Neutralizing immunity induced against the Omicron BA.1 and BA.2 variants in vaccine breakthrough infections

Noah Brazer[1#], Mary Kate Morris[2#], Venice Servellita[1], Khamal Anglin[3], Prachi Saldhi[1], Miguel Garcia-Knight[4], Sutana Bethancourt[2], Alicia Sotomayor-Gonzalez[1], Baolin Wang[1], Abiodun Foresythe[1], Jenny Nguyen[1], Amelia S. Gliwa[1], Jesus Pineda-Ramirez[3], Ruth Diaz Sanchez[3], Yueyuan Zhang[1], Melanie Ott[5,6,7,8], Debra A. Wadford[2], Raul Andino[4], J. Daniel Kelly[3], Carl Hanson[2*], Charles Chiu[1,7,8*]

[1]Department of Laboratory Medicine, University of California, San Francisco, San Francisco, CA, USA
[2]Viral and Rickettsial Disease Laboratory, California Department of Public Health, Richmond, CA, USA
[3]Department of Epidemiology and Biostatistics, University of California, San Francisco, CA, USA
[4]Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA, USA
[5]California Institute for Quantitative Biosciences (QB3), University of California, Berkeley, CA, USA
[6]Gladstone Institutes, San Francisco, CA, USA.
[7]Innovative Genomics Institute, University of California Berkeley, CA, USA
[8]Department of Medicine, University of California San Francisco, San Francisco, CA, USA

#Equal contribution
*Correspondence:

Charles Y. Chiu (**Lead Contact**)
UCSF China Basin campus
185 Berry Street, Box #0134
San Francisco, CA, USA
charles.chiu@ucsf.edu

Carl Hanson
Viral and Rickettsial Diseases Laboratory, California Department of Public Health
850 Marina Bay Parkway
Richmond, CA, USA
carl.hanson@cdph.ca.gov

All authors listed have contributed significantly to the study, have seen, and have approved the manuscript. This manuscript has neither been published elsewhere nor is being considered for publication by a journal other than *Journal of Infectious Diseases*.

Running Title: Neutralizing immunity against Omicron

Key Points: Neutralizing immunity was stronger against BA.2 than BA.1, regardless of infecting variant. Cross-variant neutralization was minimal in unvaccinated infections but moderate to strong in vaccine breakthrough infections. Observed differences in neutralizing immunity between unboosted and boosted breakthrough infections were comparable.

Abstract

Background

As of early 2022, the Omicron variants are the predominant circulating lineages globally. Understanding neutralizing antibody responses against Omicron BA.1 and BA.2 following vaccine breakthrough infections will provide insights into BA.2 infectivity and susceptibility to subsequent re-infection.

Methods

Live virus neutralization assays were used to study immunity against Delta and Omicron BA.1 and BA.2 variants in samples from 86 individuals, 24 unvaccinated (27.9%) and 63 vaccinated (72.1%), who were infected with Delta (n=42, 48.8%) or BA.1 (n=44, 51.2%). Among the 63 vaccinated individuals, 39 were unboosted (45.3%), while 23 were boosted (26.7%).

Results

In unvaccinated infections, neutralizing antibodies (nAbs) against the three variants were weak or undetectable, except against Delta for Delta-infected individuals. Both Delta and BA.1 breakthrough infections resulted in strong nAb responses against ancestral wild-type and Delta lineages, but moderate nAb responses against BA.1 and BA.2, with similar titers between unboosted and boosted individuals. Antibody titers against BA.2 were generally higher than those against BA.1 in breakthrough infections.

Conclusions

These results underscore the decreased immunogenicity of BA.1 as compared to BA.2, insufficient neutralizing immunity against BA.2 in unvaccinated individuals, and moderate to strong neutralizing immunity induced against BA.2 in Delta and BA.1 breakthrough infections.

Word count: 197

Key Words: Omicron BA.2, Omicron BA.2, and Delta SARS-CoV-2 variants; COVID-19; breakthrough infection; neutralizing antibodies; vaccine boosting

Introduction

Following its emergence in November 2021, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Omicron (B.1.1.529) variant spread rapidly to become the predominant variant globally by early 2022 [1-3]. Akin to the evolution of most SARS-CoV-2 variants of concern/variants of interest (VOC/VOI), continual mutations gave rise to multiple Omicron sub-lineages, including BA.1 and BA.2 [4]. At the end of February 2022, the World Health Organization (WHO) issued a statement of concern regarding the increasing prevalence of the BA.2 sub-lineage worldwide. BA.2 is more transmissible than BA.1 without causing more severe clinical outcomes [5-7]. Despite being classified as the same variant, the BA.1 and BA.2 sub-lineages differ by 40 mutations, and the two sub-lineages are antigenically distinct [8].

Unvaccinated individuals infected with BA.1 generate weak cross-neutralizing antibody responses against other VOCs [9, 10], while vaccinated individuals with BA.1 or Delta breakthrough infections generate moderate to strong cross-neutralizing responses [11-13]. Whether this induced broad-based immunity extends to the BA.2 sub-lineage remains largely unexplored. Either vaccination alone without prior or subsequent infection or infection alone without vaccination fails to generate a robust neutralizing antibody response against BA.2 in convalescent samples [14]. However, little is known regarding the “hybrid” immunity induced against BA.2 in vaccinated individuals who are subsequently infected with BA.1 or Delta. This

study used live virus neutralization assays to evaluate differential neutralizing antibody responses against BA.1, BA.2, Delta, and ancestral WA-1 wildtype (WT) lineages in unvaccinated and vaccinated individuals infected with either Delta or BA.1.

Methods

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human Subjects

Human subjects in this study included patients hospitalized with COVID-19 at University of California, San Francisco (UCSF) and consenting SARS-CoV-2 infected individuals enrolled in one of two longitudinal prospective studies at UCSF. The first UMPIRE (UCSF EMPloyee and community member Immune REsponse) study focused on collection of prospective whole blood and plasma samples to evaluate the immune response to COVID-19 vaccination [11], while the second study of SARS-CoV-2 household transmission at UCSF has been federally designated as a public health surveillance project [15] **(Table 1).** For hospitalized UCSF patients, remnant samples were biobanked and retrospective medical chart reviews for demographic and clinical metadata were performed under a waiver of consent and according to protocols approved by the UCSF Institutional Review Board (protocol numbers 10-01116 and 11-05519). Informed consent for participation in the UMPIRE study and protocols for data and sample collection were approved by the UCSF Institutional Review Board (protocol number 20-33083). For the household transmission study, written informed consent was obtained from all subjects and protocols for sample and data collection were approved by the UCSF Institutional Review Board.

Cell Lines

For SARS-CoV-2 isolation in cell cultures and live virus assay, Vero E6-TMPRSS2-T2A-ACE2 and Vero-81 derived from African green monkey kidney, were cultured at 37°C in Modified Eagle Medium (MEM) supplemented with 1x penicillin-streptomycin (Gibco), glutamine (Gibco), and 10% fetal calf serum (Hyclone). The Vero E6-TMPRSS2-T2A-ACE2 were supplemented with 10ug/mL puromycin. The Vero-81 cell line was authenticated by and ordered from the American Tissue Culture Collection (ATCC). Re-authentication of this cell line was not conducted prior to use.

METHOD DETAILS

Human Sample Collection

Blood samples were collected through three different protocols. First, remnant plasma samples from patients of any age and gender hospitalized with COVID-19 at UCSF were retrieved from UCSF Clinical Laboratories . Clinical data from hospitalized UCSF patients were extracted through retrospective chart review. Second, plasma samples were collected through the UMPIRE study from unboosted and boosted subjects. Consenting participants came to a UCSF Clinical Research Service Laboratory for blood collection at approximately 1, 2, and 6-month intervals following breakthrough infection. Demographic and clinical metadata from UMPIRE participants were obtained through Qualtric surveys performed at enrollment and each visit. Finally, blood samples and patient clinical metadata were obtained through the household transmission study: a field team collected blood samples from non-hospitalized participants at their residences and interviewers administered questionnaires by phone to collect sociodemographic and clinical data. Subjects were enrolled within 5 days of symptom onset of

the first SARS-CoV-2-positive case in the household and samples and metadata were collected during weekly visits for 28 days (25).

Clinical Chart Review

Moderately severe infections included hospitalization for COVID-19 pneumonia with an oxygen requirement of >2 L by nasal cannula or another infectious complication of the disease. Severe infections included COVID-19 pneumonia with severe hypoxemia with an oxygen requirement of >6 L, the need for CPAP, BIPAP, intubation with mechanical ventilation, COVID-19 associated end-organ failure, and/or death. Outpatients and hospitalized patients not meeting criteria for moderate or severe infection were classified as having a mild or asymptomatic infection.

Immunocompromised patients included patients on immunosuppressive therapy due to active malignancies, patients on immunosuppressive medication following solid organ or bone marrow transplantation, and patients with any disease resulting in a severe immunodeficiency.

SARS-CoV-2 Whole-Genome Sequencing

Viral whole-genome sequencing of SARS-CoV-2 was performed as previously described [11]. Remnant clinical nasopharyngeal/oropharyngeal (NP/OP) swab samples collected in universal transport media or viral transport media (UTM/VTM) were diluted with DNA/RNA shield (Zymo Research, # R1100-250) in a 1:1 ratio (100 µl primary sample + 100 µl shield) prior to viral RNA extraction. The Omega BioTek MagBind Viral DNA/RNA Kit (Omega Biotek, # M6246-03) and the KingFisherTM Flex Purification System with a 96 deep-well head (ThermoFisher, 5400630) were used for viral RNA extraction. Extracted RNA was reverse transcribed to complementary DNA and tiling multiplexed amplicon PCR was performed using

Artic version 3 and/or VarSkip SARS-CoV-2 primers (New England Biolabs). Adapter ligation was performed using the NEBNext® ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®)(New England Biolabs, # E7658L). Libraries were barcoded using NEBNext Multiplex Oligos for Illumina (96 unique dual-index primer pairs) (New England Biolabs, # E6440L) and purified with AMPure XP (Beckman-Coulter, #63880). Amplicon libraries were sequenced using Illumina MiSeq or NextSeq 550 as 2x150 base pair paired-end reads.

Genome Assembly, Variant Identification and Mutation Analysis

Raw sequencing data were demultiplexed, converted to FASTQ files, and screened for SARS-CoV-2 sequences using BLASTn (BLAST+ package 2.9.0). Reads containing adapters, ARTIC and/or VarSkip primer sequences and/or of low-quality were filtered using BBDuk (version 38.87) and mapped to the Wuhan-Hu-1 SARS-CoV-2 reference genome (National Center for Biotechnology Information (NCBI) GenBank accession number NC_045512.2) using BBMap (version 38.87) [16]. Consensus sequences were generated using iVar (version 1.3.1) [17] and lineages were assigned using Pangolin (version 3.1.17) [18] and NextClade (version 1.11.0) [19] softwares.

For PCA plot generation, 756 available full-length SARS-CoV-2 genomic sequences from California were downloaded from the Global Initiative on Sharing All Influenza Data (GISAID) database. The datasets included \459 Delta genomes from samples collected in San Francisco County from September, 2021 – November 2021, 214 BA.1. genomes from samples collected in California from November, 2021 – February, 2022, and 83 BA.2 genomes from samples collected in California in April, 2022. Individual genomes corresponding to the ancestral wild-type lineage (GenBank accession number NC_045512.2) and representative Beta, Alpha, Gamma, Epsilon, Lambda, and Mu variants were included. Coding mutations in the full-length

genome and spike gene were called using Nextclade (version 1.11.0) [19]. PCA was performed using the FactoMineR (version 2.4) package in R (version 4.0.2). Coordinates of the centroids and selected genomes were extracted, and Euclidean distances were calculated using custom R scripts.

SARS-CoV-2 Isolation in Cell Cultures

BA.1, BA.2, and Delta lineages were isolated from de-identified patient NP swabs sent to the California Department of Public Health. To isolate Delta, 200 µl of a NP sample previously identified as Delta was diluted 1:3 in PBS, supplemented with 0.75% bovine serum albumin (BSA-PBS) and added to confluent Vero-81 cells. Following a 1-hour absorption period, additional media was added, and the flask was incubated at 37˚C with 5% CO2 with daily monitoring for cytopathic effect (CPE). When 50% CPE was detected, the contents were collected, clarified by centrifugation, and stored at -80˚C as passage 0 stock. Passaged stock of Delta was made by inoculation of Vero-81 confluent T150 flasks with 1:10 diluted p0 stock and harvested at approximately 50% CPE. Omicron viral stock for the two lineages of interest was similarly produced from a sequence confirmed NP sample using Vero E6-TMPRSS2-T2A-ACE2. All viral stocks were sequenced to confirm lineage and TCID50 (tissue culture infective dose 50, or the dose at which 50% of inoculated cells in culture are infected) was determined by titration.

Live Virus Neutralization Assay

CPE endpoint neutralization assays were done following the limiting dilution model using p1 stocks of BA.1, BA.2 and Delta in Vero E6-TMPRSS2-T2A-ACE2. Patient plasma was diluted 1:10 in 0.75% BSA-PBS and heat inactivated at 56C for 30 minutes. Serial 3-fold

dilution of plasma were made in BSA-PBS. Plasma dilutions were mixed with 100 TCID50 of each virus diluted in BSA-PBS at a 1:1 ratio and incubated for 1 hour at 37C. Final plasma dilutions in plasma-virus mixture ranged from 1:40 to 1:84480. 100ul of the plasma-virus mixtures were added in duplicate to 96-well plates pre-seeded with Vero E6-TMPRSS2-T2A-ACE2 at a density of 2.5x104/well and incubated in a 37˚C incubator with 5% CO2 until consistent CPE was seen in the virus control wells. Positive and negative controls were included with cell control wells and a viral back titration to verify TCID50 viral input. Individual wells were scored for CPE as having a binary outcome of "infection" or "no infection" and the ID50 (inhibitory dose 50, the concentration of plasma needed to inhibit virus-induced CPE by 50%), was calculated using the Spearman-Karber method.

Quantification and Statistical Analysis

Statistical analyses and data visualization were performed using R (version 4.0.2) with the ggplot2 package (version 3.3.5) [20]. Fisher's exact test was used to evaluate associations of demographic and clinical variables with variant-specific breakthrough infections. Wilcoxon signed-rank and Wilcoxon-Mann-Whitney U tests were used to determine significance for paired and unpaired samples, respectively. All tests were conducted as two-sided at the 0.05 significance level.

Data Repository Submission

The SARS-CoV-2 genomes used in this study were deposited into the GISAID database. P-values relevant to the study, coordinates for the PCA plots, and custom scripts used for plot

generation were deposited into a publicly accessible Zenodo database repository (doi: 10.5281/zenodo.6485708).

Results

Study cohort

This study included individual plasma samples from 86 patients, including 24 (27.9%) unvaccinated, 39 (45.3%) vaccinated with a primary series of either two doses of an mRNA vaccine or one dose of an adenovirus vector vaccine ("unboosted"), and 23 (26.7%) vaccinated with an additional booster dose ("boosted"). All patients with breakthrough infections contracted COVID-19 ≥ 14 days after their last vaccine dose.

Among the 42 Delta infections **(Table 1)**, 37 (88.1%) were sequenced and classified as Delta, while the remaining 5 (11.9%) were presumed Delta because they were collected from patients infected with SARS-CoV-2 when Delta comprised 98.0-99.1% of the circulating variants in California (CDPH, 2022). Of the 42 Delta infected patients, 22 (52.4%) were immunocompromised, and 29 (69.0%) had moderate to severe COVID-19. 14 (33.3%) were unvaccinated, 25 (59.5%) were unboosted, and 3 (7.1%) were boosted. Among the 28 vaccinated patients, sample collection dates ranged from 14 to 49 days (median = 26 days) following symptom onset date or PCR positivity, whichever came earlier.

Among the 44 Omicron BA.1 infections **(Table 1)**, 32 (72.7%) were sequenced and classified as BA.1, while the remaining 12 (27.3%) were presumed BA.1 because they were collected from patients infected with SARS-CoV-2 when BA.1 comprised 97.4-99.8% of the circulating variants in California (CDPH, 2022). Of the 44 BA.1infected patients, 19 (43.2%) were immunocompromised, and 20 (45.5%) had moderate to severe COVID-19. 10 (22.7%)

were unvaccinated, 14 (31.8%) were unboosted, and 14 (45.5%) were boosted. Among the 28 vaccinated patients, sample collection dates ranged from 15 to 43 days (median = 24 days) following symptom onset date or PCR positivity, whichever came earlier.

Neutralizing antibody responses in patients infected with Delta

Among the 14 unvaccinated patients out of 42 infected with Delta, median neutralizing antibody responses against Delta were strong (median NT_{50}=1,871, where NT_{50} refers to the neutralization test titers associated with a ≥50% inhibition of the plasma sample), with titers significantly higher than that against BA.1 (48X, p=0.0011) and BA.2 (27X, p=0.0017) **(Figure 1A).** In comparison, neutralizing antibody responses against BA.2 and BA.1 were weak (median NT_{50}<100), with titers against BA.2 >1.8X higher than against BA.1 (p=0.12) **(Figure 1A)**.

Like the unvaccinated cases, neutralizing antibody responses against Delta in unboosted Delta breakthrough infections (n=25) were strong, with titers (median NT_{50}=3,240) significantly higher than against BA.1 (16X, p<0.001) and BA.2 (5.2X, p<0.001) **(Figure 1A)**. Although moderate (median NT_{50} 100-1,000), neutralizing antibody responses against BA.1 and BA.2 in unboosted Delta-infected individuals were 5.3X (p=0.0037) and 9.0X (p=0.024) higher, respectively, compared to those in unvaccinated individuals **(Figure 1A, 2A, and 2B)**. Median neutralizing titers against BA.2 were 3.0X higher than against BA.1 (p=0.15). The pattern of neutralizing antibody responses in the 3 boosted patients with Delta breakthrough infections, all of whom were immunocompromised, was comparable to that in unboosted patients **(Figure 1A).**

Neutralizing antibody responses in patients infected with Omicron BA.1

Among the 10 unvaccinated patients out of 44 infected with BA.1, neutralizing antibody responses against BA.1 were low to moderate (median NT_{50}=164), but titers were still >4.2X those of Delta (p=0.034) and 3.0X those of BA.2 (p=0.080) **(Figure 1A).** Titers against both Delta and WA-1 were below the limit of detection **(Figure 1A)**, consistent with the previously observed lack of cross-variant neutralization responses in unvaccinated individuals infected with BA.1 [9, 10].

In contrast, BA.1 vaccine breakthrough infections (n=14) induced moderate to strong antibody responses against BA.1 (NT_{50}=852) and BA.2 (NT_{50}=1,080), with 1.3X higher median neutralizing antibody titers against BA.2 than BA.1 (p=0.34) **(Figure 1A).** Compared to unvaccinated infections, these titers were 5.2X higher against BA.1 (p=0.087) and 19.8X higher against BA.2 (p=0.0081) **(Figure 2A and 2B).** In boosted individuals, the increases in titers compared to unvaccinated BA.1 infections were similar (3.8X and 15.6X higher titers against BA.1 and BA.2, respectively), with 1.4X higher titers against BA.2 than BA.1 (p=0.22). Interestingly, median neutralizing antibody titers were slightly lower for boosted compared to unboosted individuals against BA.1 and BA.2, although these differences were not significant (p=0.55 and p=0.63, respectively). Thus, BA.1 breakthrough infections were found to generate higher neutralizing antibody titers against BA.2 than against BA.1.

When patients in each vaccination/breakthrough infection category were further stratified based on immunocompromised status, disease severity, and type of vaccine received, only a few significant differences were observed **(Table S2; Figure 2E and 2F).** Titers against BA.1 and BA.2 were significantly higher in unvaccinated patients with moderate to severe Delta infections compared to those with mild or asymptomatic infections. Because no significant differences

were observed in median neutralizing antibody titers between BA.1 unboosted and boosted breakthrough infections **(Figure 2A-D; Table S2)**, we combined all BA.1 breakthrough infections and further stratified the data based on immunocompromised status, disease severity, and type of vaccine received **(Figure 1B and C).** Titers against BA.2 were significantly higher than BA.1 for (1) immunocompetent, (2) mild or asymptomatic, and (3) immunocompetent, mild or asymptomatic subgroups (p=0.039, p=0.0053, and p=0.0038, respectively). All other pairwise comparisons were not significant. Titers against Delta in immunocompetent patients were significantly higher than those in immunocompromised patients (p=0.0039) while titers against Delta in patients with mild or asymptomatic infections were significantly higher than those with moderate to severe infections (p=0.021). Similar outcomes were observed with the titers against WT with immunocompetent versus immunocompromised patients (p=0.018) and mild or asymptomatic versus moderate to severe infections (p=0.018).

We sought to investigate why observed neutralizing antibody titers against BA.2 were comparable to, albeit slightly higher than BA.1 for BA.1 breakthrough infections. Principal component analysis (PCA) plots revealed that BA.1 was more closely related to WT and Delta based on antigenic distance as compared to BA.2 **(Figure 3A).** In addition, the antigenic distance between BA.2 and BA.1 was large, similar to that between BA.2 and the ancestral WT lineage targeted by the vaccine (**Figure 3A,** 6.56 versus 6.53 and **Figure 3B,** 4.93 versus vs. 4.99). However, as expected, the centroid of the BA.1 cluster, representing an approximation of the infecting BA.1 variant, was positioned much closer to the cultured BA.1 virus used in the neutralization experiments than the BA.2 virus (**Figure 3A,** 0.92 versus 6.53 and **Figure 3B,** 0.20 versus 4.93). Thus, antigenic similarity alone did not explain why titers of BA.2 were found to be higher than BA.1 in BA.1 breakthrough infections.

Discussion

This study employed live virus assays to quantify neutralizing antibody titers in 86 Delta or BA.1 infected subjects who were either unvaccinated, vaccinated but unboosted, or boosted. Notably, neutralizing antibody responses against BA.2 were mostly higher, albeit slightly, than those against BA.1, regardless of vaccination status or infecting variant (BA.1 or Delta). In unvaccinated BA.1 and Delta infections, cross-variant neutralizing responses were weak or non-existent, while in vaccinated breakthrough infections, neutralization responses were either strong (against Delta) or moderate (against BA.1 and BA.2). We also did not detect any significant differences in neutralizing antibody titers between unboosted and boosted breakthrough infections. Taken together, these findings indicate that breakthrough infections in vaccinated but not unvaccinated individuals elicit moderate to strong neutralizing responses against BA.2, and that prior boosting does not significantly enhance this response.

Our finding of consistently higher neutralizing antibody responses against BA.2 in the setting of BA.1 breakthrough infection is unexpected. The failure to mount an enhanced response to BA.1 in BA.1 breakthrough infections relative to BA.2, which is antigenically distinct **(Figure 3)** [8], suggests at least three non-mutually exclusive possibilities. First, BA.1 infection, whether in unvaccinated or vaccinated individuals, may be inherently less immunogenic, including against itself (BA.1), than infection from other variants [9-11]. Interestingly, neutralizing antibody levels were not substantially different in a primate study comparing the original approved WT vaccine to an updated vaccine specifically targeting the BA.1 variant [21]. Another study looking at boosted but uninfected individuals found higher neutralizing antibody titers against BA.2 as compared to BA.1 [22]. The apparent decreased immunogenicity of BA.1 is possibly related to different conformational changes in the S (spike) protein of BA.1 that may

affect fusion of the virus to the cell membrane and thus influence antigenicity and the humoral immune response [23, 24]. Second, hybrid neutralizing immunity may be primarily driven through vaccination and not the infecting variant, consistent with our results and those from another study [25]. Third, here we used live virus to evaluate neutralization, in contrast to other reports typically using pseudoviruses [26]. Differences in capsid proteins other than the S (spike), including the E (fusion), M (matrix), and N (nucleoprotein) and their potential involvement during infection may explain the differences in antibody response to BA.1 compared to other variants. Importantly, the observed boost in immunity against BA.1 and BA.2 induced by BA.1 breakthrough infection will not necessarily protect against future infection as the Omicron subvariants BA.2.12.1 and BA.4/BA.5 have continued to evolve with increasing escape from neutralizing antibodies [32].

Vaccinated patients with mild or asymptomatic BA.1 breakthrough infections had significantly higher neutralizing antibody titers against BA.2 than BA.1 (Figure 1B), regardless of their immune status. Additionally, BA.1 breakthrough infections in immunocompetent, but not immunocompromised patients, had higher neutralizing antibody titers against BA.2 than BA.1. Taken together, these results suggest that immunocompromised patients hospitalized with BA.1 are less likely to mount effective antibody responses against BA.2 after recovery. As expected, neutralizing antibody titers against Delta and wild-type lineages in immunocompetent patients were significantly higher than those in immunocompromised patients. However, there was no significant difference in titers against BA.1 or BA.2 between immunocompetent and immunocompromised patients. These findings are consistent with the effectiveness of the vaccine in boosting immune protection against more less divergent lineages such as Delta and WT but decreasing effectiveness against the more divergent Omicron lineages.

In this study, we did not detect significant differences in antibody titers against BA.1 or BA.2 between unboosted and boosted individuals following BA.1 breakthrough infection, consistent with findings from another report [27]. This may be due in part to the timing of the breakthrough infection relative to when vaccinated individuals received their last dose. Antibody titers have been shown to wane over time, with boosted individuals generally starting at a higher baseline [11]. Alternatively, boosting with a vaccine targeted against wild-type ancestral virus, while further enhancing protection against hospitalizations and death from severe COVID-19 [28, 29] as well as immunity against closely related strains such as Delta [11, 30], may contribute much less towards neutralizing immunity against highly divergent variants such as Omicron BA.1 and BA.2.

Limitations of the Study

There are several limitations of the study. We analyzed remnant Delta or BA.1 positive biobanked samples, and thus the number of convalescent samples was limited by availability. The analyses stratified by clinical severity were likely underpowered to detect small effects. We were unable to definitively confirm the variant identification for a small percentage of samples. Vaccination status and other clinical metadata were gathered using retrospective chart review rather than prospectively; thus, any inconsistency or error in the electronic medical records would lead to inaccuracies in the extracted clinical metadata.

Author Contributions

N.B., M.K.M., C.H., and C.Y.C. conceived and designed the study. N.B., V.S., K.A., P.S., A.S.-G., J.N., J.P.-R., R.D.S., and D.A.W. identified and collected patient samples and clinical metadata. B.W., K.F., A.S.G., and Y.Z. sequenced SARS-CoV-2 genomes for variant identification. M.K.M. and S.B. ran the live virus neutralization assays. N.B., M.K.M., V.S., C.H., and C.Y.C analyzed the neutralization, sequencing, and clinical data. M.O., D.A.W., R.A., J.D.K., C.H., and C.Y.C. provided administrative, technical, or material support. C.H. and C.Y.C. supervised the study. N.B., M.K.M., and C.Y.C. wrote the manuscript and prepared the figures. All authors read and edited the manuscript and agree to its contents.

Conflict of Interest Disclosures: M.O. is a scientific advisory board member for Invisishield Technologies, Ltd. The other authors declare no conflicts of interest.

Acknowledgements

We would like to acknowledge staff members at the UCSF Clinical Laboratories and the UCSF Clinical Microbiology Laboratories for their help in identifying and retrieving patient whole blood samples.

Funding

This work was funded in part by US CDC Epidemiology and Laboratory Capacity (ELC) for Infectious Diseases Grant 6NU50CK000539 to the California Department of Public Health

(COVIDnet) (M.K.M., C.H., D.A.W., and C.Y.C.), the Innovative Genomics Institute (IGI) at UC Berkeley and UC San Francisco (C.Y.C.), the James B. Pendleton Charitable Trust (M.O.), US Centers for Disease Control and Prevention contracts 75D30121C10991 (C.Y.C.) and 75D30121C10991 (J.D.K.), National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID) grant K23AI146268 (J.D.K.), National Institutes of Health (NIH)/National Institute of Child Health and Human Development (NICHD) grant R61HD105618 (C.Y.C.). The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views or opinions of the California Department of Public Health or the California Health and Human Services Agency.

1 **Tables**

Characteristic		Unvaccinated Delta	Unvaccinated Delta (%)	Unvaccinated BA.1	Unvaccinated BA.1 (%)	p-value
Reported sex	Female	4	28.6%	6	60.0%	0.21
	Male	10	71.4%	4	40.0%	
Age	>65	2	14.3%	4	40.0%	0.19
	18-65	12	85.7%	6	60.0%	
Disease Severity	asymptomatic	1	7.1%	3	30.0%	-
	mild	4	28.6%	2	20.0%	
	moderate	5	35.7%	2	20.0%	
	severe	4	28.6%	3	30.0%	
Status	immunocompetent	9	64.3%	6	60.0%	1
	immunocompromised	5	35.7%	4	40.0%	
	Total	**14**		**10**		

Characteristic		Vaccine Breakthrough Delta	Vaccine Breakthrough Delta (%)	Vaccine Breakthrough BA.1	Vaccine Breakthrough BA.1 (%)	p-value
Reported sex	Female	6	24.0%	7	50.0%	0.16
	Male	19	76.0%	7	50.0%	
Age	>65	12	48.0%	5	35.7%	0.52
	18-65	13	52.0%	9	64.3%	
Disease Severity	asymptomatic	1	4.0%	2	14.3%	-
	mild	7	28.0%	6	42.9%	
	moderate	4	16.0%	1	7.1%	
	severe	13	52.0%	5	35.7%	
Vaccine type	Moderna	9	36.0%	4	28.6%	-
	Pfizer	11	44.0%	7	50.0%	
	J&J	4	16.0%	2	14.3%	
	unk	1	4.0%	1	7.1%	

Status	immunocompetent	17	68.0%	9	64.3%	1
	immunocompromised	8	32.0%	5	35.7%	
	Total	**25**		**14**		
Characteristic		**Booster Breakthrough Delta**	**Booster Breakthrough Delta (%)**	**Booster Breakthrough BA.1**	**Booster Breakthrough BA.1 (%)**	
Reported sex	Female	2	66.7%	9	45.0%	0.59
	Male	1	33.3%	11	55.0%	
Age	>65	2	66.7%	5	25.0%	0.21
	18-65	1	33.3%	15	75.0%	
Disease Severity	asymptomatic	0	0.0%	1	5.0%	-
	mild	0	0.0%	10	50.0%	
	moderate	1	33.3%	0	0.0%	
	severe	2	66.7%	9	45.0%	
Booster type	Moderna	3	100.0%	11	55.0%	0.25
	Pfizer	0	0.0%	9	45.0%	
Status	immunocompetent	0	0.0%	13	65.0%	0.068
	immunocompromised	3	100.0%	7	35.0%	
	Total	**3**		**20**		

Table 1. Clinical and demographic metadata in unvaccinated, vaccinated, and boosted patients infected with Delta or BA.1. P-values were calculated using Fisher's exact test for two-sided analysis of categorical contingency tables. The table includes all individuals in the study (n=86).

FIGURE LEGENDS

Figure 1. Comparison of neutralizing antibody titers within subgroups of unvaccinated, vaccinated but unboosted, or boosted patients infected with BA.1 or Delta. The bar plots show median neutralizing antibody titers against BA.1 (lavender), BA.2 (dark purple), Delta (red), and ancestral wild-type (WT) (green) lineages. **(A)** Median neutralizing antibody titers against BA.1, BA.2, Delta, and WT. **(B)** Median neutralizing antibody titers against BA.1, BA.2, Delta., and WT for combined unboosted and boosted patients with BA.1 breakthrough infection, stratified by immunocompromised status, disease severity, and type of vaccine received. **(C)** Neutralizing antibody titers for individual patients are plotted. The neutralizing antibody response is defined as weak (NT_{50}<100), moderate (NT_{50}≥100 and <1,000), or strong (NT_{50}≥1000). P-values are calculated using the Wilcoxon signed-rank nonparametric test for paired samples or using the Wilcoxon-Mann-Whitney nonparametric test for unpaired samples. Abbreviations NS, non-significant, *, p<0.05, **, p<0.01, ***, p<0.001.

Figure 2. Comparison of neutralizing antibody titers between subgroups of unvaccinated, vaccinated but unboosted, or boosted patients infected with BA.1 or Delta. Bar plots show median neutralizing antibody titers against BA.1 (lavender), BA.2 (dark purple), Delta (red), and ancestral WT (green) lineages. Median neutralizing antibody titers against BA.1 **(A)**, BA.2 **(B)**, Delta **(C)**, and ancestral wild-type (WT) **(D)** lineages are shown. **(E,F)** Median neutralizing antibody titers against BA.1 **(E)** or BA.2 **(F)** in patients infected with BA.1 or Delta, stratified by immunocompromised status, disease severity, and type of vaccine received. P-values are calculated using the Wilcoxon-Mann-Whitney nonparametric test for unpaired samples. Abbreviations NS, non-significant, *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Figure 3. Principal component analysis plots showing the antigenic relatedness of the SARS-CoV-2 variants based on coding mutations. (A) Antigenic relatedness between SARS-CoV-2 variants based on all coding mutations in the viral genome. **(B)** Antigen relatedness based on coding mutations in the spike gene. The 95% confidence ellipses associated with datasets of viral genomes representing the Delta (red), BA.1 (lavender), and BA.2 (purple) variants are shown, along with the centroid of each cluster (white outlined circle). Individual genomes associated with other variants (ancestral wild-type (WT), Beta, Alpha, Gamma, Epsilon, Lambda, and Mu) are denoted by colored circles, while genomes associated with the Delta, BA.1, and BA.2 cultures used in the neutralization assay are denoted by colored circles highlighted with a black outline. Lines connecting the individual genomes and/or centroids related to the neutralizing antibody response against BA.1 (solid) and BA.2 (dotted) are shown.

REFERENCES

1. Elbe S, Buckland-Merrett G. Data, disease and diplomacy: GISAID's innovative contribution to global health. Glob Chall **2017**; 1:33-46.

2. Lambrou AS, Shirk P, Steele MK, et al. Genomic Surveillance for SARS-CoV-2 Variants: Predominance of the Delta (B.1.617.2) and Omicron (B.1.1.529) Variants - United States, June 2021-January 2022. MMWR Morb Mortal Wkly Rep **2022**; 71:206-11.

3. Mullen JL, Tsueng G, Latif AA, et al. outbreak.info. Available at: https://outbreak.info/. Accessed 4-23-22 2022.

4. Arora S, Grover V, Saluja P, et al. Literature Review of Omicron: A Grim Reality Amidst COVID-19. Microorganisms **2022**; 10.

5. Araf Y, Akter F, Tang YD, et al. Omicron variant of SARS-CoV-2: Genomics, transmissibility, and responses to current COVID-19 vaccines. J Med Virol **2022**; 94:1825-32.

6. Qassim SH, Chemaitelly H, Ayoub HH, et al. Effects of BA.1/BA.2 subvariant, vaccination, and prior infection on infectiousness of SARS-CoV-2 Omicron infections. medRxiv **2022**.

7. Wolter N, Jassat W, Walaza S, et al. Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. Lancet **2022**; 399:437-46.

8. Mykytyn AZ, Rissmann M, Kok A, et al. Omicron BA.1 and BA.2 are antigenically distinct SARS-CoV-2 variants. medRxiv **2022**.

9. Rossler A, Knabl L, von Laer D, Kimpel J. Neutralization Profile after Recovery from SARS-CoV-2 Omicron Infection. N Engl J Med **2022**.

10. Suryawanshi RK, Chen IP, Ma T, et al. Limited Cross-Variant Immunity after Infection with the SARS-CoV-2 Omicron Variant Without Vaccination. medRxiv **2022**.

11. Servellita V, Syed AM, Morris MK, et al. Neutralizing immunity in vaccine breakthrough infections from the SARS-CoV-2 Omicron and Delta variants. Cell **2022**.

12. Walls AC, Sprouse KR, Bowen JE, et al. SARS-CoV-2 breakthrough infections elicit potent, broad, and durable neutralizing antibody responses. Cell **2022**; 185:872-80 e3.

13. Wratil PR, Stern M, Priller A, et al. Three exposures to the spike protein of SARS-CoV-2 by either infection or vaccination elicit superior neutralizing immunity to all variants of concern. Nat Med **2022**; 28:496-503.

14. Yamasoba D, Kimura I, Nasser H, et al. Virological characteristics of SARS-CoV-2 BA.2 variant. bioRxiv **2022**.

15. Kelly JD, Lu S, Anglin K, et al. Magnitude and determinants of SARS-CoV-2 household transmission: a longitudinal study. (under review) **2022**.

16. Deng X, Gu W, Federman S, et al. Genomic surveillance reveals multiple introductions of SARS-CoV-2 into Northern California. Science **2020**.

17. Grubaugh ND, Ladner JT, Kraemer MUG, et al. Genomic epidemiology reveals multiple introductions of Zika virus into the United States. Nature **2017**; 546:401-5.

18. Rambaut A. Phylodynamic analysis of 176 genomes. In: virological.org, ed. Vol. 2020. Edinburgh, UK, **2020**.

19. Aksamentov K, Roemer C, Hodcroft EB, Neher RA. Nextclade: clade assignment, mutation calling and quality control for viral genomes. J. Open Source Softw. **2021**; 6:3773.

20. Team" RC. R: A Language and Environmental for Statistical Computing. In: Computing RFfS, ed. Vienna, Austria, **2018**.

21. Gagne M, Moliva JI, Foulds KE, et al. mRNA-1273 or mRNA-Omicron boost in vaccinated macaques elicits similar B cell expansion, neutralizing responses, and protection from Omicron. Cell **2022**.

22. Marking U, Havervall S, Norin NG, et al. High rate of BA.1, BA.1.1 and BA.2 infection in triple vaccinated. medRxiv **2022**.

23. Suzuki R, Yamasoba D, Kimura I, et al. Attenuated fusogenicity and pathogenicity of SARS-CoV-2 Omicron variant. Nature **2022**; 603:700-5.

24. Tan CW, Chia WN, Young BE, et al. Pan-Sarbecovirus Neutralizing Antibodies in BNT162b2-Immunized SARS-CoV-1 Survivors. N Engl J Med **2021**; 385:1401-6.

25. Seaman MS, Siedner MJ, Boucau J, et al. Vaccine Breakthrough Infection with the SARS-CoV-2 Delta or Omicron (BA.1) Variant Leads to Distinct Profiles of Neutralizing Antibody Responses. medRxiv **2022**.

26. Chmielewska AM, Czarnota A, Bienkowska-Szewczyk K, Grzyb K. Immune response against SARS-CoV-2 variants: the role of neutralization assays. NPJ Vaccines **2021**; 6:142.

27. Quandt J, Muik A, Salisch N, et al. Omicron breakthrough infection drives cross-variant neutralization and memory B cell formation. bioRxiv **2022**.

28. Andrews N, Stowe J, Kirsebom F, et al. Effectiveness of COVID-19 booster vaccines against COVID-19-related symptoms, hospitalization and death in England. Nat Med **2022**; 28:831-7.

29. Arbel R, Hammerman A, Sergienko R, et al. BNT162b2 Vaccine Booster and Mortality Due to Covid-19. N Engl J Med **2021**; 385:2413-20.

30. Perez-Then E, Lucas C, Monteiro VS, et al. Neutralizing antibodies against the SARS-CoV-2 Delta and Omicron variants following heterologous CoronaVac plus BNT162b2 booster vaccination. Nat Med **2022**; 28:481-5.

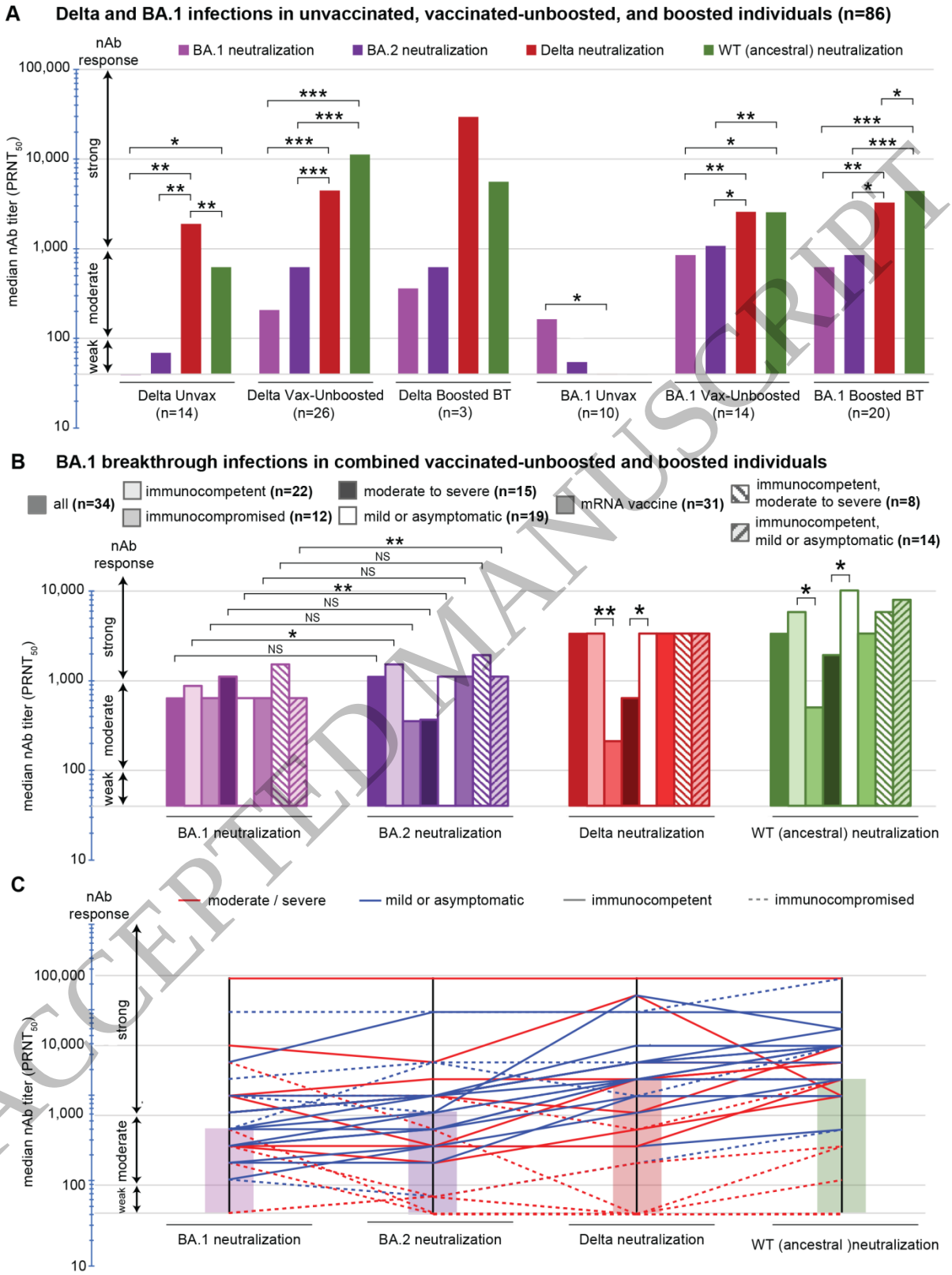

Figure 1
193x251 mm (x DPI)

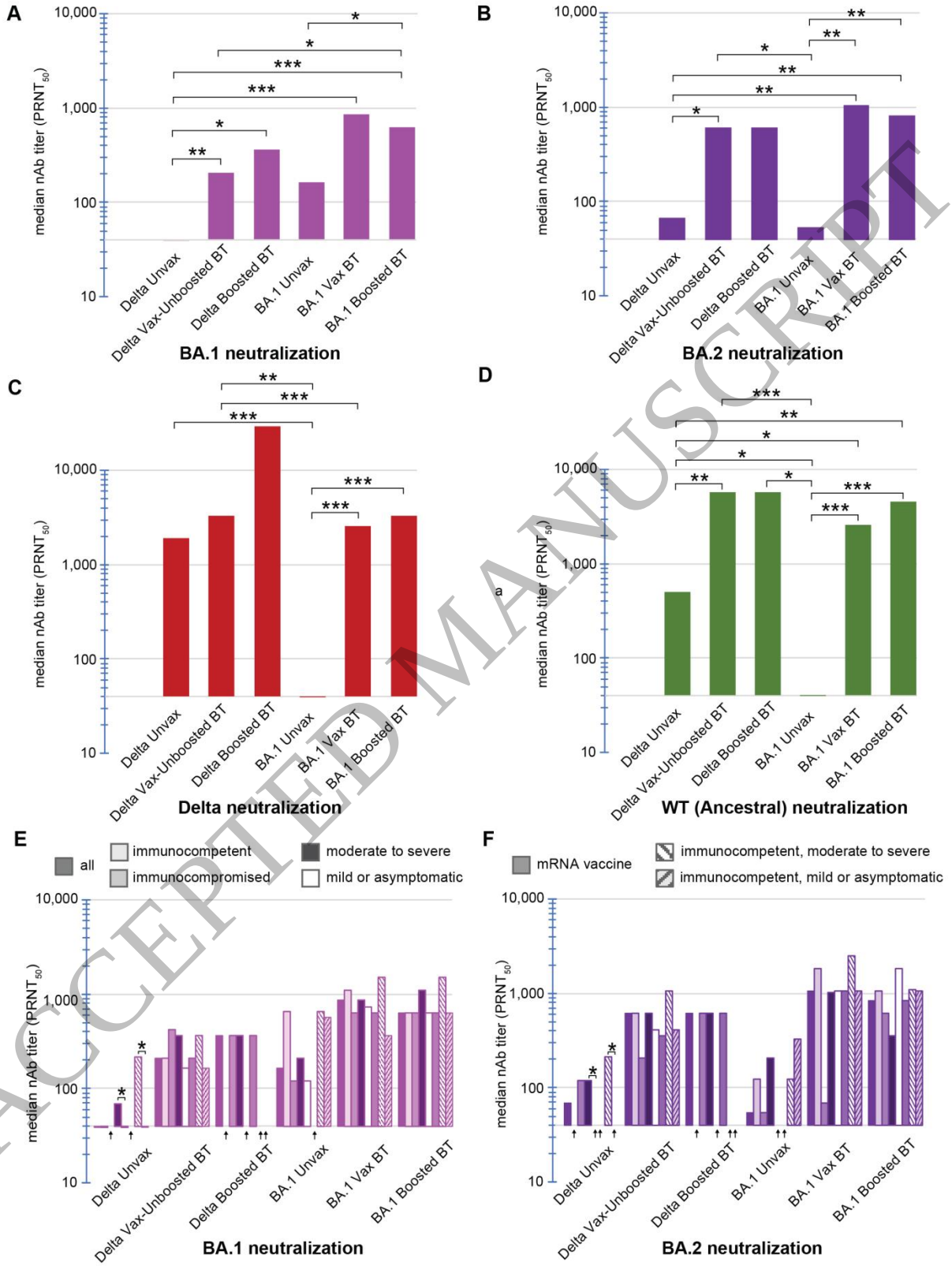

Figure 2
195x254 mm (x DPI)

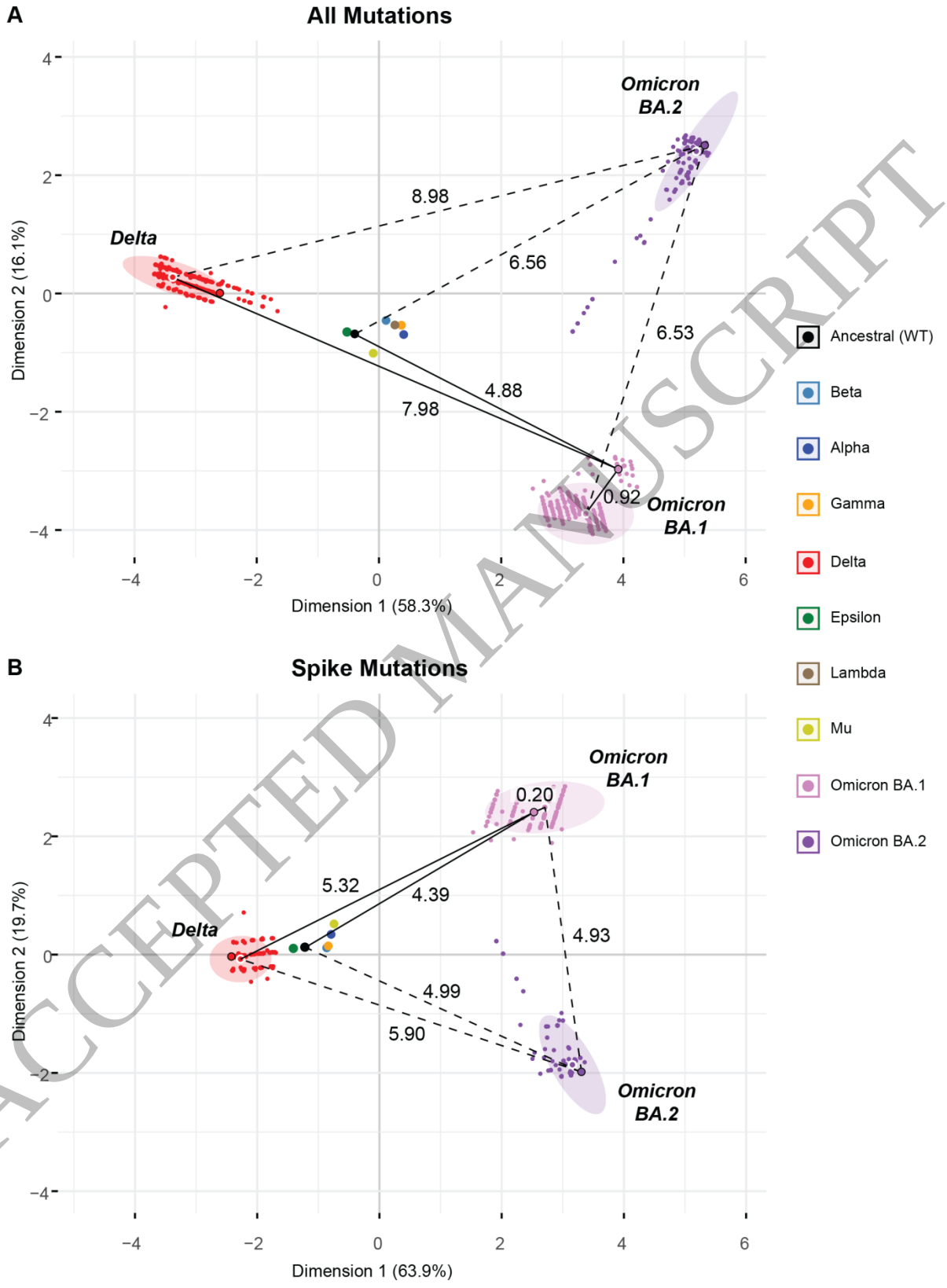

Figure 3
180x236 mm (x DPI)