Passive immunization against COVID-19 by anti-SARS-CoV-2 spike IgG in commercially available immunoglobulin preparations in severe antibody deficiency

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Conflict of interest statement
JH, SW, JF, MLD, SE, MS, GK, MP, MR, and CTB declare no conflict of interest related to this work. A.C.W. is an employee of ROCHE, Switzerland. OH is an employee of ADR-AC GmbH, Switzerland. K.W. has received speakers’ fees from CSL Behring.

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Clinical Implications

Recent immunoglobulin preparations contain high anti-SARS-CoV-2-specific antibodies resulting in passive COVID immunization. The steady-state anti-spike-IgG serum levels in recipients achieve pharmacokinetically predictable serum levels and virus neutralization capacities comparable to those of mRNA-vaccinated subjects.
Patients with antibody deficiency are at risk for bacterial and viral infections (1, 2). Regular immunoglobulin replacement therapy by intravenous or subcutaneous infusions reduces infection susceptibility. Immunoglobulin preparations containing highly enriched polyclonal IgG are derived from pooled plasma from >10^3 donors to cover a broad range of pathogen-specific antibodies. The amounts of pathogen-specific IgG within immunoglobulin preparations depend on the seroprevalence in the plasma donor population and are influenced by high pathogen circulation or high vaccination rates against a given pathogen. SARS-CoV-2, the causative agent of COVID-19, encountered an immunologically naïve population in late 2019 and rapidly spread worldwide. The high number of SARS-CoV-2 infections and, from 2021 on, the vaccination campaign continuously built up population immunity. Hence, immunoglobulin preparations are expected to cumulatively contain substantial anti-SARS-CoV-2 antibodies. This potentially allows for passive immunization in patients with severe antibody deficiency without the need for additional therapeutic monoclonal anti-SARS-CoV-2 antibodies. Here, we tested for the presence, function, and composition of anti-SARS-CoV-2 antibodies in different commercially available immunoglobulin preparations collected overtime during the pandemic. In a proof-of-concept study, we then investigated whether the anti-SARS-CoV-2 antibodies in immunoglobulin preparations are sufficient to build up assumed protective anti-SARS-CoV-2 serum levels in a patient with severe antibody deficiency who failed to mount humoral anti-SARS-CoV-2 following repeated mRNA vaccinations.

First, we measured anti-SARS-CoV-2 spike antibodies (anti-S-IgG) using the Roche Elecsys® anti-SARS-CoV-2-Spike-IgG/M assay in 14 immunoglobulin preparations (Table I). Anti-S-IgG levels were above assay positivity threshold in 9/14 (64.3%) immunoglobulin preparations. In 7/9, the measured concentrations were >150 IU/mL. In addition, we measured anti-nucleoprotein (anti-NP-IgG) and anti-S1-receptor-binding domain(RBD)-IgG
using Luminex technology (*Table 1*). The antibody levels in these assays highly correlated with the anti-S-IgG levels in the Roche assay: anti-S1-RBD-IgG ($p<0.001$, spearman $r=0.94$) and the anti-NP-IgG ($p<0.001$, $r=0.95$). Anti-S-IgM antibodies were absent in all tested products.

There were no substantial differences between immunoglobulin manufacturers. However, the time of plasma pooling of intravenous (IVIG) and subcutaneous (SCIG) preparations, indirectly reflected by the expiration date, was associated with the presence of anti-S-IgG in the products (*Table 1*).

To assess whether the detected anti-S-IgG may result in potentially protective serum antibody levels *in vivo*, we next performed a proof-of-concept study in a 34-years old male with severe antibody deficiency due to NFKB1 insufficiency. The patient, including the *NFKB1* genotype (heterozygote frameshift mutation c.1071-1074del AGAA), has previously been published in a cohort study (3). The patient has almost absent peripheral B cells (4/μl) and low T cell and NK cell numbers. After the first two doses of an mRNA COVID-19 vaccine (Spikevax®, Moderna), he had a non-detectable immune response to the spike and nucleocapsid antigen defined by negative SARS-CoV-2-specific antibodies and virus-specific B and T cell activation in a commercial lymphocyte activation assay (ADR-AC, Switzerland). Four weeks after a third dose of Spikevax®, CD8 T cell reactivity to the spike protein could be detected in the commercial T cell assay, but virus-specific antibodies were still absent on October 12th 2021. At that time, he was on SCIG replacement therapy (16-20g weekly) with a SCIG preparation without detectable anti-S-IgG (SCIG #1 in *Table 1*). We switched to a SCIG product from the same company (Hizentra, CSL Behring) with the, at that time, highest levels of anti-S-IgG (i.e., SCIG#2 = 12413 IU/mL) in our study (*Table 1*) and longitudinally assessed serum anti-S-IgG levels in the patient. After four, six, and seven weeks, anti-S-IgG serum antibody levels raised to 435 IU/mL, 534 IU/ml, and 571 IU/mL, respectively (*Figure*)
Total IgG levels remained stable between 11.7-11.9g/l after switching the SCIG product. Luminex testing confirmed that the anti-S-IgG, anti-RBD-IgG, and anti-NP-IgG increased in parallel. Virus-specific antibodies were predominantly of the IgG1 subclass, as measured by IgG subtype-specific Luminex (*not shown*). To assess the functional capacity of the serum antibody levels, we performed SARS-CoV-2 neutralization assays against the Wuhan-Hu-1-like original strain (B.1), the Alpha (B.1.1.7), and Delta variants (B.1.617.2) (see online repository text). The SCIG#2 preparation demonstrated high neutralization titers against the B.1 and B1.1.7 strains (median NT50 2519 and 2436, respectively), and reduced but measurable neutralization against the Delta variant (NT50 909) (*Figure E1*). After four weeks of treatment with SCIG#2, the patient’s serum neutralized all three SARS-CoV-2 variants (NT50 146, 114, and 81, respectively) in the range of convalescent plasma(4).

We then applied pharmacokinetic (PK) modeling to predict steady-state anti-S-IgG serum antibody levels based on the total IgG and the anti-S-IgG concentrations in the SCIG preparations. In the first step, we build a linear one-compartment PK model parameterized with IgG clearance, volume at steady state, and absorption parameter, which is only required for subcutaneous administration. Further details can be found in the online repository text. Typical PK parameter values were derived from the literature (5, 6). The clearance was calculated from the patient’s total IgG trough concentration (C<sub>trough</sub>) at steady state by dividing the total weekly dose of SCIG with the measured IgG C<sub>trough</sub> at steady-state multiplied by seven days. Using this base PK model, simulations of the anti-S-IgG serum levels were conducted and compared to the measured *in vivo* data. PK parameters of the base model were updated to match the observed data accordingly. The refined PK model predicted C<sub>trough</sub> levels at steady state for anti-S-IgG to reach ~665 IU/mL with SCIG#2 (*Figure 1*).

Twenty-three days following the switch to SCIG#3 containing 39783 IU/mL anti-S-IgG (*Table 1*), we observed a further increase of the anti-S-IgG serum level to 1533 IU/mL,
which was in good agreement with the model predicting a level of 1425 IU/mL (Figure 1).

Using SCIG#3, we predicted a steady-state in vivo serum level of ~2100 U/mL after about four weeks. Indeed, in a follow-up sample after 20 weeks on SCIG#3, the serum anti-S-IgG level increased to 2278 IU/mL. We repeated neutralization assays on this serum sample against B.1, B.1.617.2, and the by then dominating- B.1.1.529 Omicron variant. We observed a further increase in the serum neutralization capacity against B.1 and B.1.617.2 compared to the sample taken after four weeks (NT50 426 vs. 146 for B.1 and 137 vs. 81 for B.1.617.2).

Neutralization against B.1.1.529 Omicron was poor (NT50 25), as expected based on the literature on serum of convalescent and vaccinated subjects (7). However, we detected cross-reactive non-neutralizing antibodies against Omicron using Luminex (Figure E2).

Our data indicate that more recently collected IVIG and SCIG preparations contain substantial levels of anti-SARS-CoV-2 IgG. These levels are sufficient to increase the serum antibody levels in severely antibody deficient patients to values found in vaccinated or convalescent individuals associated with protection from severe COVID-19 disease. Given that immunoglobulin preparations also contain high non-neutralizing antibodies against the nucleoprotein and cross-reactive non-neutralizing antibodies against the Omicron spike protein, protection from SARS-CoV-2 may expand beyond neutralizing antibodies only.

Our data confirm recent reports of anti-SARS-CoV-2 antibodies against the spike or nucleocapsid protein in immunoglobulin preparations (8,9). However, compared to the most recent SCIG preparations used in our study, the reported anti-viral antibody levels were substantially lower, and these studies did not address whether the antibody levels in the IVIG/SCIG preparations translate into high anti-SARS-CoV-2 IgG in recipient serum.

Currently, manufacturers provide no information on the anti-S-IgG content in their immunoglobulin preparations or on the time of plasma collection. Plasma pools are collected 7-12 months before the production date indicated in Table 1. Thus, plasma of the products
tested in our study were collected prior to the COVID mass-vaccination campaign. A further increase in the anti-S-IgG levels in immunoglobulin preparations can be expected for the near future. The proposed PK modeling approach is suited to predict the anticipated steady-state levels of anti-S-IgG at a given level in the SCIG preparation. However, this approach implies that there are negligible endogenous IgG levels and that the clearance derived from steady-state concentration is in the same range as the clearance of the anti-S-IgG. Further studies will be required to define whether our data can be directly applicable to patients with higher endogenous IgG levels. Once IVIG/SCIG with high anti-S-IgG are broadly available, the method can be refined to enable applicability also to immunosuppressed patients with relevant endogenous IgG levels. In conclusion, our findings suggest that immunodeficient patients failing to develop vaccine-induced anti-SARS-CoV-2 antibodies may be passively immunized by immunoglobulin replacement therapy. The achievable anti-viral antibody levels *in vivo* may be pharmacokinetically predicted and may guide personalized dosing. The observed reduced neutralization against the Omicron variant suggests that patients with inborn errors of immunity may still be in need to receive prophylactic monoclonal antibodies with retained neutralization capacity against all circulating SARS-CoV-2 variants to be optimally protected.
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**Ethics approval**

The study was approved by the ethical committee of the northwest and central Switzerland (EKNZ 2015-187) as part of the prospective cohort study of the functional and genetic architecture of primary immunodeficiencies.

**Data availability**

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

**Authors’ Contribution Statements**

All authors contributed to the study. Material preparation, data collection and analysis were performed by Julia R. Hirsiger, Sebastian Weigang, Antje-Christine Walz, Jonas Fuchs, Mary-Louise Daly, Stefan Eggimann, Oliver Hausmann, Mike Recher and Christoph T. Berger.
Study design, conception, interpretation, and funding were performed by Martin Schwemmle, Georg Kochs, Marcus Panning, Klaus Warnatz, Mike Recher, and Christoph T. Berger. The first draft of the manuscript was written by Julia R. Hirsiger, Antje-Christine Walz, and Christoph T. Berger, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.
Table 1: Anti-SARS-CoV-2-IgG in IVIG and SCIG preparations

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<th>Batch ID</th>
<th>Production date</th>
<th>Expiration date</th>
<th>Product (Manufacturer)</th>
<th>%</th>
<th>Lot#</th>
<th>Spike IgG/M (U/ml)</th>
<th>S1-IgG (MFI)</th>
<th>S1 RBD-IgG (MFI)</th>
<th>NP-IgG (MFI)</th>
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*Production date indicates the date the pooled plasma is bottled. The collection of the plasma samples occurs >7-12 months prior to this date. Exact dates are not released by the manufacturers. 1All values measured at the same time in the Roche Elecsys® Anti-SARS-CoV-2 assay (lower level of detection <0.7 U/ml; upper level of detection >2500 U/ml);

2Luminex assay in median fluorescence intensity (MFI) (background level <50). IVIG= intravenous immunoglobulin; SCIG= subcutaneous immunoglobulin. The three batches used in the in vivo study are in bold.
References


Figure legend

Figure 1: Anti-spike antibodies and pharmacokinetic modeling of anti-S-IgG immunity.

Observed and predicted anti-S-IgG serum concentrations using SCIG#2 with 12413 IU/mL anti-S-IgG (blue line; day 0-56), and SCIG#3 with 39783 IU/mL (red line; day 57-144). The blue dotted line indicates the predicted anti-S-IgG steady-state through levels with SCIG#2 (predicted C_\text{through} \approx 665 IU/mL). Simulations show a predicted further increase in anti-S-IgG serum concentration after switching to the SCIG#3 (red line) (predicted C_\text{through} \approx 2100 IU/mL). Serum anti-S-IgG measurements on days 53 (under SCIG#2; blue dots), 85, and 144 (under SCIG#3; red triangles) confirmed a good agreement with the model. Black dotted lines indicate the upper and lower prediction intervals (dotted black lines).
**Online Repository Text**

*Luminex anti-SARS-COV-2-antibody binding testing.* Magnetic MagPlex-microspheres (Luminex Corporation, Austin, TX, USA) were coated using 10 ng SARS-CoV-2 spike protein (eENZYME LLC, Gaithersburg, MD, USA) according to xMAP® cookbook (Luminex corporation). Bovine serum albumin (BSA)-coated beads were the negative control. To measure anti-S-specific-IgG, M, and IgG subclass specific antibodies, serum or the SCIG/IVIG preparations were mixed with spike protein-coated or BSA-coated control beads for one hour at RT on a plate shaker at 800 rpm. The beads were washed twice with PBS-TBN buffer and then incubated with a PE-labeled mouse anti-human detection antibody (anti-IgG, -IgM, -IgA, -IgG1-4; all SouthernBiotech, Birmingham, AL, USA) for another 45 minutes. Binding of spike-specific detection antibodies was measured on a Luminex® 100 analyzer running on xPonent® software (Luminex corporation). The extent of antibody-binding is represented by the PE median fluorescence intensity (MFI).

We found that serum after 4, 6, and 7 weeks on SCIG#2 showed increasing amounts of anti-S1(B.1)-RBD-IgG (median fluorescence intensity (MFI): 2740, 3692, and 4212) and anti-NP (median MFI: 1107, 1503 and 1834) levels. Using different recombinant spike proteins of the B.1 (Wuhan), B.1.617.2 (Delta), and B.1.1.529 (Omicron) variants, we also tested for variant-specific binding antibodies, as shown in Figure E2.

Immunoglobulin IgG1-4 subset specific anti-S-IgG measurement in the SCIG products showed that the anti-S-IgG were predominantly of the IgG1 subset (70-80%) and less of the IgG2 subset. Similarly, the patient's serum contained predominantly anti-S-IgG of the IgG1 subclass. Interestingly, the RBD response showed a higher amount of IgG2 subclass antibodies in the serum.

*SARS-CoV-2 in vitro neutralization assay.* Virus neutralization experiments with SARS-CoV-2 were performed under Biosafety Level 3 (BSL3) protocols at the Institute of Virology, Freiburg. Adherent African green monkey kidney VeroE6 cells (ATCC CRL-1586™) were cultured in 1x Dulbecco’s modified Eagle medium (DMEM) containing 5% or 10% fetal calf serum (FCS). The following SARS-CoV-2 isolates were used to test for neutralization: Delta variant B.1.617.2 (EPI_ISL_2535433) isolated from a patient in Freiburg, Germany; Muc-IMB-1, lineage B.1 (EPI_ISL_406862 Germany/BavPat1/2020); Alpha variant B.1.1.7 (EPI_ISL_751799); and Omicron variant BA.1 (EPI_ISL_6959868). Neutralizing antibody titers were determined by a plaque reduction assay. Serial 2-fold dilutions of the sera or the SCIG preparations were incubated for 1 h with 100 pfu of the respective SARS-CoV-2 isolates. The serum-virus mixture was then used to infect VeroE6 for 90 min at 37°C. The inoculum was removed and the cells were overlaid with 0.6% oxoid agar for 48 to 72h at 37 °C. Cells were fixed with 3.7% formaldehyde and stained with crystal violet. The reduction in counted plaque numbers was determined compared to an untreated mock-infected control without serum. Each experiment was performed in three independent replicates. A least-square non-linear regression was calculated (constraints: 0 and 100 %), and the NT_{50} values were determined based on the curve fits.

**Pharmacokinetic modeling.** The patient received an average total dose of ~20g SCIG per week, resulting in a trough concentration at steady state (Css,trough) of 11.5 g/L IgG. From this observation, an estimated Clearance of 0.25L/Day was derived according to the
following formula: $CL = \text{total weekly dose}/(C_{ss,\text{trough}} \times 7\text{day})$. Two different batches were administered, batch 1 (Charge P100360850) had a concentration of 12’413 IU/mL, and batch 2 (Charge P100388734) had a concentration of 39’783 IU/mL of the anti-S-IgG. For PK prediction, a one-compartmental model was used with a 1st order absorption rate constant $ka$ of 0.34 1/day (5), a central Volume $Vc$ of 7.4L, a Clearance of 0.22L/day, and an estimated halflife of ~ 25 days in the range of reported values for total IgGs(6). The simulations were conducted in Berkeley Madonna v8.3.18.
Supplementary Figures

Figure E1: Virus neutralization against different SARS-CoV-2 variants of concern. Virus neutralization capacity of the SCIG#2 product and the patients’ serum 4 and 20 weeks after immunoglobulin substitution with products containing increasing anti-S-IgG are shown. Data represent the mean and standard deviation of three independent experiments. SCIG#2 shows high neutralization NT$_{50}$ against the original strain (B.1), B.1.1.7 (Alpha), and about 3-fold reduced neutralization against B.1.617.2 (Delta). The patient’s serum showed virus neutralization against all three SARS-CoV-2 variants, albeit at lower NT$_{50}$. In steady-state after 20 weeks, neutralization capacity increased further. However, the newly emerging Omicron variant was only very poorly neutralized. PRNT$_{50}$ = 50% reduction in the plaque reduction neutralization test. Data derive from three independent experiments. Bars indicate median and range. n/a indicates that the respective variant was not tested for the timepoint.

Figure E2: Non-neutralizing strain-specific antibody response. Antibody binding to the spike protein (S1, left) and the receptor-binding domain of the spike protein (RBD, right) of the B.1 (Wuhan), B.1.1.617.2 (Delta), and B.1.1.529 (Omicron) were measured. We compared the binding of patient serum (20 weeks on the anti-SARS-CoV-2 containing SCIG), serum of five mRNA vaccinated (B.1 vacc) subjects collected four weeks after the second dose of an mRNA vaccine (Spikevax; Moderna) and of five subjects with Omicron breakthrough infection despite two doses of an mRNA vaccine (B.1 vacc + B.1.1.529). RBD IgG are considered a surrogate for virus neutralization, and total anti-S1-IgG also contain the non-neutralizing antibodies. Data indicates cross-reactive anti-S1-IgG that recognize Omicron in all tested subjects, whereas only Omicron breakthrough infection associated with high anti-RBD-IgG against Omicron.