

Omicron Severe Acute Respiratory Syndrome Coronavirus 2 Neutralization by Immunoglobulin Preparations Manufactured From Plasma Collected in the United States and Europe

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After >2 years of the coronavirus disease 2019 (COVID-19) pandemic, immunoglobulins (IGs) contain highly potent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing antibodies, based on the large proportion of United States (US) plasma donors who have gone through COVID-19 or vaccination against the virus. Neutralization of Omicron SARS-CoV-2 by antibodies generated after non-Omicron infection or vaccination has been lower though, raising concerns about the potency of IG against this new virus variant. Also, as plasma collected in the US remains the main source of IG, the neutralization of SARS-CoV-2 for plasma collected elsewhere has been less well studied. Here, we confirm Omicron neutralization by US as well as European Union plasma-derived IG lots.

Keywords. SARS-CoV-2 antibody potency; intravenous immunoglobulin; neutralizing antibodies; primary immunodeficiency; prophylaxis.

After several months of the coronavirus disease 2019 (COVID-19) pandemic, highly potent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralization by intravenous immunoglobulin (IG) manufactured from plasma collected in the United States (US), including post-COVID-19 and COVID-19-vaccinated donors, has been reported [1]. The functional antibody neutralization assay used in this report was based on the original Wuhan SARS-CoV-2 strain. Several other variants of concern have since emerged [2], and the most recent Omicron variant may even have

established a new serotype, with—by definition—consequences on the level of virus cross-neutralization [3].

Plasma donors with residence in the US provide a quantitatively dominant contribution to the world's supply of IG products, and earlier investigations into the potency of IGs against SARS-CoV-2 have thus focused on IG manufactured from US plasma. Several European countries also contribute sizeable volumes of plasma to the production of IG. The increase of COVID-19 case numbers as well as the number of vaccine doses administered in Europe has somewhat been behind the US [4], with unclear consequences on the development of SARS-CoV-2 neutralization potency in IGs produced from plasma collected there.

As immunocompromised people, either due to their oncological conditions, after organ transplantation, or with certain immunodeficiencies, have a higher risk of severe COVID-19 consequences [5], the levels of SARS-CoV-2 neutralizing antibodies in their IG treatment are of critical importance and were thus revisited in the above context. Beyond the IG preparations for intravenous use tested in earlier studies [1, 6], preparations suitable for the increasingly widely used subcutaneous application were also included in the current investigation.

METHODS

Measurement of SARS-CoV-2 Antibodies

Wuhan SARS-CoV-2 wild-type (WT) (strain BavPat1/2020) and Omicron SARS-CoV-2 (strain hCoV-19/Netherlands/NH-RIVM-71076/2021, lineage B.1.1.529) neutralizing antibody titers were determined essentially as previously reported [6]. The reciprocal sample dilution resulting in 50% virus neutralization (μNT_{50}) was determined using the Spearman-Kärber formula, and the calculated μNT_{50} neutralization titer was normalized to an internal assay control, therefore reported as μNT_{50} [norm. 1:X]. For Wuhan SARS-CoV-2 WT, a qualified analytical method was used that included the National Institute of Biological Standards and Control (Potters Bar, United Kingdom) World Health Organization International Standard 20/136 [7], and the concentration of neutralizing antibodies therefore is also reported in IU/mL. Both Wuhan SARS-CoV-2 and Omicron neutralization assays included several validity criteria (ie, confirmatory titration of input virus infectivity, cell viability, and neutralization testing of an internal reference standard), all of which had to comply with defined ranges.

Immunoglobulin Preparations and Anti-SARS-CoV-2 Hyperimmune Globulin

To determine Wuhan SARS-CoV-2 WT and Omicron SARS-CoV-2 neutralization by immunoglobulin, 6 commercial IG, 10% lots fractionated from US plasma collected prior to the

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pandemic (prepandemic; Gammagard Liquid, Baxalta US Inc, Lexington, Massachusetts), 10 COVID-19 hyperimmune globulin (HIG) preparations manufactured exclusively from early-pandemic COVID-19 convalescent donors (donations collected from April 2020 for approximately 6 months) [1], and 100 commercial IG, 10% lots fractionated from US or European Union (EU) plasma collected during the pandemic (Gammagard Liquid, Baxalta US Inc or KIOVIG, Takeda Manufacturing Austria AG, respectively; released March till April 2022) were tested. To evaluate the development of SARS-CoV-2 neutralizing antibody content in IG lots manufactured from US- and EU-sourced plasma over time, all IG, 10% lots and all lots of immunoglobulin for subcutaneous application (SCIG), 20% (Cuvitru, Baxalta US Inc; US plasma) released between May 2021 and April 2022 were analyzed for original Wuhan SARS-CoV-2 WT neutralization potency. Of these, the IG, 10% lots released between November 2021 and April 2022 were also analyzed for Omicron SARS-CoV-2 neutralization.

Graphs and Statistical Analysis

Graphical illustrations and statistical analysis (paired and unpaired *t* tests) were done in GraphPad Prism version 9.2.0 software.

RESULTS

Wuhan and Omicron SARS-CoV-2 Neutralization by Immunoglobulin Preparations From Plasma Collected Before the Pandemic, or After COVID-19 Caused by Wuhan-Like Virus Variants

Immunoglobulin lots that were fractionated from plasma collected prior to the COVID-19 pandemic (prepandemic; $n = 6$) had no neutralizing activity against either of the 2 SARS-CoV-2 strains (Figure 1), fully consistent with earlier findings [8]. The investigational COVID-19 hyperimmune (HIG; $n = 10$) preparations had high neutralization capacity (geometric mean titer \pm standard error of the mean μNT_{50} [norm. 1:X]) against the Wuhan SARS-CoV-2 WT (947 ± 109), yet a significantly ($P < .0001$) lower neutralization titer against the recent Omicron strain (24 ± 1), that is, an approximately 40-fold difference in potency. Regular IG lots released in March and April 2022, produced from US plasma (5057 ± 281 ; $n = 85$) or from EU plasma (3214 ± 382 ; $n = 15$), had 3- to 5-fold greater Wuhan SARS-CoV-2 WT neutralization capacity than even the HIG. These IG lots also had a quite potent neutralization capacity for the Omicron strain (US: 394 ± 27 ; EU: 116 ± 19) (Figure 1), although this was on average 20-fold lower than their respective Wuhan neutralization titer.

Wuhan and Omicron SARS-CoV-2 Neutralization by Immunoglobulin Preparations From Plasma Collected During the Course of the Pandemic and Vaccination Campaigns, in the US and EU

Regular IG, 10% released to the US market between May 2021 and April 2022 had continuously and quite rapidly increasing neutralization titers against the Wuhan SARS-CoV-2 WT,

which seemed to reach plateau levels toward the end of 2021, at levels approximately 25-fold higher than in May 2021 (Figure 2A). This increase in titers was somewhat delayed for EU plasma-derived IG lots, which did finally reach antibody levels approximately within only a factor of 2 from the US lots, that is, a single dilution step as used in the neutralization assay. By April 2022, consistent and high neutralization capacity for the Wuhan SARS-CoV-2 WT virus was seen for all IG lots (US: 6168 ± 511 ; EU: 4476 ± 430), with Omicron neutralization 12-fold (US: 510 ± 50) and 22-fold (EU: 199 ± 14) lower (Figure 2A).

SARS-CoV-2 Antibody Levels in IG Preparations for Intravenous Versus Subcutaneous Application

When normalized to 10% protein content, lots of SCIG, 20% for subcutaneous application, released to the US market between May 2021 and April 2022, had near equivalent and similarly increasing neutralization potency against the Wuhan SARS-CoV-2 WT as seen for lots of IG, 10% for intravenous application (Figure 2B). IG preparations for subcutaneous application also reached similar consistent plateau levels toward the end of 2021, at approximately 25-fold higher potency than in May 2021 (Figure 2B). There was no difference ($P = .71$) in anti-SARS-CoV-2 potency development over time nor at levels reached between these 2 classes of IG preparations.

DISCUSSION

In line with a previous report for Wuhan SARS-CoV-2 WT virus [8], IG fractionated from plasma collected prior to the COVID-19 pandemic also did not neutralize the Omicron SARS-CoV-2 variant (Figure 1). COVID-19 HIG preparations, fractionated from COVID-19 convalescent donor plasma collected at the beginning of the pandemic and therefore representative of the antibody response to infection only, provided for some Wuhan as well as Omicron neutralization, with the latter, however, considerably lower than against the Wuhan SARS-CoV-2 WT, in line with previous reports of 16-fold and up to 40-fold lower neutralization of the Omicron versus Wuhan strain by post-COVID-19 sera or convalescent plasma [9, 10].

The difference in Wuhan versus Omicron neutralization potency was somewhat less pronounced for pandemic IG preparations (Figure 1), manufactured from plasma collected from a mixed convalescent, COVID-19-vaccinated and partially even reexposed donor population. The highly potent and heterogeneous SARS-CoV-2 antibody specificities contained in these commercial IG lots apparently provide for better Omicron neutralization, reflective of the greater antibody response to COVID-19 vaccination rather than SARS-CoV-2 infection only [1, 11] and broader Omicron neutralization following repeat vaccination and infection exposures [12].

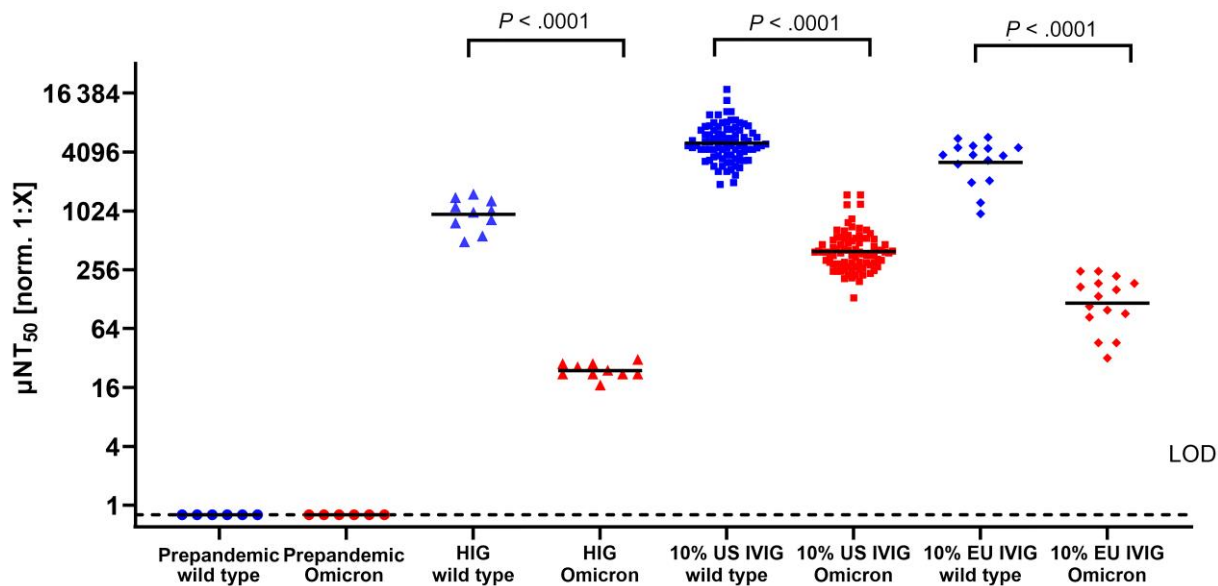


Figure 1. Wuhan severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) wild-type (blue) and Omicron SARS-CoV-2 (red) normalized neutralizing antibody content of 10% intravenous immunoglobulin lots manufactured from plasma collected prior to the coronavirus disease 2019 (COVID-19) pandemic (prepandemic; $n = 6$), exclusively from COVID-19 convalescent donors (hyperimmune immunoglobulin; $n = 10$), and in the United States and European Union ($n = 85$ and $n = 15$, respectively; released in March and April 2022), illustrated as scatter dot plot; line indicates geometric mean on base-2 logarithmic scale. Abbreviations: HIG, coronavirus disease 2019 hyperimmune immunoglobulin; IVIG, intravenous immunoglobulin; LOD, limit of detection; Omicron, severe acute respiratory syndrome coronavirus 2 strain hCoV-19/Netherlands/NH-RIV-M-71076/2021, lineage B.1.1.529; μNT_{50} [norm. 1:X], normalized 50% virus neutralization; wild-type, severe acute respiratory syndrome coronavirus 2 strain BavPat1/2020.

Still, the observed differences in SARS-CoV-2 variant cross-neutralization by the IG preparations support the proposal to consider the Omicron SARS-CoV-2 variant a distinct serotype [3].

A dramatic upward change in anti-SARS-CoV-2 potency was previously reported for US plasma-derived IG lots released in May 2021 [1], a trend that continued until the end of 2021 and seems to have plateaued since then (Figure 2A), irrespective of whether IG preparations for intravenous or subcutaneous application were analyzed (Figure 2B). Analysis of EU plasma-derived IG lots indicated that SARS-CoV-2 neutralization titers against the Wuhan virus strain were lower in May 2021, reflective of the lower COVID-19 incidence as well as slower uptake of vaccination in Europe as compared to the US [4]. By the end of 2021, Wuhan SARS-CoV-2 WT neutralizing titers in EU plasma-derived IG lots had reached equivalently high levels as in US plasma-derived IG lots.

The first Omicron cases were reported in November 2021 from the US and EU [13, 14], and the variant then rapidly became dominant in both geographies. With a typical period of approximately 6 months between plasma collection and IG lot release [6], Omicron-induced antibodies are not yet likely present in the IG preparations investigated here. An increase in (cross-)neutralization titer also against the new variant is evident in US and EU plasma-derived IG lots released in March and April 2022, which is expected to further increase significantly in the IG lots released in the upcoming months. This

outlook is of relevance for people with immunodeficiencies or immunosuppression, who depend on the antibody specificities contained in their IG treatment for protection from COVID-19. In the US, the COVID-19–Associated Hospitalization Surveillance Network (COVID-NET) has analyzed factors associated with severe outcomes in >22 000 adults hospitalized with COVID-19. Of these, 12.2% were immunocompromised, although they only constitute an estimated 2.7% of the entire US population [5].

More specifically to the Omicron virus variant, plasma samples from patients with X-linked agammaglobulinemia, who receive regular IG as prophylaxis, were found to contain relatively low plasma concentrations of SARS-CoV-2 antibodies, with no potential to neutralize the Omicron variant in vitro, and 3 of 4 patients had symptomatic COVID-19 during the Omicron wave [15]. The most current IG lot used in this study was produced in August 2021, that is, at a time when SARS-CoV-2 neutralization titers of US plasma-derived lots were 4–5 times lower than reported here for current IG lots, and lower yet for EU plasma-derived lots (Figure 2A).

One limitation of the study reported here is the use of an Omicron BA.1 variant strain for IG screening. This variant has meanwhile been displaced by the Omicron BA.4 and BA.5 variants from circulation, both of which are considered neutralization escape mutants relative to BA.1 [16]. The continued emergence of new virus variants may quickly outdate IG potency screening data, while at the same time the

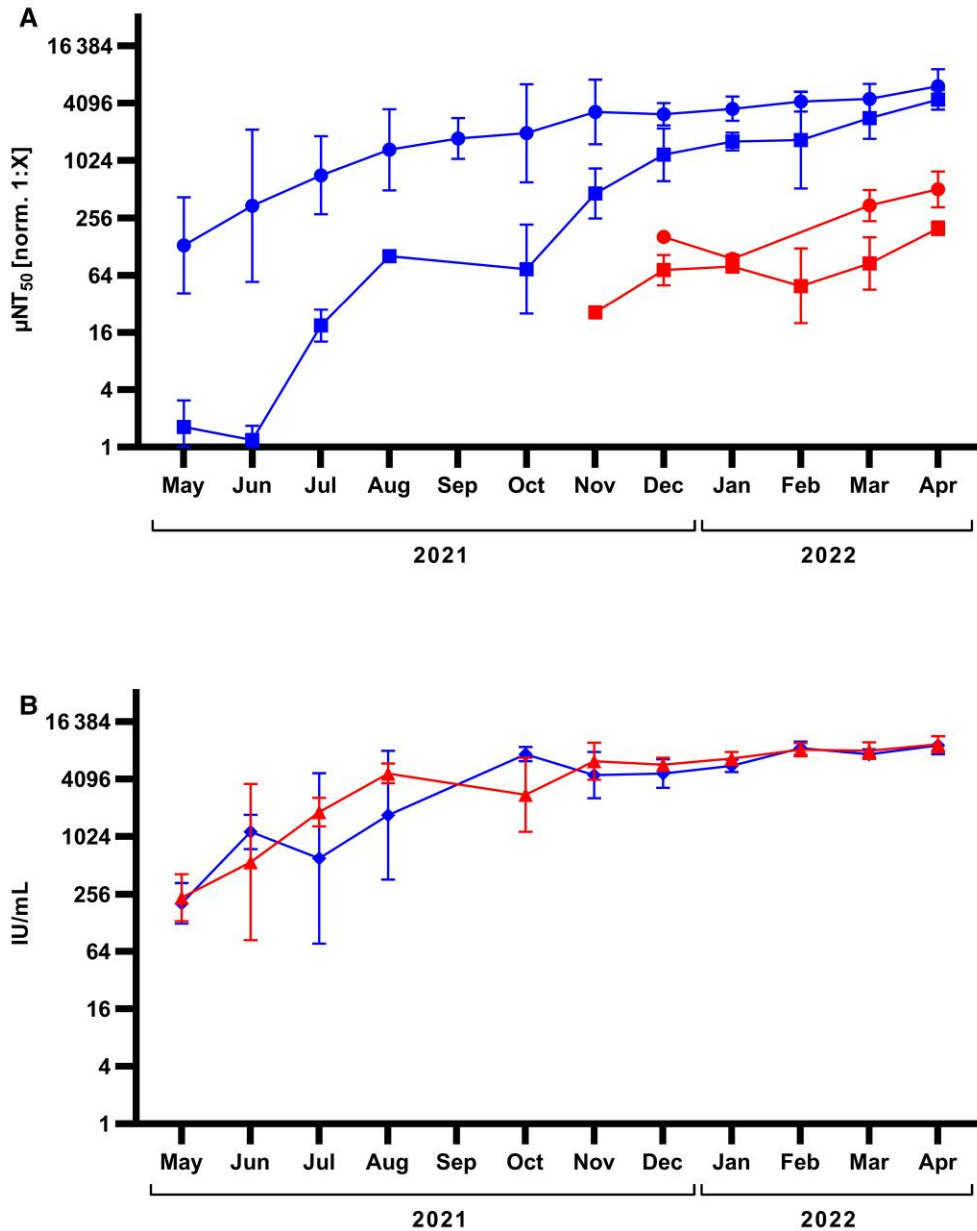


Figure 2. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing antibody content of immunoglobulins (IGs) released between May 2021 and April 2022. *A*, Wuhan SARS-CoV-2 wild-type (blue) and Omicron SARS-CoV-2 (red) normalized neutralizing antibody content of IG, 10% lots manufactured from plasma collected during the coronavirus disease 2019 pandemic in the United States (US, ●) and European Union (EU, ■), illustrated as geometric means with geometric standard deviation on base-2 logarithmic scale. *B*, Wuhan SARS-CoV-2 wild-type neutralization potency of 2 commercial IG products produced from US plasma for intravenous (IG, 10%; ◆) or subcutaneous (IG, 20%; ▲) (values normalized to 10% protein content) application, illustrated as geometric means with 95% confidence interval on base-2 logarithmic scale. Abbreviation: μNT_{50} [norm. 1:X], normalized 50% virus neutralization.

protective level of Omicron neutralizing antibodies in a prophylactic setting is not yet known. Whether the more potent antibody levels of pandemic IG, now also against the Omicron virus variant, driven by additional COVID-19 cases and even more so effective vaccination campaigns, will be able to afford clinically meaningful levels of protection remains to be determined.

Notes

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