A Phase II, Open Label, Randomized Controlled Trial to Assess the Safety and Efficacy of Convalescent Plasma to Limit COVID-19 Associated Complications in Moderate Disease.

Abstract:

The novel coronavirus disease (COVID-19), which began in Wuhan, China, in December 2019, has been declared to be a pandemic by the World Health Organization (WHO). Currently, there are no proven therapy for COVID-19. Management is primarily supportive with oxygen and mechanical ventilation along with a multitude of drugs like Remdesivir, Lopinavir/Ritonavir, Lopinavir/Ritonavir with interferon beta, and hydroxychloroquine. Convalescent plasma has been historically used in many viral infections such as poliomyelitis, measles, mumps and influenza. More recently, convalescent plasma has been used with variable benefit in cases of H1N1 influenza, SARS, Ebola. Considering the lack of efficacious treatments for COVID 19 and the epidemic situation with high mortality rate, US FDA has approved convalescent plasma for COVID-19 for clinical trials, expanded access and single patient emergency investigational new drugs (IND). This multi-centric, open label, randomized control trial has been planned to assess the efficacy and safety of convalescent plasma collected from recovered COVID-19 patients. The primary outcome to be measured is a composite of avoidance of progression to severe ARDS (P/F ratio <100) or all-cause mortality at 28 days. We plan to enroll 452 patients of moderate COVID-19 patients over next 6 months. The adverse events related to plasma transfusion will be regularly monitored.

Introduction

The novel coronavirus disease (COVID-19), which began in Wuhan, China, in December 2019, has been declared to be a pandemic by the World Health Organization (WHO).[1] Caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), COVID-19 has resulted in 1,781,127 cases and 108,994 deaths globally (till 12th April, 2020), affecting 199 countries and 2 international conveyances.[2] In India, it has caused 8,356 confirmed cases, 716 recoveries, and 273 deaths from COVID-19 (till 12th April, 2020).[3]

The clinical manifestations of COVID-19 range from fever, cough, fatigue, sore throat, shortness of breath and less common symptoms such as headache, nausea and diarrhea.[4,5] The most common abnormalities in vital signs are increased temperature and tachypnea. The most common radiological findings are bilateral pulmonary infiltrates, ground glass opacities and consolidation. The most common findings associated with severe disease are older age, d-dimer levels greater, higher SOFA score, elevated IL-6, increased Lactate Dehydrogenase, hyperferritinemia and lymphopenia on admission.[6,7] The most common complications are sepsis, respiratory failure, acute respiratory distress syndrome (ARDS), cardiac injury and acute kidney injury.

Although not completely understood, multiple pathophysiological mechanisms have been hypothesized for the cause of mortality in COVID-19.[8] The plausible mechanisms of respiratory failure are hyperinflammation due to cytokine storm causing ARDS.[9] Another plausible mechanism of respiratory failure is occlusion and micro-thrombosis in small pulmonary vessels.[10]

Secondary hemophagocytic lymphohistiocytosis (sHLH) is a hyperinflammatory condition characterized by hypercytokinemia with multi-organ failure.[11] sHLH is usually triggered by viral infections and sepsis.[12] Infections account for 6-28% of causes triggering sHLH.[12–14] Severe COVID resembles sHLH characterized by cardinal features like fever, cytopenia, hyperferritinemia and increased Interleukins.[9]

Significantly abnormal coagulation parameters were noted in people who succumbed to COVID-19, with higher levels of D-dimer and fibrin degradation products (FDP), and lower levels of the fibrinogen and AT levels.[15] Increased levels of D-dimer are commonly reported in one third of patients with severe illness.[16] Occlusion and micro-thrombosis formation in pulmonary small vessels in such patients have also been reported.[10] The endothelial cell dysfunction, induced by infection, results in excess thrombin generation and fibrinolysis shutdown in patients with infection.[17,18] In addition, hypoxia stimulates thrombosis through not only increasing blood viscosity, but also a hypoxia-inducible transcription factor-dependent signaling pathway.[19]

According to another hypothesis, COVID-19 attacks the 1-beta chain of hemoglobin to dissociate the iron to form the porphyrin. This will cause reduced ability of the lungs to effectively exchange Oxygen and Carbon Dioxide. This inability causes inflammation in lungs causing ground glass opacities on radiological imaging.[20]

Convalescent Plasma in COVID-19

Currently, there are no approved treatments for COVID-19. The management plan is supportive care with supplemental oxygen and mechanical ventilation.[21] Multiple trials are being done across the globe to assess the efficacy of various treatment strategies. WHO initiated the SOLIDARITY trial in several countries to compare the effectiveness of the following regimens against COVID-19: Remdesivir, Lopinavir/Ritonavir, Lopinavir/Ritonavir with interferon beta, and hydroxychloroquine.[22] In a clinical trial, Lopinavir/Ritonavir did not demonstrate any benefit over Standard of care.[23] US FDA has recently approved Convalescent Plasma from patients recovered from COVID 19 for the treatment of severe or life threatening COVID-19 infections.[24]

In a small case series, five critically ill COVID-19 patients with ARDS were treated with convalescent plasma containing neutralizing antibodies. Infusion of plasma was followed by improvement in clinical status in all five patients, with no deaths and the study reported that three patients were discharged, whilst two continued to be stable on mechanical ventilation.[25]

In another small case series of four patients, including one pregnant woman, it was seen that all four recovered eventually.[26]

In another feasibility study of convalescent plasma therapy, 10 severely ill patients were transfused with 200 ml of convalescent plasma.[27] It was well tolerated with significant increase in neutralizing antibodies and disappearance of viremia in 7 days. Clinical symptoms rapidly improved in 3 days.

Historically, it has been used in viral diseases such as poliomyelitis, measles, mumps and influenza before vaccines became available.[28–32] A meta-analysis of 1703 patients with H_1N_1 influenza during the Spanish Flu of 1918 suggested that patients who received convalescent plasma had lower mortality.[33] Conversely, in a double blind, randomized, placebo-controlled trial, convalescent plasma was not found to be superior to placebo in patients infected with Influenza A.[34–36] Furthermore, 84 patients with Ebola virus disease who were transfused with convalescent plasma without known levels of neutralizing antibodies did not have a survival benefit.[37] Convalescent plasma was also studied during the previous coronavirus outbreak of SARS in 2002 -2004. In a retrospective study of 80 patients by Cheng et al, it was observed that patients who received convalescent plasma before day 14 of illness had better outcomes, defined as early hospital discharge, compared to patients who received it after day 14 of illness (15.6% vs 58.3%; $P<0.001$).[38] Considering the lack of efficacious treatments for COVID 19 and the epidemic situation with high mortality rate, US FDA has approved convalescent plasma for COVID-19 for clinical trials, expanded access and single patient emergency investigational new drugs (IND).[39]

Majority of the adverse effects associated with plasma transfusion are non-lethal; medically treatable adverse effects commonly associated with transfusion of plasma include TRALI; transfusion associated circulatory overload (TACO); allergic/anaphylactic reactions; transfusion related transmission of infections (TTI); febrile non-hemolytic transfusion reactions (FNHTR); hemolytic transfusion reactions (HTR); and rarely RBC allo-immunization.[40] Another theoretical risk of using convalescent plasma includes antibody dependent enhancement of infection.[41]

Hypothesis and Objectives

We hypothesize that the use of convalescent plasma will improve the clinical outcomes in patients with moderate COVID-19 infection.

We designed this phase II, open label, randomized clinical trial with the primary objective to assess the safety and efficacy of the therapy in moderate disease.

Efficacy Objective: To assess the efficacy of convalescent plasma to limit progression to severe disease in COVID-19 patients.

Safety Objective: To evaluate the safety of treatment with anti SARS-CoV-2 plasma in patients with COVID-19.

Method

Study Design

This is a generic protocol for a multi-center, two-arm, prospective, Phase II, open label, randomized controlled trial.

Study Population

Hospitalized COVID-19 patients fulfilling the inclusion and exclusion criteria, will be eligible for inclusion in the trial.

Inclusion Criteria

1. Patients admitted with RT-PCR confirmed COVID-19 illness.
2. Age > 18 years
3. Has any of the two
    a. PaO2/ FiO2: 200-300
    b. Respiratory Rate > 24/min and SaO2 $\leq$ 93% on room air
4. Availability of matched donor plasma at the point of enrolment

Exclusion criteria

1. Pregnant women
2. Breastfeeding women
3. Known hypersensitivity to blood products
4. Receipt of pooled immunoglobulin in last 30 days
5. Critically ill patients:
    a. P/F ratio <200 (moderate - severe ARDS)
    b. Shock (Requiring Vasopressor to maintain a MAP >= 65mmHg or MAP below 65)
6. Participating in any other clinical trial
7. Clinical status precluding infusion of blood products

Assessment of donor for eligibility

COVID-19 convalescent plasma will be collected from recovered individuals if they are eligible to donate blood. Considerations for assessment of donor for eligibility are outlined in the section on convalescent plasma.

Sample Size

For sample size calculation, we considered that given the standard of care, 18% of patients would succumb to COVID-19 infections.[5] We considered a relative effectiveness of 50% for sample size calculations. We calculated sample size using the PASS v11.0 software.[42]

Group sample sizes of 226 in the intervention group and 226 in the control group achieve 80% power to detect an absolute difference of -0.09 between the group proportions. The proportion of participants not meeting the primary outcome in group one (the treatment group) is assumed to be 0.18 under the null hypothesis and 0.09 under the alternative hypothesis. The proportion of participants in group two (the control group) who will not meet the primary outcome is assumed to be 0.18 based on current evidence.[5] The test statistic used is the two-sided Z test with pooled variance. The significance level of the test was targeted at 0.05, with a power of 0.8.

Duration of Study: 6 months

Study sites: This will be a multi-centric trial. The sites (at least 50 or more, each site to enroll 10-15 patients) are being shortlisted and the list will be submitted as an addendum. There may be some sites which will enroll more than 10 patients and some less. ICMR, New Delhi will act as the coordinating centre.

Informed consent and Randomization:

The patients who meet the inclusion criteria and do not have any exclusion criteria will be invited to part of this study. The study and intervention will be explained to patient and written informed consent will be obtained. The experimental nature of the intervention will be explained, highlighting the potential risks and benefits. If the patient is unable to consent for any reason, the patient's family member will be contacted personally or over the phone and consent sought after explaining the study and reading the participant information sheet in a language they can understand. The patient's or the patient's family's right to rescind the consent and leave the trial at any point, without any punitive effects on the standard of care will also be mentioned.

After obtaining written, informed consent the patient will be enrolled in the study and randomized into either the intervention or control arm according to the randomization list prepared by independent investigator at National Institute of Epidemiology, Chennai.

Stratified randomization will be done based on the following strata:

a. Trial site

In case of unavailability of eligible patients in any strata, more patients can be enrolled from the other strata.

Intervention arm: the patients in this group will receive two doses of 200mL each of convalescent plasma. The details are given under the heading of 'Convalescent plasma' later.

Control arm: The patients randomized to the control arm will receive the usual care for COVID 19 disease as stated later under the heading 'Details of usual care' later.

Investigations and follow-up:

The enrolled patients will undergo examinations and investigations as outlined in Table 1. Follow-up will be done on days 1, 3, 5, 7, 14 and 28 days from the transfusion of first dose of convalescent plasma in case of intervention arm or enrolment in case of control arm (day 0).

Primary Outcomes

The primary outcome is a composite measure of the avoidance of -

1. Progression to severe ARDS (P/F ratio <100) or
2. All-cause Mortality at 28 days

Secondary Outcomes

1. Time to symptom resolution
    a. Fever
    b. Shortness of Breath
    c. Fatigue
2. Length of hospital stay
3. Change in SOFA pre and post transfusion
4. Change in oxygen requirement post transfusion
5. Duration of respiratory support required
    a. Duration of Invasive Mechanical Ventilation
    b. Duration of Non-Invasive
6. Radiological improvement
7. Adverse events (AE) associated with transfusion
8. To measure the change in RNA levels (Ct values) of SARS-CoV-2 from RT-PCR [Time Frame: Days 0, 3, and 7 after transfusion]
9. Correlation between viral neutralization titer and Elisa antibody assay in donor plasma
10. Correlation between IgG antibody in donor plasma and recipient plasma after transfusion.
11. Levels of bio-markers (CRP, IL6, Ferritin) pre and post transfusion
12. Need of Vasopressor use
13. Pre and post transfusion antibody titers (IgG) in recipient plasma

End Point of study period for each patient: 28 days post enrolment or death whichever comes earlier

Analysis Plan

Baseline data about the demography, clinical presentations, ongoing medical therapy, and clinical history of participants in both arms will be collected and compared. Response to convalescent plasma will be coded as a binary outcome – based on whether the composite primary end point is met or not. Adverse events associated with infusion of convalescent plasma will also be descriptively summarized and compared with the adverse events experienced by participants receiving standard of care.

The objective response rate, defined as the proportion of participants successfully meeting the primary end points, will also be reported, along with a two-sided 95% confidence interval calculated using the Clopper-Pearson method.[45] This will be compared between the intervention and control groups.

Participants who drop off from the treatment arm prior to evaluation of post-intervention response will be considered as non-responders, regardless of the discontinuation reasons, and will be included in the intention to treat analysis. Comparison between group response rates will be analyzed and reported.

Table 1: Study Procedures

	Screen	**Enrolment/plasma transfusion**	**Follow-up**					
Day	**-1 to 0**	**0**	**1**	**3**	**5**	**7**	**14**	**28**
Pre-enrolment Screening								
Eligibility screening and Informed Consent	x							
Demographic and Medical history	x							
SARS-CoV-2 RT-PCR test for eligibility	x							
Pregnancy test	x							
ABO	x							
After enrolment in study								
Baseline patient particulars		x						
Randomization		x						
Symptom checking		x	x	x	x	x	x	x
Physical examination		x	x	x	x	x	x	x
SOFA score		x	x	x	x	x	x	x
Screening for TTIs i.e HIV, HCV and HBV by ELISA or NAT.		x						
Convalescent Plasma Infusion		x	x					
Vital signs	x	xxxx[1]	x	x	x	x	x	x
Adverse event monitoring		x	x	x	x	x	x	x
Recording details in CRF		x	x	x	x	x	x	x
Lab Investigations								
CBC and CMP		x	x	x	x	x	x	
Arterial blood gas	x	x	x	x	x	x	x	
PT/INR/aPTT/Fibrinogen		x	x	x	x	x	x	
Biomarkers (Ferritin, LDH, CRP D-Dimer)		x		x		x		
IL – 6		x		x				

SARS-CoV-2 RT-PCR		x		x		x		
SARS-CoV-2 antibody (IgG titre)		x		x		x		
Blood for testing and archiving		x	x		x	x	x	
Imaging (Chest Xray, AP or PA as per the patient's condition)		x		x		x		
Others	As clinically indicated							

Vital signs need to be tested immediately prior to infusion, within 15 minutes after initiation of infusion, at the completion of infusion and within one hour of end of infusion.

CBC: complete blood count; to include Hb, hematocrit, total leucocyte count, differential count, platelet.

CMP: comprehensive metabolic panel; to include blood glucose, Calcium, Sodium, potassium, carbon dioxide, and chloride, Bilirubin, Albumin, Total protein, ALP (alkaline phosphatase), ALT (alanine transaminase), and AST (aspartate aminotransferase), BUN (blood urea nitrogen) and Creatinine.

SOFA score: sequential organ failure assessment score

Convalescent plasma:

Eligibility of Donor

The following criteria should be met for potential donors [39]:

- 18 years to 65 years of age
- Males or nulliparous female donors of weight >50Kg
- Prior diagnosis of COVID-19 documented by a laboratory test (RT-PCR) with symptomatic disease with at least fever and cough and
- Complete resolution of symptoms at least 28 days prior to donation

or

Complete resolution of symptoms at least 14 days prior to donation and two negative real time PCR test for COVID-19 from nasopharyngeal swab, collected 24 hours apart.

In addition, donor eligibility criteria for whole blood donation will be followed in accordance to the Drugs & Cosmetics Act 1940 and rules 1945 therein (as amended till March 2020).[44]

These individuals fulfilling the above criteria will be contacted telephonically and explained the details of the study and their extent of participation. They will be encouraged to visit blood

bank for further evaluation towards eligibility for blood donation. If requested, they will be provided transport for the same.

Recruitment of Donor

At the time of discharge, all recovered COVID-19 patients will be counselled. They will be motivated towards donation of convalescent plasma and its probable beneficial effects in the management of COVID-19 patients. Such patients will be briefed in detail regarding their extent of participation in the study. They will be encouraged to visit the Blood Bank for further evaluation towards eligibility for blood donation. If need arises, the participant will be provided conveyance for facilitating transport. The donors will be provided INR 500 as travel and wage compensation.

Screening of eligible donor

1. Donor will be screened, followed by brief physical examination.
2. Donors not fit to donate blood based on the history and examination will be deferred and excluded from plasma donor pool for a time period specified by country regulation.
3. Donors who have had transfusion of blood products in last 12 months will be excluded.
4. Donors who have had COVID diagnosis more than 4 months will be excluded from donation.
5. Two EDTA samples (5 ml each) and one plain sample (5 ml) will be drawn for the following pre-donation tests as required for convalescent plasmapheresis.
    a. Blood group (ABO grouping and Rh phenotyping) and antibody screening for clinically significant antibodies (Extended Rh, Kell, Duffy, Kidd, MNS) – Antibody screen positive donors will be deferred.
    b. Complete blood count including Hb, Hct, Platelet count, Total and differential leucocyte count. Donors with Hb>12.5g/dl, platelet count >1,50,000 per microliter of blood and TLC within normal limits will be accepted.
    c. Screening for HIV, HBV and HCV by serology or NAT. Donor negative by either test will be included.
    d. Screening for syphilis and malaria by serology. Negative donors will be included.
    e. Total serum protein. Donors with total serum protein > 6gm/dl will be accepted (as per Drugs and Cosmetics (Second Amendment) Rules, 2020)

6. Titration of anti-COVID-19 (both IgG and IgM) antibodies and SARS-CoV-2 neutralizing antibodies may be done depending on availability of facilities at the time of testing. Unavailability of antibody titres will not preclude convalescent plasma transfusion. Desired titers for IgG antibodies is 1:1024 and for neutralizing antibodies is 1:40. If not done at the time of plasma collection, the donor samples will be stored in aliquots at <-80° C to be tested later at NIV, Pune.

Plasmapheresis of donors

Donors will be explained the procedure of plasma donation and the adverse events associated with the process. Among the consenting donors and based on the results, accepted donors will be asked to return on a specified date for plasma donation.

Plasma collection will be done by centrifugal separation using any of the apheresis equipment available at the facility. Volume collected will not exceed 500 ml per sitting (as per Drugs and Cosmetics (Second Amendment) Rules, 2020). Throughout the procedure the extracorporeal volume of blood will never exceed >15% of the total blood volume of the donor. Donor adverse events will be managed as per trial site's protocol for Apheresis donations.

A unique donor identification number will be provided to the collected unit as per attached SOP and the unit will be stored as per attached SOP or issued for patient use. The collected plasma will be divided into smaller packs of 200 ml each for easy storage and transfusion and frozen within 8 hours. The plasma will be stored at <-40 degree Celsius.

No pooling of plasma from different donors will be done.

Successful plasma donors will be requested to repeat the donation. If the donor agrees for a repeat donation, such donation will be scheduled after at least 2 weeks of the first plasma donation. If there was a loss of red cells at the time of first donation owing to any procedural problems or otherwise the donor will be deferred for a period of 3 or 4 months for male or female donors, respectively. All the donor selection guidelines described above will apply to repeat donation as well.

In repeated plasmapheresis:

1. Total serum protein will be tested before the third procedure if done within four weeks and it should be 6 gm/dl.
2. The quantity of plasma separated from the blood of donor will not exceed 500 ml per sitting and once in a fortnight or shall not exceed 1000 ml per month.

Infusion of Blood Products

For infusion of plasma, standard SOP for transfusion of FFP should be followed with special care to monitor these patients during and post-24 hours of transfusion. All such transfusions must be done using blood transfusion sets. The clinician will send a request for plasma component specifically mentioning the diagnosis and that convalescent plasma is required. An ABO compatible plasma bag of approx. 200mL will be issued maintaining all the blood bank records after thawing at 37 degree Celsius.

Dose of convalescent plasma: After randomization to intervention arm, one dose of 200 mL convalescent plasma will be transfused. This will be first dose. If the first dose does not lead to any adverse event which contraindicates plasma transfusion, a second dose of 200mL of convalescent plasma will be transfused after an interval of 24 hours from the first infusion.

Hence, the cumulative dose of convalescent plasma for each patient will be 400mL. [25] The second plasma unit will preferably be from a different donor depending on the availability of another ABO compatible plasma unit or else plasma unit from the same donor will be issued. [45]

Details of Usual Care for COVID 19

COVID-19 is an evolving disease and the management of the disease is rapidly changing as the understanding of pathogenesis is getting better. The drugs that are currently used in the management of COVID -19 are Hydroxychloroquine, Lopinavir/Ritonavir, Remdesevir, and Favipiravir. The management may also require immunomodulators like steroids, Tocilizumab, Sarilumab. Common manifestation of COVID is pneumonia, which may require antibiotics for superimposed bacterial infection, invasive and non-invasive respiratory support. It will also require on a case to case basis fluid resuscitation, analgesia, thromboprophylaxis and ulcer prophylaxis.

The other complications of COVID include cardiac arrythmias which will require treatment in the form of either rhythm or rate control agents depending on the nature of the arrhythmia. It may also require cardioversion. Hemodynamic support in form of vasopressors may also be required for cardiogenic or septic shock.

The Ministry of Health and Welfare has issued detailed guidelines for the management of COVID-19 based on varying grades of severity.[46] For the management of ARDS or sepsis, the respective guidelines issued by ARDSNet and Surviving Sepsis campaign will be followed. Other institutional protocols for supportive management will be implemented.

Safety Assessments and Reporting

Definition of adverse event

An Adverse Event/Experience [AE] is any untoward medical occurrence in a participant undergoing convalescent plasma therapy, is one which does not necessarily have a causal relationship with the convalescent plasma therapy procedure. It can be any unfavorable and unintended sign, symptom, or disease, which is temporally associated with the use of convalescent plasma, whether or not considered to be causally related to convalescent plasma.

A Serious Adverse Event [SAE] is any untoward medical occurrence, at any point during convalescent plasma, results in:

- Death

- A life-threatening condition. This means any event in which the participant is at risk of death; it does not refer to a condition, which, with increasing severity, may result in death.
- Significant disability

Other medically significant outcomes, which may not result in any of the above, but may jeopardize the well-being of the participant, requiring greater degrees of medical or surgical interventions to prevent one of the outcomes listed above.

Expected adverse events (AE) [47,48]

- Donor-related adverse events: They are divided into local reactions and systemic reactions. AEs were classified according to severity into mild, moderate, and severe and according to etiology in a donor into hypotensive reactions, citrate reactions, hematomas, loss of consciousness, seizures, and allergy.

- Kit/ Equipment-related adverse events: These are secondary to improper disposable sets. These are hemolysis, thrombus formation, air embolism, leakage, infection, improper mounting of the kits etc.

- Recipient related adverse events: A transfusion-related adverse reaction is a response or effect in a patient temporally associated with the administration of blood or blood components. Majority of these are non-lethal and medically treatable. Adverse effects commonly associated with transfusion of plasma include transfusion related acute lung injury (TRALI); transfusion associated circulatory overload (TACO); allergic/anaphylactic reactions; transfusion related transmission of infections (TTI); febrile non-hemolytic transfusion reactions (FNHTR); hemolytic transfusion reactions (HTR); and rarely RBC allo-immunization.

Management of Adverse Events:

1. TRALI – Discontinue transfusion. Notify the blood bank. Treatment is supportive care with respiratory support either with Non-Invasive or Invasive Mechanical Ventilation depending on the clinical scenario.
2. TACO – Stop the transfusion. Notify the blood bank. Respiratory support with supplementary O2 and assisted ventilation. Fluid mobilization, typically done with diuretics along with careful monitoring of renal function and electrolytes.
3. Allergic reactions – Discontinue transfusion. Notify the blood bank. Observe for resolution. Give diphenhydramine 25-50mg orally. Can continue with rest of the transfusion depending on the clinical scenario.
4. Anaphylactic reactions – Discontinue transfusion. Notify the blood bank. The management includes epinephrine 0.3mL of a 1:1000 solution IM. Resuscitation with fluids, O2, vasopressors.

5. FNHTR – Discontinue transfusion. Notify the blood bank. Administer paracetamol. Evaluate for other causes of fever.
6. HTR – Discontinue transfusion. Notify the blood bank. Diagnosis should be confirmed quickly and repeat ABO and Rh compatibility testing. Along with that, vitals should be monitored every 15 min. Aggressive hydration of the patient. If needed vasopressors should be initiated to maintain stable vital signs. Along with this electrolytes, hemoglobin and cardiac rhythm should be monitored.

Protocol Deviations

Any changes or deviations from protocol-specified procedures and study-related SOPs occurring during the conduct of the trial will be documented as protocol deviation(s). For example, if a participant already enrolled into the trial is found to have been noncompliant with inclusion and exclusion criteria, the case will be documented as a protocol violation.

The severity of the protocol violation and protocol deviation will be graded as minor if the violation/deviation is not altering the integrity of the study plan or its safety and efficacy outcome, as major if the violation/deviation is altering the integrity of the study plan or its safety and efficacy outcome. Serious or repeated protocol deviations and violations will constitute grounds to interrupt the trial at a study site and will be reported to the corresponding IEC/IRBs.

Data Management

Data Management Responsibilities

The Investigators will be responsible for assuring completeness, accuracy and timely collection of data. Case Record Forms (CRFs) should support the data entered in the electronic data capture system. They will be signed and dated by the person filling out the forms, and the investigator reviewing the same. Data will be reviewed by the Investigator and co-signed. Data will be entered in REDCap software (Vanderbilt, USA) by the study sites and Case Record forms will be scanned in REDCap (Vanderbilt, USA). Data management will be undertaken by ICMR-National Institute of Epidemiology.

Data Capture Methods

Paper CRFs will be used to collect data. The data will then be entered on an electronic system. A data entry system with electronic tracking, password restricted access, audit trail, with time and date stamps on data entry and edits, will be developed. SOP for data collection will be provided. This SOP may be adopted by each site depending on their capacity and will have to be submitted to ICMR and get approved by ICMR.

Types of Data

Data source would include, but are not limited to, participant CRFs, Informed Consent Forms (ICFs), participant medical records, laboratory reports, ECG tracings, x-rays or other radiologic scans, radiologist's reports, any biopsy or microbiology reports, ultrasound photographs, progress notes, pharmacy records, and any other similar reports or records of procedures performed during the subject's participation in the study, which is deemed to hold informational value by the investigator.

Access to Source Data/Documents

All data source documents, including clinical reports and records necessary for the evaluation and reconstruction of the clinical trial, will be stored securely, with a focus on ensuring the patient's confidentiality.

Record Retention

The investigator will be responsible for retaining all essential documents as outlined in the GCP Guidelines for a period of at least 5 years. All stored records will be kept confidential as outlined by applicable law.

Ethical Principles and Informed Consent

Informed Consent Process

If participants are compos mentis, they will provide their informed consent, and sign the document prior to any procedures being done specifically for the study. If the patient is unable to provide written consent for any reason e.g impaired consciousness, on mechanical ventilation etc. the family members (next of kin) will be contacted. If the family members are not available in person, they will be contacted telephonically. The study will be explained in detail and written informed consent will be obtained. In cases where the family members are being contacted over video-calling, the conversation will be recorded with permission of both parties (Audio-visual recording).

The participants or their representatives may withdraw consent at any time throughout the course of the trial. The welfare and rights of the participants will be protected by providing them assurance that their medical care will not be impaired if they refuse to participate in the trial. The experimental nature of the treatment will also be emphasized to the participants or their consenting LARs.

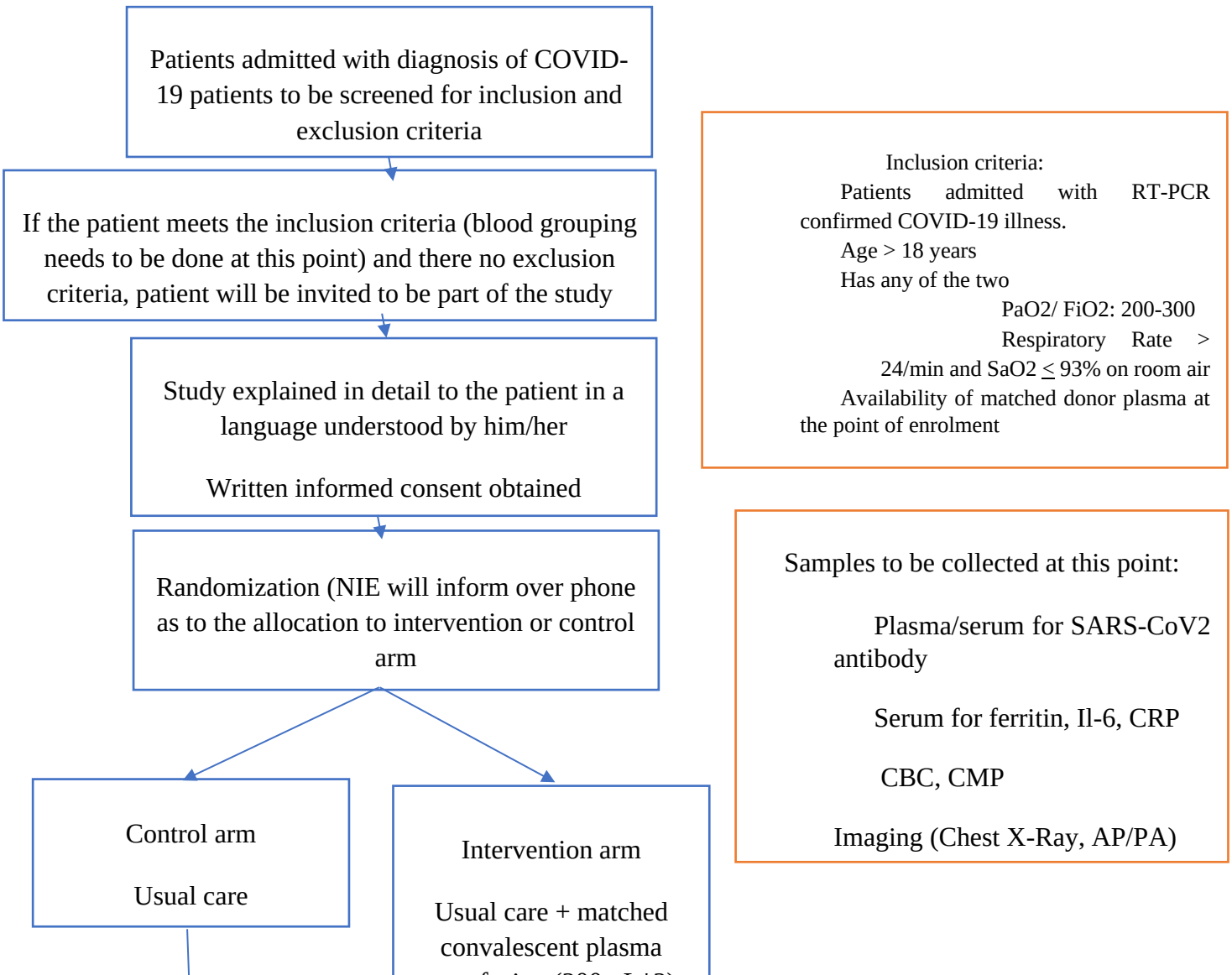
A copy of the informed consent/assent document will be given to the participants or their family members/LAR for their records. The completion of the signing of the informed consent form will be noted in the participants' medical records by the trial physicians. SOP for informed consent is attached. This SOP may be adopted by each site depending on their and will have to be submitted to ICMR and get approved by ICMR.

Participant Confidentiality

All records and CRFs will be kept confidential, with access only to study staff, monitors, other authorized representatives who may inspect any documents which are required to be maintained by the investigator. Paper forms will be stored in locked cabinets and computer entered data will be maintained on password protected, access restricted computers.

Study flow:

Figure 1: The flow of enrolment and follow-up of patients



Definitions:

1. Hypertension – Blood pressure greater than 140/90mmHg or on anti-hypertensives for previously diagnosed hypertension.
2. Diabetes Mellitus – Fasting Blood Sugar > 126mg/dL or Plasma Glucose >200mg/dL two hours after a 75 grams oral glucose load or HbA1c > 6.4 DCCT% on medications for Diabetes Mellitus for previously diagnosed Diabetes Mellitus.
3. Obesity – BMI >30.
4. CKD Stage IIIA – GFR less than 60.

Moderate COVID-19 Disease: In this protocol moderate COVID 19 disease is defined by patients who either have PaO2/FiO2 ratio between 200- 300 or are tachypneic to more than 24 breaths/min and have SaO2$\leq$93%.

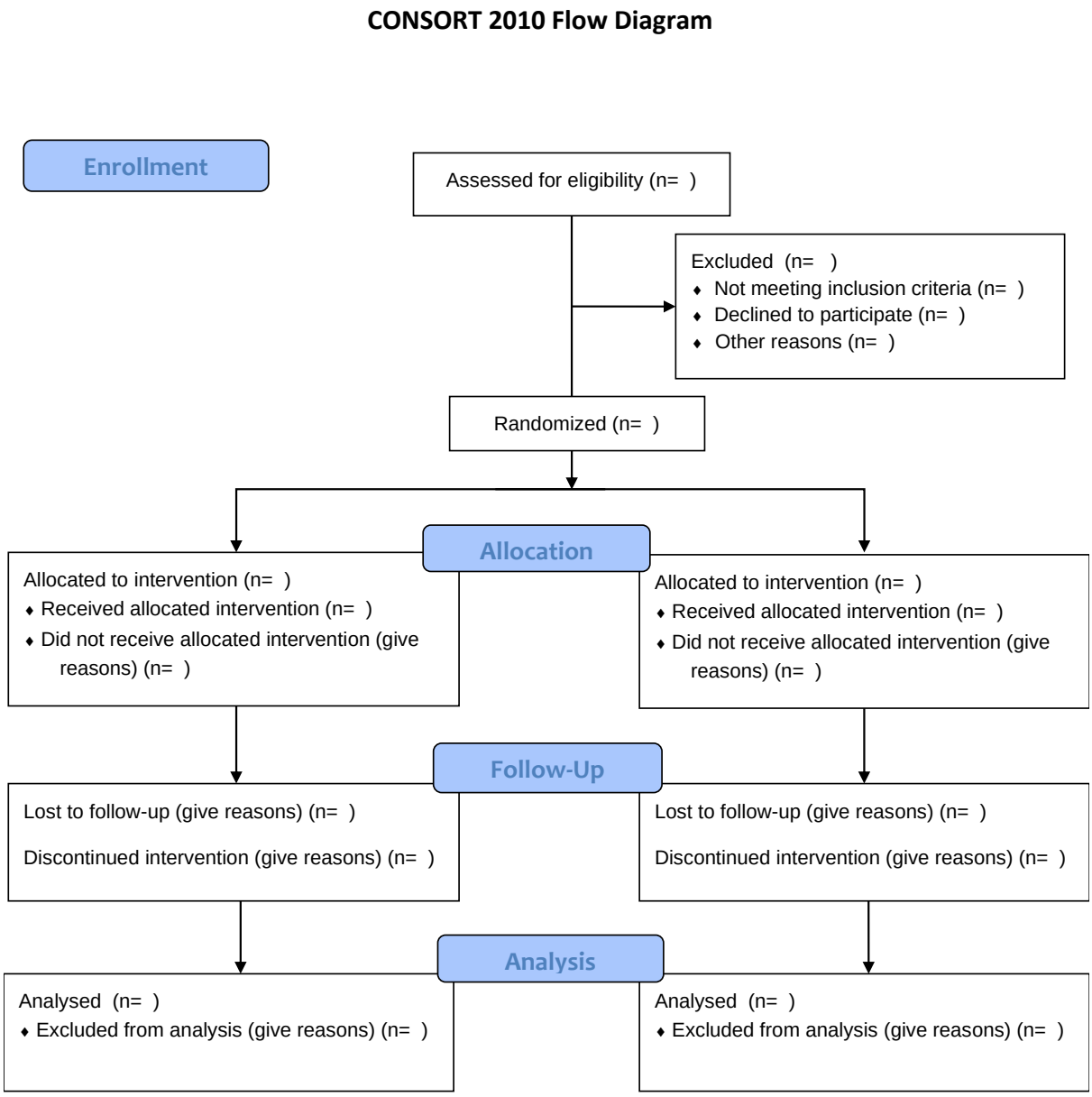
Transfusion related adverse events: Adverse effects commonly associated with transfusion of plasma include transfusion related acute lung injury (TRALI); transfusion associated circulatory overload (TACO); allergic/anaphylactic reactions; transfusion related transmission of infections (TTI); febrile non-hemolytic transfusion reactions (FNHTR); hemolytic transfusion reactions (HTR); and rarely RBC allo-immunization

Trial deviate: Enrolled patient will be considered a trial deviate if

1. If the two doses of convalescent plasma could not be completed.
2. If the 28 days follow-up could not be done.
3. Withdrawal of consent after randomization

CONSORT Flow Diagram

Each study site to provide the details in the following:



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