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Immunoadsorption as a method of antibody donation during the COVID-19 pandemic

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Abstract

Background and Objectives: Initial therapeutic efforts to treat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) included the use of plasma from convalescent donors containing anti-SARS-CoV-2 antibodies. High-neutralizing antibody titres are required for therapeutic efficacy. This study aims to show that immunoadsorption followed by tangential flow filtration can be used to obtain antibody concentrates with high-neutralizing capacities.

Materials and Methods: Eligible donors ($n = 10$, five males and three females) underwent immunoadsorption using adsorber columns specific for human antibodies. Glycine-washed out eluates of 1.5 L volume were further concentrated by tangential flow filtration using 30 kDa ultrafiltration membranes. The same membranes were applied for diafiltrations to exchange residual glycine for 0.9% normal saline.

Results: Antibody concentrates were obtained within 8 h from the start of donation and had 4.58 ± 1.95, 3.28 ± 1.28 and 2.02 ± 0.92 times higher total IgG, IgA and IgM concentrations, 3.29 ± 1.62 and 3.74 ± 0.6 times higher SARS-CoV-2 N and S antibody concentrations and 3.85 ± 1.71 times higher SARS-CoV-2 S-specific IgG concentrations compared to the donors' peripheral blood. The specific SARS-CoV-2 virus neutralization capacities increased in all but one concentrate. All antibody concentrates (50–70 mL final volume) passed microbiological tests, were free of hazardous glycine levels and could be stored at −80°C and 4°C for 1 year with 20 ± 3% antibody loss.

Conclusion: Immunoadsorption followed by tangential flow filtration is a feasible procedure to collect IgG, IgA and IgM as well as SARS-CoV-2 N- and S-specific antibody concentrates of low volume, free of albumin and coagulation factors. Whether these concentrates can be used as passive immunisation in infected patients remains to be elucidated.

Keywords
antibody donation, COVID-19 convalescence, immunoadsorption, virus neutralization

Highlights

- Combining immunoadsorption and tangential flow filtration is a feasible method to obtain antibodies from convalescent donors. We have successfully collected IgG, IgA and IgM antibodies as well as SARS-CoV-2 N- and S-specific antibodies from peripheral blood in all 10 donations. A median of 2586 ± 741 mg IgG, 271 ± 83 mg IgA, 138 ± 73 mg IgM have been obtained per donation.
- The resulting antibody concentrates had on average three to four times higher SARS-CoV-2-specific antibody concentrations compared to the peripheral blood of the donor and showed increased neutralization capacity in all but one sample.
- This is a safe method for manufacturing antibody concentrates of approximately 50 mL within 1 day that can be stored at 4°C and −80°C for over 2 years and are free of albumin and coagulation factors.

INTRODUCTION

Several treatments against COVID-19 are available to help patients with severe symptoms. One of them is the use of COVID-19 convalescent plasma (CCP) [1]. This approach relies on the hypothesis that antibodies are formed in sufficient amounts in the previously infected individuals to be donated as passive immunisation.

Studies on the effectiveness of CCP in treating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have identified the importance of high neutralization titres [2–4].

In this study, we present a method of antibody donation that can obtain high neutralization titres, by using one-time plasmapheresis including immunoadsorption (IA) followed by tangential flow filtration (TFF) for the production of antibody concentrates (ACs) as an alternative to CCP. The ACs are of low volume and free of plasma components like albumin and coagulation factors that potentially could, if still present, lead to unwanted side-effects during transfusion. Side effects possibly related to volume overload, when CCP doses are increased, are also circumvented.

IA is an established alternative to plasma exchanges for the treatment of a wide range of autoimmune diseases and is considered to be safe for patients [5, 6]. Its efficacy is based on the removal of immunoglobulins from patients' plasma and simultaneously returning most of the antibody-cleared plasma to the patients whereby removed antibodies are usually discarded. Instead of throwing these antibodies out, we collected them from our convalescent donors during in-process elutions of the adsorbers [7].

TFF is commonly used in the downstream processes of pharmaceutical products, for example in the concentration of immunoglobulins, vaccines and recombinant proteins [8–10], using a porous membrane of defined pore size that traps the product in a loop while constantly removing liquids plus particles smaller than the defined pore size. In addition, it can be used to exchange liquids by continuously adding a new solution to the loop at the same rate the previous one is removed, a process called diafiltration. TFF has already been successfully applied in the downstream process of SARS-CoV-2 neutralizing antibodies (nAb-SARS-CoV-2) derived from cell cultures [11].

Our first two IA for the production of ACs with reusable adsorbers, specific for IgG (subclasses 1–4), IgA and IgM antibodies, were quite promising [12]. Here, we present the extension of our initial case report, with additional quantitative data and focus on (1) using one-time adsorbers, (2) assessing the concentration process in detail and (3) comparing two different storage conditions, 4°C versus −80°C.

MATERIALS AND METHODS

Sampling

In all donations, samples for analyses were drawn from the peripheral blood of the donors before and after the IA as well as in the final ACs directly after preparation. Once COVID-19 antibody assay availability was significantly improved, immunoglobulins and specific antibodies were also monitored in eluates, during concentration and diafiltration steps of the TFF, as well as on several time points during storage at 4°C and −80°C for 2 years (3, 6, 9, 12, 18 and 24 months).

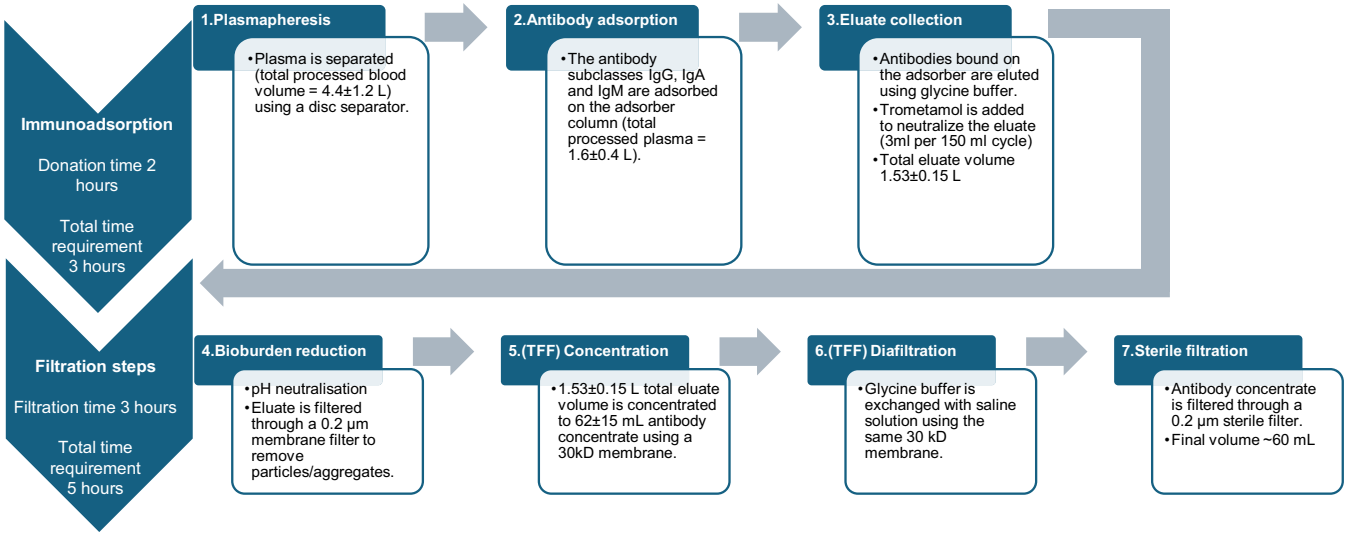

FIGURE 1 Schematic representation of the process steps during immunoadsorption and tangential flow filtration. Immunoadsorption is divided into three steps. Plasma donation (separates the antibody containing plasma from the remaining blood), antibody adsorption (binds the antibodies on the adsorber) and eluate collection (using a glycine buffer to elute the antibodies). Tangential flow filtration is divided into four steps. Bioburden reduction filtration (to remove particles larger than 0.2 µm), concentration (to concentrate the antibody solution), diafiltration (to exchange the glycine buffer with saline solution in water) and final sterile filtration (to remove contaminants).

Immunoadsorption

Five male and three female donors participated in this study after medical assessment, as previously described [7]. Informed consent was obtained and documented from all donors. Two of the donors (1 and 2) agreed to donate twice with approximately 1 year gap between the first and second donation. Each donor had recovered from a previously diagnosed SARS-CoV-2 infection prior to the antibody donation. Due to initial non-availability, the first three donors had not been vaccinated before IAs. IAs were performed as previously described [12] and were tolerated by all donors very well. A schematic presentation of each step of antibody donation and concentrate preparation can be found in Figure 1. Each donor was tested for serum protein, SARS-CoV-2 antibodies, immunoglobulins and blood count before inclusion in the study, before and after IA.

Tangential flow filtration

To remove excessive volume, column eluates obtained from the IA containing IgG-, IgA-, IgM- and COVID-specific antibodies were first filtered through a 0.2 µm membrane filter with 260 cm^2 filter area (Supor EAV, Cytiva Dreieich, Germany) to reduce the bioburden and then concentrated using a TFF system as previously described [7]. A total of 0.9% (w/v) NaCl solution in water, subsequently referred to as saline solution, was used to exchange the remaining glycine [12]. Finally, sterile filtration with 5.6 cm^2 filter area (Supor ECV, Cytiva Dreieich, Germany) was performed before aliquoting the ACs in 1.5 mL tubes. Samples were stored at 4°C and −80°C. A schematic representation of each step during TFF can be found in Figure 1.

Antibody determination and virus neutralization

The Tina-Quant IgG/IgA/IgM Gen.2 immunoassays (Roche Diagnostics GmbH, Mannheim, Germany) are immunoturbimetric assays and were used in combination with the Cobas 501 analyser to determine the IgG/IgA/IgM concentrations. The assays are based on goat-derived anti-human IgG that reacts by forming an antigen/antibody complex. Polyethylene glycol was then added to determine concentrations using a turbidimeter.

SARS-CoV-2 N-specific antibodies were determined using the quantitative Elecsys Anti-SARS-CoV-2 electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics GmbH, Mannheim, Germany) on the Cobas e801 analyser. Biotinylated SARS-CoV-2 specific recombinant antigen and ruthenylated SARS-CoV-2 specific recombinant antigen combine to form a sandwich complex. This complex represents the nucleocapsid (N) antigen of SARS-CoV-2. Sample is added to the mixture and the SARS-CoV-2 N specific antibodies present in the sample bind to the sandwich complex. This complex then subsequently binds to streptavidin-coated microparticles. Inside the measuring cell, the microparticles are magnetically captured onto the surface of the electrode. Chemiluminescent emissions are induced and measured using a photomultiplier. Results are reported as cut-off indices (COI) comparing the electrochemiluminescent signal with cut-off values previously determined via calibration. Calibration curves were added by the manufacturer in a later assay version and used from donation 3 onwards.

The Elecsys Anti-SARS-COV-2 S ECLIA (Roche Diagnostics GmbH, Mannheim, Germany) is a quantitative assay to determine antibodies against the receptor binding domain (RBD) of the spike (S)-protein (SARS-CoV-2 S-specific antibodies). This assay was used as soon as it became available starting from the third donation in

combination with the Cobas system. It is based on a recombinant protein that represents the RBD from the spike (S)-antigen resulting in a chemiluminescent sandwich ELISA analogous to the Sars-Cov2 N assay. A 2-point calibration curve is used to determine the concentration of SARS-CoV-2 S-specific antibodies, reported in IU/mL (1 IU/mL is equivalent to 1 binding antibody unit [BAU]/mL).

To detect IgG levels against the SARS-CoV-2 spike S1 subunit (S), the anti-SARS-CoV-2 QuantiVac-ELISA (Euroimmun, Luebeck, Germany) was applied, and the results are reported in BAU/mL. Values are plotted against a 6-point calibration curve using World Health Organisation reference serum.

To determine the antiviral efficacy, in-house full virus neutralization tests were performed as previously described using the Wuhan-Hu1 WT Isolate (GISAID Accession number: EPI_ISL_425126) [12].

The Elecsys anti-thyroglobulin (Tg) and anti-thyroid-peroxidase (TPO) immunoassays (Roche Diagnostics GmbH, Mannheim, Germany) were used to determine the anti-Tg and anti-TPO antibody concentrations in donation 5. Both antibodies are disease-triggering autoantibodies in Hashimoto thyroiditis [13]. Antibodies from the sample compete with antibodies from the reagents for limited binding sites. The mixture is analysed on the Cobas e801 analyser. The results are determined using a 2-point calibration curve and converted into IU/mL.

Glycine, albumin and protein analyses

Glycine concentrations were measured during the IA and in the AC using the Biochrom 30+ amino acid analyser (Biochrom GmbH, Berlin, Germany). This ion exchange chromatography is based on five lithium citrate buffers with post column derivatization using Ninhydrin. The measurements were normalized according to the glycine HCl solution used during the IA.

The Albumin Gen.2 and the Total Protein Gen.2 test (Roche Diagnostics GmbH, Mannheim, Germany) were used to determine the albumin and total protein concentrations according to the manufacturer's instructions.

Microbiological tests

Microbiological controls were performed according to the Institute for Transplant Diagnostics and Cell Therapeutics standard operational procedures equivalent to tests on cellular products as demanded in the national guidelines for the production and administration of haematopoietic stem cell products [14].

For aerobic and anaerobic bacterial cultures, test tubes were inoculated with 1 mL each per product on the day of production after the last filtration step and repeated after 2 years in storage. Bacterial colonies were determined on day 8 after inoculation. In case of positive bacterial contamination detected by Virtuo (BioMerieux), bacterial colonies are analysed on agar plates.

Statistics

Statistical analysis to calculate median, standard deviation and significance (paired *t*-test) were performed using Microsoft Excel 365.

Ethics statement

The Ethics Committee of the University Clinic Duesseldorf has reviewed this study and voted positive (study number 2022-1132). Informed consent was obtained and documented from all donors.

RESULTS

IgG, IgA and IgM concentrations and absolute values

The median antibody concentrations of IgG, IgA and IgM in donors' peripheral blood prior to donations were 947 ± 211 mg/dL, 141 ± 40 mg/dL and 118 ± 82 mg/dL, respectively (Table 1). Antibody serum concentrations in the peripheral blood decreased after IA on average by 29 ± 7% (IgG), 27 ± 7% (IgA) and 23 ± 6% (IgM). Final concentrations in the AC compared with the peripheral blood increased 4.6 ± 2.0 times for IgG, 3.3 ± 1.3 times for IgA and 2.0 ± 0.9 times for IgM. A median of 2586 ± 741 mg IgG, 271 ± 83 mg IgA, 138 ± 73 mg IgM have been obtained per donation.

SARS-CoV-2 antibody concentrations and absolute values

SARS-CoV-2 nucleotide (N)-specific antibodies in donors' sera prior to the IA ranged between 1.7 COI and 154 COI (Table 1). After IA, the SARS-CoV-2 N antibody serum concentrations decreased on average by 32 ± 15%. SARS-CoV-2 N antibodies in the final AC resulted in 1.3–6.8 times higher values compared with the peripheral blood.

In addition to the SARS-CoV-2 N antibodies, antibodies against the spike protein (S) were determined by two different test systems except donations 1 and 2 in the final AC ranging between 1773 and 180,000 IU/mL.

Once the test was available on a routine basis, SARS-CoV-2 S-specific IgG was measured between 597 and 8162 BAU/mL (Table 1) and decreased on average by 24% ± 11% after IA in peripheral blood of the donors. In the final AC, IgG specific for SARS-CoV-2 S lay 3.8 ± 1.8 times higher compared with the peripheral blood.

Comparing the neutralization titres from the peripheral blood of the donor to the AC, all but one titre (donation 4) had increased by at least one titre level (two-fold serial dilution) (Table 1). On average, an increase of 2 ± 1.3 titre levels was determined. Most prominent titre increases were seen in donation 5 (increased from 1:1280 to 1:10240) and donation 6 (from 1:1280 to 1:20480) (Table 1). Median absolute SARS-CoV-2 specific IgG content ranged from 36,134 to 1,203,482 BAU.

TABLE 1 Summary of antibody concentrations, neutralization titres, glycine rest and final product volume.

	Donor blood before IA					Donor blood after IA					Antibody concentrate						
	IgG, IgA, IgM	CoV2 (N)	CoV2 (S)	CoV2 (S IgG)	NT	IgG, IgA, IgM	CoV2 (N)	CoV2 (S)	CoV2 (S IgG)	NT	IgG, IgA, IgM	CoV2 (N)	CoV2 (S)	CoV2 (S IgG)	NT	Glycine	Volume
Donation	mg/dL	COI	IU/mL	BAU/mL	1/x	mg/dL	COI	IU/mL	BAU/mL	1/x	mg/dL	COI	IU/mL	BAU/mL	1/x	µmol	mL
1	899, 119, 84	30.5[a]	t.n.a.	t.n.a.	n.t.	600, 83, 66	t.n.a.	t.n.a.	t.n.a.	n.t.	5591, 523, 212	106[b]	t.n.a.	738[b]	160	11.7	49
2	879, 113, 111	42.5[a]	t.n.a.	t.n.a	20	558, 74, 89	25.2[a]	t.n.a.	t.n.a.	n.t.	7696, 603, 372	148[b]	t.n.a.	937[b]	80	67.5	61
3	1551, 197, 251	154	597	597	80	996, 127, 163	114	n.t.	442	80	4956, 456, 367	204	1773	623	160	12.6	58
1.1	919, 120, 85	1.7	>2500[c]	3535	2560	632, 90, 61	1.3	>2500[c]	2707	2560	6176, 661, 333	11.6	>2500[c]	25,058	10,240	1.63	44
4	1212, 175, 148	91	>2500[c]	3681	2560	946, 146, 120	73.6	>2500[c]	3224	1280	2534, 274, 140	177	>2500[c]	10,706	2560	13.1	101
5	962, 177, 325	53	>2500[c]	3280	1280	752, 144, 274	37.8	>2500[c]	3140	640	3628, 444, 430	171	49,046	15,600	10,240	18.5	72
2.1	931, 130, 124	14.6	>2500[c]	1882	640	700, 98, 97	11	>2500[c]	1224	n.t.	2753, 338, 164	45	53,227	6400	1280	12.5	63
6	1166, 152, 72	112	55,052	8162	1280	904, 117, 59	80	39,631	5564	1280	3904, 370, 116	140	180,000	20,398	20,480	11.1	59
7	1110, 87, 169	60	6304	1206	320	830, 67, 135	43	4655	802	320	4517, 248, 271	184	29,013	6021	2560	10.8	50
8	802, 217, 55	7	23,451	2558	1280	469, 136, 36	2	5622	678	640	3755, 721, 115	36,8	96,894	10,466	5210	12.2	61
Median	947, 141, 118	48	23,451	2919	1280	726*, 108*, 93*	38*	5622	1966	640	4211, 450, 242	144	51,137	8433	2560	12	60
Std. dev.	211, 40, 82	47	20,191	2174	906	168, 28, 66	37	16,265	1651	770	1516, 153, 112	65	57,535	8066	6249	17	15

Note: Shown are concentrations of IgG-, IgA-, IgM- and COVID-specific antibodies (Sars-CoV-2 N and S) and neutralization titres (NT) in the peripheral blood of donors before and after the immunoadsorption (IA) and in the final antibody concentrate.

a: Determined with a qualitative test, due to lack of better options. b: Determined on back up sample as soon as a quantitative test was available (6 months). c: Sample was not diluted. Data points not included in median; t.n.a.: test not available.; n.t.: not tested.

*Significant difference between before and after IA.

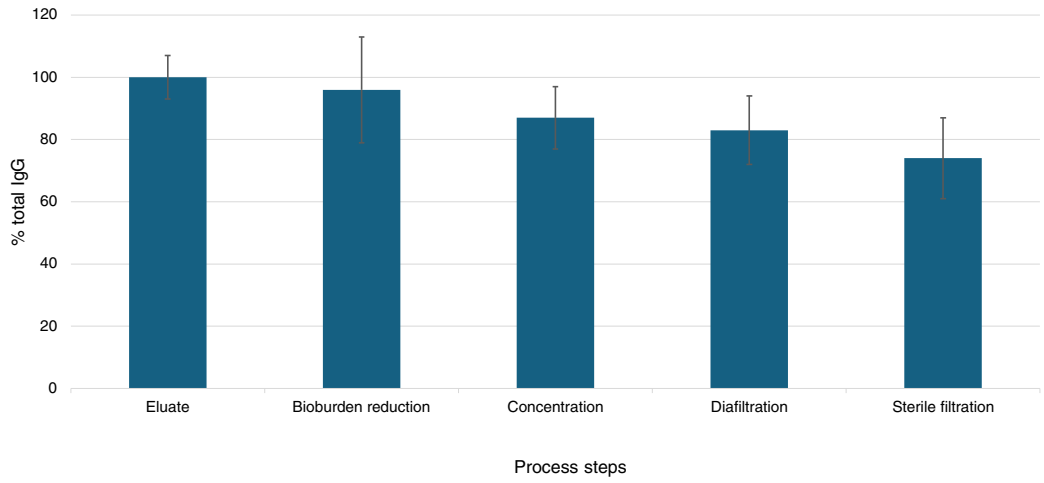

FIGURE 2 Recovery of IgG at every filtration step. Average IgG recovery, shown as a percentage of the baseline, were monitored during every step of the process (after bioburden reduction, concentration, diafiltration and sterile filtration) in donations 2.1, 5, 6, 9 and 10. Total IgG content of the eluate served as baseline 100% and was calculated using the IgG concentration in mg/dL multiplied by the volume at that stage. Recovery for donation 5 at the bioburden step was not measured.

Monitoring TFF

In five donations (2.1, 5, 6, 9 and 10), product loss was monitored during TFF. Small amount of IgG was lost during each step of the process (Figure 2). On average, 13.0% ± 7.7% were lost during concentration, 4.2% ± 3.9% during diafiltration and 8.6% ± 5.2% during sterile filtration. The highest loss of IgG was found in donation 2.1 with 25% during the concentration step. The cumulated IgG loss over all process steps was 26% ± 8%.

Safety and storage

Glycine is necessary for the removal of bound antibodies from adsorber matrices allowing a quick in-process recovery of matrix-bound anti-human IgG for following adsorber cycles. Using diafiltration, glycine was successfully removed from our ACs and replaced by saline solution. In every AC, the final glycine concentration was below 1% of the original value (Table 1), and final microbiological tests showed that all ACs passed microbiological tests after donation.

The end volume of every AC was separated into 1.5 mL tubes and then stored at 4°C and −80°C. ACs 1 and 2 from the first two donations were stored for 2 years already. Looking at SARS-CoV-2 N specific antibodies, samples frozen at −80°C compared with those stored at 4°C show a slightly better outcome in frozen specimen (donation 1 at 176 COI [−80°C] compared with 133 COI [4°C] and donation 2 at 228 COI [−80°C] compared with 217 COI [4°C] [Table SI]).

Comparison of Ig flex and Ig omni 1

Antibody concentrates 1 and 2 were produced by using Ig flex columns for IA. Interestingly, the values of SARS-CoV-2 N specific antibodies started to increase during storage at −80°C for 12 months in the first two ACs adsorbed with Ig flex. The SARS-CoV-2 N specific antibodies from donation 1 almost doubled from 106 to 196 COI and increased from 148 to 246 COI in the AC from donation 2. Consecutively, in the second year of storage, the values decreased to 158 COI (donation 1) and 174 COI (donation 2) after 24 months of storage. This was different in those ACs collected using the Ig omni 1 adsorbers. Though not all the donations using Ig omni 1 adsorbers have been stored for more than 1 year yet, SARS-CoV-2 N- and S-specific antibodies remained stable after 6 and 12 months (Table SI).

SARS-CoV-2 S-specific antibodies show a similar trend under storage conditions. Freezing at −80°C preserved slightly more antibodies for donation 1 compared to storage at 4°C with 453 IU/mL (−80°C) compared to 350 IU/mL (4°C) after 18 months. Concentrations of donation 2 were equal in samples stored at −80°C and 4°C (783 IU/mL) after 18 months of storage (Table SII). The values for the first two donations also increased during the second year of storage from 426 to 449 IU/mL (donation 1) and 730 to 783 IU/mL (donation 2). Donations of ACs obtained by Ig omni 1 adsorbers did not show concentration changes during storage.

Hashimoto thyroiditis

Donor 4 turned out to have a positive history of autoimmune Hashimoto thyroiditis, which had not been communicated during the enrolment in this study but was detected afterwards by coincidence. Of course, anti-Tg and anti-TPO antibodies were also collected and concentrated by our method. All antibodies and immunoglobulins remained stable with the exception of anti-Tg which doubled during 1 year of storage (Figure S1).

DISCUSSION

IA is an effective therapeutic approach to reduce circulating autoantibodies [5, 15]. To our knowledge, we are the first to use IA to obtain

viral-specific antibodies with the objective to achieve an AC for potential therapeutic use. Using IA followed by TFF, we succeeded in the production of 10 ACs from convalescent donors. The results show that the ACs contain IgG, IgA and IgM as well as the SARS-CoV-2 N and S-specific antibodies in high concentrations. In addition, we were able to show that the antibodies' neutralizing capacities for the SARS-CoV-2 virus was not lost, but instead increased in all but one sample; the neutralization capacity in that particular sample remained stable.

In contrast to CCP therapy, ACs obtained from IA are concentrated to a volume of 50 mL and do not contain plasma components, like albumin and coagulation factors. Though no severe side effects were reported in trials using CCP [16], our low-volume ACs could potentially be applied in patients with heart or renal decompensation, who had been excluded from CCP trials.

We tested two different adsorber pairs, namely the Ig flex used for donation 1 and 2 and—as production of the Ig flex was discontinued—changed to Ig omni 1 in the following procedures. Ig flex uses polyclonal sheep anti-human IgG and binds to multiple epitopes on the Fc and F(ab) fragments. Ig omni 1 columns are coated with recombinant camelid anti-human immunoglobulins and bind only to the constant region of the light chains. Ig omni 1 and Ig flex are similar in removing IgG and IgA, whereas the design of Ig flex suggests a preference for IgG accompanying a lesser efficiency in IgM removal [17].

Considering that 75% removal of IgG was observed during one-time IA on other studies and can be increased to 95% when repeated on two consecutive days [15], the median 29 ± 7% IgG removal in this study is comparatively low. Increasing the blood volume during antibody donation using plasmapheresis is possible and could further increase the output. Afterwards, patients' IgG levels recovered to 75% of the pre-treatment values 2 months after IA with a low risk for infectious complications without immunoglobulin substitutions [18]. Likewise, no clinical diagnosis of SARS-CoV-2 reinfection was reported to the investigators after donation.

Antibody concentrates gained by Ig omni 1 seemed to be more stable, whereas ACs produced with Ig flex demonstrated continuous increases in SARS-CoV-2 specific antibodies during storage, while total amounts of immunoglobulins (IgG, IgA and IgM) remained stable at the same time. One explanation could be that plasmatic sheep antibodies used in Ig flex initially blocked SARS-CoV-2 binding sites and were partially removed upon storage. Another explanation could be conformational changes of these eluted antibodies by freeze-thawing. This might also explain the increase of anti-thyreoglobulin, as observed in one donor.

In contrast, camelid-coated Ig omni 1 adsorbers resulted in stable COVID-19 ACs. This stands in line with findings on CCPs, in which antibody concentrations and neutralization titres remained stable for 42 days at 4°C as well as after freezing and thawing [19].

In the beginning of the COVID outbreak in 2019, initial studies using CCP revealed that patients treated with high (≥1:250) neutralization titres have a low but significant 1% (95% CI = 0.24, 1.78) absolute reduction in 7-day mortality and 1.5% (95% CI = 0.43, 2.67) absolute reduction in 28-day mortality [20].

As stated by Focosi et al., in order to achieve a measurable (two-fold) increase in the SARS-CoV-2 antibody titre of patients, one 200 mL plasma unit has to contain an approximately 10 times higher antibody titre compared with the patients' blood, because it will be diluted in about 2.5 L of patient plasma volume thereby possibly reducing therapeutic efficacy [4]. Increasing the amount of plasma-transfused means more volume load and coagulation factors co-administered to the patient, with potentially unwanted side effects like a transfusion-related circulatory overload, allergic, febrile or other reactions of incompatibility. In contrast, IA returns these coagulation factors to the donors during the process of donation while still preserving or even increasing the antibody neutralization capacities.

Our ACs had neutralization titres of ≥1/10240 in 3 of 10 donations. Comparing with titres in the peripheral blood of donors to titres in the final AC, we can see a 2 ± 1.3 increase in titration endpoints including an increase of four titration levels in donation 6 as the highest increase. SARS-CoV-2 S-specific IgG content increased by 3.8 ± 1.8 times and up to 7.1 times in donation 1.1. This means that ACs manufactured by our method resulted in high titre levels independent of the initial blood values, thereby widening the range of suitable donors to those with low neutralization capacities [21].

Apart from CCP, monoclonal antibodies (mAbs) against COVID-19 supported patients' recovery; however, the new variants SARS-CoV-2 BQ and XBB have evasive properties against mAbs, so that the COVID-19 Treatment Guidelines Panel from the National Institutes of Health does not recommend the use of mAbs in its last update from 6 March 2023 [22]. In contrast, ACs obtained with our approach are derived from convalescent donors and can therefore be efficient against the newest variants as soon as a suitable donor is identified.

Studies have shown that the neutralization effect of CCPs can decrease down to the point of not being detectable if the CCP dose from an older variant is used in a patient suffering from a new variant [23]. It is therefore important to obtain antibodies as quickly as possible from the region of transmission for an efficient treatment [20]. The development of COVID-19 monoclonal antibodies (mAbs) is an extensive and time-consuming process. For example, it took 16 months and an emergency use authorization to get the first mAbs (Sotrovimab manufactured by GSK) ready to use [24]. This developmental process paired with a risk of failing regulatory requirements culminates in high prices per dose for mAbs. In contrast, our ACs can be produced within 1 day, as soon as a donor is available. IA is performed within 3 h, while the necessary preparation for the following downstream processing can be done in parallel. Bioburden filtration, concentration, diafiltration and sterile filtration took less than 5 h to finalise ACs production. Taking into account the fast development of new virus variants our approach provides new specific antibodies quicker than the development of monoclonal antibodies. Considering EU pharmaceutical legislation, our ACs may be considered as an Investigational Medicinal Product and not a Blood Product, depending on jurisdiction. This does imply clinical trials for dosing, safety, comparative efficacy and so forth similar to the development of monoclonal antibodies against viral infections. The possibility of compassionate use in urgent clinical situations remains as a last onsite option after ethical approval.

To assess the safety of the donated AC, all concentrates passed microbiological tests. Only the first donation was found to be positive for *Staphylococcus hominis* after 2 years of storage at −80°C, most likely due to handling contamination during test bottle inoculation. A retained sample from the same donation was retested and was found to be negative for aerobic microbes. Bound IgG, IgA and IgM as well as SARS-CoV-2 N- and S-specific antibodies were washed out from the adsorbers using a high molecular glycine solution. The latter was effectively removed and exchanged with saline solution during the diafiltration process leaving only small amounts of non-biohazardous residuals. The glycine buffer needed to be removed because of its high osmolarity (200 mmol/L), although it is termed as non-biohazardous (EG 1272/2008). The no adverse event level lies at 2 g/kg body weight. A total of 100 mL glycine buffer contains 1.5 g glycine, meaning that a person with an average body weight of 70 kg would have to be infused with 9.3 L of glycine buffer in order to develop potential side effects. As seen in one donor concealing his Hashimoto thyroiditis, unwanted autoimmune antibodies were also concentrated. However, we doubt that co-infusion of highly concentrated beneficial antiviral antibodies together with disease-triggering antibodies will lead to strong or permanent side effects, since autoimmunity cannot be transferred by blood donations. Nevertheless, this event calls for a comprehensive pre-donation scrutiny.

Pathogen inactivation has not been assessed in our ACs, but we want to point out that the process already contains two steps, which help to reduce pathogens. First, the adsorbers specifically bind to anti-human Fc of human immunoglobulins and not to virus membranes potentially present in plasma. Second, the glycin-HCL buffer used for eluting bound immunoglobulins from the adsorbers at each collection cycle is performed at low pH comparable to an acid elimination of potentially infectious particles. Once large-scale production from pooled eluates will be anticipated, additional pathogen inactivation procedures might come into practice.

In conclusion, IA combined with TFF proved to be a feasible method for the manufacturing of IgG, IgA and IgM as well as SARS-CoV-2 N and S ACs. These could be produced within 1 day, passed microbiological tests and were free of hazardous glycine levels. Final antibody neutralization titres were found to be as high or even higher than in the peripheral blood. However, we recognize that unwanted autoimmune antibodies will be concentrated to the same extent as the intended ones calling for careful donor screenings beforehand. Tests on clinical efficacy, for example, in animal models remain to be performed. With new viral replicants like those of H5N1 birds' influenza on the horizon, our strategy of on-site delivery of ACs could serve as a model in future pandemics.

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J.R. performed the research and wrote the first draft of the manuscript; J.R. and A.M. designed the research study; S.R.B. contributed essential reagents, tools, collection design and operational support; L.M., P.N.O. and D.H. performed the analytical methods on the samples; A.M., J.C.F and J.S. supervised the research and reviewed and edited the manuscript. Open Access funding enabled and organized by Projekt DEAL.

[Correction added on 25 May 2024, after first online publication: In addition to minor grammatical errors throughout the paper, the last author's ORCiD, Correspondence affiliation, Figures 1 and 2, Table 1, Reference 14, the 2nd last sentence of Materials and Methods: Immunoadsorption and the 1st sentence of paragraph 2 of the Acknowledgements were corrected.].

CONFLICT OF INTEREST STATEMENT

J.R. is employed by Cytiva; S.R.B. is an employee of Miltenyi Biotec. L.M., P.N.O., J.F., D.H., J.S. and A.M. have nothing to disclose.

DATA AVAILABILITY STATEMENT

Data and methods presented in this study are available in Transfusion Apheresis Science at DOI: 10.1016/j.transci.2021.103193 and in Transfusionsmedizin 2022; 12: 1–6 (ISSN 2191-8805) at DOI: 10.1055/a-1720-8203.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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