



Limited neutralisation of the SARS-CoV-2 Omicron subvariants BA.1 and BA.2 by convalescent and vaccine serum and monoclonal antibodies

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Summary

Background In recent months, Omicron variants of SARS-CoV-2 have become dominant in many regions of the world, and case numbers with Omicron subvariants BA.1 and BA.2 continue to increase. Due to numerous mutations in the spike protein, the efficacy of currently available vaccines, which are based on Wuhan-Hu 1 isolate of SARS-CoV-2, is reduced, leading to breakthrough infections. Efficacy of monoclonal antibody therapy is also likely impaired.

Methods In our *in vitro* study using A549-AT cells constitutively expressing ACE2 and TMPRSS2, we determined and compared the neutralizing capacity of vaccine-elicited sera, convalescent sera and monoclonal antibodies against authentic SARS-CoV-2 Omicron BA.1 and BA.2 compared with Delta.

Findings Almost no neutralisation of Omicron BA.1 and BA.2 was observed using sera from individuals vaccinated with two doses 6 months earlier, regardless of the type of vaccine taken. Shortly after the booster dose, most sera from triple BNT162b2-vaccinated individuals were able to neutralise both Omicron variants. In line with waning antibody levels three months after the booster, only weak residual neutralisation was observed for BA.1 (26%, $n = 34$, o median NT₅₀) and BA.2 (44%, $n = 34$, o median NT₅₀). In addition, BA.1 but not BA.2 was resistant to the neutralising monoclonal antibodies casirivimab/imdevimab, while BA.2 exhibited almost a complete evasion from the neutralisation induced by sotrovimab.

Interpretation Both SARS-CoV-2 Omicron subvariants BA.1 and BA.2 escape antibody-mediated neutralisation elicited by vaccination, previous infection with SARS-CoV-2, and monoclonal antibodies. Waning immunity renders the majority of tested sera obtained three months after booster vaccination negative in BA.1 and BA.2 neutralisation. Omicron subvariant specific resistance to the monoclonal antibodies casirivimab/imdevimab and sotrovimab emphasizes the importance of genotype-surveillance and guided application.

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Research in context

Evidence before this study

The SARS-CoV-2 Omicron variant was first described in South Africa in November 2021 and displaced the previously dominant Delta variant in short time suggesting increased infectivity. Multiple substitutions in Omicron Spike (S), particularly in the immunological relevant receptor-binding-domain (RBD), raised concerns about resistance to previously existing immunity (Pulliam et al., 2021, Tegally et al., 2021) and reduced vaccine efficacy (Majumbar et al., 2021, Wang et al., 2021). December 2021, our preliminary study provided early comprehensive data on reduced neutralisation of Omicron subvariant BA.1 to neutralising antibody-mediated neutralisation (Wilhelm et al. 2021). However, most recently, a descendent of the Omicron variant termed BA.2 was about to spread. Due to distinct mutations in S, both SARS-CoV-2 Omicron subvariants BA.1 and BA.2 were assumed to significantly limit the antibody-mediated neutralisation and increases the risk of (re)infections. At the beginning of this study, however, the neutralisation capacity of vaccine-elicited or convalescent sera as well as monoclonal antibodies against the barely described Omicron variant s BA.1 and BA.2 remained unclear. In addition, whether administered monoclonal antibodies including sotrovimab retain sufficient activity against both variants (BA.1 and BA.2) has not yet been conclusively determined.

Added value of this study

Previous work has shown reduced neutralisation of Omicron subvariant BA.1 by vaccine-elicited and convalescence sera as well as by monoclonal antibodies casirivimab/imdevimab. The results of this study extend that data and demonstrate that both SARS-CoV-2 Omicron subvariants BA.1 and BA.2 escape neutralisation by antibodies elicited by vaccination, previous infection with SARS-CoV-2 or the monoclonal antibodies casirivimab/imdevimab and sotrovimab used in clinics at the time of the study. Moreover, three months after booster vaccination, waning antibody levels renders the majority of tested sera negative in BA.1 and BA.2 neutralisation.

Implications of all the available evidence

Due to the rapid global spread of the SARS-CoV-2 Omicron sub-variants BA.1 and BA.2, public health related questions about the duration and effectiveness of vaccine protection and the need for a vaccine booster against these variants has arisen. Using authentic SARS-CoV-2 isolates we have gathered highly relevant in vitro data that contributes to the assessment of the pandemic situation after the emergence of the Omicron variants BA.1 and BA.2. Given the controversial data on the efficacies of monoclonal antibodies against Omicron sub-lineages, this study revealed a BA.1 and BA.2 specific resistance to the monoclonal antibodies casirivimab/imdevimab and sotrovimab, respectively. Omicron subvariant specific resistance to the monoclonal

antibodies casirivimab/imdevimab and sotrovimab emphasizes the importance of genotype surveillance and guided application.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by infection with the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). The origin of the SARS-CoV-2 outbreak was described in the metropolis of Wuhan (China) in December 2019 and rapidly evolved into a global pandemic.^{1,2} Since the onset of the pandemic, over 507 million infections and a total of 6.2 million SARS-CoV-2 associated deaths have been reported globally,³ (04-27-2022). The emergence of new viral variants continues to pose a challenge to public health.

SARS-CoV-2 Spike (S) binds the human angiotensin-converting enzyme 2 (ACE2) receptor and is subsequently cleaved by the transmembrane protease serine subtype 2 (TMPRSS2) to enter the host cell and initiate replication.⁴ During viral replication nucleotide substitution, insertion, or deletion might arise in S, but only certain mutations are able to change the viral properties in a significant manner affecting transmissibility, susceptibility to monoclonal antibodies (mAb) used for treatment and prophylaxis, and to antibodies from convalescent and vaccine-elicited sera.⁵

The SARS-CoV-2 variant Omicron (B.1.1.529) was first identified in South Africa on November 9, 2021⁶ raising concerns about reduced vaccine efficacy and increased risk of reinfection⁷ due to multiple substitutions S (Figure 1, Supplementary Table 1).^{8,9} Consequently, this variant was classified by the World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC) as a variant of concern (VoC) on 11/26/2021.

Recently, Omicron has been classified into subvariants including BA.1 and BA.2 sharing common but also a set of unique mutations.^{10,11} Compared to the parental variant (B.1), Omicron BA.1 S has 30 non-synonymous substitutions, three small deletions and an insertion (Figure 1, Supplementary Table 1). Fifteen of these mutations are in the receptor-binding-domain (RBD), a major target of neutralising antibodies (NAbs).^{5,12} Compared to BA.1, which has 13 unique mutations in S, subvariant BA.2 has 7 exclusive amino acid exchanges¹⁰ (Figure 1, Supplementary Table 1) raising the demand to evaluate the neutralisation profile of this subvariant. Several of the S mutations observed in Omicron BA.1 were reported in preceding variants of concern like Alpha, Beta, Gamma, Delta as well as in variants of interest (VoI) such as Kappa, Zeta, Lambda, and Mu that were associated with higher transmissibility and immune escape (Supplementary Table 1). Due

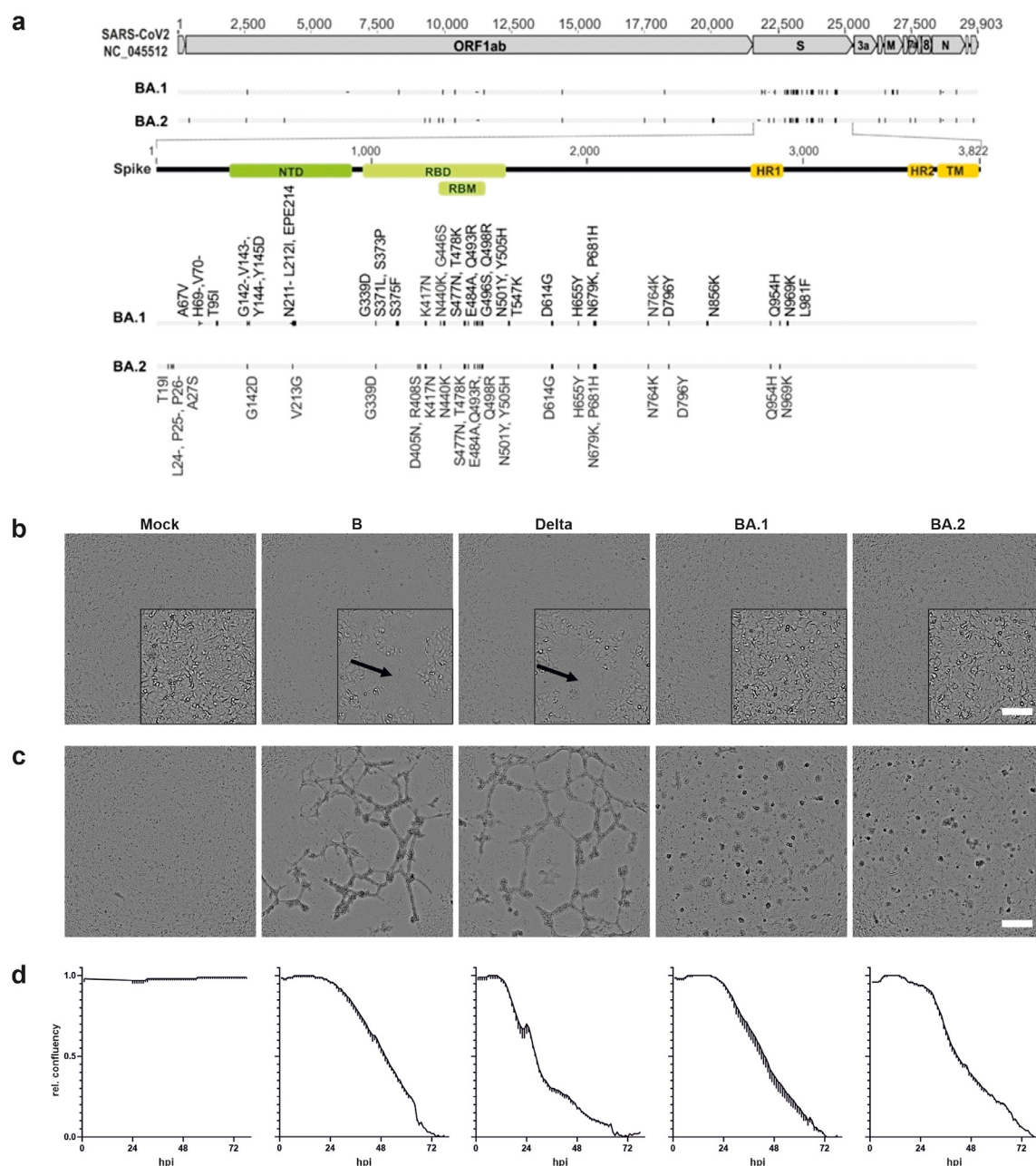


Figure 1. Genotypic and phenotypic features of SARS-CoV-2 variants used in this study. **a**) Schematic drawing of SARS-CoV-2 BA.1 and BA.2 genomes compared to isolate Wuhan-Hu-1 (NC_045512) indicating spike positions common and unique within each of the indicated subvariant genome. The numbers denote nucleotide positions based on the reference strain NC_045512. ORFs based on reference sequence NC_045512 are shown as grey boxes. The receptor-binding domain (RBD), receptor-binding motif (RBM) as well as the N-terminal domain (NTD) are highlighted by green boxes. Heptad repeat domains 1 (HR1) and 2 (HR2), and the transmembrane region (TM) are indicated by orange boxes. Nucleotide substitutions compared to the reference sequence are indicated in the lower section. **b-c**) Representative cytopathic effect (CPE) formation and **d**) growth kinetics of SARS-CoV-2 variants in A549-AT cells (at least $n = 12$ biological replicates). CPE formation was analysed at **b**) peak syncytia formation and **c**) at the onset of cell lysis. Hours post infection (hpi). Scale bar in low-magnification images, 500 μm . Scale bar in high-magnification images, 180 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to the high accumulation of mutations in Omicron S, which have been already reported in previous VoCs and Vols, a reduction in the neutralisation activity at peak-immunity is expected.^{13–15} So far, Beta and Mu had exhibited the most severe immune evading capacities.^{13,14} Omicron BA.1 exhibits limited neutralisation by sera after vaccination, but also breakthrough infections.^{15–22} In countries where Omicron variants were introduced, rapid spread was observed despite advancing vaccination campaigns, indicating increased infectivity and resistance to pre-existing immunity for both variants.²³ It is of particular interest whether prior immunity protects against breakthrough infections.

In addition, there is only limited knowledge about the duration of protection against these subvariants by neutralising antibodies, as anti-Spike IgG levels induced by vaccination or natural infection decrease over time.^{24–26} Vaccine antibody levels start to wane continuously leading to reduced protection against reinfections and breakthrough infections four months after the thirds dose.²⁷ However, it is still unclear whether this applies equally to both Omicron subvariants.

In this study, we sought to perform direct comparison between BA.1 and BA.2 Omicron variants in terms of their ability to escape antibody-mediated neutralisation *in vitro*. Using authentic SARS-CoV-2 variants, we systematically examined neutralisation efficiencies using 165 sera collected cross-sectional at different time points from individuals who received homologous, heterologous vaccine regimens or had breakthrough infections. Since treatment with single monoclonal antibodies against variants carrying certain mutations including E484K and K417N.^{14,28} were ineffective, we also evaluated the activity of single and combined monoclonal antibodies against Omicron variants.

Methods

Ethics statement

The retrospective study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the Ethics Committee of the Faculty of Medicine at Goethe University Frankfurt (2021-201, 20-864, and 250719).

Human sera

Peripheral blood was collected from vaccinated individuals before and 0.5 (two weeks) or three months after the booster vaccination with BNT162b2 (Pfizer-BioNTech). Samples from double BNT162b2-vaccinated (2xBNT_{6m}) and sera from double BNT162b2-vaccinated and SARS-CoV-2 infected individuals (2xBNT/SARS-CoV-2 infection_{4m}) were obtained from the Impfcare study.²⁹ Sera from triple BNT162b2-vaccinated individuals (2xBNT/BNT_{0.5m} and 2xBNT/BNT_{3m}) and sera from double mRNA-1273 vaccinated and additionally

BNT162b2-boosted (2xMOD/BNT_{0.5m}) individuals were obtained from an internal study at the University Hospital Frankfurt (Frankfurt am Main, Germany). Sera from double mRNA-1273 vaccinated (2xMOD_{6m}) individuals were obtained from an internal study at the University Hospital Frankfurt (Frankfurt am Main, Germany) and a heterologous vaccination study (unpublished). Sera from heterologous ChAdOx1 and BNT162b2-vaccinated (1xChAd/1xBNT_{6m}) and BNT162b2-boosted (1xChAd/2xBNT_{0.5m}) donors were obtained from a heterologous vaccination study (unpublished). See Supplementary Table 3 for detailed information on sample donors. Sera were isolated by centrifugation 2000 x g for 10 min. All sera were inactivated at 56°C for 30 min and stored at –20°C until use. All samples available and relevant for comparison were included for analysis.

Virus identification and sequencing

SARS-CoV-2 isolates were obtained from nasopharyngeal swabs of travel returnees from South Africa and Zimbabwe as screened by the Public Health Office of the City of Frankfurt am Main, Germany. Swab material was suspended in 1.5 mL phosphate-buffered saline (PBS) and split for RNA-isolation and a viral outgrowth assay. RNA was isolated using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. RNA was subjected to variant specific RT-qPCR genotyping and Oxford Nanopore sequencing.

Library preparation, sequencing and bioinformatic analysis

RNA samples extracted from swabs were used for library preparation according to NEBNext ARTIC Standard Protocol (New England Biolabs Ipswich, Massachusetts, USA) (dx.doi.org/10.17504/protocols.io.budxns7n) using the Artic nCoV-2019 V4 primers (IDT, Coralville, Iowa, USA). Libraries were generated using ligation sequencing kit SQK-LSK109, native barcoding expansion kit EXP-NBD104 and FLO-MIN106D R9.4.1 flow cell according to the standard protocol (Oxford Nanopore Technologies, UK) and sequenced on MinION Mk1c (Oxford Nanopore Technologies, UK) for 8 h with basecalling and demultiplexing options enabled. The obtained FASTQ files were filtered and analysed using ARTIC pipeline (<https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html>). Primers (Supplementary Table 2) were spiked in to avoid dropouts while using the ARTIC protocol. First the alternative primers were pooled into two spike-in pools and then 0.2 µL per 4 µL (10 µM ARTIC V4 pool) were added into the respective master mix for a final per oligo concentration of ~15 nM.

See Figure 1 for schematic representation of the SARS-CoV-2 genome indicating spike positions for BA.1 and BA.2. Sequences are available on GISAID and GenBank under the following accession numbers: SARS-CoV-2

B.1.1.529 (BA.1) FFM-SIM0550/2021 (EPI_ISL_6959871; GenBank ID: OL800702), SARS-CoV-2 B.1.1.529 (BA.1) FFM-ZAF0396/2021 (EPI_ISL_6959868; GenBank ID: OL800703), SARS-CoV-2 B.1.617.2 FFM-IND8424/2021 (GenBank ID: MZ315141), SARS-CoV-2 BA.2 FFM-BA.2-3833/2022 (GenBank ID: OM617939), SARS-CoV-2 B FFM5/2020 (GenBank ID: MT358641), and SARS-CoV-2 B.1 FFM7/2020 (GenBank ID: MT358643).^{14,28,30}

Cell culture and virus propagation

A549-AT cells³¹ (based on A-549 derived from DSMZ, Braunschweig, Germany, no: ACC 107) stably expressing ACE2 and TMPRSS2 and Caco-2 cells (DSMZ, Braunschweig, Germany, no: ACC 169) were maintained in Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS), 4 mM L-glutamine, 100 IU/mL of penicillin, and 100 µg/mL of streptomycin at 37°C and 5% CO₂. All culture reagents were purchased from Sigma (St. Louis, MO, USA). Additional information regarding the cell lines are available at DSMZ (<https://www.dsmz.de>). Mycoplasma testing has been performed routinely. As described previously SARS-CoV-2 isolates were propagated using Caco-2 cells, which were selected for high permissiveness to SARS-CoV-2 infection by serial dilution and passaging.^{32,33} Cell-free cell culture supernatant containing infectious virus was harvested after complete cytopathic effect (CPE) and aliquots were stored at -80°C. Titres were determined by the median tissue culture infective dose (TCID₅₀) method as described by Spearman³⁴ and Kaerber³⁵ using Caco-2 cells. All cell culture work involving infectious SARS-CoV-2 was performed under biosafety level 3 (BSL-3) conditions. Sample inactivation for further processing was performed with previously evaluated methods.³⁶

Neutralisation and antiviral assays

SARS-CoV-2 anti-Spike IgG antibody concentrations were determined using the SARS-CoV-2 IgG II Quant assay and the Alinity I device (Abbott Diagnostics, Wiesbaden, Germany) with an analytical measurement range from 2.98–5680 binding antibody units per mL (BAU/mL). All sera were serially diluted (1:2) and incubated with 4000 TCID₅₀/mL of SARS-CoV-2 Delta or Omicron subvariants BA.1 and BA.2. Infected cells were monitored for cytopathic effect (CPE) formation 48 h post inoculation. Monoclonal antibody solutions containing imdevimab and casirivimab alone or in combination in equal ratios (1:1) were serially diluted (1:2) and incubated with 4000 TCID₅₀/mL of the indicated SARS-CoV-2 variant. After 48 h CPE formation was evaluated microscopically. Assays were performed testing each sample in a parallel approach comparing Delta and Omicron neutralisation. Representative result of the reduced susceptibility of convalescent and vaccine-

elicited sera against SARS-CoV-2 Delta compared with the parental strain harbouring D614G (Supplementary Figure 1).²⁸ Evaluation of monoclonal antibodies was quantified using SparkCyto 400 multimode imaging plate reader (Tecan) as described before.^{28,31} The term efficacy was used to refer to the neutralizing activity and the results are referred to immunogenicity.

Statistics

Figures and statistical analysis were generated with GraphPad Prism (version 9.3.1, GraphPad Software, LLC) and IBM SPSS Statistics (version 28.0.1.1, IBM). Statistical differences between the groups was calculated using the tests indicated in each figure legend. For statistical analysis the groups were assessed as independent. Statistical significance for IgG antibody responses after SARS-CoV-2 booster vaccination and age distribution of the groups was calculated by one-way ANOVA with Tukey's multiple comparison test (Figure 2). Statistical significance for antibody-mediated neutralisation of authentic SARS-CoV-2 variants was calculated by two-tailed, Wilcoxon Rank Sum Test (Figure 3). Further on, multivariate linear regression analysis was used to determine differences in waning antibody responses for SARS-CoV-2 variants Delta, BA.1 and BA.2 taking into account the demographic covariates gender and age (Figure 4 and Supplementary Table 4).

Role of funders

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Results

Cultivation of omicron subvariants BA.1 and BA.2

For the cultivation of both Omicron subvariants BA.1 and BA.2 we used Caco-2, which is an immortalized cell line of human colorectal adenocarcinoma cells. These cells have been selected for high permissiveness to SARS-CoV-2 infection by serial dilution and passaging as described previously.^{32,33} Original swab-derived material and the cultivated viral stocks were sequenced and the amino acid substitution of both isolates were compared with the parental SARS-CoV-2 sequence (Figure 1a, Supplementary Table 1). For the subsequent characterization of the isolates, lung epithelial derived A549-AT cells overexpressing the human angiotensin-converting enzyme 2 (ACE2) receptor as well as the transmembrane protease serine 2 (TMPRSS2) in a favourable ratio required for optimal SARS-CoV-2 entry^{4,31} were used. In comparison to the parental isolate harbouring the D614G mutation (B.1, FFM7)³⁰ and Delta²⁸ both resulting in large syncytia formation, we observed a distinct cytopathogenic effect (CPE) for

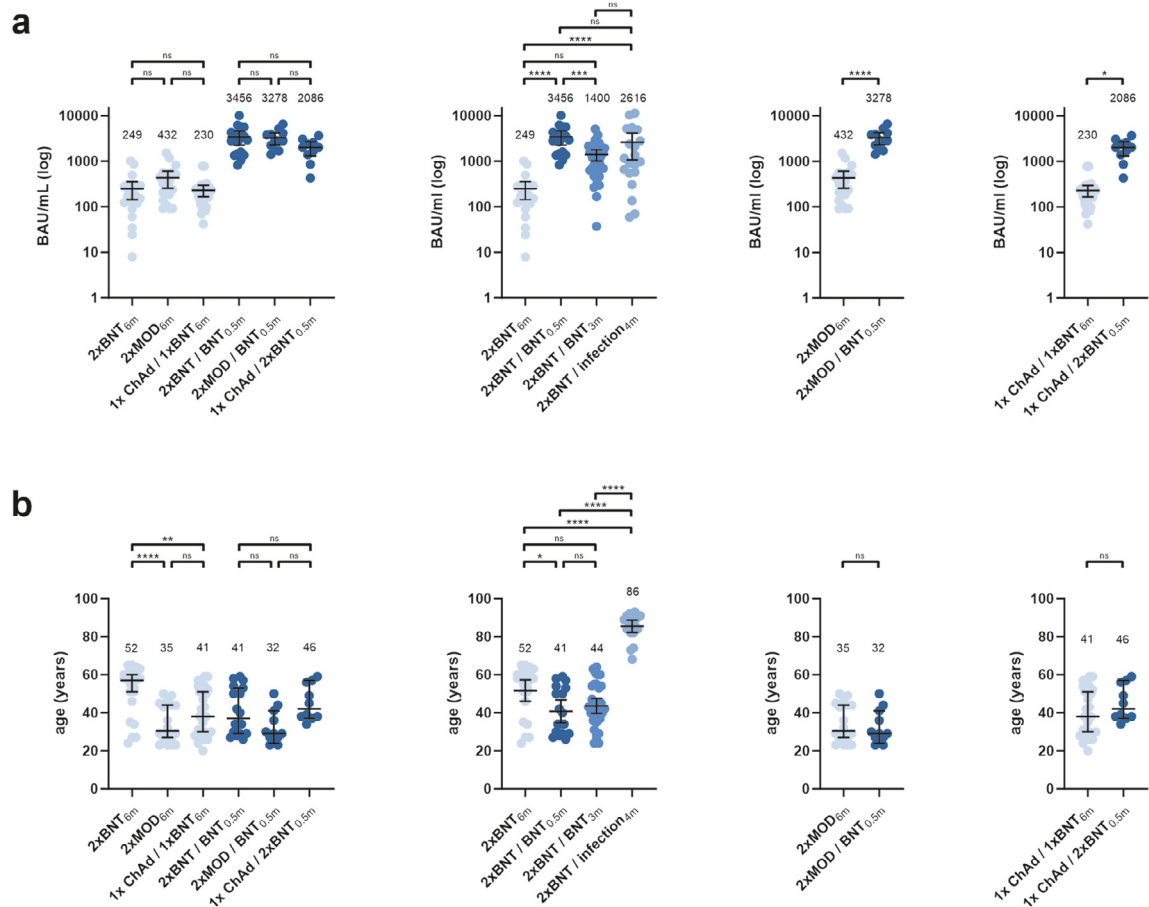


Figure 2. IgG antibody responses after SARS-CoV-2 booster vaccination and age distribution of the groups used in this study. a SARS-CoV-2 IgG antibody concentrations were determined and indicated as binding antibody units per millilitre (BAU/mL). **b** Age distribution of the serum donors grouped by vaccination scheme and sampling date. Bars indicate mean values with SD. BNT=BNT162b2; MOD=mRNA-1273; ChAd= ChAdOx1. Statistical significance was calculated by one-way ANOVA with Tukey's multiple comparison test. Asterisks indicate *p*-values as * (*p* < 0.05), ** (*p* < 0.01), *** (*p* < 0.001) and **** (*p* < 0.0001).

Omicron BA.1 and BA.2 showing small syncytia only (Figure 1b-c). This observation was in agreement with previous reports indicating a limited fusogenicity of the two Omicron subvariants.³⁷ Despite its small size, the CPE formation was suitable for semi-automated, microscopical characterisation of infected cells and neutralisation assays (Figure 1d).

Anti-Spike IgG levels after booster vaccination

Booster vaccinations have been recommended to overcome declining protection after a two-dose schedule.^{24–26} In this first part of the study, we investigated the increase in anti-Spike IgG titres resulting from booster vaccination with different vaccine regimen and examined the course after three months. To this end, 165 sera from individuals vaccinated with different vaccination regimens (pre and post booster vaccination) were tested for anti-Spike IgG antibody titres (Table 1,

Figure 2, and Supplementary Figure 2). All sera tested from individuals who had received two doses of either mRNA vaccines (BNT162b2 or mRNA-1273) or a heterologous vaccination (ChAdOx1 nCov-19 /BNT162b2) had comparably moderate anti-Spike IgG titres (230 – 432 mean BAU/mL) after 6 months (6.2 mean months (SD: 0.4) for 2xBNT162b2; 6.6 mean months (SD: 0.7) for 2xMOD; 5.8 mean months (SD: 0.2) for ChAdOx1 nCov-19 /BNT162b2) (Figure 2a, Table 1). Regardless of the used vaccine, booster vaccination resulted in increased antibody titres compared with individuals who had received only two vaccinations. At peak immunity, 0.5 months after the last dose (Figure 2a), all sera had a comparable increase of approx. 10 fold in anti-Spike IgG levels (2086 – 3456 mean BAU/mL) when compared with pre-booster titres.

In particular, sera from individuals who had received two doses of BNT162b2 (Table 1) had relatively low titres (249 mean BAU/mL), 6 month after last vaccination

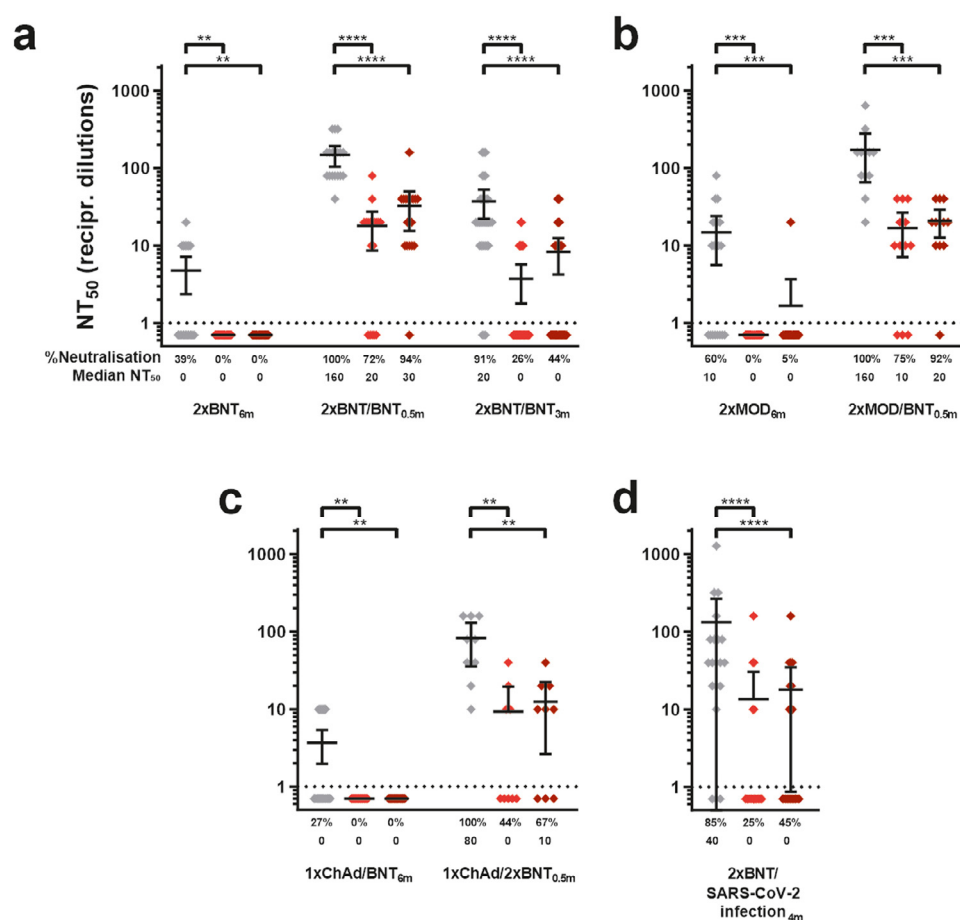


Figure 3. Antibody-mediated neutralisation of authentic SARS-CoV-2 Delta and Omicron subvariants BA.1 and BA.2. Values represent reciprocal dilutions micro-neutralisation titres resulting in 50% virus neutralisation (NT_{50}) of SARS-CoV-2 variants Delta (grey) and Omicron BA.1 (red), BA.2 (dark red). a) Neutralisation assays were performed using serum samples obtained from vaccinated individuals receiving the indicated vaccine schemes (sampling time after last vaccination/booster indicated in subscript): double BNT162b2-vaccinated ($2xBNT_{6m}$), and sera from triple BNT162b2-vaccinated individuals ($2xBNT/BNT_{0.5m}$ and $2xBNT/BNT_{3m}$) b) Neutralisation assays with sera from double mRNA-1273 vaccinated ($2xMOD_{6m}$) and additionally BNT162b2-boosted ($2xMOD/BNT_{0.5m}$) individuals. c) Neutralisation titres for sera from heterologous ChAdOx1 and BNT162b2-vaccinated ($1xChAd/1xBNT_{6m}$) and BNT162b2-boosted ($1xChAd/2xBNT_{0.5m}$) donors. d) Neutralisation assays performed with sera from double BNT162b2-vaccinated and SARS-CoV-2 infected individuals ($2xBNT/SARS-CoV-2$ infection $_{4m}$). Information regarding the sera donors (sex, age, antibody titres test and time after vaccination) are summarised in Table 1 and Supplementary Figure 2. Median titres and relative portion (%) of neutralising sera against each variant are indicated below each panel. Mean values of two technical replicates per sample are depicted with 95% confidence intervals and SD. Statistical significance was calculated by two-tailed, Wilcoxon Rank Sum Test. Asterisks indicate p -values as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and **** ($p < 0.0001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(6.2 mean month, SD: 0.4). Sera from individuals receiving a booster vaccination with an additional dose of BNT162b2 had 0.5 month later (SD: 0.1 month) significantly higher titres (3456 mean BAU/mL, $p < 0.0001$) (Figure 2). Anti-Spike IgG levels significantly decreased by the factor of 2.5 (1400 mean BAU/mL, $p < 0.01$) in sera obtained three months after the third dose (3.4 mean month, SD: 0.4), which was in agreement with previous observations demonstrating the waning immunity over time.^{24–26} In addition, a breakthrough infection in individuals who received two BNT162b2 doses

resulted in increased anti-Spike IgG levels when compared to vaccinated without infection (2616 mean BAU/mL, $p < 0.0001$). The mean anti-Spike IgG titre after booster was not significantly different than levels after two vaccinations and subsequent infection with SARS-CoV-2 (Figure 2a).

In our study, the cohorts of BNT162b2 double-vaccinated and double-vaccinated individuals with a breakthrough infection had a significant ($p < 0.0001$) higher age (85.5 mean years, SD: 6.9) compared to the boosted groups (40.8 mean years, SD: 12.1 and 43.6 mean years,

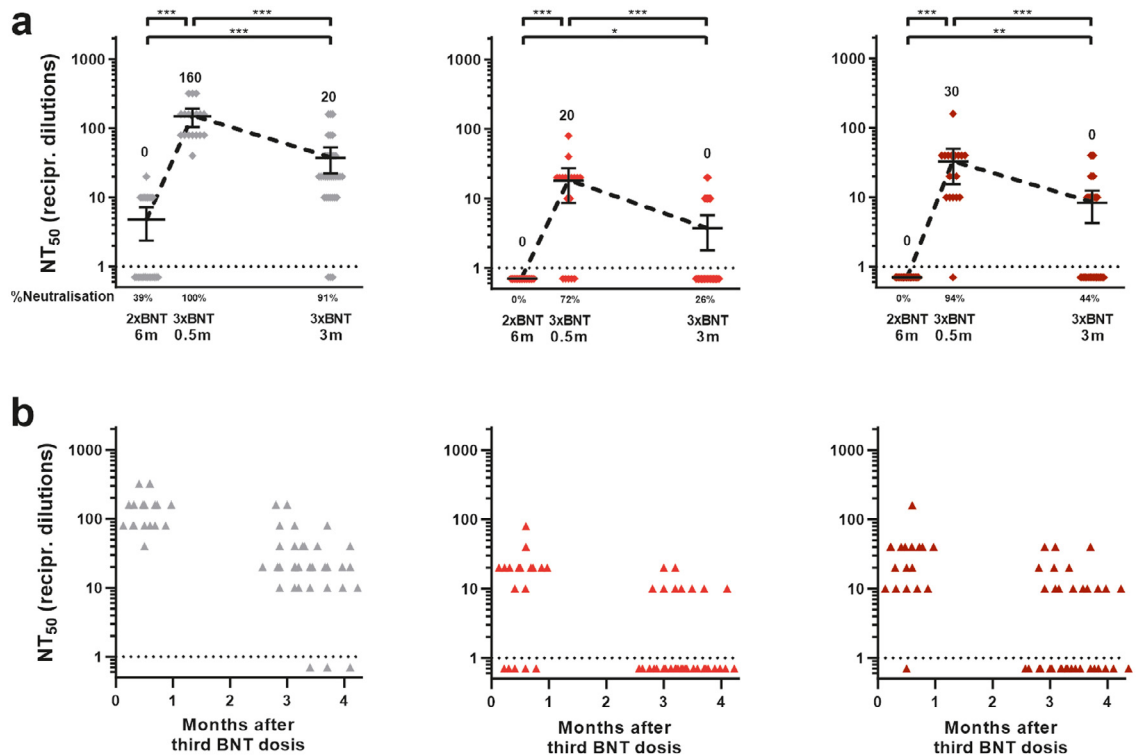


Figure 4. Waning antibody responses elicited by SARS-CoV-2 vaccination over time. a) Neutralising antibody responses were compared in BNT162b2-vaccinated individuals (2xBNT_{6m}, *n* = 23; 2xBNT/BNT_{0.5m}, *n* = 18; and 2xBNT/BNT_{3m}, *n* = 34). Values represent reciprocal dilutions micro-neutralisation titres resulting in 50% virus neutralisation (NT₅₀) of SARS-CoV-2 variants Delta (grey), Omicron BA.1 (red), and BA.2 (dark red). Median titres and relative portion (%) of neutralising sera against each variant are indicated above or below each panel, respectively. Mean values of two technical replicates per sample are depicted. b) Decrease in variant-specific neutralisation titres from panel A over time. Statistical significance was calculated by multivariate analysis including demographic covariates (Supplementary Table 4). Asterisks indicate *p*-values as * (*p* < 0.05), ** (*p* < 0.01) and *** (*p* < 0.001). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

SD: 11.3, respectively), as these samples derived from a study (No 20-864) conducted in long-term care facilities for the elderly.²⁹ The mean age of all other groups was comparable (Table 1, Figure 2b).

Taken together, these data indicate that mRNA vaccine based boosters generate strong anti-Spike IgG antibody levels in adults, however, titres significantly (*p* < 0.001) decline three months after the last dose.

Reduced neutralisation of SARS-CoV-2 omicron subvariants BA.1 and BA.2 by vaccine-elicited sera

We and others have previously shown that vaccine sera from double-vaccinated individuals have reduced protection against certain SARS-CoV-2 variants of concern such as Beta and Delta.^{13,28,38–44} In order to assess the susceptibility of SARS-CoV-2 subvariants BA.1 and BA.2 to neutralizing antibodies, we tested convalescent and vaccine-elicited sera samples obtained from individuals that are either double-vaccinated with mRNA (BNT162b2 and mRNA-1273) or heterologous-vaccinated (ChAdOx1/BNT162b2) followed by a BNT162b2

booster, respectively. Antibody-mediated neutralisation efficacy against Omicron was determined *in vitro* using authentic SARS-CoV-2 BA.1 and BA.2 isolates described above (Figure 1) and compared to the efficacy against Delta, the predominant variant preceding Omicron. As demonstrated previously,²⁸ neutralising antibody titres against SARS-CoV-2 Delta were significantly (*p* < 0.05) lower when compared to the parental variant B.1 harbouring the D614G substitution (Supplementary Figure 1). Since Delta represents the currently relevant reference variant, the data on BA.1 and BA.2 presented in this study were compared to Delta, implicating that differences shown in this study would be even more pronounced when compared to the parental isolate B.1.

Almost no neutralisation of Omicron BA.1 and BA.2 was observed using sera from individuals vaccinated with two vaccine doses irrespective of the applied vaccine, while a partial protection against Delta (39% [2xBNT_{6m}], 60% [2xMOD_{6m}], and 27% [1xChAd/BNT_{6m}]) was detected (Figure 3a-c, Table 1). A third vaccination with BNT162b2 resulted in complete neutralisation of Delta 0.5 months after the last dose in all

Group	Original Study	Immunization scheme	Group size N	Age mean (SD)	Sex (f/m)	BAU/mL mean (SD)	months after last vaccination mean (SD)	months after SARS-CoV-2 infection mean (SD)	NT Delta median (range)	NT Omicron BA.1 median (range)	NT Omicron BA.2 median (range)
2xBNT _{6m}	IC	2x BNT162b2	23	51.7 (13)	16 / 7	248.5 (244.4)	6.2 (0.4)	0	0 (0 - 20)	0 (0 - 0)	0 (0 - 0)
2xBNT / BNT _{0.5m}	IS	2x BNT162b2 + 1x BNT162b2	18	40.8 (12.1)	15 / 3	3456 (2362.9)	0.5 (0.1)	0	160 (40 - 320)	20 (0 - 80)	30 (0 - 160)
2xBNT / BNT _{3m}	IS	2x BNT162b2 + 1x BNT162b2	34	43.6 (11.3)	22 / 12	1400 (1122.1)	3.4 (0.4)	0	20 (0 - 160)	0 (0 - 20)	0 (0 - 40)
2xMOD _{6m}	IS / HVS	2x mRNA-1273	20	34.6 (10)	11 / 9	431.5 (375.2)	6.6 (0.7)	0	10 (0 - 80)	0 (0 - 0)	0 (0 - 20)
2xMOD / BNT _{0.5m}	IS	2x mRNA-1273 + 1x BNT162b2	12	31.9 (8.9)	5 / 7	3278 (1529.7)	0.5 (0)	0	160 (20 - 640)	10 (0 - 40)	20 (0 - 40)
1x ChAd / 1xBNT _{6m}	HVS	1x ChAdOx1 + 1x BNT162b2	29	40.5 (12.4)	21 / 8	230.1 (170.9)	5.8 (0.2)	0	0 (0 - 10)	0 (0 - 0)	0 (0 - 0)
1x ChAd / 2xBNT _{0.5m}	HVS	1x ChAdOx1 + 2x BNT162b2	9	45.9 (9.7)	8 / 1	2086 (1013.5)	0.5 (0.1)	0	80 (10 - 160)	0 (0 - 40)	10 (0 - 40)
2xBNT/ infection _{4m}	IC	2x BNT162b2 + SARS-CoV-2	20	85.5 (6.9)	17 / 3	2616 (3304.3)	7 (0.5)	4.0 (1.8)	40 (0 - 1280)	0 (0 - 160)	0 (0 - 160)

Table 1: Patient characteristics and overview of sera used in this study. Overview of sera used in this study indicating the relative overlaps between the groups is illustrated in Supplementary Figure 2.
 IC = Impfcare (Delbrück et al. 2022); IS= internal study (unpublished); HVS = heterologous vaccination study (unpublished).

groups and residual neutralisation of BA.1 and BA.2 (72% and 94% [2xNT/BNT_{0.5m}], 75% and 92% [2xMOD//BNT_{0.5m}], and 44% and 67% [1xChAd/2xNT_{0.5m}]; range 0–160 NT₅₀) (Figure 3).

Neutralisation assays performed with sera from double BNT162b2-vaccinated individuals who have undergone SARS-CoV-2 breakthrough infection on average 7 months after last vaccination (SD: 0.5 months) confirmed a significantly reduced neutralisation activity against BA.1 ($p < 0.0001$) and BA.2 ($p < 0.0001$). While 85% (40 median NT₅₀, range 0–1280 NT₅₀) of the sera were able to neutralise Delta, only 25% and 45% (range 0–160 NT₅₀) were able to neutralize the Omicron subvariants BA.1 and BA.2, respectively (Figure 3d, Table 1).

These data demonstrate a comparable reduction of the neutralisation activity of vaccine-elicited and convalescence sera against the two subvariants BA.1 and BA.2, although BA.2 was neutralised moderately more efficient.

Declining SARS-CoV-2 omicron neutralisation titres after booster vaccination

Considering that Omicron BA.1 and BA.2 are associated with a significant reduction of the neutralizing activity of vaccine-elicited antibody immunity, one crucial question remaining is how long neutralising antibodies against both infection with Omicron subvariants persists. While all sera from triple BNT162b2-vaccinated individuals were able to neutralize Delta at peak immunity 0.5 months after booster vaccination ($n = 18$, 160 median NT₅₀), 91% of sera were still able to neutralize the virus after three months ($n = 34$, 20 median NT₅₀) (Figure 4a and 4b). The majority of sera tested from triple BNT162b2 vaccinated individuals were able to neutralize Omicron BA.1 (72%, $n = 18$, 20 median NT₅₀) early after booster vaccination, however, after three

months, neutralisation was observed in only a small proportion of sera (26%, $n = 34$, 0 median NT₅₀) (Figure 4a and b). BA.2 could be neutralized by 94% of sera at peak immunity (2 weeks after the third vaccination) ($n = 18$, 30 median NT₅₀), and three months after the last vaccination dose by only 44% of sera ($n = 34$, 0 median NT₅₀) (Figure 4a and b). A multivariate analysis including demographic covariates revealed three month after booster vaccination a significant decline of neutralisation for SARS-CoV-2 Delta ($p < 0.001$), BA.1 ($p < 0.001$) and BA.2 ($p < 0.001$) (Figure 4a and Supplementary Table 4).

SARS-CoV-2 omicron subvariants exert distinct resistance against monoclonal antibodies

Neutralizing mAbs are recombinant proteins that might be vulnerable to the emergence of resistance and immune escape mutations in SARS-CoV-2 S as they may reduce antibody binding. Hence, in this study we additionally evaluated the neutralisation capacity of mAbs casirivimab/imdevimab and sotrovimab against authentic SARS-CoV-2 Omicron subvariants BA.1 and BA.2 in comparison to the parental isolate B/FFM5³⁰. While casirivimab/imdevimab (1:1) was still potent against the parental strain (EC₅₀ 0.00096 µg/mL), strong resistance against BA.1 was observed, which was outside the measurable range of the assay (Figure 5). Against BA.2, casirivimab/imdevimab exhibited neutralisation, even though the effect was significantly reduced (EC₅₀ 10.24 µg/mL). Compared with casirivimab/imdevimab, sotrovimab was able to neutralize the parental strain (EC₅₀ 0.2712 µg/mL) and in particular BA.1 (EC₅₀ 11.96 µg/mL) to a limited extent. BA.2 could not be neutralised by sotrovimab within the measuring range of the assay. The results indicate that subtype determination may be useful prior to monoclonal antibody administration, as monoclonal antibodies

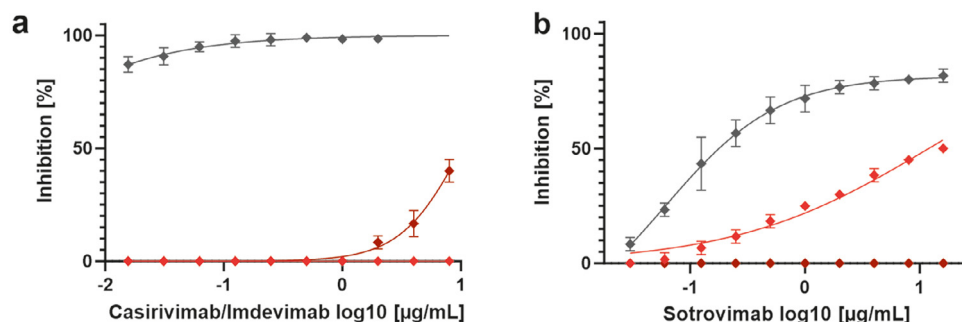


Figure 5. Neutralisation of SARS-CoV-2 subvariants by mAbs. Comparison of EC₅₀ values by monoclonal antibodies **a)** imdevimab/casirivimab (applied in a 1:1 ratio) and **b)** sotrovimab against parental SARS-CoV-2 strain B/FFM5³⁴ (grey), BA.1 (red), and BA.2 (dark red). Experiments were performed in three biological replicates using A549-AT cells. Readout was performed two days after infection. Bars indicate mean values with SD. The dotted line indicates the upper quantification limit of the assay. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

currently in clinical use show distinct efficacy against the omicron BA.1 and BA.2 subtypes.

Discussion

Our *in vitro* study using authentic SARS-CoV-2 Omicron subvariants BA.1 and BA.2 indicate that, in contrast to the previously circulating Delta variant, the neutralisation efficacy of vaccine-elicited sera against both subvariants was significantly reduced. In particular, booster vaccinations provided temporary humoral neutralizing efficacy against BA.1 and BA.2 infection at peak immunity, but it was significantly reduced three months after the third dose.

Understanding the evolution of SARS-CoV-2 variants at the functional level is currently of great value to public health. To initiate the coronavirus typical replication cycle,⁴⁵ an initial binding of the human cellular receptor angiotensin-converting enzyme 2 (ACE2) is essential but an interaction with the transmembrane protease serine 2 (TMPRSS2) was described as a further prerequisite for SARS-CoV-2 entry.^{4,31} Recent animal studies have shown that the BA.1 causes less severe disease and replicate less efficiently in the lower respiratory tract when compared to preceding variants of concern but show efficient replication in human primary nasal epithelial cultures.^{46–48} A comprehensible explanation for this observation could be a switched tropism for Omicron using the endosomal entry route not engaging TMPRSS2 as efficiently as the earlier isolates.^{37,47} Considering that TMPRSS2 facilitates syncytia formation by accelerating the glycoprotein-mediated membrane fusion,⁴⁹ our data showing a reduced ability of both Omicron subvariants BA.1 and BA.2 in inducing syncytia formation (Figure 1) might at least partly reflect the outcome of Omicrons tropism shift. However, further studies are needed to demonstrate a tropism shift of BA.2 compared to previous variants. Moreover, BA.1 harbours specific amino acids forming hydrogen bonds and salt bridges that might compensate for immune escape substitutions like K417N known to reduce ACE2 binding affinity.⁵⁰ Indeed, comparable biochemical ACE2 binding affinities for Delta and Omicron variants were determined. Interestingly, several studies reported that Omicron BA.1 does not replicate as well as other variants in Vero and Calu-3 cells,^{15,37} which however, we could neither observe using A549 cells overexpressing ACE2 and TMPRSS2 (A549-AT³¹) nor Caco-2^{32,33} cell lines.

In contrast to the previously circulating SARS-CoV-2 Delta variant, Omicron BA.1 exhibited high resistance to antibody-mediated neutralisation by vaccine-elicited antibodies as well as antibodies derived after breakthrough infection with previous SARS-CoV-2 lineages. The latter group, in our study, was limited by considerable higher age of the study subjects, and results may be altered in a younger cohort.^{15–22}

In a preliminary study comparable neutralisation titres for SARS-CoV-2 Omicron BA.1 and BA.2 variants have been observed.⁵¹ However, this study was performed with pseudoviruses spiked with SARS-CoV-2 S and may not reflect the whole mutational profile of the authentic viruses. In agreement with these early observations, using authentic SARS-CoV-2 we found a reduced sensitivity of both Omicron variants BA.1 and BA.2 to antibody neutralisation, even though the NT₅₀ levels against BA.2 were marginally higher compared to BA.1 (Figure 3). The observation that BA.2 was slightly better neutralised than BA.1 in our experiments, in contrast to previous reports, may be related to the fact that the BA.1 variant used in this study also contains the K417N substitution (Figure 1a), which is, among other substitutions at positions 452, 484, and 501, described in a recent preprint for the severe immune evasion.⁵²

In our study, we could demonstrate that neutralisation of both Omicron subvariants BA.1 and BA.2 was still effective using booster-vaccine-elicited sera obtained at peak immunity (0.5 months after receiving the third dose) although NT₅₀ values determined for both variants were largely reduced when compared to Delta (Figures 3 and 4). No neutralisation of BA.1 or BA.2 was observed in sera obtained from double vaccinated individuals 6 months after the second dose, which was in line with a recent study investigating the effectiveness of mRNA-1273 against SARS-CoV-2 Omicron BA.1 and Delta variants.⁵³ Hence, the higher the S antibody titre (e.g. after booster vaccinations), the more likely SARS-CoV-2 Omicron is neutralized. Consequently, in contrast to Delta, long-term neutralisation against Omicron is significantly reduced due to waning antibody titres^{24–26} already three months after booster vaccination (Figure 4).

We further found that Omicron BA.1 was resistant to imdevimab/casirivimab mediated neutralisation but was still susceptible to sotrovimab (VIR-7831), which is in line with previous publications and preprints.^{18,54,55} Importantly, in agreement with recent pseudovirus-derived preprint data, our study using authentic viruses confirmed that BA.2 considerably escapes neutralisation by sotrovimab mono treatment.⁵⁴ Previously, the E340A and E340K substitutions were described to greatly reduce the effect of sotrovimab. Additionally, S371F primarily affecting RBD-directed antibodies was also shown to confer sotrovimab resistance.⁵⁶ However, these mutations were not found in the BA.2 isolate used in this study (Figure 1). In conclusion, since Omicron subvariants exert distinct resistance against monoclonal antibodies, genotyping may be needed to guide mAb treatment. It would also be interesting to test whether a mixture of the three antibodies tested here (casirivimab/imdevimab/sotrovimab) might be effective against both Omicron subvariants.

This study had several limitations. We only measured a limited sample size at no more than two time-points. The vaccine Ad26.COV2.S was not considered

in this study as it accounted for less than 3% of all vaccinations in Germany until March 2022. The samples showing waning immunity (Figure 4) represent single participants at single time points but individual dynamics of nAb titres after vaccination may vary. Nevertheless, the cohort reflects a highly significant decrease of nAbs regardless of the individual course. The NT₅₀ values determined in this study appear low, but as demonstrated in Supplementary Figure 1 are a representative result of the already reduced susceptibility of convalescent and vaccine-elicited sera against SARS-CoV-2 Delta (compared with the parental strain harbouring D614G).²⁸ The age differences of the individual groups, especially regarding the group of double vaccinated and individuals who have experienced a SARS-CoV-2 infection (Table 1, Figure 2, and Supplementary Figure 2), may affect the comparability of the neutralisation titres. Furthermore, a part of this cohort represents young and healthy persons. However, regardless of the mRNA vaccine administered for the basic immunization, the use of a booster BNT162b2 dose achieved a comparable increase in anti-Spike IgG antibodies.

Factors such as age and gender, but also body composition and ethnicity have an impact on the humoral immune response. However, demographic and medical data were only available to a limited extent for all groups (age, gender, and IgGs) while additional data (BMI, comorbidities) was available for certain groups only (Supplementary Table 3). Multivariate linear regression analysis confirmed significant differences in neutralisation titres against SARS-CoV-2 variants between BNT162b2-vaccinated individuals including demographic covariates age and gender (Figure 4, Supplementary Table 4). In this study we analysed longitudinal data with missing values since only few sera represent follow-up samples (Supplementary Figure 2). Hence, for statistical analysis regarding differences in IgG antibody responses and age distribution the groups were considered as independent. The robustness of the statistical analysis was confirmed by a sensitivity analysis (Supplementary Figure 3). Furthermore, serum samples used in this study were not tested for generalisability and thus were not used for vaccine efficacy associations, but rather to show differential neutralising capacities against Omicron and Delta variants at different times after booster vaccination.

Another limitation of this *in vitro* study results from the strongly differing tendencies towards CPE formation (Figure 1). The exact timing of the CPE readout is essential, as relative inhibition must be adequately detected for both virus isolates to be compared. A too early or too late readout might significantly influence the EC₅₀ values determination. This issue may also have caused the discrepancies in early publications and preprint on the neutralising capacity of sotrovimab against SARS-CoV-2 VoCs and Omicron subvariants. In particular, the values determined using pseudoviruses,

which do not have a comparable CPE formation, deviate significantly from the values determined using authentic viruses in some studies.

Protection through vaccination is not limited to neutralizing antibodies alone and also the quality of the antibodies plays a role. In particular, the non-neutralizing effect of vaccine-elicited sera also stimulates cellular immunity since antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) may also contribute to the clinical efficacy of vaccines.^{57–59} Furthermore, T cell-mediated immunity represents an essential barrier to prevent severe COVID-19 upon Omicron infection.^{60–62} Of note, T cell responses in older adults were generally lower when compared to young cohorts, however, after receiving a third vaccination T-cell responses are comparably high.⁶³

In conclusion, our data indicate that both SARS-CoV-2 Omicron subvariants BA.1 and BA.2 escape antibody-mediated neutralisation elicited by vaccination and previous infection with SARS-CoV-2. In particular, three months after booster vaccination, this reduction renders the majority of tested sera negative in BA.1 and BA.2 neutralisation. Omicron subvariant specific resistance to the monoclonal antibodies casirivimab/imdevimab and sotrovimab emphasizes the importance of genotype-surveillance and guided application.

Contributors

A.W., M.W., K.G., T.T., B.S., C.P. and M.M. conducted experiments and verified the data in this study. N.K., S.H., F.A.H., T.W. and U.G. provided essential resources. A.W., M.W., K.G., T.T., E.H. and B.S. performed data analysis. M.W. drafted the first version. A.W. and M.W. performed writing, reviewing and editing. M.W. and S.C. conceptualized and administered the project. T.T. performed software based NGS analysis. T.T. and R.M. provided methodological support. All authors have read and acknowledged the final manuscript. M.W. and A.W. contributed equally.

Data sharing statement

All data analysed in this study are available in the article, the supplementary materials or are available on GISAID and GenBank under the following accession numbers: SARS-CoV-2 B.1.1.529 FFM-SIM0550/2021 (EPI_ISL_6959871; GenBank ID: OL800702), SARS-CoV-2 B.1.1.529 FFM-ZAF0396/2021 (EPI_ISL_6959868; GenBank ID: OL800703), SARS-CoV-2 B.1.617.2 FFM-IND8424/2021 (GenBank ID: MZ315141), SARS-CoV-2 BA.2 FFM-BA.2-3833/2022 (GenBank ID: OM617939), SARS-CoV-2 B FFM5/2020 (GenBank ID: MT358641), and SARS-CoV-2 B.1 FFM7/2020 (GenBank ID: MT358643).^{14,28,30} The related data are available for sharing upon request to the corresponding author.

Declaration of interests

S.H. has received research support from Roche Diagnostics and a speaker's fee from Sanofi Genzyme. T.W. has received speaker and consultancy fees from Gilead Sciences, Merck Sharp & Dohme, and Janssen Pharmaceuticals. N.K. received speaker fees from Abbott. S.C. was a member of a clinical advisory board for Biontech. All other authors declare no conflicts of interest, financial or otherwise.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.ebiom.2022.104158](https://doi.org/10.1016/j.ebiom.2022.104158).

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