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Neutralisation sensitivity of the SARS-CoV-2 omicron (B.1.1.529) variant: a cross-sectional study

Daniel J Sheward, Changil Kim, Roy A Ehling, Alec Pankow, Xaquin Castro Dopico, Robert Dyrdak, Darren P Martin, Sai T Reddy, Joakim Dillner, Gunilla B Karlsson Hedestam, Jan Albert, Ben Murrell

Summary

Background The SARS-CoV-2 omicron (B.1.1.529) variant, which was first identified in November, 2021, spread rapidly in many countries, with a spike protein highly diverged from previously known variants, and raised concerns that this variant might evade neutralising antibody responses. We therefore aimed to characterise the sensitivity of the omicron variant to neutralisation.

Methods For this cross-sectional study, we cloned the sequence encoding the omicron spike protein from a diagnostic sample to establish an omicron pseudotyped virus neutralisation assay. We quantified the neutralising antibody ID\textsubscript{50} (the reciprocal dilution that produces 50% inhibition) against the omicron spike protein, and the fold-change in ID\textsubscript{50} relative to the spike of wild-type SARS-CoV-2 (ie, the pandemic founder variant), for one convalescent reference plasma pool (WHO International Standard for anti-SARS-CoV-2 immunoglobulin [20/136]), three reference serum pools from vaccinated individuals, and two cohorts from Stockholm, Sweden: one comprising previously infected hospital workers (17 sampled in November, 2021, after vaccine rollout and nine in June or July, 2020, before vaccination) and one comprising serum from 40 randomly sampled blood donors donated during week 48 (Nov 29–Dec 5) of 2021. Furthermore, we assessed the neutralisation of omicron by five clinically relevant monoclonal antibodies (mAbs).

Findings Neutralisation antibody responses in reference sample pools sampled shortly after infection or vaccination were substantially less potent against the omicron variant than against wild-type SARS-CoV-2 (seven-fold to 42-fold reduction in ID\textsubscript{50} titres). Similarly, for sera obtained before vaccination in 2020 from a cohort of convalescent hospital workers, neutralisation of the omicron variant was low to undetectable (all ID\textsubscript{50} titres <20). However, in serum samples obtained in 2021 from two cohorts in Stockholm, substantial cross-neutralisation of the omicron variant was observed. Sera from 17 hospital workers after infection and subsequent vaccination had a reduction in average potency of only five-fold relative to wild-type SARS-CoV-2 (geometric mean ID\textsubscript{50} titre 495 vs 105), and two donors had no reduction in potency. A similar pattern was observed in randomly sampled blood donors (n=40), who had an eight-fold reduction in average potency against the omicron variant compared with wild-type SARS-CoV-2 (geometric mean ID\textsubscript{50} titre 369 vs 45). We found that the omicron variant was resistant to neutralisation (50% inhibitory concentration [IC\textsubscript{50}] >10 μg/mL) by mAbs casirivimab (REGN-10933), imdevimab (REGN-10987), etesevimab (Ly-CoV016), and bamlanivimab (Ly-CoV555), which form part of antibody combinations used in the clinic to treat COVID-19. However, S309, the parent of sotrovimab, retained most of its activity, with only an approximately two-fold reduction in potency against the omicron variant compared with ancestral D614G SARS-CoV-2 (IC\textsubscript{50} 0·1–0·2 μg/mL).

Interpretation These data highlight the extensive, but incomplete, evasion of neutralising antibody responses by the omicron variant, and suggest that boosting with licensed vaccines might be sufficient to raise neutralising antibody titres to protective levels.

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Research in context

Evidence before this study
Towards the end of 2021, the novel SARS-CoV-2 omicron (B.1.1.529) variant rapidly replaced the highly transmissible delta (B.1.617.2) variant in many countries. Sequencing showed that the omicron variant was extensively diverged from all other previously known lineages and harboured a number of mutations in the viral spike protein, including many mutations known or predicted to confer resistance to neutralising antibodies. However, the combined effect of these mutations, and the phenotypic effects of a number of novel omicron mutations, were unknown, and no experimental data were available on the resistance of omicron to neutralising antibodies at the onset of this study. We searched PubMed from database inception to Dec 19, 2021, without language restrictions, for articles using the search terms: “((SARS-CoV-2) AND ((Neutralisation) OR (Neutralisation)) AND ((Omicron) OR (B.1.1.529)))”. Our search yielded 17 articles, of which four were directly relevant. Two preprints evaluated neutralisation of five omicron isolates for recipients of the BNT162b2 mRNA vaccine (Pfizer-BioNTech), reporting an approximate 36-fold to 40-fold reduction in sensitivity relative to ancestral SARS-CoV-2 isolates. Two preprints reported resistance of omicron spike pseudotyped viruses to neutralisation by serum from convalescent and vaccinated individuals (BNT162b2, mRNA-1273 [Moderna], and Ad26.COV2.S [Johnson & Johnson] vaccine recipients), demonstrating low or undetectable titres against the omicron variant after infection or primary vaccination but substantial cross-neutralisation of omicron after an additional mRNA vaccine dose. An additional preprint not identified in the literature search reported on the neutralisation of the omicron variant by a panel of monoclonal antibodies (mAbs), showing that the omicron variant evaded neutralisation by approximately 85% of mAbs tested.

Added value of this study
This study provides in-vitro data on the sensitivity of the omicron variant to antibody-mediated neutralisation and quantifies the loss of neutralisation potency by vaccine and convalescent serum standards, as well as by five mAbs incorporated in licensed antibody therapies. Furthermore, we assessed neutralising activity against the omicron variant in sera from two Swedish cross-sectional cohorts, providing insight into population-level immunity against the omicron variant in Sweden at present. The initial serum neutralisation results from this study were disseminated just 13 days after the omicron variant was first reported to WHO.

Implications of all the available evidence
In agreement with several studies done in parallel, we identified that most mAbs incorporated into therapeutics licensed for human use have little or no neutralising activity against the omicron variant, which is likely to undermine their efficacy. One mAb, S309 (the parent of sotrovimab), retained potency against omicron, suggesting that sotrovimab might retain clinical utility. Furthermore, the results of this study and others have shown that the omicron variant displays profound escape from neutralising antibodies in serum samples obtained after infection or vaccination, which is likely to underpin the reductions in vaccine effectiveness observed in real-world settings. However, in a real-world cohort of blood donors from Stockholm (Sweden), reduced but detectable cross-neutralisation of the omicron variant was evident, suggesting that loss of protection at the population level might be less substantial in certain groups. We showed that although little or no cross-neutralisation of the omicron variant was identified in the sera of convalescent individuals, individuals who had been infected before being vaccinated had considerably higher neutralising potency against the omicron variant, highlighting the benefit of vaccination in individuals who have been previously infected. This finding is in line with other studies that emphasised the benefits of an additional vaccine dose in broadening neutralising antibody responses, including against the omicron variant.

Methods

Serum samples
For this cross-sectional study, we studied two cohorts. The first cohort comprised serum samples from 40 blood donors (anonymised and therefore with unknown exposure and vaccination status), donated during week 48 of 2021 (Nov 29–Dec 5), in Stockholm, Sweden. The second cohort comprised serum samples from previously infected hospital workers at the Karolinska University Hospital (Stockholm, Sweden; as described previously) who were confirmed to be SARS-CoV-2 positive by PCR in April or May, 2020. 17 previously infected hospital workers had serum sampled in November, 2021, after vaccine rollout, and nine had serum sampled in June or

positions 484, 417, and 501 are common to multiple variants of concern, and these three mutations alone (but E484K instead of E484A in the omicron variant) explain the majority of resistance exhibited by the beta (B.1.351) variant,1 which has no other receptor binding domain mutations. Deep mutational scanning data suggest that E484A and K417N, in addition to G446S and Q493R (which are not present in other variants of concern) are the largest

484, 417, and 501 are common to multiple variants of concern, and these three mutations alone (but E484K instead of E484A in the omicron variant) explain the majority of resistance exhibited by the beta (B.1.351) variant,1 which has no other receptor binding domain mutations. Deep mutational scanning data suggest that E484A and K417N, in addition to G446S and Q493R (which are not present in other variants of concern) are the largest contributors to the resistance profile of the omicron variant.1 Such substantial antigenic drift might undermine protection afforded by currently licensed vaccines and monoclonal antibodies (mAbs) used in the clinic. We therefore aimed to characterise, using a pseudotyped virus assay, the sensitivity of the omicron variant to neutralisation by relevant monoclonal antibodies, pooled serum from vaccine recipients and convalescent individuals, serum samples from previously infected and previously infected-then-vaccinated hospital workers, and serum from a random sample of blood donors.

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July 2020, before vaccination (defined as convalescent samples hereafter). Informed consent was obtained from all participants included in the hospital worker and convalescent cohorts as part of an ethics approval (decision number 2020-01620, with amendments 2020-02881, 2020-05630, and 2021-04377) from the Swedish Ethical Review Authority. The blood donor cohort and the omicron-positive sample from which the spike was cloned were anonymised and thus not subject to ethical approvals, as per advisory statement 2020–01807 from the Swedish Ethical Review Authority.

Plasma and serum pooled standards were also studied, including the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin (20/136), pooled from convalescent patients in 2020. Additionally, pooled serum standards from vaccinated individuals (including BEI Resources NRH-17727, NRH-17846, and NRH-20012) were provided by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health. For the three pools, serum was isolated approximately 3 months after completion of the primary vaccination series (mean 69 days [SD 14-9] for recipients of the BNT162b2 mRNA vaccine [Pfizer–BioNTech], 102 days [13-1] for recipients of the mRNA-1273 [Moderna] vaccine, and 83 days [19-0] for recipients of the Ad26.COV2.S [Johnson & Johnson] vaccine) from six donors with no history of infection who were aged between 18 and 55 years (mean age 44·2 years (16-0)).

Individual ID₅₀ values for each sample against each variant were calculated in GraphPad Prism (version 9.0) by fitting a four-parameter logistic curve to neutralisation by serial three-fold dilutions of serum. For comparisons of titres across variants, we used non-parametric Wilcoxon matched-pairs tests. To assess whether fold reductions were normally distributed, we used a Shapiro-Wilk test in Prism. We assayed all available samples and no sample size calculations were performed.

The omicron spike protein was molecularly cloned from an anonymised diagnostic sample, suspected to contain omicron due to S-gene target failure, which was subsequently confirmed by sequencing. The approach and primers used for the construction of the omicron spike expression plasmid are described in the appendix (pp 2–3, 5). The resulting spike plasmid had an amino acid sequence identical to the omicron consensus, using native codons from amino acids 43–1000, and was codon optimised outside of this region. Plasmids encoding the spikes from the B.1 (D614G), mu (B.1.621), and delta variants were obtained from the G2P-UK National Virology consortium.¹⁻³

HEK293T cells (CRL-3216; ATCC, Manassas, VA, USA) and HEK293T-ACE2 cells (stably expressing human ACE2) were cultured in Dulbecco’s Modified Eagle Medium (high glucose, with sodium pyruvate) supplemented with 10% fetal calf serum, 100 units per mL penicillin and 100 μg/mL streptomycin. Cultures were maintained in a humidified 37°C incubator (5% CO₂).

The pseudovirus neutralisation assay was done as previously described.¹⁻³ Spike-pseudotyped lentivirus particles were generated by the co-transfection of HEK293T cells with a relevant spike plasmid, an HIV gag-pol packaging plasmid (8455; Addgene, Watertown, MA, USA), and a lentiviral transfer plasmid encoding firefly luciferase (170674; Addgene) using polyethyleneimine.

Neutralisation was assessed in HEK293T-ACE2 cells. Briefly, pseudoviruses sufficient to produce approximately 30000 relative light units were incubated with serial three-fold dilutions of serum for 60 min at 37°C in a black-walled 96-well plate. 10000 HEK293T-ACE2 cells were then added to each well, and plates were incubated for 48 h. Luminescence was measured using Bright-Glo Luciferase Assay System (Promega, Madison, WI, USA) on a GloMax Navigator Microplate Luminometer (Promega). Neutralisation was calculated relative to the average of eight control wells infected in the absence of serum. All fold-changes reported use titres from neutralisation assays run in parallel.

Statistical analysis

Individual ID₅₀ values for each sample against each variant were calculated in GraphPad Prism (version 9.0) by fitting a four-parameter logistic curve to neutralisation by serial three-fold dilutions of serum. For comparisons of titres across variants, we used non-parametric Wilcoxon matched-pairs tests. To assess whether fold reductions were normally distributed, we used a Shapiro-Wilk test in Prism. We assayed all available samples and no sample size calculations were performed.

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Results

The WHO International Standard Immunoglobulin (20/136) had approximately 40-fold lower ID₅₀ titres

See Online for appendix
against omicron compared with wild-type SARS-CoV-2 (from 1765 [0·6 IU/ml] to 43 [23·4 IU/ml]; figure 2A), indicating substantial resistance to antibodies elicited by infection with early variants of SARS-CoV-2.

We assessed neutralisation of the omicron variant, delta variant, and wild-type SARS-CoV-2 by pooled serum standards from recipients of BNT162b2 (Pfizer–BioNTech), mRNA-1273 (Moderna), and Ad26.COV2.S (Johnson & Johnson) vaccines. We found that neutralisation of omicron was substantially reduced (by seven-fold to >40-fold), across the vaccine standard serum pools (figure 2B–D; appendix p 9).

While neutralising activity in serum sampled shortly following vaccination provides critical information about the antibody responses elicited and boosted by vaccines, immunity at the population level and real-world vaccine protection incorporates not just vaccination but a variety of previous and subsequent exposures, as well as waning14,15 of the responses to these. Therefore, to provide a snapshot of immunity at the population level before the introduction of omicron, we assessed neutralisation by sera from two cohorts from Stockholm, Sweden. Geometric mean neutralising ID50 titres for the blood donors were approximately eight-fold lower against the omicron variant than against wild-type virus (figure 3A). However, the reduction in neutralising activity was heterogeneous, with some sera nearly 90-fold less potent and others having no significant reduction in potency, indicating the presence of cross-neutralising antibodies in a subset of donors. Similarly, geometric mean ID50 titres from hospital workers were around five-fold lower against the omicron variant than against wild-type virus (figure 3B), and considerable inter-individual variation was observed (figure 3C; table 1). Fold changes in neutralising ID50 titres in both cohorts were consistent with a log-normal distribution (appendix p 10), and the potency with which a sample neutralised the omicron variant correlated with its ability to cross-neutralise other variants (appendix p 8).

Historical samples obtained from nine hospital workers after confirmed SARS-CoV-2 infection, but before vaccination, showed a near-complete loss of neutralising activity against the omicron variant (figure 3D). However, for seven hospital workers who received two doses of
the BNT162b2 vaccine after infection, robust cross-neutralisation of the omicron variant was evident in a number of individuals (figure 3E), highlighting the improvement in the neutralisation of the omicron variant afforded by vaccination in previously infected individuals.

We assessed the sensitivity of the omicron variant to neutralisation by several clinically relevant mAbs that have previously been licensed or authorised for human use. Casirivimab (REGN-10933), etesevimab (REGN-10987), bamlanivimab (LY-CoV555) did not neutralise the omicron variant at the highest concentration tested (10 μg/mL; table 2). However, the parent antibody of sotrovimab, S309, maintained its activity, with only a two-fold reduction in potency against the omicron variant compared with ancestral B.1 (D614G) virus (table 2), which is likely to be attributable to the location of the epitope that sotrovimab binds to being outside of the highly mutated receptor binding motif.

Discussion
Neutralising antibodies are a mechanistic correlate of SARS-CoV-2 vaccine protection. Although other components of the immune system contribute to protection from severe disease, the significant reduction in neutralisation sensitivity observed in this study is likely to translate into a reduction in vaccine-mediated protection against infection. This hypothesis is supported by the rapid spread of the omicron variant in countries with high vaccine coverage.

We found that there was a marked reduction in neutralisation potency against the omicron variant for serum pools from convalescent donors and recently vaccinated individuals, and from individual convalescent donors sampled soon after initial infection. This finding is consistent with several other contemporaneous studies. However, sera from a high-exposure cohort of hospital workers who had been infected, then vaccinated, achieved substantial cross-neutralisation of the omicron variant, which correlates with their ability to cross-neutralise other variants. This finding suggests that responses to the SARS-CoV-2 spike protein broaden with increasing antigenic exposure, which has been shown for other variants in the context of both previous infections and three doses of vaccination.

Serum samples from a cohort of blood donors in Stockholm also showed substantial cross-neutralisation of the omicron variant. On average, the fold reduction in neutralising antibody titres against the omicron variant was only marginally greater than that of the previously infected, then vaccinated, hospital worker cohort. Such cross-neutralisation in a real-world cohort would not have been predicted from the responses observed soon after vaccination. At the time of sampling, most individuals in Sweden had only received two vaccine doses, thus this breadth of antibody response might be explained by the frequency of exposure to SARS-CoV-2 before, or following, vaccination in Stockholm. At the time of anonymous blood donor sampling, 78% of adults (≥18 years) had received two vaccine doses. Testing recommendations in Sweden make it difficult to directly estimate the proportion of the population who had been infected with SARS-CoV-2. In early 2021, before mass vaccination, the seroprevalence of anti-SARS-CoV-2 antibodies in blood donors in Stockholm was estimated at around 20%, and there have subsequently (before sampling of the current blood donor cohort) been two waves of SARS-CoV-2 infection, dominated by the highly transmissible alpha (B.1.1.7) and delta variants.
Previous infection rates are thus expected to have been high in the blood donor cohort, which could contribute to cross-neutralisation responses. Alternatively, systematic differences might exist in cross-neutralisation for samples obtained immediately after a second immunisation compared with those sampled later. Affinity maturation of antibody lineages over the course of months after SARS-CoV-2 infection enabled the cross-neutralisation of heterologous sarbecoviruses and SARS-CoV-2 variants of concern, which has also been demonstrated for the omicron variant.

From a global health perspective, the marked reduction in neutralisation of the omicron variant by serum obtained from previously infected, but unvaccinated individuals raises the question as to whether such individuals can be considered immune. Vaccine effectiveness afforded by two doses seems to be significantly reduced against infection with the omicron variant. Furthermore, the cross-neutralising antibody responses in individuals who had been infected then vaccinated in the hospital worker cohort indicate that vaccination of previously infected individuals is of considerable value.

mAbs represent important treatment and prophylactic options for certain categories of patients, and can significantly reduce morbidity in those otherwise at risk for severe COVID-19. Considering the complete resistance of the omicron variant to several clinically relevant mAbs, now supported by several studies, treatment options should be informed by rapid SARS-CoV-2 genotyping in regions where the omicron variant and other variants are co-circulating. This finding also highlights the need for rapid diversification of our clinical mAb portfolio to protect against unpredictable reduction in potency against future variants and for the further development of small-molecule antivirals against more conserved targets. There is also a need to rapidly screen variants for their sensitivity to clinical antibody therapeutics.

Methodologically, the current standard practice for generating pseudovirus spike expression plasmids for novel variants relies on site-directed mutagenesis when only a small number of mutations differ from an existing plasmid construct or gene synthesis to generate entire spike genes. Exceptional urgency is demanded by the emergence of a rapidly spreading novel variant with a large number of spike mutations. Molecular cloning from a diagnostic sample allowed us to circumvent gene synthesis delays and share pseudovirus neutralisation data just 8 days after receipt of the diagnostic samples suspected to contain the omicron variant and 13 days after the variant was first reported to WHO. One risk associated with this approach is that if the expression of a non-codon-optimised spike protein is too low, pseudovirus entry into target cells might be too inefficient to accurately quantify neutralisation. For this reason, our cloning strategy retained as much of the C-terminal region of the spike, which is not mutated in the omicron variant. It is not clear whether such a strategy would universally succeed with all variants; therefore, a dual approach that attempts gene synthesis and direct cloning (when samples are available) would mitigate this risk.

**Table 1:** ID₅₀ titres against omicron and wild-type SARS-CoV-2 in hospital workers infected with SARS-CoV-2 in early 2020

| ID₅₀ (μg/mL) | D614G IC₅₀ | Omicron IC₅₀ | Fold change in IC₅₀ | Mutations* |
|-------------|-------------|-------------|---------------------|------------|
| Casirivimab (REGN-10933) | 0.009 | >10 | >1100 | 4171, 4841, 4931, 477, 478 |
| Imdevimab (REGN-10987) | 0.008 | >10 | >1200 | 4401, 4461 |
| Bamlanivimab (LY-CoV555) | 0.007 | >10 | >1400 | 4841, 4931, 478 |
| Etesevimab (LY-CoV16) | 0.04 | >10 | >270 | 4171, 493, 501, 505, 477 |
| Sotrovimab (S309) | 0.1 | 0-2 | 2 | 339 |

IC₅₀=50% inhibitory concentration. *Mutated amino acid positions modelled on the omicron receptor binding domain, proximal to the antibody interface, are listed. 1Functional evidence (from deep mutational scanning data) for an effect on antibody binding of mutations at that amino acid position.

**Table 2:** Neutralising potency of clinically relevant monoclonal antibodies against the SARS-CoV-2 omicron (B.1.1.529) variant.
Limitations of this study include the absence of vaccination and infection information for the anonymous blood donors and the heterogeneity in vaccination histories of the hospital workers. As a result, the drivers of the observed variability in the ability to cross-neutralise the omicron variant could not be assessed. Furthermore, although we evaluated serum pools post-vaccination with BNT162b2, mRNA-1273, and Ad26.COV2.S vaccines, each of these were pools of serum from only six individuals, and a single individual with cross-neutralising antibodies could influence the apparent cross-neutralisation for the entire pool. Nevertheless, the use of these standardised reagents enables future efforts to calibrate vaccine titres, across assays and variants. Additionally, we have not yet assessed the relative sensitivity of other members of the omicron clade, including BA.2, nor of emerging sublineages with additional mutations that might have a substantial impact on neutralising antibodies.

Ultimately, long-term protection against SARS-CoV-2, including antigenic variants that will arise, might require updated vaccines or vaccines that elicit more broadly cross-neutralising antibodies. Until such vaccines are available, our data from two different cohorts suggest that there is incomplete loss of neutralisation against the omicron variant. It has previously been shown with other variants that a third dose of an unmodified vaccine might provide a broadening effect on the antibody response.\(^{25}\) Even in the absence of a broadening effect, in many donors, the magnitude of reduction in neutralisation observed against the omicron variant suggests that it might be possible to boost antibody titres into a protective range using currently licensed vaccines. Indeed, emerging evidence suggests that a third dose of licensed vaccines enhances protection against symptomatic infection, hospitalisation, and death associated with the omicron variant.\(^{30}\)

Contributors

DJS, DPM, GBKH, JA, and BM conceptualised the study. DJS and BM did the formal analysis. DJS, CK, XCD, and RD conducted the study. DJS, DPM, GBKH, JA, and BM provided resources. DJS, GBKH, STR, and BM oversaw the study. DJS and BM wrote the initial draft. DJS, DPM, GBKH, JA, and BM reviewed and edited the manuscript. DJS and BM have accessed and provided resources. DJS, GBKH, JA, and BM oversaw the study. DJS, CK, XCD, and RD conducted the study. DJS, DPM, GBKH, JA, and BM provided resources. DJS, GBKH, STR, and BM oversaw the study. DJS and BM wrote the initial draft. DJS, DPM, GBKH, JA, and BM reviewed and edited the manuscript. DJS, GBKH, JA, and BM were responsible for the figures and tables. RAE, STR, JD, GBKH, and JA were responsible for the decision to submit the manuscript for publication. Requests should be directed to the corresponding author (benjamin.murrell@ki.se).

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