Safety and Efficacy of Convalescent Plasma to Treat Severe COVID-19: Protocol for the Saudi Collaborative Multi-center Phase II Study

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Abstract

Background: Coronavirus disease 2019 (COVID-19) pandemic is expected to cause significant morbidity and mortality. The development of an effective vaccine will take several months to become available, and its affordability is unpredictable. Transfusion of convalescent plasma (CP) may provide passive immunity. Based on initial data from China, a group of hematologists, infectious disease specialists, and intensivists drafted this protocol in March 2020.

Objective: To test the feasibility, safety, and efficacy of CP in treating patients with COVID-19 across Saudi Arabia.

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COVID-19 has proven to be a moving target as far as its management is concerned, we will use current definitions according to the Ministry of Health (MOH) guidelines for diagnosis, treatment, and recovery. All CP recipients will receive supportive management including all available recommended therapies according to the available MOH guidelines. Eligible CP donors will be those COVID-19 patients who have fully recovered from their disease according to MOH recovery criteria as detailed in inclusion criteria. CP donors have to qualify as blood donors according to MOH regulations except for the history of COVID-19 in the recent past. We will also test the CP donors for the presence of SARS-CoV-2 antibodies by a rapid test and aliquots will be archived for future antibody titration. Due to the perceived benefit of CP, randomization was not considered. However, we will compare the outcome of the cohort treated with CP with those who did not receive CP due to a lack of consent or lack of availability. In this national collaborative study, there is a likelihood of not finding exactly matched control group patients. Hence, we plan to perform a propensity score (PS) matching of the CP recipients with the comparator group patients for the major characteristics. We plan to collect demographic, clinical, and laboratory characteristics of both groups and compare the outcomes. A total sample size of 575 patients: 115 convalescent plasma recipients and 460 matched controls (1:4 ratio), would be sufficient to detect a clinically important hospital stay & thirty-day mortality difference between the two groups with 80% power and a 5% level of significance.

Results: At present, patient recruitment is still ongoing, and the interim analysis of the first 40 patients will be shared soon.

Conclusions: Here, we present a protocol for a national collaborative multicenter phase II study in Saudi Arabia for assessing feasibility, safety, and potential efficacy of convalescent plasma in treating COVID-19 patients with severe disease. We plan to publish an interim report of the first 40 CP recipients and their matched comparators soon. Clinical Trial: ClinicalTrials.gov Identifier: NCT04347681
https://clinicaltrials.gov/ct2/show/NCT04347681?term=NCT04347681&draw=2&rank=1

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Keywords:

Coronaviruses; SARS-CoV-2; COVID-19; Antibodies; Convalescent plasma

Introduction

Coronavirus disease 2019 (COVID-19) pandemic, caused by SARS-CoV-2, is a major health and economic concern worldwide due to its morbidity and mortality. Coronaviruses (CoV) are a large family of RNA viruses that cause illnesses ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome - Corona Virus (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV) [1]. The new strain of coronavirus identified in December 2019 in Wuhan city, Hubei province of China, was called the 2019 novel coronavirus (2019-nCoV). The International Committee on Taxonomy of Viruses (ICTV) determined that SARS-CoV-2 is the same species as SARS-CoV and has been named as Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2). The World Health Organization (WHO) has named the disease associated with SARS-CoV-2 infections as “COVID-19”.

Clinical features of SARS-CoV-2 infection typically include fever and respiratory symptoms like cough, and shortness of breath; in severe cases, the infection can cause pneumonia, severe acute respiratory distress syndrome (ARDS), kidney failure, and even death. SARS-CoV-2 has a higher transmission rate (TR) with an approximate fatality rate of 3% [2]. The final diagnosis of SARS-CoV-2 infection depends on laboratory detection of the SARS-CoV2 viral RNA by real-time reverse transcription-polymerase chain reaction (rRT-PCR) [1-4].

The concept of using convalescent plasma (CP) is not new since It has been tried in limited numbers of patients during recent viral crises, including the 2003 SARS (severe acute respiratory syndrome) epidemic, the 2009 "swine flu" epidemic, and the 2012 outbreak of MERS (Middle East respiratory syndrome) [5]. CP treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection [6]. Patients with a resolved viral infection typically develop a polyclonal antibody immune response to different viral antigens. Some of these polyclonal antibodies will likely neutralize the virus and prevent new rounds of infection. Patients who recovered from COVID-19 can donate plasma, and then this plasma can be transfused into actively infected patients [7]. Indeed, the same rationale was used in the treatment of several Ebola patients with convalescent serum during the outbreak in 2014–2015 [8].

Therefore, CP therapy is expected to improve the clinical, laboratory, and radiological features of the patients severely affected by COVID-19. Decrease in morbidity & mortality of COVID-19 disease in a cost-effective manner, leading to improved quality of healthcare and self-sufficiency in the treatment of serious diseases affecting the masses, is in line with the top priorities in Vision 2030 of the Kingdom of Saudi Arabia.

SARS-CoV-2 specific immunoglobulins containing convalescent plasma:

Among the most attractive intuitive options, during this fast-kinetic pandemic, is treating the sick COVID-19 patients with SARS-CoV-2 specific immunoglobulins found in patients who have fully recovered from COVID-19 and are considered no more infective. We know from prior research that antibodies against viral antigens render people immune, but we do not know yet how long the immunity will last. However, Zhao et al, showed seroconversion in 173 COVID-19 patients appeared for total antibody, IgM, & IgG in 11, 12, & 14 days [9]. Presence of antibodies was <40% in the first 7 days & then rapidly increased to 100%, 94.3%, & 79.8% for antibodies, IgM, & IgG by day 15. In contrast, viral RNA decreased from 66.7% before day 7 to 45.5% in days 15-39. Moreover, a higher titer of antibodies was independently associated with a clinically worse COVID-19 ($p = 0.006$) [9].

Antibodies detection by a rapid serological method and their kinetics:

In those patients who have passed the viremic phase, the presence of antibodies is highly desirable and provides evidence of immunity to combat COVID-19 (**Table 1**). Fortunately, recently Saudi Food and Drug Authority (SFDA) has approved a highly needed rapid test kit made by BIOZEK company (Inzek B.V. Vissenstraat 327324 AL, Apeldoorn The Netherlands) and other brands in the Saudi market that detect qualitatively IgG and IgM antibodies against SARS-CoV2 from whole blood, serum or plasma using a single-use cassette. This kit utilizes lateral flow chromatographic immunoassay and can produce results within 10 minutes only. The combination use of the IgM and IgG tests can reflect virus infection and the immune status of the body effectively (**Table 1**).

Table 1. How to interpret the results of PCR and Antibody results

Test Results			Clinical Significance
PCR	**IgM**	**IgG**	
+	-	-	The patient may be in the window period of infection.
+	+	-	The patient may be in the early stage of infection.
+	+	+	The patient is in the active phase of infection.
+	-	+	The patient may be in the late or recurrent stage of infection.
-	+	-	The patient may be in the early stage of infection. PCR result may be false-negative.
-	-	+	The patient may have had a past infection and has recovered.
-	+	+	The patient may be in the recovery stage of an infection, or the PCR result may be false-negative.

Adapted from:

1. Lauer, S. et al., 2020. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported

Confirmed Cases: Estimation and Application. *Annals of Internal Medicine (Lauer et al., 2020)*

2. National Health Commission of the People's Republic of China, New Coronavirus Pneumonia Diagnosis and Treatment Program (Trial Version 7)

Role of convalescent plasma in treating severe COVID-19 disease:

Casadevall and Pirofski suggested that convalescent sera from COVID-19 individuals may be an option for treating the highest risk COVID-19 patients and possibly for prophylaxis of infection in individuals at high risk of COVID-19 disease [10]. This passive antibody administration concept to prevent disease is already used in patients exposed to hepatitis B and rabies viruses, and to prevent severe respiratory syncytial virus (RSV) disease in high-risk infants [10]. The proposed use of convalescent sera in the COVID-19 epidemic would rely on preparations of high titers of neutralizing antibodies against the SARS-CoV-2.

In the first pilot Chinese study reported by Kai Duan et al. CP therapy in 10 patients showed a potential therapeutic effect and low risk in the treatment of severe COVID-19 patients [11]. One dose of CP with a high concentration of neutralizing antibodies can rapidly reduce the viral load and tends to improve clinical outcomes [11]. The optimal dose and treatment time point, as well as the definite clinical benefits of CP therapy, need to be further investigated in randomized clinical studies. 39/40 donors (recovered COVID-19 patients) showed a high antibody titer of at least 1:160. After receiving CP therapy, 9/10 recipients were found to have neutralizing antibody titers of >1:640 [11]. Although small, but it is a pivotal study to prove the safety and efficacy to prove the efficacy of CP therapy. All

patients showed an increase in oxygen saturation within 3 days. Other parameters that improved were increased absolute lymphocyte counts (ALC) and decreased C-reactive protein. Varying degrees of resolution of lung lesions were also seen on radiological examinations within 7 days. In seven patients who previously had viremia, the viral load was undetectable after transfusion [11].

Currently available therapeutic options for COVID-19:

According to WHO, the management of COVID-19 has mainly focused on infection prevention, case detection, and monitoring, and supportive care. Although there are reports on the potential efficacy of new potential therapeutic agents (**Figure 1**). However, no specific anti-SARS-CoV-2 treatment is recommended because of conflicting evidence. Evidence shows that CP from patients who have recovered from viral infections can be used as a treatment without the occurrence of severe adverse events. Therefore, it might be worthwhile to test the safety and efficacy of CP transfusion in SARS-CoV-2-infected patients [12].

Our study aims to test the feasibility, safety, and efficacy of CP in treating patients with COVID-19 across Saudi Arabia.

Methodology

This is a national, phase II, multicenter trial evaluating the safety and potential efficacy of CP to treat severe COVID-19 and patients at high risk of developing severe COVID-19. Detailed bilingual informed consent forms (ICF) approved by MOH / institutional IRBs will be used for both CP donors and CP recipients.

A. Time for the study:

Proposed duration: 3 months was considered ideal for recruiting the first 40 CP recipients for our proposed prospective study. If interim analysis will show the benefit of CP, we will increase the sample size and extend the trial period.

B. Inclusion criteria:

1. **Recipients:**
    - **A.** 18 years of age or older.
    - **B.** COVID-19 patients with POSITIVE rRT PCR test for SARS-CoV-2 “using one of the SFDA approved kits used in KSA” as per current MOH guidelines.
    - **C.** Must have been requiring ICU care, severe or immediately life-threatening care:

i. Patient requiring ICU care and/or admission.

ii. Severe disease is defined as:

1. Dyspnea
2. Respiratory frequency ≥ 30/min
3. Blood oxygen saturation ≤ 93%
4. The partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300, and/or Lung infiltrates > 50% within 24 to 48 hours

iii. The life-threatening disease is defined as:

1. Respiratory failure
2. Septic shock, and/or
3. Multiple organ dysfunction or failure

2. Donors:

i. 18 years of age or older.

ii. Prior confirmed COVID-19 diagnosis as per current MOH guidelines.

iii. Complete Clinical Recovery from COVID-19 before donation (at least 14 days from the last SARS-CoV-2 negative PCR or 28 days from the initial symptoms) [13-16].

iv. All MOH criteria for blood donation will be followed.

v. All Transfusion Transmissible Infections (TTI) markers on the donor's blood are negative as per current MOH routine blood donor screening regulations.

vi. Positive rapid serology test for antibodies (IgG) against SARS-CoV-2 indicating immunity against COVID 19.

C. Exclusion criteria

1. Recipients:
   a- Negative or non-conclusive test COVID-19 rRT PCR test for SARS-CoV-2
   b- Mild symptoms
   c- Hospitalization not requiring ICU care and/or admission
2. Donors:
   a. Unfit for blood donation
   b. Multiparous or Pregnant females

D. Collection of convalescent plasma infusion for treatment for COVID-19

As antibody kinetics show IgG levels are highest around day 28 and decline around day 42 to complete disappearance in many months, we will try to get plasma donation from fully recovered patients soon after day 28 of onset of symptoms (that usually last for 7-10 days). For the recovery definition, we will continue to follow the Ministry of Health's current precautionary protocol to prevent the spread of the virus causing COV1D-19 that require blood donor abstinence from donation for 28 days from exposure [15, 16].

Plasmapheresis to collect plasma from the donors (using Trima, Hemonetics, or alike machines) is a commonly used procedure. Donors are required to be in an acceptable health state and pass through a multistep screening (donor history questionnaire, vital signs check, laboratory tests, etc.) before CP donation. The arrangement for plasmapheresis and collection will start after obtaining the donor's informed consent. The collected plasma will undergo an additional safety step of pathogen reduction using Mirasol or Intercept Pathogen Reduction Technology, which is SFDA and CE approved (**Figure 2**). The Mirasol system uses vitamin B2 (Riboflavin) and Ultraviolet Light. Mirasol-treated fresh frozen plasma (FFP) maintains a good quality of therapeutic proteins as demonstrated in multiple external validation studies [17-19]. After passing through the pathogen reduction system, the CP will be sent to the COVID-19 recipients or it can be stored in a dedicated FFP freezer at ≤ -18˚C. The shelf-life of frozen CP should be similar to normal FFP according to the storage conditions (one year at ≤ -18 ˚C or 24 hours at 1-6 ˚C). The CP units will be labeled, stored, and shipped as per Central Board for Accreditation of Healthcare Institutes (CBAHI) and American Association of Blood Banks (AABB) and Joint Commission International (JCI) guidelines for blood products handling and management.

CP will be used only for eligible patients who have COVID-19. The common side effects of FFP transfusion include side effects of blood product transfusion e.g. allergic/febrile reactions, transfusion related acute lung injury (TRALI), and transfusion-associated circulatory overload (TACO), while the infectious risk is minimal. Plasma volume to be collected from each donor can be up to 15% of total blood volume (Total Blood Volume/TBV=Weight in Kg x 70 ml). For example, from a CP donor who weighs 65 Kgs, we can collect up to ~ 682 ml plasma (15/100 x 65 x 70). CP donors can donate more than once as per the regulations of the CBAHI and AABB, which allows healthy donors to donate plasma twice in a month up to a maximum of 24 donations in a year. Special ISBT labels will be affixed on the plasma bags indicating COVID-19

CP (**Figure 2**).

E. Informed Consent Form (ICF) will be used for both the CP donor and the recipient:

- Approved Donor ICF (Arabic and English)
- Approved Recipient ICF (Arabic and English)

F. Transfusion of convalescent plasma for treatment for COVID-19

After obtaining informed consent, Eligible Patients who have severe COVID-19 and have not recovered yet will be infused with the donated CP 300 ml (200-400 ml/ treatment dose) at least once and if needed daily for up to 5 sessions (**Figure 3**).

Other supportive and therapeutic measures should continue according to the locally approved protocols with due diligence. Patients will be monitored after FFP transfusion for the usual side effects of blood product transfusion e.g. allergic/febrile reactions, TRALI, and circulatory overload. We will then assess the response after the infusion of the plasma in these patients as detailed below (section H).

As with other plasma therapies, attention should be given to ABO compatibility. For plasma selection, we will consider ABO compatibility (**Table 2**) regardless of Rh status. To minimize the risk of TRALI, preference will be given to plasma from male donors and nulliparous women.

Table 2. ABO group selection order for transfusion of plasma.

Patient's ABO Group	Fresh Frozen Plasma
O	
1st Choice	O
2nd Choice	A or B
3rd Choice	AB
A	
1st Choice	A
2nd Choice	AB
3rd Choice	B*
B	
1st Choice	B
2nd Choice	AB
3rd Choice	A*
AB	
1st Choice	AB
2nd Choice	A*
3rd Choice	B*

*Tested and Negative for high titer Anti-A and Anti-B (should be less than 1:64)

Adapted from: The ABO System. In: Norfolk D, editor. Handbook of Transfusion Medicine. 5th ed. UK: TSO Information & Publishing Solutions; 2013. https://www.transfusionguidelines.org/transfusion-handbook/2-basics-of-blood-groups-and-antibodies/2-4-the-abo-system

G. Data collection

Clinical information of all enrolled patients will be retrieved from the hospital electronic/paper records system, including the:

- Baseline demographic data
- Days of illness duration
- Presenting symptoms
- Radiological findings (CXR and CT scan chest, if possible)
    - on the day of Hospital Admission
    - on the day of ICU admission
    - on the Day of CP infusion (Day 0)
    - then on days 3, 7, 14, 30
- Laboratory infectious marker testing results like culture from respiratory, urinary or blood culture

- Laboratory inflammatory markers:
    - CRP (Day of CP infusion (Day 0), then on days 3, 7, 14, 30
- Application of assisted mechanical ventilation and their modes, intranasal oxygen inhalation, number of days of intubation or nasal oxygen support
- Medication regimen (e.g. hydroxychloroquine, azithromycin, any anti-viral therapies, steroids, tocilizumab, etc.)
- Complications (including acute renal failure, acute coronary syndrome, myocarditis, acute respiratory distress syndrome, GI complication, and nosocomial infection)
- The SARS-CoV-2 RNA from the serum sample will be monitored during treatment and at day 14 of recovery or discharge, whatever is later.

We plan to test for all of the following parameters for COVID-19 patients:

1. CBC differential to include percent & absolute lymphocyte count (ALC) and percent & absolute neutrophil count
2. Chemistry panel to include Total protein, Albumin, Lactate Dehydrogenase (LDH), Aminotransferases (ALT, AST), Procalcitonin
3. Cardiac biomarkers (e.g. cardiac troponins)
4. CPK
5. Ferritin
6. Full coagulation profile to include PT, APTT, fibrinogen & D-dimer
7. C Reactive Protein (CRP)
8. Oxygen saturation
9. Radiological examination
10. ABO RhD grouping and antibody screening
11. rRT PCR test for SARS-CoV-2
12. Test for IgG & IgM Antibodies against SARS-CoV-2

CP Donors will essentially undergo routine blood donation processes: donor history questionnaire, clinical examination, and testing for the infectious marker (serology and NAT methods) along with ABO RhD grouping, antibody screening, and CBC. They will also undergo a test for IgG & IgM antibodies against SARS-CoV2 (should be positive for IgG)

Data collection forms have been developed to collect data for CP donors, recipients, and

controls (comparators).

H. Response assessment:

a- Daily clinical assessment by a physician
b- Vital signs including temperature, blood pressure, respiratory rate, heart rate.
c- Oxygen saturation.
d- Oxygen requirement.
e- Ventilator requirement and the modes employed
f- Inotrope medications requirement.
g- Complete blood counts, liver function tests, urea, creatinine, and electrolytes daily
h- Apache score
i- SOFA score
j- Fluid balance.
k- X-ray / CT changes, repeated every 3-5 days
l- Organs functions assessment.
m- Plasma doses and frequency requirement.
n- Transfusion-related side effects including TRALI, TACO, etc.
o- SARS-CoV-2 RNA will be tested on recovery (or deterioration to determine alternative etiology)

I. Study endpoint and outcome measures

Our primary endpoints are:

1. ICU (or designated area for critical patients) length of stay
2. Safety of CP and reporting of serious adverse reactions such as anaphylaxis, TRALI, and TACO.

Secondary endpoints will include:

1. Number of days on mechanical ventilation
2. 30 days mortality
3. Days to clinical recovery as defined by MOH

J. Study Population:

A total sample size of 575 patients: 115 CP recipients and 460 matched controls (1:4 ratio),

would be sufficient to detect a clinically important difference of 11.6% between two groups (CP recipients vs. matched controls) in 30 days mortality using a two-tailed z-test of proportions / Chi-Square test with 80% power and a 5% level of significance. This 11.6% difference represents a 12.4% mortality in CP recipient group and 24.4% mortality in matched control patients [20].

- Treatment group (CP recipients Group):
  - 115 patients who have COVID 19 but have not recovered yet as per the inclusion criteria.
- Control group (Comparator Group):
  - 460 Patients who are either not consenting to receive CP, or those who will not be able to receive CP due to non-availability, will serve as a control group to compare the efficacy of the CP. Control group patients will be subjected to propensity score matching based on age, gender, DM, HTN, and intubation.

K. Statistical Analysis:

Descriptive and inferential statistics will be used to characterize the study sample and test hypotheses. Descriptive results for all quantitative variables (e.g., age) will be presented as mean ± standard deviation (SD; for normally distributed data), or median with inter-quartile range (for data not normally distributed), while numbers (percentage) will be reported for all qualitative variables (e.g., gender).

To assess the independent effect of CP transfusion safety and survival, we will conduct a propensity score-matching (based on age, gender, DM, HTN, and intubation) analysis. Among the predictors, exact matching will be enforced to achieve the balance for all predictors between the plasma and control groups.

The bi-variate analysis will be performed using Independent sample t-test, Mann Whitney U-test, Pearson Chi-Square test or Fisher Exact test whenever appropriate to compare the demographic characteristics (e.g. age, gender, nationality) and clinical characteristics (improvement in oxygenation, laboratory parameters, radiological findings, complications and length of hospital stay) between those who will receive the CP and those who will not receive this therapy.

Multiple binary logistic regression model will be used to assess the effect of CP transfusion on 30 days mortality after adjusting for potential confounding factors compared to matched controls patients. The adjusted Odds ratio and 95% confidence interval for the adjusted odds ratio will be

reported. The Hosmer-Lemeshow goodness-of-fit statistics will be used to determine whether the model adequately describes the data.

The time to event analysis will be measured from the date of diagnosis. The overall survival (OS) at 30 days and 3 months will be evaluated using the Kaplan-Meier estimator, and compare between the two groups (plasma recipients vs. matched control patients) using the log-rank test. A Cox proportional hazard model will also be used to estimate the hazard ratio for in-hospital 30 days mortality for the plasma group compared with matched control group patients after adjusting for potential confounding factors. In addition, interactions between CP administration and all the predictors will be tested to see if the plasma effects will be the same in subgroups. A "P" value <0.05 (two-tailed) will be considered statistically significant. All statistical analyses will be performed using the Statistical Package for Social Sciences Version 24 (SPSS).

L. Interim Analysis:

An interim analysis will be performed after enrolling 40 CP recipients and propensity score-matched 40 controls. A similar statistical analysis will be performed as described above.

M. Monitoring & Safety:

Plasma infusion is a routine practice in health care facilities. All known adverse events (AE) and serious adverse events (SAE) of CP infusion such as anaphylaxis, TRALI, and TACO, will be collected as per the SFDA reporting standard. SAE or death of a study participant due to any cause will be reported by the study team to the IRB chairman and PI within 24 hours of the event.

N. Confidentiality Statement:

All subject related personal information will be saved in password-protected files which will only be accessed by the study research team. All data will be archived in our archiving facility within the hospital once the study has come to an end. This will be in accordance with the standard requirement for the clinical trial archival.

O. Patient/Family Education and Donor Recruitment Strategy:

A variety of methods will be used to recruit CP donors for the study. These include referrals of the recovered patients from various hospitals, dissemination of messages to the public through social media platforms, and a website set up specifically for the study for general information and communication. The website address is: https://plasmaforcovid.com

We will be using the following Twitter account: @Plasma4CovidKSA for public information only. Approved videos will be used on the website and twitter account. Also, multi-lingual advertisement statements and links to the videos by international physicians may be shared with non-Arabic speaking patients. Detailed bilingual informed consent forms approved by MOH / institutional IRBs will be used for both CP donors and CP recipients.

Results:

We are still collecting data and recruiting patients for our ongoing national clinical trial. We will soon share the results of an interim analysis of the first 40 CP recipients and PS matched controls. Once the study is completed, the final results will be published.

Approvals of the study plan and the schedule:

The following approvals have been obtained in addition to institutional IRB approvals of the participating centers:

MOH Central IRB log No: 20-COVID-19-01M – approval date: 2nd April 2020

SFDA SCTR No. 20041102 – approval date: 14th April 2020

ClinicalTrials.gov Identifier: NCT04347681 (updated and under process)

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Figures:

Figure 1. Therapeutic options for treating COVID-19.

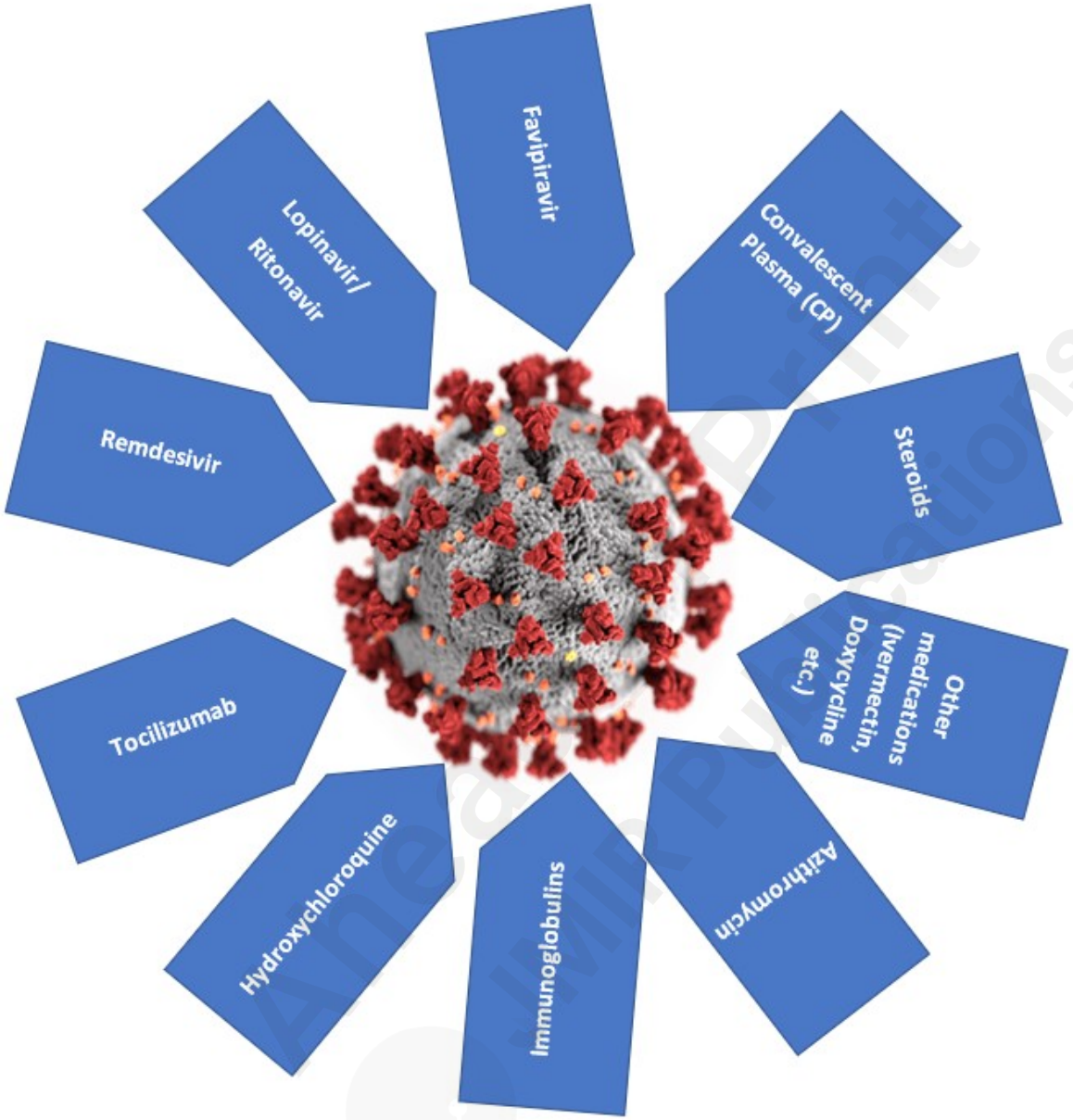


Figure 2. Logistic cycle of CP procurement from donor, processing, and infusion to COVID-19 patient

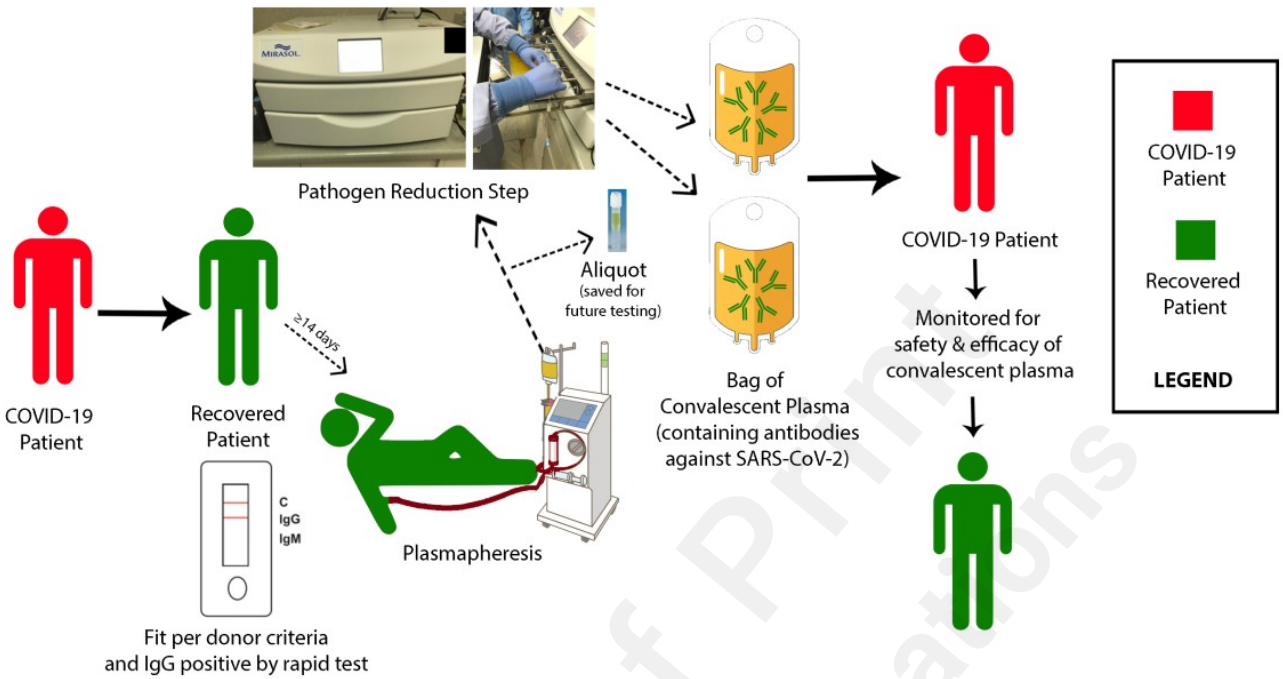
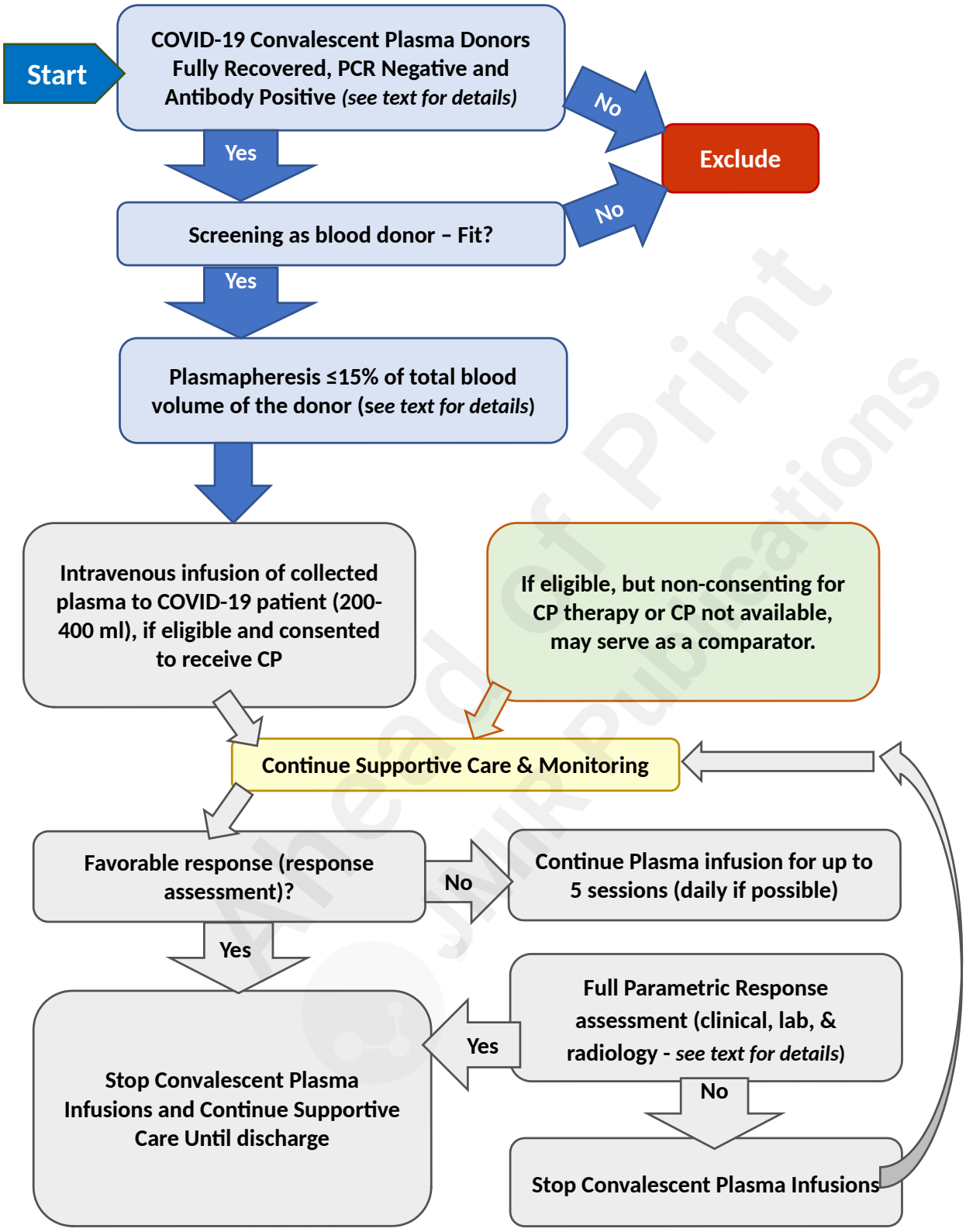


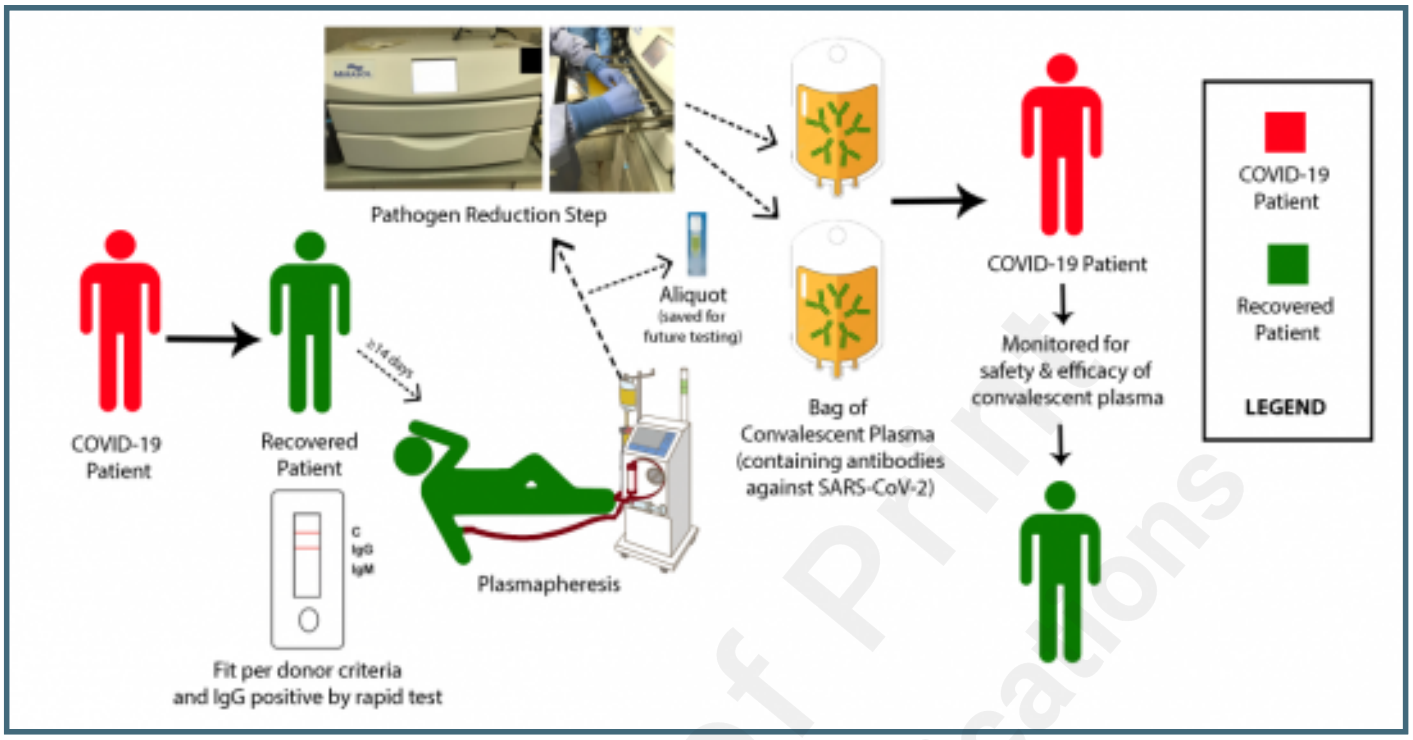
Figure 3. Schema for the proposed process of CP donation and infusion



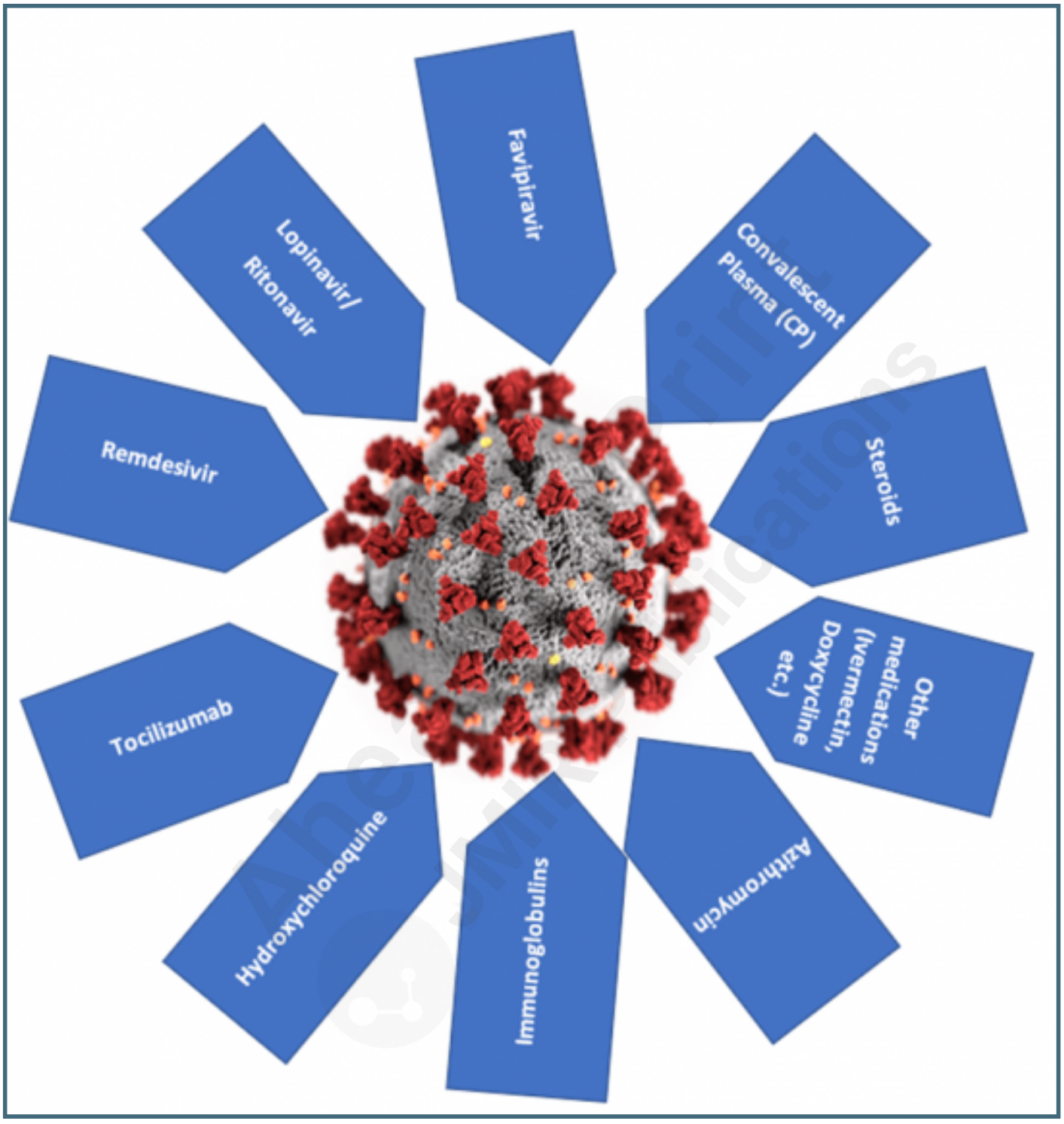
Supplementary Files

Figures

Logistic cycle of CP procurement from donor, processing, and infusion to COVID-19 patient.



Some therapeutic options for treating COVID-19.



Schematic for the proposed process of CP donation and infusion.