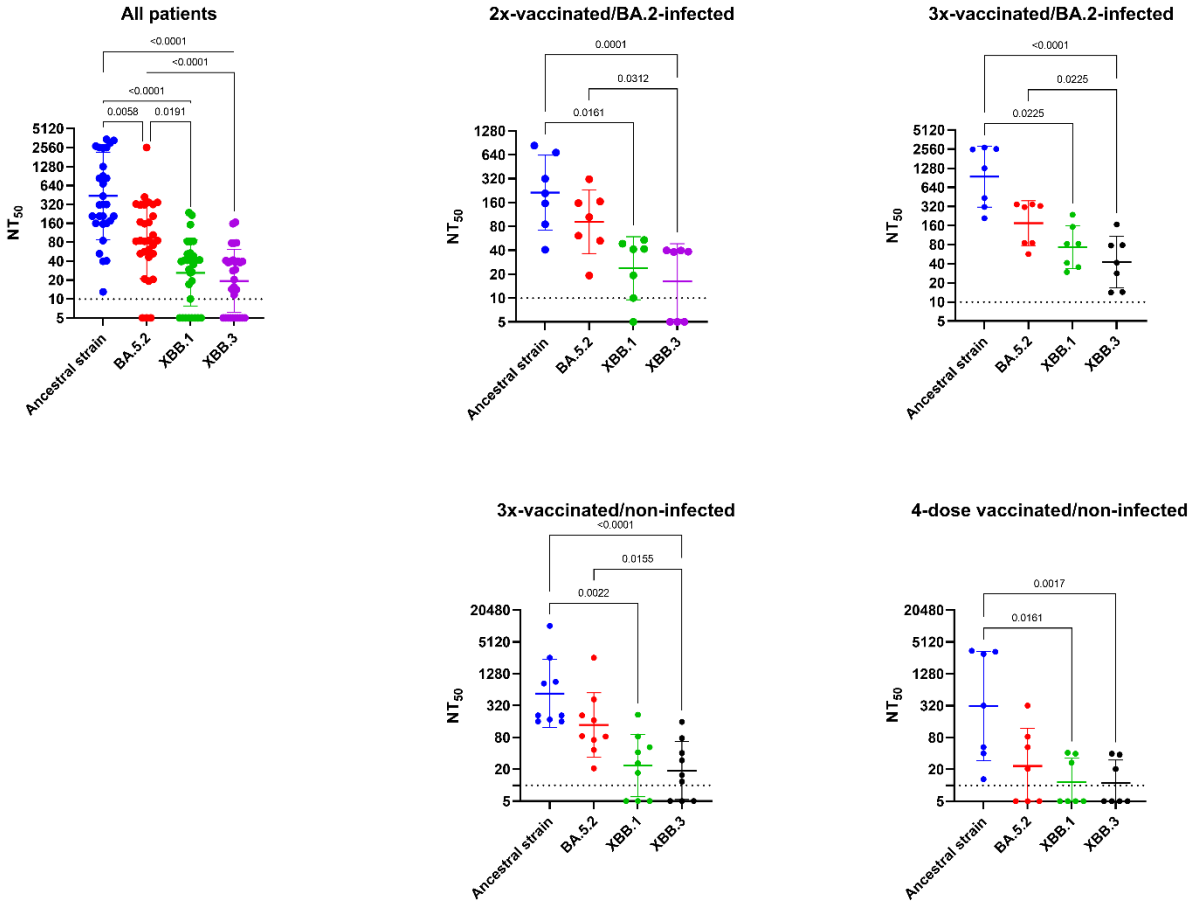
Supplementary appendix

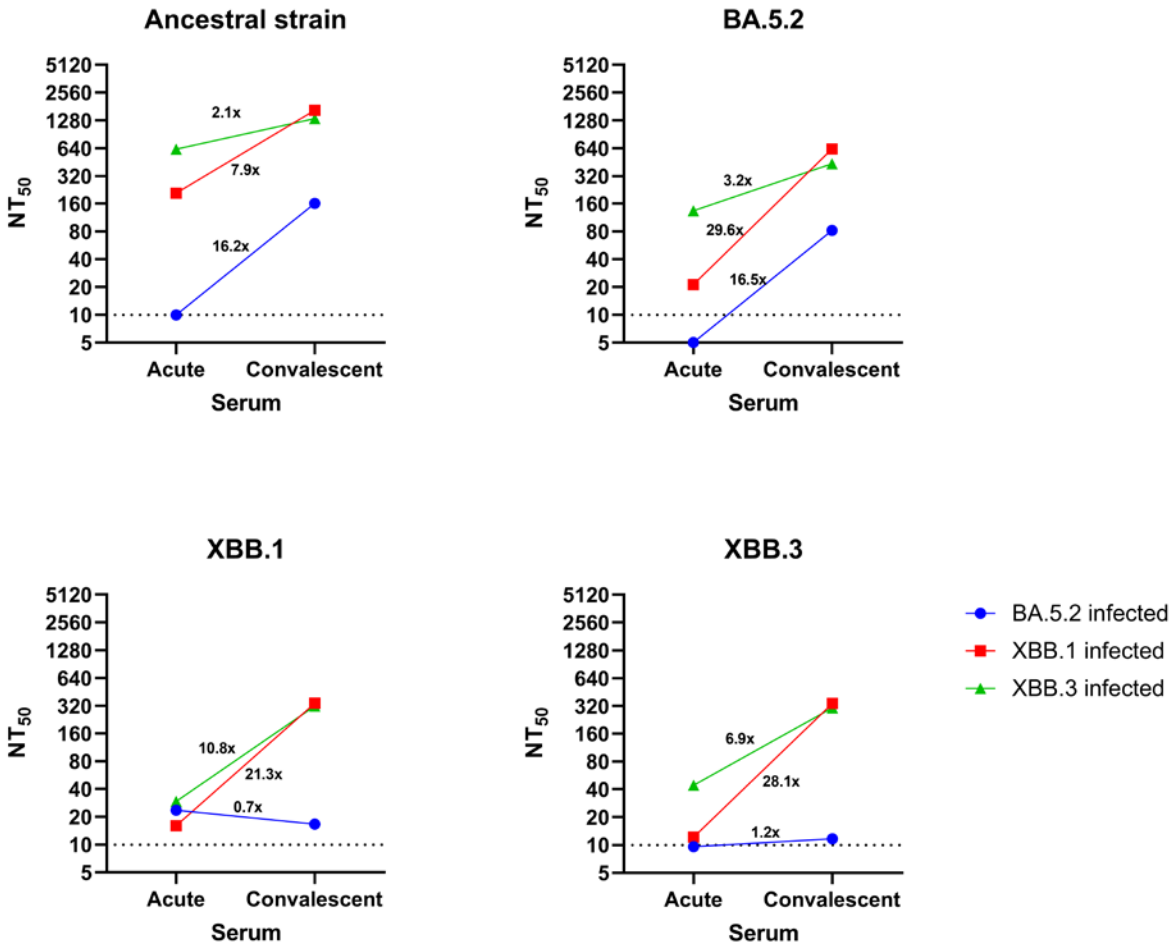
This appendix formed part of the original submission. We post it as supplied by the authors.

Supplement to: Zhang, X, Chen L-L, Ip JD, et al. Omicron sublineage recombinant XBB evades neutralising antibodies in recipients of BNT162b2 or CoronaVac vaccines. *Lancet Microbe* 2022; published online December 6. https://doi.org/10.1016/S2666-5247(22)00335-4.

Supplementary Appendix



Supplementary Figure S1. Comparison of live virus neutralising antibody titers against ancestral strain and Omicron sublineages BA.5.2, XBB.1 and XBB.3 for individuals with different history of vaccination and infection. Dotted horizontal lines represent the lower limit of detection. P values were shown if <0.05. NT_{50}, 50% neutralisation titer



Patient	Collection date (Days after symptom onset)		No. of vaccine doses received	Type of vaccine
	Acute serum	Convalescent serum		
BA.5.2 infected	3	39	2	BNT162b2
XBB.1 infected	5	18	3	BNT162b2
XBB.3 infected	3	18	4	BNT162b2

Supplementary Figure S2. Comparison of live virus neutralising antibody titers against ancestral strain and Omicron sublineages BA.5.2, XBB.1 and XBB.3 for the 3 patients with acute and convalescent serum specimens available. Details of the 3 infected patient are shown in the table. The fold increase 50% neutralisation titer between acute and convalescent sera is shown. Dotted horizontal lines represent the lower limit of detection. NT50, 50% neutralisation titer

Supplementary Table S1. Characteristics of study participants

	2x-vaccinated/ BA.2 infected (n=7)	3x-vaccinated/ BA.2 infected (n=7)	3x-vaccinated/ non-infected (n=9)	4x-vaccinated/ non-infected (n=7)	Total (n=30)
Demographics					
Age in years, median (range)	52 (44-59)	51 (41-59)	58 (53-73)	72 (70-86)	57 (49-71)
Female sex	6 (85.7)	5 (71.4)	6 (66.7)	4 (57.1)	21 (70)
Vaccine type					
BNT162b2	4 (57.1)	4 (57.1)	6 (66.7)	3 (42.9)	17 (56.7)
CoronaVac	3 (42.9)	3 (42.9)	3 (33.3)	4 (57.1)	13 (43.3)
Interval between vaccination/infection and serum collection date					
1st vaccine dose and serum collection	350 (336-365)	350 (344-372)	364 (315-391)	491 (450-494)	N/A
2nd vaccine dose and serum collection	322 (311-344)	329 (311-344)	339 (287-366)	464 (429-470)	N/A
3rd vaccine dose and serum collection	N/A	116 (85-148)	126 (92-146)	215 (197-232)	N/A
4th vaccine dose and serum collection	N/A	N/A	N/A	89 (82-111)	N/A
Infection[a] and serum collection	140 (135-150)	104 (21-130)	N/A	N/A	N/A

Data are expressed as no. (%) unless otherwise specified

N/A, not applicable

[a] Defined as the date of diagnosis

METHODS

Serum specimens

Serum specimens were collected in our ongoing prospective cohort study which enrolled COVID-19 vaccine recipients and COVID-19 patients in Hong Kong[1]. Details these demographics, vaccine type, interval between vaccine doses and serum collection, and interval between infection and serum collection were documented. The study has been approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 21-214 and UW 13-265). Written informed consent were obtained from all study participants.

Viral culture

Viral culture was performed in a biosafety level 3 facility as we described previously with slight modifications [2, 3]. Briefly, TMPRSS2-expressing VeroE6 (VeroE6/TMPRSS2) cells (JCRB Cat#JCRB1819) were seeded with 1 mL of minimum essential medium (MEM) (Gibco®, Thermo Fisher Scientific) with 10% fetal bovine serum (FBS) and 5 mg/mL G418 (Gibco®, Thermo Fisher Scientific) at 1 x 10^5 cells in a shell vial (Diagnostic Hybrids, Inc). The plates were incubated at 37°C in a carbon dioxide incubator until confluence for inoculation. Each well was inoculated with 100 μL of clinical specimen. One hour after incubation, the clinical specimen was removed and cells were replenished with 1 mL of MEM medium with 1% FBS, 100 U/ml penicillin-streptomycin, 100 U/ml of nystatin, and 25 mM HEPES (Gibco®, ThermoFisher Scientific). The cells were incubated at 37°C with 5% CO_2 and observed 2-3 times per day for virus-induced cytopathic effect (CPE). Cultures with more than 50% virus-induced CPE were expanded to large volume in VeroE6/TMPRSS2 cells with the same culture condition. The 50% tissue culture infective doses ($TCID_{50}$) were determined in

VeroE6/TMPRSS2 cells. The whole genome sequence of the culture isolates was determined using nanopore sequencing (Oxford Nanopore Technologies).

Live virus neutralising antibody assay

Live virus neutralising antibody (nAb) assay was performed as we described previously [4-6]. The SARS-CoV-2 BA.5.2 isolate (GISAID accession number EPI_ISL_13777658), XBB.1 isolate (GISAID accession number EPI_ISL_15602393), and XBB.3 isolate (GISAID accession number EPI_ISL_15602394) were isolated in this study. The ancestral strain (GISAID accession number: EPI_ISL_434571) was isolated from previous studies [3]. Briefly, serum samples were heat-inactivated at 56°C for 30 min and were serially diluted in 2-folds with MEM containing 1% FBS. Duplicates of each diluted serum were mixed with a SARS-CoV-2 virus isolate to reach a final concentration of 100 $TCID_{50}$ and were incubated at 37°C for 1 hour. After incubation, 100 μL of the serum-virus mixture was then added to VeroE6/TMPRSS2 cells that were seeded in 96-well plates 24 hours before infection. The cells were incubated with the mixture at 37°C. After incubation for 3 days, CPE was visually scored for each well by two independent observers. The 50% neutralisation titer (NT_{50}) was determined by using log (inhibitor) vs normalized response- variable slope in GraphPad PRISM version 9.4.0. For statistical analysis, a value of 5 was assigned if the live virus neutralising antibody titer is <10.

Statistical analysis

We did statistical analysis using PRISM 9.4.0. NT_{50} against ancestral strain, BA.5.2, XBB.1 and XBB.3 were compared using one way ANOVA and corrected for multiple

comparison using Dunn's multiple comparisons test. A *P* value of less than 0.05 was judged statistically significant.

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