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(RESEARCH ARTICLE)

Gravity-driven crossflow membrane filtration and mechanical centrifugation yield plasma with equivalent total and COVID-19-specific immunoglobulin content

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Abstract

While the administration of convalescent plasma is a promising therapy for coronavirus disease 2019 (COVID-19), upfront costs and technological barriers of conventional plasmapheresis have limited the collection and processing of COVID-19 convalescent plasma (CCP) in Low- and Middle-Income Countries (LMICs). We previously reported that bedside plasma separation using gravity-driven microfiltration has made CCP therapy accessible as a treatment option in Suriname. However, the question remains whether the gravity-driven microfiltration method yields similar amounts of immunoglobulins as compared to conventional plasmapheresis. Here, we compared the gravity-driven microfiltration method with conventional plasma centrifugation for the total and COVID-19-specific immunoglobulin content of the obtained CCP. Blood donations from 10 donors recovered from PCR-confirmed COVID-19 were processed using both methods. Samples were collected pre- and post-processing for analysis to allow direct comparison of both methods. There were no differences in COVID-19-specific IgG levels between convalescent plasma obtained by microfiltration and centrifugation for 4 of the 5 assays used. Anti-RBD-Omicron IgG levels were slightly higher in the plasma obtained after filtration (median 274, range 69 to 1258) than after centrifugation (median 249, range 67 to 1175), Wilcoxon P = 0.0488. No significant differences were detected between the two methods for levels of total albumin, total cholesterol, total IgA, IgM and IgG levels. These results indicate that gravity-driven microfiltration and conventional centrifugation yield CCP with equivalent amounts of total and COVID-19-specific antibodies. This makes the gravity-driven microfiltration method a viable option for the collection and treatment with CCP in the LMICs setting.

Keywords: Convalescent plasma; COVID-19; HemoClear; SARS-CoV-2

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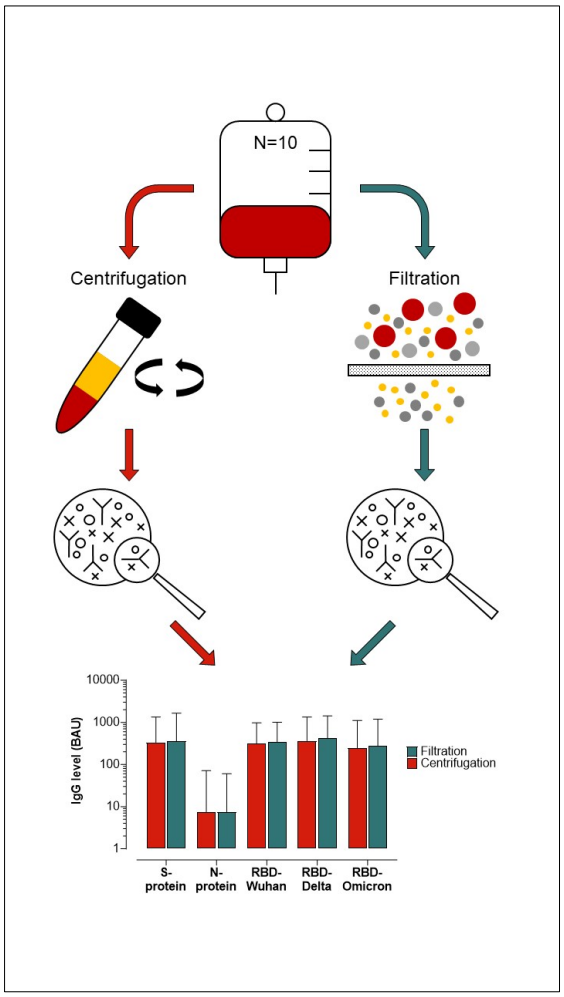
Graphical Abstract



1. Introduction

Plasmapheresis is the removal, treatment, and return or exchange of blood plasma or components thereof from and to the blood circulation (1). This procedure can either benefit recipients receiving the collected blood plasma (components), or donors by removing and/or replacing plasma (components). Therapeutic plasma exchange can decrease the level of circulating antibodies, antigen-antibody complexes, cytokines, abnormal plasma proteins, cholesterol, metabolic waste products, and plasma-bound toxins causing severe symptoms in several diseases and disorders (2,3). Additionally, in case of a donor cured after COVID-19, the collected plasma - COVID-19 convalescent plasma (CCP) - contains SARS-CoV-2-specific polyclonal antibodies that may be used for treatment of COVID-19 patients (4,5).

Emerging evidence supports the effective treatment of COVID-19 using CCP (6,7). Unfortunately, high upfront costs and technological barriers of conventional centrifugal plasmapheresis have limited the collection and processing of CCP in Low- and Middle-Income Countries (LMICs) (8). Previously, we have successfully used a novel gravity-driven crossflow blood filter to obtain CCP and treat ICU admitted patients in Suriname (9).

Since CCP is a non-standardized medicinal product, containing a mixture of polyclonal antibodies and other pro- and anti-inflammatory proteins, the question remains whether the methods used to produce CCP result in the same plasma composition. Production methods may affect the collected plasma content of the various plasma components, which may have different effects on virus neutralization *in vivo*. Here, we present a comparison of the gravity-driven microfiltration method with conventional plasma centrifugation for the total and COVID-19-specific immunoglobulin content of the obtained CCP.

2. Material and Methods

2.1. Trial design and clinical procedures

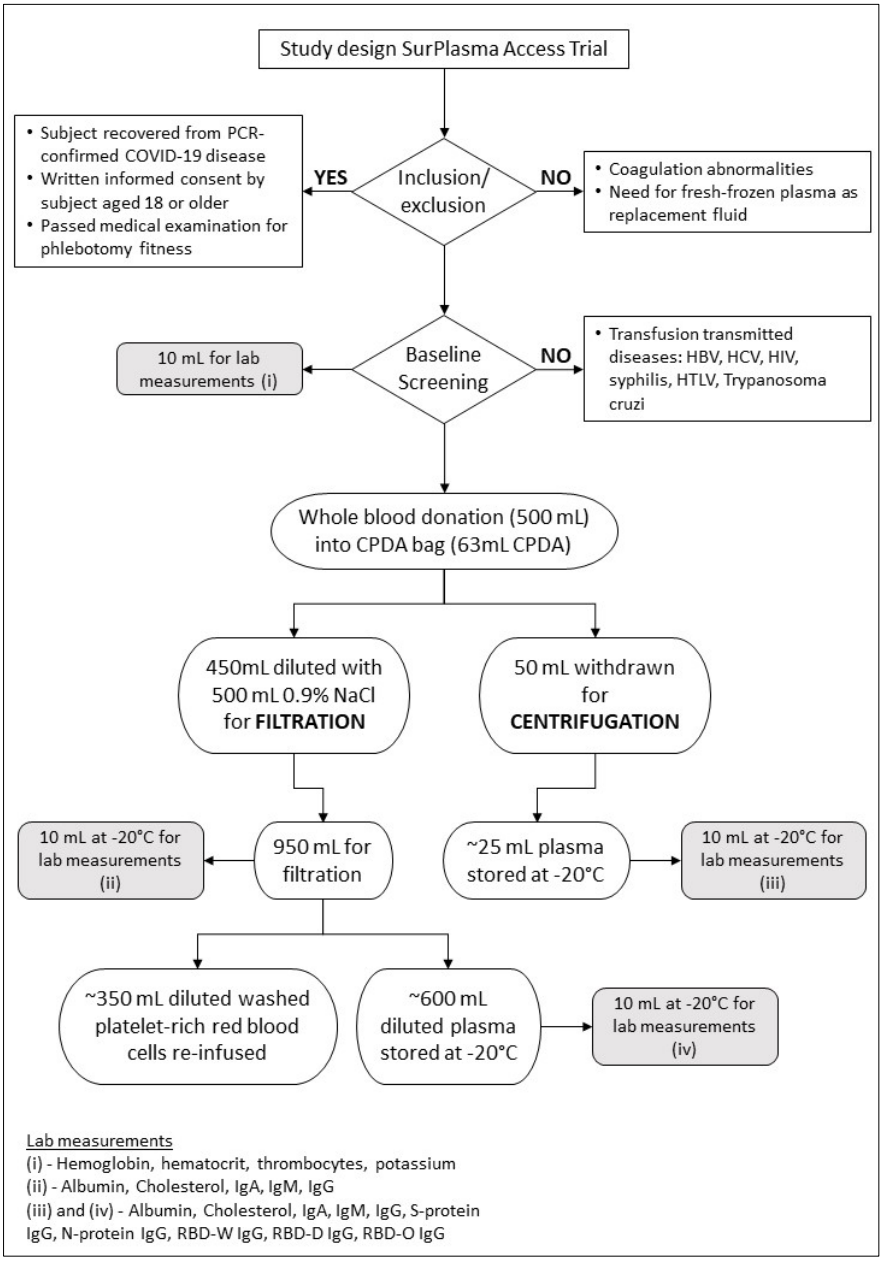


Figure 1 Patient selection flowchart with study enrolment and experimental design. HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV, human T lymphotrophic virus.

This work is reported in adherence to the "Strengthening the Reporting of Observational studies in Epidemiology" (STROBE) guidelines. In this prospective paired sample study, performed at the Academic Hospital Paramaribo in Suriname, in December 2021 (ISRCTN21941624, https://doi.org/10.1186/ISRCTN21941624), we compared gravity-driven crossflow membrane and centrifugal convalescent COVID-19 plasma production procedures. Ten participants aged 18 or older and recovered from PCR-confirmed COVID-19 disease were recruited to the study. After obtaining written informed consent and passing medical examination for phlebotomy fitness, baseline screening for transfusion transmitted diseases was performed according to standard procedures (10–13). The blood donation procedure was performed by the anesthesiologist of the Academic Hospital of Paramaribo in the recovery or operating room environment. The donor was installed and monitored on vital signs. An arterial and peripheral line were placed for donation and re-infusion of the cellular components after the procedure. The donor received a 500 mL infusion of

Ringer's solution (Baxter) prior to donation to compensate for the procedural blood withdrawal. The bedside microfiltration procedure allowed for direct re-infusion of the red blood cells. Since conventional centrifugal plasmapheresis was not possible in the Academic Hospital of Paramaribo where the blood donation procedure was performed, this procedure was performed at the National Blood Bank of the Surinamese Red Cross. As it was considered unethical to discard red blood cells from a COVID-19 recovered donor and centrifugation results were not considered to be affected by the volume to be centrifuged, only 50 mL of whole blood was processed at the National Blood Bank of the Surinamese Red Cross. This was performed by centrifugation of 50 mL of whole blood at 3400 rpm for 10 minutes (11), which is comparable with the longer clinical centrifugal therapeutic plasmapheresis procedure that applies centrifugation between 2000-2500 rpm to separate the contents of the anticoagulated blood (14). For the microfiltration procedure, 500 mL of 0.9% NaCl was added to 450 mL of whole blood prior to passing over the HemoClear device according to the manufacturer's instructions (15). Samples were collected pre- and post-processing for analysis to allow direct comparison of both methods. The study flow chart in **Figure 1** illustrates the study enrolment and experimental design.

Ethical approval was granted by the Suriname Ministry of Health's Ethics Review Board (registration number: CMWO 01/2022; ISRCTN21941624). The data used to support the findings of this study are available from the corresponding author upon request.

2.2. Donor population

Healthy volunteers aged 18 years and older recovered from PCR-confirmed COVID-19 disease who were free of COVID-19 symptoms for at least 2 weeks, i.e. absence of coughing, fever, cold symptoms, dysosmia and dysgeusia were enrolled in the study in the period from June until December 2021. The eligibility criteria included written informed consent given by the donor and passed medical examination for phlebotomy fitness. Participants weighed at least 50 kg, had never received blood or plasma transfusions in the past, and were hepatitis B virus, hepatitis C virus, human immunodeficiency virus, human T lymphotropic virus, *Treponema pallidum* and *Trypanosoma cruzi* negative (tested according to the American Red Cross blood donation guidelines: https://www.redcrossblood.org/biomedical-services/blood-diagnostic-testing/blood-testing.html). Participant characteristics at the time of blood donation are described in **Table 1**.

2.3. Lab measurements

Samples collected at baseline screening were tested directly for hemoglobin, hematocrit, thrombocytes, and potassium levels. Samples collected on the day of the blood donation pre- and post-processing were stored at -20°C or below for analysis at a later time point (**Figure 1**, box i). Albumin, cholesterol, as well as total IgA, IgM and IgG levels were measured prior to starting the convalescent plasma procedure (**Figure 1**, box ii).

COVID-19 antigen-specific IgG load was measured in convalescent plasma produced either by centrifugation (**Figure 1**, box iii) or filtration (**Figure 1**, box iv) using a validated spike (S), nucleocapsid (N) and receptor binding domain (RBD) protein Luminex assay, as previously described (16).

2.4. Procedure measurements

Procedure times: Filtration procedure time, including setup and priming of the microfiltration filter, was measured as the time between the start of set-up and finishing the convalescent plasma procedure. Centrifugation procedure time was 10 minutes of centrifugation plus the time required to transfer the plasma to a new tube.

Procedure volumes: Processed volumes were obtained by weighing the blood donation bag before and after the filtration procedure and subtracting the weight of the bag (56 g).

2.5. Statistical analysis

Differences between the filtration and centrifugation methods were analyzed using paired t tests for parametric continuous variables and Wilcoxon matched-pairs signed rank tests for non-parametric continuous variables in GraphPad Prism (version 10.1.0).

3. Results

3.1. Participants

A total of 10 participants were enrolled in the study with an average of 66 days (range 24 to 115 days) between COVID-19 infection and blood donation. The mean age of the participants was 43±14 years and three patients (30%) were male. The demographic, clinical and hematological characteristics are described in **Table 1**.

Table 1 Blood donor characteristics [a]

Characteristic	**Donor (N=10)**	
DEMOGRAPHICS (mean)		
Age (SD) - year	43	(14)
Male sex (%) - no.	3	(30)
Weight (SD) - kg	77	(13)
Length (SD) - cm	167	(8)
COVID vaccinations (SD) - no.	2.1	(0.9)
COVID booster (%) - no.	3	(30)
Interval between COVID infection and blood donation (SD) - days	66	(31)
HEMATOLOGICAL PARAMETERS (mean)		
Hemoglobin (SD) - mmol/L	8	(0.8)
Hematocrit (SD) - %	40	(3)
Thrombocytes (SD) - 10^9/L	265	(55)
Potassium (SD) - mmol/L	3.7	(0.4)
PROCEDURE TIME AND VOLUMES (median)		
Filtration time (IQR) - minutes	15	(14-16)
Whole blood donation volume (IQR) - mL [b]	444	(409-444)
Pre-filtration volume (IQR) - mL [c]	894	(859-894)
Post filtration volume (IQR) - mL [d]	620	(593-640)
Theoretical maximal plasma yield (IQR) - mL [e]	230	(216-237)

[a] SD, standard deviation; IQR, interquartile range.
[b] Volume obtained by subtracting weight of blood bag (56 g) from total weight of whole blood donation.
[c] Volume obtained by subtracting volume used for centrifugation procedure (50 mL) and weight of blood bag (56 g) from total weight of blood donation and adding volume of 0.9% NaCl used to dilute whole blood prior to filtration (500 mL).
[d] Volume obtained by subtracting weight of blood bag (50 mL) from total weight of filtrate.
[e] Volume calculated by subtracting volume used for centrifugation procedure (50 mL) and weight of blood bag (56 g) from total weight of blood donation and correcting for the respective donor Hematocrit level.

Median time for the filtration procedure was 16 minutes (range 13 to 26 minutes), which is similar to the time needed for the centrifugation procedure (10 minutes of centrifugation plus transfer of the plasma to a new tube). The median whole blood donation volume was 444 mL (range 394 to 444 mL). After removal of 50 mL of whole blood for the centrifugation procedure and addition of 500 mL of 0.9% NaCl, each donation was filtered using the gravity-driven microfiltration device to yield a median 620 mL of convalescent plasma (range 520 to 680 mL), see also **Table 1**.

3.2. Primary outcome: total COVID-19 antigen-specific antibody load

Table 2 Comparison of parameters after centrifugation and filtration (N=10) [a]

Characteristic	Pre-treatment		Centrifugation		Filtration		*P*-value
HAEMATOLOGY (mean)							
Total Albumin (SD) - g	7.5	(1.5)	6.9	(1.1)	6.4	(1.0)	0.2808 [b]
Total Cholesterol (SD) - mmol	0.85	(0.19)	0.64	(0.11)	0.59	(0.14)	0.2940 [b]
Total IgA (SD) - g	0.30	(0.048)	0.43	(0.11)	0.38	(0.10)	0.0678 [b]
Total IgM (SD) - g	0.083	(0.016)	0.20	(0.11)	0.19	(0.11)	0.5674 [b]
Total IgG (SD) - g	1.3	(0.29)	1.9	(0.46)	1.7	(0.58)	0.3446 [b]
COVID-19 specific (median)							
Total S-protein (IQR) - BAU	N/T	N/T	333	(182-449)	358	(198-435)	0.3359 [c]
Total N-protein (IQR) - BAU	N/T	N/T	7.5	(2-22)	7.5	(2.8-26)	0.8750 [c]
Total RBD-Wuhan (IQR) - BAU	N/T	N/T	310	(148-470)	337	(173-456)	0.4766 [c]
Total RBD-Delta (IQR) - BAU	N/T	N/T	364	(221-596)	416	(246-561)	0.3086 [c]
Total RBD-Omicron (IQR) - BAU	N/T	N/T	249	(101-334)	274	(114-331)	**0.0488** [c]

[a] SD, standard deviation; IQR, interquartile range; BAU, binding antibody unit. P-values <0.05 are in bold.

[b] Paired t test.; c Wilcoxon matched-pairs signed rank test

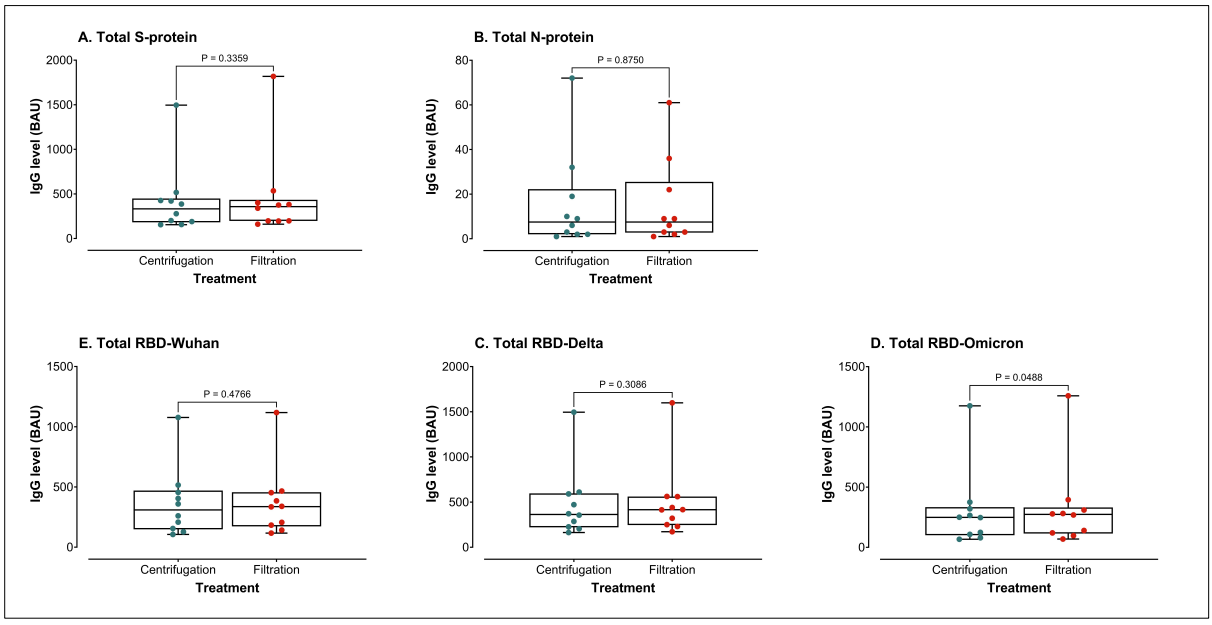


Figure 2 Total COVID-19 antigen-specific antibody load. (A-E) COVID-19 antigen specific IgG levels were measured using validated spike (S), nucleocapsid (N) and receptor binding domain (RBD) protein Luminex assays (16). Graphs depicts total specific binding antibody unit (BAU) levels in convalescent plasma obtained after centrifugation (teal) or filtration (red). Wilcoxon matched-pairs signed rank test *P*-values are shown.

A multiplex immunoassay was used to measure COVID-19 antigen-specific IgG levels, for S protein, N protein and three variant-specific RBD proteins: Wuhan, Delta, and Omicron. There were no differences in binding antibody unit (BAU) levels between convalescent plasma obtained by filtration and centrifugation for 4 of the 5 assays (see **Figure 2** and **Table 2**). A very small but statistically significant difference was observed for anti-RBD-Omicron IgG in plasma obtained

after filtration (median 274, range 69 to 1258) versus centrifugation (median 249, range 67 to 1175), Wilcoxon *P* = 0.0488 (see **Table 2**).

3.3. Secondary outcome: total albumin, cholesterol, IgA, IgM and IgG levels

In addition to the COVID-19 antigen specific content of the convalescent plasma, the two plasma collection methods were compared for the following analytes: total albumin, total cholesterol as well as total IgA, IgM and IgG levels. No significant differences were detected for any of these analytes between the two methods (see **Figures 3** and **Table 2**).

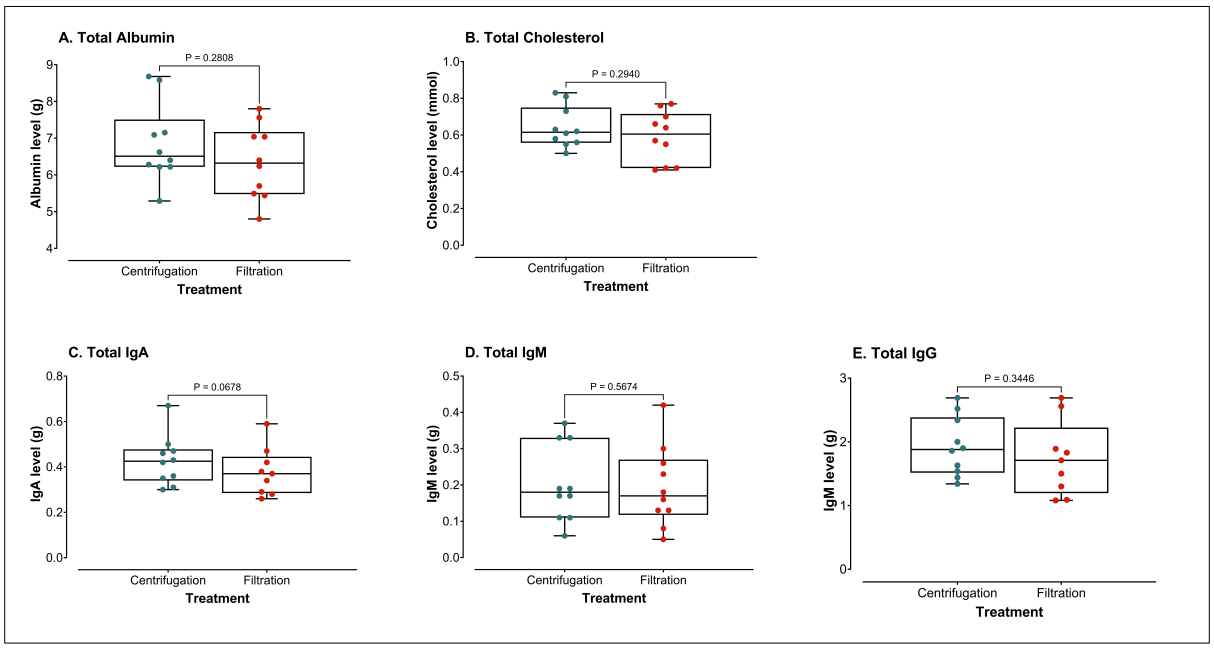


Figure 3 Non COVID-19 specific plasma content. (A-E) Total albumin, cholesterol, IgA, IgM and IgG levels were measured using standard assays. Graphs depict levels after centrifugation (teal) or filtration (red). Paired t test *P*-values are shown.

4. Discussion

This study compares the gravity-driven crossflow microfiltration method with conventional plasma centrifugation for the total and COVID-19-specific immunoglobulin content of the obtained CCP. With the exception of anti-RBD-omicron IgG, where a very small, borderline significant difference was observed, the two plasma collection methods were similar with regards to the measured outcomes.

When administered early to outpatients, high-titer antigen-specific CCP has been shown to result in a remarkable reduction in COVID-19 hospitalizations (17). Our finding that the gravity-driven crossflow microfiltration method results in at least equivalent levels of COVID-19 specific immunoglobulin content would also support its use in this indication.

In our study, we did not measure thrombocytes levels in CCP as the pre- and post-processing samples were stored at -20°C prior to analysis, which affects both thrombocyte recovery and activation. Given the potential immune-modulatory and protective effects of thrombocytes (18,19), it will be important to also compare the thrombocyte content from CCP obtained by both methods in future studies.

With significant barriers for bedside production of CCP in primary health care facilities in low-resource settings using conventional plasmapheresis, CCP produced by the gravity-driven crossflow microfiltration method may represent a first line of defense against new virus mutants not responsive to existing vaccines in LMICs.

5. Conclusion

This study compares total and COVID-19-specific immunoglobulin content of plasma obtained using bedside microfiltration versus conventional centrifugation. There were no differences in COVID-19-specifc IgG levels between convalescent plasma obtained by microfiltration and centrifugation for 4 of the 5 assays used and slightly higher levels using microfiltration for 1 assay. In addition, no significant differences were detected between the two methods for levels of total albumin, total cholesterol as well as total IgA, IgM and IgG levels. With plasma production in primary health care facilities in local communities limited by the upfront costs and technological barriers of conventional plasmapheresis, this method could be an accessible and rapid line of defense against new virus mutants that are not responsive to existing vaccines in LMICs.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Arno P. Nierich is the inventor of the HemoClear filter and holds stock ownership in HemoClear BV, Dr. Stolteweg 70, 8025 AZ Zwolle, Netherlands. No involvement in actual patient treatment Suriname.

All other authors declare no conflict of interest.

Statement of ethical approval

Ethical approval was granted by the Suriname Ministry of Health's Ethics Review Board (registration number: CMWO 01/2022; ISRCTN21941624).

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Winters JL. Plasma exchange: concepts, mechanisms, and an overview of the American Society for Apheresis guidelines. Hematology [Internet]. 2012 Dec 8;2012(1):7–12. Available from: https://ashpublications.org/hematology/article/2012/1/7/84346/Plasma-exchange-concepts-mechanisms-and-an

[2] Kambic HE, Nosé Y. Historical Perspective on Plasmapheresis. Therapeutic Apheresis. 1997 Feb 14;1(1):83–108.

[3] Rososhansky S, Szymanski IO. Clinical Applications of Therapeutic Hemapheresis. J Intensive Care Med. 1996 May 1;11(3):149–61.

[4] Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proceedings of the National Academy of Sciences. 2020 Apr 28;117(17):9490–6.

[5] Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. JAMA. 2020 Apr 28;323(16):1582.

[6] Focosi D, Franchini M, Pirofski L anne, Burnouf T, Paneth N, Joyner MJ, et al. COVID-19 Convalescent Plasma and Clinical Trials: Understanding Conflicting Outcomes. Clin Microbiol Rev. 2022 Sep 21;35(3):1–29.

[7] Fonseca-González G, Alamilla-Sánchez M, García-Macas V, Herrera-Acevedo J, Villalobos-Brito M, Tapia-Rangel E, et al. Impact of plasmapheresis on severe COVID-19. Sci Rep. 2023 Dec 1;13(1).

[8] Bloch EM, Goel R, Montemayor C, Cohn C, Tobian AAR. Promoting access to COVID-19 convalescent plasma in low- and middle-income countries. Transfusion and Apheresis Science. 2020 Sep;102957.

[9] Bihariesingh-Sanchit R, Bansie R, Ramdhani N, Mangroo R, Bustamente D, Diaz E, et al. Reduced Mortality among COVID-19 ICU Patients after Treatment with HemoClear Convalescent Plasma in Suriname. Casadevall A, editor. mBio. 2023 Feb 23;

[10] Smid WM, Burnouf T, Epstein J, Kamel H, Sibinga CS, Somuah D, et al. Points to consider in the preparation and transfusion of COVID-19 convalescent plasma in low– and middle– income countries. Africa Sanguine. 2020 Aug 20;22(2):5–7.

[11] AABB. AABB Technical Manual. 15th ed. Brecher ME, editor. 2005. 1–931 p.

[12] FDA. Investigational COVID-19 Convalescent Plasma. Guidance for Industry. Food and Drug Administration. 2020.

[13] Gordon-Smith TC. Making a blood transfusion safe: vigilance and audit. Qual Saf Health Care. 2008 Aug 1;17(4):236–7.

[14] Williams ME, Balogun RA. Principles of separation: indications and therapeutic targets for plasma exchange. Clin J Am Soc Nephrol [Internet]. 2014 Jan 7;9(1):181–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24178973

[15] Osemwengie D, Lagerberg JW, Vlaar R, Gouwerok E, Go M, Nierich AP, et al. Recovery of platelet-rich red blood cells and acquisition of convalescent plasma with a novel gravity-driven blood separation device. Transfusion Medicine. 2022 Feb 10;32(1):53–63.

[16] Fröberg J, Koomen VJCH, van der Gaast-de Jongh CE, Philipsen R, GeurtsvanKessel CH, de Vries RD, et al. Primary Exposure to SARS-CoV-2 via Infection or Vaccination Determines Mucosal Antibody-Dependent ACE2 Binding Inhibition. J Infect Dis. 2023 Jan 12;

[17] Levine AC, Fukuta Y, Huaman MA, Ou J, Meisenberg BR, Patel B, et al. Coronavirus Disease 2019 Convalescent Plasma Outpatient Therapy to Prevent Outpatient Hospitalization: A Meta-Analysis of Individual Participant Data From 5 Randomized Trials. Clinical Infectious Diseases. 2023;76(12):2077–86.

[18] Koupenova M, Clancy L, Corkrey HA, Freedman JE. Circulating Platelets as Mediators of Immunity, Inflammation, and Thrombosis. Circ Res [Internet]. 2018 Jan 19;122(2):337–51. Available from: https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.117.310795

[19] Sciaudone A, Corkrey H, Humphries F, Koupenova M. Platelets and SARS-CoV-2 During COVID-19: Immunity, Thrombosis, and Beyond. Circ Res [Internet]. 2023 May 12;132(10):1272–89. Available from: https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.122.321930