Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Transfusion and Apheresis Science 60 (2021) 103193

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

COVID-19 antibody donation using immunoadsorption: Report of two cases

Jannik Rothenburg [a,b], Silke Rink-Baron [c], Lisa Mueller [d], Philipp Niklas Ostermann [d], Johannes Fischer [a], Johannes Stegbauer [e], Anja Moldenhauer [a,f,*]

[a] Institute for Transplantat Diagnostics and Cell Therapeutics, University Hospital Düsseldorf, Heinrich-Heine-University, Germany
[b] Pall Corporation, Dreieich, Germany
[c] Miltenyi Biotec, Bergisch Gladbach, Germany
[d] Institute of Virology, Medical Faculty, University Hospital Düsseldorf, Heinrich-Heine-University, Germany
[e] Department of Nephrology, Medical Faculty, University Hospital Düsseldorf, Heinrich-Heine-University, Germany
[f] Saarland University, Homburg, Germany

ARTICLE INFO

Keywords:
COVID-19 convalescence
Antibody donation
Immunoadsorption
Virus neutralization

ABSTRACT

For more than a year the whole world is suffering from the COVID-19 pandemic with no treatment option in sight. Administration of plasma from convalescent donors containing anti-SARS-CoV-2 antibodies, though promising according to case reports, failed to show a clear benefit in a greater number of trials.

One reason could be varying and low antibody contents in a majority of plasma units hampering standardization and clinical efficacy. Besides, other plasma components unnecessarily transfused like coagulation factors might promote hypercoagulation seen in severe COVID-19 etiopathology.

We therefore hypothesized that instead of collecting whole plasma units, convalescent donors could donate solely immunoglobulins by undergoing immunoadsorption, a mode of therapy regularly applied in autoimmune diseases. Here, we report the results of the first two antibody donations performed at the University Hospital Düsseldorf.

In both cases, immunoadsorptions were very well tolerated with no side effects. Collected and neutralized eluates were concentrated using tangential flow filtration increasing the concentration of immunoglobulins 10fold as compared to peripheral blood and leading to probably eight times more neutralizing antibodies than in one plasma unit.

Therefore, immunoadsorption can be used as a method of antibody donation. Whether these donated antibodies can be used as passive immunization in acutely infected patients remains to be elucidated.

1. Introduction

Despite beginning vaccination campaigns, the treatment of SARS-CoV-2 (COVID-19) positive patients remains a worldwide challenge. One promising therapeutic option is the administration of monoclonal antibodies, which are currently applied in clinical trials [1]; another approach is the transfusion of plasma units from convalescent donors [2]. Though plasma had been applied at varying dosages ranging from 200 mL to 1.200 mL – dependent on the stage of disease - [3], the appropriate dosage remains unknown [4]. These units, however, contain not only beneficial antibodies, but also coagulation factors, albumin and fluids unnecessarily co-administered during transfusions. In light of an increased risk for thromboembolic events [5] or transfusion associated circulatory overload [2], avoidance of these extra and unnecessary components might be beneficial.

During immunoadsorption antibodies are removed during extracorporeal apheresis, a therapeutic modality frequently used in patients suffering from autoimmune diseases [6]. In the past, we evaluated immunoadsorption in patients suffering from multiple sclerosis (MS) not solely for its clinical efficacy, but also for the adsorbed components in adsorber eluates [7]. This enabled the detection of until then unknown autoantigens, namely Neurofascin [8] and Contactin-2 [9], which turned out to play a decisive role in MS pathogenicity.

Therefore, we hypothesize that immunoadsorption can be used in covalent plasma donors to obtain high antibody concentrates exempt from other plasma components.

* Corresponding author at: Institute for Transplantat Diagnostics and Cell Therapeutics, University Hospital Düsseldorf, Heinrich-Heine-University, Germany.
E-mail address: moldenha@hhu.de (A. Moldenhauer).

https://doi.org/10.1016/j.transci.2021.103193
Received 21 March 2021; Received in revised form 1 June 2021; Accepted 11 June 2021
Available online 15 June 2021

2. Methods

2.1. Immunoadsorption

Two convalescent plasma donors (PL-75 and PL-107) underwent 7 (PL-75) and 18 (PL-107) cycles of immunoadsorption using an affinity column adsorber pair (TheraSorb - Ig flex, Miltenyi Biotec, Bergisch-Gladbach, Germany) connected to a LIFE 21 plasmapheresis platform (Miltenyi Biotec) and single-needle venipuncture after informed consent had been obtained and health evaluations as recommended in [10] had been performed. Both had been tested positive for COVID-19 two months prior to antibody donations suffering from mild symptoms for less than 10 days like loss of taste, fatigue and febrile temperatures (PL-75) or coryza (PL-107). The immunoadsorptions were tolerated very well with no side effects even seven and eight months afterwards, which were ruled out by personal interrogation via phone calls. Column eluates containing adsorbed IgG, IgA and IgM were collected during each glycine wash step after 80 mL of glycine had been loaded upon the column. Upon each wash cycle, 80 mL of immunoglobulins in glycine were collected in clinical grade infusion bags (5 L EVA, Hegewald Corp., Lichtenberg, Germany) instead of disposal. 2.2 mL 3 M Trometamol (THAM Koehler, Alsbach-Hähnlein, Germany) were added to each 80 mL of adsorber eluates for neutralization.

A total of 1050 mL plasma from donor PL75 were apheresed in 144 min, adsorbed in 7 cycles leading to an eluate volume of 1.1 L. Another 2 mL of Trometamol had to be added for optimum neutralization. Donor PL-107 was apheresed in 248 min thereby processing 2700 mL plasma in 18 adsorber cycles. This resulted in an eluate volume of 1.3 L (no addition of extra Trometamol was necessary).

After immunoadsorptions, donors were retested for serum protein, immunoglobulins and blood count. Heart rates and blood pressures were determined before discharge. During follow-up, donors were personally contacted by a medical doctor via phone the next day, one, seven (PL-107) and eight (PL-75) months after the antibody donations and asked for any health issues. The final interrogation after 12 months has yet to be done.

2.2. Tangential flow filtration

A detailed description of tangential flow filtration can be found in [11]. Our target was to concentrate the eluate volumes at least 15fold. The eluate from PL-75 was 15.5fold concentrated using an Omega 30 kDa polyethersulfone membrane (Omega™ 30 kD TFF Membrane, Pall Biotech Dreieich, Germany) on a Quattroflow 150S pump (Pana-Sonic, PSG, Duisburg). A transmembrane pressure of 1.15 bar was used for the concentration. The membrane was operated at a crossflow flux of 230 LMH and an average permeate flux of 21.6 LMH was achieved during concentration. After concentration, the retentate was diafiltrated with 0.9 % NaCl over 10 diafiltration volumes using a transmembrane pressure of 2 bar. During diafiltration, the membrane was operated at a crossflow flux of 230 LMH and an average permeate flux of 20.6 LMH was achieved during diafiltration.

The eluate form PL-107 was 21.3fold concentrated with a transmembrane pressure of 1.15 bar. The membrane was operated at a crossflow flux of 204 LMH and an average permeate flux of 43.4 LMH was achieved during concentration. In contrast to PL-75, the bioburden was reduced using a 0.2 μm polyethersulfone filter (Supor® EAV, KA02EAVS, Pall Biotech) before concentration (total throughput of 50 L/m^2). The retentate was diafiltrated with 0.9 %NaCl over 7.7 diafiltration volumes using a transmembrane pressure of 2.3 bar. During diafiltration, the membrane was operated at a crossflow flux of 345 LMH and an average permeate flux of 24.9 LMH was achieved. Including pre- and post- cleaning, the whole procedure took 5 h consisting of 3 h of actual process time for concentration and diafiltration and an additional 2 h of pre- and post-cleaning.

One reason for glycine removal lies in its high osmolarity (200 mmol/L). As glycine can function as inhibiting neurotransmitter, side effects include nausea, headache, muscle weakness and cramps. Usually, healthy individuals can metabolize extra glycine with no problems. In patients with non-ketotic hyperglycinemia, however, a very rare mitochondrial disorder of the newborn, accumulation occurs in blood and liquor. Glycine is termed as non-biohazardous (see EG 1272/2008). The no adverse event level (NOAEL) lies below 2 g/kg body weight. 100 mL Glycine-HCl solution contains 1.5 glycine. So, a minimum of 9.3 L had to be infused in adult donors with an average body weight of 70 kg to evoke potential side effects (personal communication Thomas Schreiner, Miltenyi Biotec).

2.3. Virus neutralisation test

To detect SARSCoV-2 specific neutralizing antibodies, the following virus neutralization test was performed. Samples were heat inactivated for 30 min at 56 °C. Two-fold serial dilutions (1:5 to 1:10240) were prepared in 50 μL volume with maintenance medium (Dulbecco's Modified Eagle Medium (Gibco, Ref 11995−065), 100 U/mL Penicillin and 100 μg/mL Streptomycin (Gibco, Ref 11995−065), 2% Fetal Calf Serum (Pan Biotech, Cat P303031)). Then, 50 μL of SARS-CoV-2 NRW-42 isolate (EPI_ISL_425126) virus solution ($TCID_{50}$/mL = 2000) was added. Cell-free plates were incubated at 37 °C for 1 h, then 100 μL Vero cell suspension containing 7×10^4 cells/mL (ATCC-CCL-81, obtained from LGC Standards) were added to the samples. Plates were incubated at 37 °C, 5% CO_2 for 4 days. The neutralization titer was determined by microscopic inspection as the highest sample dilution without a virus induced cytopathic effect. Previously evaluated samples (NT-positive and negative) as well as virus only (no serum present) and cell growth controls were run during each test [12].

3. Results and discussion

In the peripheral blood of donor PL-75, IgG, IgA and Ig M concentrations were reduced by 1/3 after immunoadsorption (Table 1).

The eluate volume of 1.1 L was reduced to a final volume of 52 mL storable antibody concentrate containing a total immunoglobulin amount of 29 g IgG, 2.7 g IgA and 1.1 g IgM (1:160 neutralizing antibody titer in the final product). Glycine was reduced to 32.2 μmol.

Like in PL-107 antibody concentrate, COVID-antibodies were enriched more than 3fold (from 30.5 to 106).

Results of PL-107 antibody donations are summarized in Table 2.

Using immunoadsorption, IgG and IgA were reduced by 1/3, IgM by 1/5 in the peripheral blood of Donor PL107. In the final product volume of 61.2 mL, a total IgG of 48.8 g, IgA 3.7 g and IgM 2.3 g antibodies were collected. Glycine was successfully exchanged by 0.9 % sodium chloride resulting in a final glycine content of non-hazardous 67.5 μmol.

Interestingly, the amount of neutralizing antibodies increased four-fold as compared to the content in peripheral blood. Sample vials were stored at 4 °C and −80 °C for now eight and seven months, respectively.

Table 1
Results of first COVID antibody donation using immunoadsorption and followed by tangential flow filtration.

	Immunadsorption		Sterile filtration		
PL-75	Donor before	Donor after	Retentate before	Retentate after	Reference
IgG (mg/dl)	899	600	6515	5591	700−1600
IgA (mg/dl)	119	83	523	523	70−500
IgM (mg/dl)	84	66	209	212	40−230
Covid-Ab	30.5	n.a.	106	106	<1.0

n.a., not analyzed. Reference refers to normal blood.

Table 2
Results of second COVID antibody donation using immunoadsorption and followed by tangential flow filtration (TFF).

	Immunadsorption		Sterile filtration		
PL-107	Donor before	Donor after	Retentate before	Retentate after	Reference
IgG (mg/dl)	879	558	7852	7696	700–1600
IgA (mg/dl)	113	74	616	603	70–500
IgM (mg/dl)	111	89	376	372	40–230
Covid-Ab	42.5	25.2	145	148	<1.0
Protein (g/dl)	7.17	6.09	n.a.	9.77	6.4–8.3
Albumine (g/dl)	4.9	4.8	n.a.	<1.0	3.5–5.3
Glycine (μmol/L)	n.a.	n.a.	Before TFF: 130,300	1103	150–325
Neutral. Ab	1:20	n.a.	1:80	1:80	

Immunoglobulins, albumin, protein and Covid-antibodies were determined in the peripheral blood of the donors before and after immunoadsorption, as well as in the retentate before and after sterile filtration. Glycine was measured before TFF and after sterile filtration. Ab, antibody; Neutral: SARS-CoV-2 neutralizing; n.a., not analyzed.

Both, the concentration of COVID antibodies and neutralization factor remained stable; COVID antibodies even increased slightly (PL-75: 162 after 8 months versus 106 freshly prepared; PL-107: 184 after 7 months versus 148 freshly prepared). Whether this increase has any impact on efficiency, is based on conformational changes induced by freezing [13] or based on temperature-dependent removal of inhibitory components remains to be elucidated. Samples were sterile in aerobic and anaerobic cultures of both antibody concentrates; no endotoxins were detected in PL-107, whereas one vial of PL-75 was tested positive for endotoxin.

Taken into account that the average plasma has a concentration of 2 ng/mL anti-SARS/CoV-2 antibodies [14], donated covalent plasma would contain on average 500 ng in one unit. With the method presented here, we could mathematically expect a final amount of 4 mg therapeutically effective antibodies in one concentrate unit, eight-fold more than in one covalent plasma unit and producible within 1–2 days.

Considering commercial aspects, at this point of development, it is very difficult to determine a price for one unit of antibody concentrate. Regarding costs, we know that single-use items are estimated at 1.200 Euro for one immunoadsorption and 900 Euro for TFF. Prices for immunoadsorption and TFF instruments including pumps are usually negotiated by the institutional sales management and can vary deeply.

In summary, immunoadsorption followed by tangential flow filtration could possibly be used to collect COVID-19 antibody concentrates for a potential therapeutic use without compromising the donor's immune system. In light of new virus mutations like the more infectious B 1.1.7 variant [15] and in sight of the debatable efficiency of convalescent plasma (see Convalescent Plasma EUA Letter of Authorization March 9, 2021 (fda.gov), accessed March 14, 2021), this rapid and effective procedure might help to obtain optimum efficiency. A further extension to a pilot study is planned in the near future.

CRediT authorship contribution statement

Jannik Rothenburg: Conceptualization, Methodology, Investigation, Resources, Data curation, Writing - review & editing, Visualization. **Silke Rink-Baron:** Methodology, Validation, Investigation, Resources, Writing - review & editing. **Lisa Mueller:** Methodology, Validation, Investigation, Data curation, Writing - review & editing. **Philipp Niklas Ostermann:** Methodology, Validation, Investigation, Data curation. **Johannes Fischer:** Validation, Resources, Funding acquisition. **Johannes Stegbauer:** Validation, Resources, Writing - review & editing, Funding acquisition. **Anja Moldenhauer:** Conceptualization, Validation, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

JR is employed by PALL Corporation; SRB is an employee of Miltenyi Biotec. LM, PNO, JF and JS have nothing to disclose. Starting June, 2021, AM will receive personal fees from Sanofi AG for lectures outside the work presented here.

Acknowledgements

We are indebted to Elena Stemmler, Claudia Hülstrunk and Thomas Dechmann-Sültemeyer for excellent operational, Gregor Leinen and Martina Müller for excellent technical assistance. PNO is supported by the Jürgen Manchot Foundation. The study was funded in part by Miltenyi Biotec, Bergisch-Gladbach, Germany, and Pall Biotech, Dreieich, Germany. Both companies were not involved in study design, data collection, analysis and interpretation of data nor in the decision to submit the article for publication. The study was approved by the ethical committee of the Heinrich-Heine-University Düsseldorf (No. 1020-1132).

References

[1] Hansen J, Baum A, Pascal KE, Russo V, Giordano S, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. Science 2020;369(6506):1010–4.
[2] Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. J Clin Invest 2020;130(6):2757–65.
[3] Xia X, Li K, Wu L, Wang Z, Zhu M, Huang B, et al. Improved clinical symptoms and mortality among patients with severe or critical COVID-19 after convalescent plasma transfusion. Blood 2020;136(6):755–9.
[4] Bose S, Adapa S, Aeddula NR, Roy S, Nandikanti D, Vupadhyayula PM. Medical management of COVID-19: evidence and experience. J Clin Med Res 2020;12(6):329–43.
[5] Bikdeli B, Madhavan MV, Gupta A, Jimenez D, Burton JR, Der Nigoghossian C, et al. Pharmacological agents targeting thromboinflammation in COVID-19: review and implications for future research. Thromb Haemost 2020;120(7):1004–24.
[6] Lipphardt M, Wallbach M, Koziolek MJ. Plasma exchange or immunoadsorption in demyelinating diseases: a meta-analysis. J Clin Med 2020;9(5).
[7] Moldenhauer A, Haas J, Wäscher C, Derfuss T, Hoffmann KT, Kiesewetter H, et al. Immunoadsorption patients with multiple sclerosis: an open-label pilot study. Eur J Clin Invest 2005;35(8):523–30.
[8] Mathey EK, Derfuss T, Storch MK, Williams KR, Hales K, Woolley DR, et al. Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 2007;204(10):2363–72.
[9] Derfuss T, Parikh K, Velhin S, Braun M, Mathey E, Krumbholz M, et al. Contactin-2/TAG-1-directed autoimmunity is identified in multiple sclerosis patients and mediates gray matter pathology in animals. Proc Natl Acad Sci U S A 2009;106(20):8302–7.
[10] Epstein J, Burnouf T. Points to consider in the preparation and transfusion of COVID-19 convalescent plasma. Vox Sang 2020;115(6):485–7.
[11] Musumeci T, Leonardi A, Bonaccorso A, Pignatello R, Puglisi G. Tangential flow filtration technique: an overview on nanomedicine applications. Pharm Nanotechnol 2018;6(1):48–60.
[12] Müller L, Ostermann PN, Walker A, Wienemann T, Mertens A, Adams O, et al. Sensitivity of anti-SARS-CoV-2 serological assays in a high-prevalence setting. Eur J Clin Microbiol Infect Dis 2021:1–9.
[13] Roy I, Gupta MN. Freeze-drying of proteins: some emerging concerns. Biotechnol Appl Biochem 2004;39(Pt 2):165–77.
[14] Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature 2020;584(7821):437–42.
[15] Galloway SE, Paul P, MacCannell DR, Johansson MA, Brooks JT, MacNeil A, et al. Emergence of SARS-CoV-2 B.1.1.7 Lineage - United States, December 29, 2020-January 12, 2021. MMWR Morb Mortal Wkly Rep 2021;70(3):95–9.