Section 1: Administrative information:

1.1 Purpose of SAP

The purpose of SAP is to provide the details of procedural methods used including inclusion, exclusion, the kind of patient enrolment, the flow of study, stopping rule and analysis used as part of the COPLA-II trial for publication.

1.2 Trial and Trial registration

Efficacy of Convalescent Plasma Therapy in Patients With COVID-19

Trial registered with ClinicalTrial.gov (identifier: NCT04425915).

1.3 SAP Version: First version written on 10th June 2020. With sample 400 (200 in each group)

1.4 Protocol Version: First version written on 10th June 2020.

1.5 SAP revisions: No

1.6 Roles and responsibility:

1.6.1.1 Dr. Shiv Kumar Sarin: Chief Coordinator/Clinical Lead

1.6.1.2 Dr. Meenu Bajpai: Chief investigator/principal investigator

1.6.1.3 Dr. Guresh Kumar: Senior Statistician

1.6.1.4 Dr. Ankit Bhardwaj: Clinical Trial Coordinator.

1.6.1.5 Dr. Ashish Maheshwari: Wrote the SAP

SECTION 2

INTRODUCTION:

2.1 Background and rationale:

The outbreak of SARS-CoV2 infection, which had originated in Wuhan, China, has become a pandemic involving more than 10 million people across the globe with almost half million deaths and still counting (1). The case-fatality rate of COVID-19 has ranged from 1.2-13% (1,

2). The current evidence-based strategy relies on providing supportive care in mild to moderate cases and providing mechanical ventilation and extracorporeal membrane oxygenation in severe cases. There is no targeted drug therapy available at present. Some studies have indicated benefits with, intravenous Remdesivir and Dexamethasone in reducing the duration and severity of illness, but not mortality (3-5). Apart from antiviral drugs, virus-specific neutralizing antibodies, which could accelerate virus clearance and prevent entry into target cells, could serve as a mechanism for the restriction and clearance of the viruses by the host. The plasma of convalescent patients who have recovered from SARS-CoV2 infection may contain such neutralizing antibodies which may accelerate virus clearance in an infected recipient and be used in the treatment of patients with COVID-19 (6). The experience of using convalescent plasma is derived from its utility in improving the survival rate of patients with SARS infection wherein the patients who had no response to intravenous corticosteroids showed improvement. Providing passive antibody therapy by convalescent plasma in COVID-19 infection could be one of the approaches towards disease mitigation in the absence of definitive treatment (5, 6). This approach can be effective in patients before they develop a humoral response to COVID-19.

2.2 Objectives:

2.2.1 **Primary objective**:

Efficacy of convalescent plasma in severe COVID 19 patients in time to clinical improvement (Clinical improvement: Reduction of two points in ordinal scale or live discharge from the intensive care unit, whichever is earlier) [Time Frame: Day 28]

The six-point scale is as follows:

a. death=6;

b. hospital admission for extracorporeal membrane oxygenation or mechanical ventilation=5;

c. hospital admission for non-invasive ventilation or high-flow oxygen therapy=4;

d. hospital admission for oxygen therapy (but not requiring high-flow or non-invasive ventilation) =3;

e. hospital admission but not requiring oxygen therapy=2;

f. discharged or having reached discharge criteria (defined as clinical recovery-ie, normalization of pyrexia, respiratory rate 94% on room air, and relief of cough, all maintained for at least 72 h) =1.

2.2.2 Secondary objectives:

1. Proportion of patients in each category according to the ordinal scale [Time Frame: 48 hours]
2. Proportion of patients in each category according to the ordinal scale [Time Frame: 7 day]
3. Proportion of patients in each category according to the ordinal scale [Time Frame: Day 14]
4. Proportion of patients in each category according to the ordinal scale [Time Frame: Day 28]
5. Duration of oxygen therapy in both groups [Time Frame: Day 28]
6. Duration of hospital stay in both groups [Time Frame: Day 28]
7. Proportion of patients on mechanical ventilation at day 7 in both groups [Time Frame: Day 7]
8. Mortality in both groups [Time Frame: Day 7]
9. Mortality in both groups [Time Frame: Day 28]

10. Duration of Intensive Care Unit stay [Time Frame: Day 28]
11. Incidence of adverse effects in both groups [Time Frame: Day 28]
12. Presence of antibodies against SARS-CoV-2 in serum after plasma administration [Time Frame: Day 0] (IgG Titres against S1, RBD antigen, and SARS CoV2 neutralizing antibody titres)
13. Presence of antibodies against SARS-CoV-2 in serum after plasma administration [Time Frame: Day 7] (IgG Titres against S1, RBD antigen, and SARS CoV2 neutralizing antibody titres)
14. Change in Cytokines in both groups
15. Change in acute phase reactants in both groups

Section 3: Study Methods

3.1 Trial design: It will be a open-labeled; phase III randomized controlled trial.

3.2 Randomization: The randomization will be done by using stratified block randomization method into two treatment groups. The allocation concealment will be done by using "Sequentially Numbered Opaque Sealed Envelopes" (SNOSE) method.

3.3 Sample size: The sample size was calculated by assuming median survival in SMT as 24 days, and convalescent plasma with 18 days giving a reduction of 25% of the median time; then with α=5% and β=20%, we need to enrol 190 participants in each treatment arm by survival analysis method. $(Z_{1-\alpha/2}+z_{\beta})^2/((\ln(\theta))^2$ where $Z_{1-\alpha/2}$=1.96 and $z_{\beta=}$ 0.84, $\theta= \lambda 1 = \log 2/24$ $\lambda 0 = \log 2/18$. Hence, we enrolled 400 cases which were equally and randomly allocated into two arms

3.3 **Framework:** it will be a comparative trial with the motive of testing the efficacy of convalescent plasma in severe COVID 19 patients with standard medical trial.

3.4 Treatment allocation:

Patients will be randomized to one of two groups by block randomization:

- **Group A:** Convalescent Plasma plus standard medical treatment
- **Group B:** Standard medical treatment only.

3.5 Statistical interim analysis and stopping guidance:

3.5.1 Statistical interim analysis:

Since it is of short duration trial no interim analysis is proposed.

3.5.2 Stopping guidance: No stopping rule is proposed until or unless ethical or IRB or designated government authority may ask for stopping the trial.

3.5.3 Details of guidelines for stopping the trial early: Nil

3.5.4 Statistical analysis:

Continuous variables will be expressed as mean (SD) or median (range) and compared by Student's t-test or Mann- Whitney U test as appropriate. The categorical data will analyzed using Chi-Square or Fisher's exact test. To compare pre and post values, a paired t-test or Wilcoxon signed-rank test will be used. To find out the predictor in survival analysis Cox-proportional hazard regression analysis will be applied. The actuarial probability of survival will be calculated by the Kaplan-Meier graph and compared by the log-rank test. The p value < 0.05 will be considered statistically significant.

3.5.5 Timing of final analysis: The final trial analysis will be performed once the trial is completed or the recruitment of patients ends or at the end of follow up (probably Jan 2021)

3.5.6 Timing of outcome assessments: Jan 2021.

Section 4: Statistical Principals:

4.1 Confidence intervals and P-values: The p value < 0.05 will be considered statistically significant.

Description and rationale for any adjustment for multiplicity:

No adjustments to the type I error will be made as their will be two groups.

Confidence intervals to be reported: Will be reported at 95%.

4.2 Adherence and Protocol deviations: No protocol deviation is expected and will adhere to the defined protocol of trial.

4.3 Analysis populations:

4.3.1 Definition of analysis populations:

Intention-to-treat (ITT) analysis of the sample/population of interest will be carried out in the severe COVID-19 infected individuals. There is no plan to do per-protocol (PP) population analysis as none will be allowed to switch to other treatment group.

4.3.2 ITT population:

This population includes all patients that will be randomized regardless of treatment adherence.

All summaries and analysis will be on the lTT population unless otherwise specified.

4.3.3 PP population:

Not applicable.

4.3.4 Analysis software

All statistical tests and graphs will be performed using Microsoft excel, and SPSS for Windows version 22 (SPSS IBM Corp. Ltd. Armonk, NY).

4.4 **SAFETY REPORTING**

All safety reporting will be performed on the ITT population as per CTCAEv5.0 guidelines.

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4.4.1 Serious adverse events

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR): any untoward medical Occurrence or effect that at any dose of plasma transfusion:

- Results in death
- Is life threatening

–refers to an event in which the patient will be at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it is more severe

- Requires hospitalization, or prolongation of existing in patients 'hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect?

Medical judgment should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other end points listed in the definition above, should also be considered serious. Information regarding serious toxicity, including Principal Investigator (Pl) assessment, CTCAEv5.0 grade, will be summarized. Frequencies of all CTCAE events reported as SAEs will be produced and sorted by total frequency (high to low) and categorized using CTCAE system.

Section 5: Trial Population:

5.1 Screening data: All cases with possible COVID-19 will be screened for possible inclusion into the study based on inclusion and exclusion criteria.

5.2 Eligibility: As per inclusion and exclusion criteria mentioned below-

5.2.1 Inclusion criteria:

Patients with severe COVID-19 will be considered for randomization and will be transfused convalescent plasma within 3 days of symptom onset (Severe COVID-19) Severe COVID -19 defined by WHO Interim Guidance and the Guideline of Diagnosis and Treatment of COVID-19 of National Health Commission of China (version 5.0) along with confirmation by real-time RT-PCR assay with severe disease i.e. meeting any 2 of the following criteria-

1. Patients on ventilator (in last 24 hours)
2. Respiratory distress, RR ≥30 beats/min
3. Oxygen saturation level less than 90 % in resting state
4. Partial pressure of oxygen (PaO2)/oxygen concentration (FiO2) ≤ 300 mmHg
5. Lung infiltrates > 50% within 24 to 48 hours

5.2.2 Exclusion criteria

1. Patient/ family members who do not give consent to participate in the study.
2. Patients with age less than 18 years
3. Patients presenting with multi-organ failure
4. Pregnancy
5. Individuals with HIV and Viral Hepatitis and Cancer
6. Extremely moribund patients with an expected life expectancy of less than 24 hours
7. Hemodynamic instability requiring vasopressors
8. Previous history of allergy to plasma
9. Cirrhosis
10. Severe renal impairment with GFR< 30ml/min or recipients of RRT, peritoneal dialysis

11. Patients with uncontrolled diabetes mellitus, hypertension, arrhythmias and unstable Angina

5.3 Recruitment: Consort flow will include as per protocol-

Patients will be assessed for eligibility >>>randomization will be done for eligible participants >>> Allocation of treatment in two groups (one arm will be given convalescent plasma with standard medical treatment and another arm will be given standard medical treatment only) >>> follow up till 28 days.

5.4 Withdrawal/ Follow-up: Withdrawal is not expected, follow up till 28 days in all enrolled patient.

5.5 Baseline patient characteristics: following variables will be considered:

- Age at screening (years) = date of screening.
- Time from initial admission to randomization(days) = date of transfusion allotment by snooze method and transfusion of plasma.
- O_2 saturation at baseline.
- PiO_2/FiO_2 ratio at baseline.
- Respiratory rate at baseline.
- Need of Mechanical ventilation at baseline.
- SOFA score at baseline.
- For those who died: Date of death – transfusion start date or date of randomization.
- For those who are alive: Date of last follow-up – transfusion start date or date of randomization.

Section 6:

Analysis

6.1 Outcome definitions:

6.1.1 Definition of primary endpoint: Time to clinical improvement (Clinical improvement: Reduction of two points in ordinal scale or live discharge from the intensive care unit, whichever is earlier)

6.1.2 Secondary endpoints:

1. The proportion of patients in each category according to the ordinal scale at 48 hours and day 7, 14, and 28 after randomization
2. Duration of oxygen therapy
3. Duration of hospital stay
4. The proportion of patients on mechanical ventilation at day 7. (after randomization)
5. Mortality at day 7 and day 28 (after randomization)
6. Duration of Intensive Care Unit stay
7. Incidence of adverse effects in both groups
8. Presence of antibodies against SARS-CoV-2 in serum after plasma administration.
9. Cytokines and acute phase reactants

6.2 Analysis methods:

Continuous variables will be expressed as mean (SD) or median (range) and compared by Student's t-test or Mann- Whitney U test as appropriate. The categorical data will analyzed using Chi-Square or Fisher's exact test. To compare pre and post values, a paired t-test or Wilcoxon signed-rank test will be used. To find out the predictor in survival analysis Cox-proportional hazard regression analysis will be applied. The actuarial probability of survival will be calculated by the Kaplan-Meier graph and compared by the log-rank test. The p value < 0.05 will be considered statistically significant.

6.3 Missing data: Not expected, if data are, we will try to impute.

6.4 Additional analyses: Not planned, if required an appropriate analysis will be carried out.

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6.5 Harms: The risks will be the same as the risks related to the transfusion of Plasma Components, which are described below in the table. No additional risks of transfusing Convalescent plasma (CP) therapy are expected as a part of enrolment in this study as per previous literature available. The patient will be managed according to the standard of care at treatment centre. All the adverse transfusion events will be recorded and managed by clinicians or investigators as per standard treatment. Risk of Transfusion transmitted Infections (TTI) like transmission of HIV, Hepatitis B and Hepatitis C, Syphilis, and Malaria are very rare due to standard TTI testing of plasma donors at ILBS, New Delhi as per Drug and Cosmetics Act and Rules.

6.6 Statistical software: All statistical tests will be performed using SPSS for Windows version 22 (SPSS IBM Corp. Ltd. Armonk, NY).

6.7 Important references:

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223):497-506.
2. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395: 507-13.
3. Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020; 382(19):1787-1799. doi:10.1056/NEJMoa2001282.
4. Grein J, Ohmagari N, Shin D, et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. N Engl J Med. 2020;382(24):2327-2336. doi:10.1056/NEJMoa2007016.
5. Shen C, Wang Z, Zhao F, et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma [published online ahead of print, 2020 Mar 27]. JAMA.

2020;323 (16):1582-1589. doi:10.1001/jama.2020.4783.

6. Chen L, Xiong J, Bao L, Shi Y. Convalescent plasma as a potential therapy for COVID-19. Lancet Infect Dis. 2020;S1473-3099(20)30141-9.