SurPlasmaAccess trial:
A prospective paired sample study comparing gravity driven cross flow membrane and mechanical centrifugal convalescent COVID-19 plasma production procedures.

Protocol, fourth version, Sunday, 6 February 2022.

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Introduction

Plasmapheresis is a technique where plasma is separated from the rest of the blood in an extracorporeal circuit and this plasma is then replaced by appropriate fluids(1). By removing plasma, it 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(2–5). In case of a donor cured after COVID-19, the produced plasma is called COVID-19 convalescent plasma (CCP), in order to collect polyclonal antibodies for further treatment of new patients(6–11).

Evidence that supports the use of CCP for treatment of COVID-19 is increasingly emerging. However, very few Low and Middle Income Countries (LMICs) countries have undertaken the collection and processing of CCP(12–15). The use of an new device, Hemoclear, in a LMIC as Suriname has proven its applicability and effectivity as a method to obtain CCP(16–19). Its treatment effect on Intensive Care (ICU) admitted patients resulted in a more than 70% reduction of mortality, although based on a matched case control study(20). This in contrast with several other studies that could not show any benefit of CCP in this type of patients(21). Since CCP is a non-standarized mixture of polyclonal antibodies and other pro- and anti-inflammatory proteins, the question remains whether the methods of producing CCP are equal. The removed substances in CCP include alloantibodies, autoantibodies, immunocomplexes, cytokines, exogenous toxins, monoclonal proteins, lipoproteins and excessively produced plasma components. And beside the antibodies, there might be other proteins that affect virus inactivation in vivo(22–25).

Methods of separation

According to the apheresis transfusion guidelines 30–40 mL/kg of plasma (1–1.5 plasma volumes) can be removed at each procedure and replaced with isotonic volume 0.9% saline or human globuline (32,33). In this study, the donor will receive an 500 ml infusion Ringer's solution (Baxter) prior donation in order to compensate on beforehand for the procedural blood withdrawal. There will be two sessions of whole blood donation. The first donation of 500 ml whole blood is processed at the National Blood Bank.The second donation of 500 ml whole blood will be filtrated by the Hemoclear device and the residual red blood cells and platelets will be reinfused into the donor.

The methods used to separate the plasma from the blood can be divided into centrifugation and filtration.

Centrifugation (cPE)

Centrifugation is the older method, based on the separation of cellular elements from the plasma by rapid spinning, in which centrifugal force separates the different components according to their density, size, and molecular weight. This method has the advantage that there is no upper limit to the molecular weight of the substances to be separated out. The main drawback of centrifugation is the risk of thrombocytopenia. Moreover, it requires anticoagulation with citrate, so it can lead to hypocalcemia. Centrifugation is the method used by blood banks; it requires sophisticated difficult-to-transport equipment that limits its use in therapeutic apheresis in critical care environments.

Filtration (hPE).

In the Hemoclear filtration, the cellular components of blood are separated from the plasma by passing the blood along a filter with large pores (2,2 Mu) that extracts all non cellular molecules through the pores and leaving the cellular components unaffected since they flow parallel over the membrane into a collection bag(26,27). The mechanism of separation consists of applying low gravity pressure to transfer the blood across a synthetic membrane that is highly permeable due to the large size of its pores. The advantages of hPE filtration are based on the low costs and complexity of the method. Also more platelets are collected into the CCP and platelet derived Abs may play a role in the therapeutic efficacy of CCP as well. Harvesting CP is best when the donor is still in hospital with a high concetration of all Abs still circulating, including IgM that normally disappears after 2 months of onset of COVID-19. Hemoclear is compared with cCP simple to handle if a COVID-19 quarantaine restricted plasmapheresis area is mandatory.

A head-to-head comparison studie between the cPE and hPE devices is not performed yet. Therefore, not only would it be of interest to understand whether the methods differ not only on relevant procedural results, but also in the components collected into the CCP. Even efficacy of the collected Abs is a question since both centrifugal as cross-flow forces can influence the function of Abs.

Thus, in this study we compare centrifugal CP aquesition based on the conventional methods such as of the Hettich Rotanta Silenta 630 RS and the Hemoclear system (Hemoclear BV) in a paired prospective in vitro study treating whole blood from 10 patients by both methods. The cPE methods use a centrifugal force of 3400 RPM with a time frame of 10.22 minutes in order to separate most of plasma from cellular components. The primary endpoint of our study is total AB load per ml(3,28–31). Secondary endpoints are procedure time (including setup and priming), blood cell losses, and IgG, IgA, IgM and fibrinogen removal efficiencies for the hCP method by Hemoclear. Also virus neutralization tests will be performed (Radboud/Erasmus MC, Netherlands).

Not only are we the first to go into detail on the analysis of a cPE versus hPE, we also are the pioneers to publish on the technical details of the Hemoclear system for producing hPE.

The aim of this study is:

1. to compare the feasibility of collecting and processing of CCP with the new Hemoclear method (hPE) with the industrial data of cPE
2. to assess the composition of the CCP in vitro from both the hPE as the cPE.

Study design

This is a prospective paired sample study where whole blood from each chosen patient is used for PE on both the Hemoclear filter device and the Centrifugal method by spinning on 3400 rpm during 10.22 minutes for production of CP as used by conventional cPE methods such as of Hettich Rotanta Silenta 630 RS(National Blood Bank). Blood samples will be collected pre- and post processing for analysis.

This set-up will allow direct comparison of both methods.

The procedure is performed by the anesthesiologist of the Academic Hospital of Paramaribo at the recovery or operation room environment. The donor is installed and monitored on vital signs, a periphereal line is placed for donation (and reinfusion of the cellular components after the hPE procedure) . The donor will receive an 500 ml infusion Ringer's solution (Baxter) prior donation in order to compensate on beforehand for the procedural blood withdrawal.

The first donated 500ml of whole blood will be processed for obtaining cPE at the National Blood Bank .The donor will receive an 500 ml infusion Ringer's solution (Baxter) prior donation in order to compensate on beforehand for the procedural blood withdrawal.

The Centrifugation (cPE), procedure will be performed in the lab of the National Blood Bank by spinning this volume for 3400 RPM during 10.22 minutes in order to obtain the separation.The red blood cells (300 ml)wil not be used.

The donor will continue with the second donation process by filtration. 500 ml whole blood will be filtrated by Hemoclear filter method. The residual red bloodcells (200-300ml) and platelets are reinfused into the donor at the end of each fitration procedure.

Materials and Methods

Donor selection

Donor patients (n=10-15) for convalescent plasma donation have to fulfill the following inclusion criteria: written consent for being subjects in this publication, and age from 18 years and older. Patients with coagulation abnormalities or with the need for fresh-frozen plasma (FFP) as a replacement fluid are excluded from the study. Potential donors will have a medical examination by the principal investigator to evaluate phlebotomy fitness. Based on National Blood bank Suriname screening protocol, the plasma will be tested for absence of the following transfusion transmitted infectious diseases:

- Hepatitis B: HBsAg en HBV-DNA (NAT)
- Hepatitis C: anti-HCV en HCV-RNA (NAT)
- HIV: anti-HIV-1/2/(O) en HIV-RNA (NAT)
- Syphilis: RPR
- HTLV I/II: Anti-HTLV I/II
- Trypanosomi cruzi (Chagas disease).

Donor information and consent

In general, donors presenting at the participating hospital after confirmed COVID-19 and possibly qualifying for participation will be informed about the trial by the treating/attending physician and asked if they are interested to participate.

Written informed consent of patients is required before enrollment in the trial and before any study related procedure takes place. ICH-GCP and other applicable regulations must be followed in informing the patient and obtaining consent. It should be taken into consideration if the donor is capable of giving informed consent. Before informed consent may be obtained, the donor should be given ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the donor.

There is no set time limit for the donor to make a decision. The investigator should inform each donor if there is a specific reason why he/she must decide within a limited time frame, for example if patients condition necessitates start of treatment or if the trial is scheduled to close for enrollment.

The content of the donor information letter, informed consent form and any other written information to be provided to donors will be in compliance with ICH-GCP and other applicable regulations and should be approved by the ethics committee in advance of use. The donor information letter, informed consent form and any other written information to be provided to donors will be revised whenever important new information becomes available that may be relevant to the donor's consent. Any substantially revised informed consent form and written information should be approved by the ethics committee in advance of use. The donor should be informed in a timely manner if new information becomes available that might be relevant to the donor's willingness to continue participation in the trial. The communication of this information should be documented.

Specific to this study protocol, hospitalized patients treated after COVID-19 infection will be informed about the study by the treating/attending physician who is involved in patient care of the patient. If the patient is interested to participate as a donor in this study, a member of

the study team will visit the patient to inform him/her about the study. In case the patient is unable to give informed consent, the appropriate relative will be contacted and informed about the study and to give written informed consent.

Variables analysis plan

First of all, 10 ml of donated whole blood will be used for a complete blood count is performed (Hemoglobin, Hematocrit, MCH, MCHC, RDW, Reticulocytes, WBCs, PLTs) with a cellular analysis system on blood samples taken from each patient before and after each procedure. Also samples are analysed on non-cellular components: Free Hb, LDH, Potassium, Complement C3, Complement C4, Fibrinogen, D-dimer.

Further, the 500 ml whole blood will be taken from the citrated bloodbag, for further in vitro processing by the lab in order to produce cCP. These samples are taken before the procedure , right before the connection of the patient to the hPE device after the setup and priming of the Hemoclear device.

From each stored blood sample, IgG, IgA, IgM and fibrinogen will be measured and removal efficiencies will be calculated. Also virus neutralization tests will be performed (Erasmus MC, Netherlands).

The total blood volume (TBV) of each patient was also calculated using the equation of Nadler and colleagues. For the hPE procedure, the setup time (time to prepare the disposables prior to the priming of the set with fluid), priming time, procedure time, and total time (sum of all previous) are recorded. The volume of replacement fluid infused, TBV processed, and plasma volume removed are recorded from the procedural data of either PE device used.

Finally, other removal efficiencies (in %) are calculated by comparing the values before and after each procedure with the formula:

Removal efficiency= (Preprocedural value-Postprocedural value/Preprocedural value)*100

Removal efficiencies are calculated for all blood cell types in addition to IgG, IgA, IgM and fibrinogen removal efficiencies. PLT loss iss calculated with the same formula.

From both CP additional samples will be stored by aliquots of 1ml and for each time moment 5 aliquots. In total for each donor, 10 samples of 1ml will be stored for further analysis in The Netherlands by Radboud and Erasmus University. These samples will be stored at -20 degree celcius as soon as possible after the production of CP in AZP.

Statistical analysis

Data are reported as mean values and their standard deviations (mean SD). Differences between groups are evaluated by a t test. Differences within each group are evaluated by a paired t test. In the rare event where data were not normally distributed, nonparametric statistics will be deployed. Differences between groups will be analyzed by a Mann-Whitney U test. Differences within each group will be analyzed by a Wilcoxon rank test. Statistics output will be generated by a software package (SPSS and R stat).

Data storage

Files and documents are recorded digitally and saved in storage system of the Academic Hospital Paramaribo for future use.

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