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Efficacy of convalescent plasma therapy in the patient with COVID-19: a randomised control trial (COPLA-II trial)

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ABSTRACT

Importance No proven treatment is available for severely ill COVID-19. Therapeutic use of COVID-19 convalescent plasma (COPLA) is under investigation.

Objective To compare the efficacy of COPLA with standard medical therapy (SMT) alone in severe COVID-19 patients.

Design, setting and participants A multicentric, open-labelled, phase-III randomised controlled trial conducted at two treatment centres with COPLA collected at the third dedicated centre in North-India, the coordinating centre during trial from June 2020 to December 2020. The study population comprised 400 participants in the ratio of 1:1 in each treatment group.

Intervention One group received COPLA with SMT (n=200), and another group received SMT only (n=200).

Main outcome measures Primary outcome was time to clinical improvement measured by a two-point reduction in the ordinal scale. Secondary outcomes included duration of O_2 therapy, the proportion of patients on mechanical ventilation at day-7, mortality, SARS-CoV-2 antibody levels, cytokine levels and incidence of adverse events.

Results The median time to a two-point reduction in the ordinal scale in both groups was 9 days (IQR=7–13) (p=0.328). The median duration of O_2 therapy was 8 days (IQR=6–12) in COPLA and 10 days (IQR=6–12) in SMT group (p=0.64). The PaO_2/FiO_2 ratio showed significant improvement at 7 days in COPLA group(p=0.036). There was no difference in mortality till 28 days in both groups (p=0.62). However, if COPLA was given within 3 days of hospital admission, a significant reduction in ordinal scale was observed (p=0.04). Neutralising antibody titres in COPLA group (80 (IQR 80–80)) were higher than SMT group (0 (IQR 0–80)) at 48 hours (p=0.001). COPLA therapy led to a significant reduction in TNF-α levels at 48 hours (p=0.048) and D-dimer at 7 days (p=0.02). Mild allergic reactions were observed in 3 (1.5%) patients in COPLA group.

Conclusion and relevance Convalescent plasma with adequate antibody titres should be transfused in COVID-19 patients along with SMT in the initial 3 days of hospitalisation for better clinical outcomes.

Strengths and limitations of this study

- Study highlights the role of testing of antibody titres in convalescent plasma.
- Patient baseline titre levels are unrelated with outcomes.
- Study emphasises that the timing of transfusion of high titre convalescent plasma is critical.
- Delayed transfusion of convalescent plasma is rather harmful and should be discouraged.
- Duration from admission to transfusion could not be regulated as most patients did not fulfil inclusion criteria on the day of admission.

Trial registration number NCT04425915.

INTRODUCTION

Since the emergence of SARS-CoV-2 infection in late 2019, no proven treatment options are available forCOVID-19. Researchers are working relentlessly to develop therapies to combat this life-threatening problem. COVID-19 convalescent plasma (COPLA) use in COVID-19 has been approved for off-label emergency use and under phase III trials by many national and international bodies. Convalescent plasma from recovered COVID-19 patients contains neutralising antibodies against the spike protein of SARS CoV-2, which may benefit severely sick COVID-19 patients by neutralising the virus and halting its replication in the host. Convalescent plasma was found effective in the treatment against the Middle East respiratory syndrome, influenza A (H1N1), Avian influenza (H5N1) and Ebola in the past.[1–3] One recent observational study conducted

by the Mayo Clinic on 20 000 patients transfused convalescent plasma has shown a good safety profile.[4] A study done by Cheng *et al* on 80 patients of SARS virus who received convalescent plasma had a lower mortality rate than the overall mortality rate. They reported encouraging outcomes in patients treated with convalescent plasma in the 2003 SARS pandemic. They further found that out of 80 patients, 33 patients transfused convalescent plasma within the 2 weeks of symptom onset showed better outcomes than those transfused later.[1] During the current pandemic (SARS CoV-2), many studies have proven that convalescent plasma can limit viral replication by providing passive neutralising antibodies to SARS CoV-2in the initial viraemia phase and thus mitigate the disease in the absence of definitive therapy.[5] In recovered patients, who are prospective COPLA donors, there are variations in antibody titres and specificities against components of the virus.[6] In this trial, we assessed the safety and efficacy of convalescent plasma transfusion in severe COVID-19 patients using an ordinal scale until 28 days for clinical outcomes.

METHODOLOGY

Study settings and trial design

The COPLA-II trial was a multicentric, open-labelled randomised controlled trial conducted at two treatment centres with convalescent plasma being collected at the third dedicated centre in India, which was also the coordinating centre during the trial. The study population comprised 400 patients with severe COVID-19, and stratified block randomisation (each block of 10) was done in the ratio of 1:1 with 200 patients in each of the treatment groups (ie, CP with standard medical therapy (SMT) as the intervention arm vs SMT only as control arm). Allocation concealment was done using the 'Sequentially Numbered Opaque Sealed Envelopes' method. Written informed consent from all the study participants was taken before their enrolment in the study. The trial protocol is available in online supplemental material 1 as study protocol and online supplemental material 2 as statistical analysis plan. The conduct of the trial was as per the Declaration of Helsinki principles.

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%, A sample size of 190 participants was needed to be enrolled 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, θ= λ1=log2/24 λ0=log2/18. Hence, we enrolled 400 cases equally and randomly allocated into two arms, as shown in figure 1. The study was started on 14 June 2020, and the last follow-up was conducted on 15 December 2020. All the authors are fully responsible for the trial design and conduct and assure the data's authenticity. The overall study was monitored regularly by an independent data and safety monitoring board.

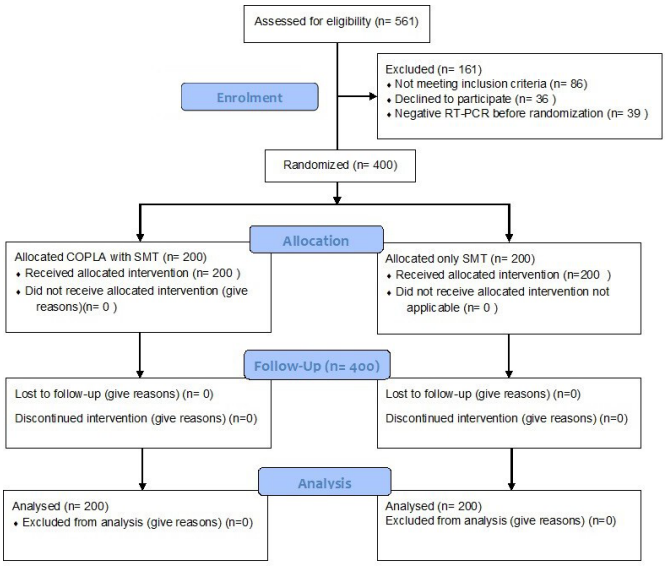

Figure 1 Consort flow diagram. COPLA, COVID-19 convalescent plasma; SMT, standard medical therapy.

Inclusion and exclusion criteria

Study participants with severe COVID-19 of age 18 or above were considered for randomisation in the study after obtaining informed consent at both the COVID-19 treatment centres. The WHO Interim Guidance defined severe COVID-19 as following any of the two criteria out of five including ventilated patient within 24 hours, respiratory rate ≥30 beats/min, oxygen saturation in the resting state level less than 90% in resting state, Partial pressure of oxygen/ Fractional inspired oxygen ratio $(PaO_2)/(FiO_2)$ ≤300 mm Hg, and lung infiltrates ≥50% within 24–48 hours. Patients with a history of allergy to plasma, pregnancy, multiorgan failure, HIV, viral hepatitis, cirrhosis, renal impairment on dialysis and Renal replacement therapy (RRT), cancer, uncontrolled hypertension and diabetes, arrhythmias, unstable angina and haemodynamically unstable patients requiring vasopressors and expected life expectancy less than 24 hours were excluded.

Intervention

Patients in the intervention arm were given two doses of 250 mL of convalescent plasma on consecutive days along with the standard of care, while in the control arm, only standard of care was given. The standard of care was based on the detailed guidelines for COVID-19 management laid down by the Ministry of Health and Family Welfare, India.[7] All institutional protocols for supportive management were implemented but not restricted. It included other investigational drugs like remdesivir and dexamethasone approved by the government authorities for COVID-19 patients irrespective of the treatment group. Guidelines issued by the ARDSNet for ARDS and the Surviving Sepsis campaign for sepsis were followed.[8] All the patients were monitored daily until clinical improvement or up to 28 days as per the ordinal scale. At the plasma collection centre, up to 500 mL of convalescent

plasma was collected from recovered COVID-19 patients as per the Drugs and Cosmetics Act 1940 and Rules 1945, amended on 11 March 2020.[9] Collected plasma was labelled appropriately and frozen below −30°C for 1 year.

Laboratory evaluation

We tested the presence of IgG antibodies and neutralising antibodies to SARS CoV-2 in the serum of study subjects. The spike protein S1 receptor-binding domain (RBD) IgG antibody titres were done by ELISA method (SARS-CoV-2 Spike S1-RBD IgG Detection Kit, Genscript, USA), directed against the SARS-CoV-2 RBD proteins. The titre was determined by the ELISA method with sample dilutions 1:80, 1:160 and 1:640 as per the manufacture's instruction. All the samples were tested in duplicate. ELISA titres were determined by the endpoint dilution. The S1 RBD IgG antibody titres were determined in recipient samples before transfusion at baselines 24, 48, 72 hours than day 7, day 10 and day 14. A fourfold rise in titres was considered useful for protection.

The determination of serum neutralisation antibodies in donors was done by the SARS-CoV-2 surrogate virus neutralisation test Kit (Genscript, USA). The minimum acceptable neutralising antibody titre of transfused convalescent plasma was 80. The test is used to detect circulating neutralising antibodies against the SARS-CoV-2 virus that can block the interaction between the RBD of the viral spike glycoprotein with the ACE2 cell surface receptor. The neutralisation antibody titres at 1:80 dilutions were tested as per the manufacturer's instruction at similar time points as S1 RBD IgG antibody titres.

Cycle threshold value

We amplified two SARS CoV-2 E-genes (for sensitivity) and RdRP genes (for specificity) by 45 cycles run by RT-PCR (real-time, reverse transcriptase-PCR, Q-Line-ER nCoV-19 RT-PCR Detecon kit, POCT services, India) method. Any amplification ≤40 cycle threshold (Ct) value for both the genes was considered as positive, and the cycle number (Ct value) was noted. Any amplification >40 Ct was considered as negative .

Other lab parameters

Patient's samples were tested for complete blood count (CBC), D-dimer, International normalized ratio (INR), Activated partial thromboplastin time (APTT), Liver function test (LFT), kidney function test (KFT), Lactate dehydrogenase (LDH), C reactive protein (CRP), serum ferritin and cytokines (IL-1, IL-6, TNF-α) (equipment/kit details are in online supplemental material 1).

Clinical outcomes

The primary outcome measure was time to clinical improvement, defined as a reduction in ordinal scale by two points or live discharge, whichever was earlier up to 28 days. Secondary outcome measures included the proportion of patients in each treatment group based on the ordinal scale at 48 hours, 7 days, 14 days and 28 days, duration of O_2 therapy, Intensive Care Unit (ICU) stay, hospital stay, the proportion of patients on mechanical ventilation at day 7, mortality in both groups at 7 days, 28 days. Incidence of adverse effect in both the groups during 28 days, presence of antibodies to SARS-CoV-2 in serum after plasma administration on the baseline, after 48 hours, day 7 and day 14 and their correlation with disease parameters, changes in cytokine levels and acute phase reactant till 28 days.

Statistical analysis

Data collected and entered into Microsoft excel sheet 2010. The Statistical analysis was conducted using SPSS software V.22 (IBM). Descriptive analysis was mean±SD or in median (IQR) as appropriate for a continuous variable. The categorical data is shown as n (%), and continuous data were analysed either by Student's t-test or Mann-Whitney test depending on the normality assumption. The categorical data is analysed by χ^2 or Fisher's exact test. Besides this Kaplan-Meier method, along with Cox regression analysis, was also used for survival analysis. Repeated measure analysis was carried out to see change over the period, followed by post hoc comparison by the least square deviation method. The significance was seen at a 5% level of significance.

Patient and public involvement

Patients and the public were not involved in the design, conduct, reporting or dissemination plans of this trial.

RESULTS

Patients

A total of 561 patients were assessed for eligibility, out of which 86 did not meet the inclusion criteria, 36 declined to participate and 39 were RT-PCR negative; finally, a total of 400 patients were randomised equally into the COPLA group (COPLA with SMT) and SMT group (SMT only). All the patients included in the COPLA group received at least one dose of 250 mL of convalescent plasma.

Baseline patient profile: both the patient groups were comparable for mean age, gender and body mass index

The patients in both the COPLA and SMT groups had a comparable ordinal scale, O_2 saturation, respiratory rate, PaO_2/FiO_2 ratios, and other laboratory parameters, as shown in table 1.In the COPLA group, 500 mL of convalescent plasma was transfused in two divided doses of 250 mL each 24 hours apart in addition to the standard medical treatment in both the groups. The median S1 RBD IgG antibody titre of all patients at baseline was 0 (0, 40). In COPLA group, it was 0 (0, 40) while in SMT group 0 (0, 80), with no significant difference between COPLA and SMT group at inclusion in the study (p=0.275). The median neutralising antibody titre of all patients at baseline was 0 (0, 40). In COPLA group it was 0 (0, 40) while in SMT group 0 (0, 80) with no significant difference between both treatment groups at inclusion in the study (p=0.322). We found that overall, 136 (34%) patients

Table 1 Baseline characteristics of study participants

Baseline parameters	Overall (n=400)	COPLA (n=200)	SMT (n=200)	P value
Age (in years)	55.52±1.17	54.73±9.48	56.31±12.6	0.158
Male (n, %)	269 (67.3%)	143 (71.5%)	126 (63%)	0.088
Chest X-ray change (n, %)	371 (92.8%)	186 (93%)	185 (92.5)	0.366
BMI (kg/m^2)	23.34±3.34	23.6±3.4	23.09±3.27	0.128
Ordinal scale	3.22±0.42	3.24±0.44	3.20±0.41	0.396
Respiratory rate (per min)	28.71±5.5	28.8±6.96	28.61±3.56	0.722
O2 Saturation (in %)	85.9±3.35	85.78±3.37	86.03±3.33	0.547
PaO2/FiO2 ratio	145.65±58.63	146.44±55.13	144.82±62.25	0.183
N/L ratio	13.48±13.39 9.64 (5.19–17.15)*	12.69±12.0 9.4 (4.53–17.06)*	14.25±14.61 10.5 (5.42–17.76)*	0.253
Ct value	25.4±5.2	25.27±5.35	25.52±5.05	0.631
SOFA score	2.61±1.06	2.68±1.12	2.54±0.99	0.228
Serum ferritin (ng/mL)	618.05±510.59 486 (235.5–833.5)*	661.34±539.56 502 (305–878)*	574.53±477.20 452.5 (206.25–815.75)*	0.101
D-dimer (mg/L)	1.08±1.59 0.27 (0.15–1.2)*	1.09±1.6 0.25 (0.014–0.89)*	1.09±1.5 0.38 (0.18–1.28)*	0.996
CRP (mg/L)	95.79±77.34 80.9 (32.8–139)*	94.26±74.13 81.9 (34.25–130.5)*	97.33±80.61 79.65 (31.57–142.75)*	0.698
IL-1 (pg/mL)	18.08±20.05 9.92 (5.0–24.3)*	17.89±17.79 9.7 (5.0–24.77)*	18.26±22.15 10.1 (5.0–23.6)*	0.866
IL-6 (pg/mL)	48.36±89.95 21.9 (9.56–59.5)*	51.97±94.32 22.25 (10.8–64.4)*	44.75±85.44 20.4 (8.18–58.4)*	0.443
TNF-α (pg/mL)	15.47±14.16 13.2 (9.56–17.27)*	15.41±16.86 12.75 (9.57–16.52)*	15.54±10.84 13.55 (9.47–18.52)*	0.932
Neutralising antibodies (mean and proportion %)	21.6±32.99 130 (32.5%)	19.6±31.3 62 (31%)	23.6±34.5 68 (34%)	0.522
S1 RBD IgG antibodies (mean and proportion %)	31.6±81.49 136 (34%)	24.0±54.84 65 (32.5%)	39.2±102.7 71 (35.5%)	0.526

*Median (IQR) value.
†Data are in mean±SD.
BMI, body mass index; COPLA, COVID-19 convalescent plasma; CRP, C reactive protein; Ct, cycle threshold; FiO2, Fractional inspired oxygen; IL-1, Interleukin-1; IL-6, Interleukin-6; N/L ratio, Neutrophil lymphocyte ratio; O2 Saturation, Oxygen Saturation; PaO2, Partial pressure of oxygen; RBD, receptor-binding domain; SOFA, Sequential Organ Failure Assessment; TNF-α, Tumour necrosis factor α.

had S1 RBD IgG antibodies at baseline due to the natural course of infection, among which 65 (32.5%) were in COPLA group while 71 (35.5%) were in SMT group and were comparable statistically (p=0.526). Similarly, overall, 130 (32.5%) had neutralising antibodies at baseline, among which 62 (31%) were in COPLA group while 68 (34%) were in SMT group and were comparable statistically (p=0.522).

Primary outcome

The median time for a two-point reduction in ordinal scale in both groups was 9 days with an IQR between0.328), as shown in table 2. On subgroup analysis after adjusting for days to randomisation and transfusion of convalescent plasma before (n=115, ≤3 days) or after (n=85, ≥4) 3 days from admission, significant improvement in ordinal scale was observed in the COPLA group patients who were transfused within 3 days of admission (n=115 vs n=85; p=0.04), as shown in figure 2. Further, patients transfused convalescent plasma after 3 days of admission showed less improvement in the ordinal scale than SMT therapy (p=0.08).

Secondary outcome

The proportion of patients in each treatment group based on the ordinal scale at 48 hours, 7 days, 14 days and 28 days were comparable with no statistically significant difference as shown in table 3. Maximum patient

Table 2 Changes in clinical parameters during treatment

Variable	COPLA (n=200)	SMT (n=200)	P value
Time for 2-point reduction in ordinal scale (days)	9.174±5.36 8 (7, 11)*	9.56±6.03 9 (6, 12)*	0.552
Duration of oxygen therapy (days)	10.28±6.85 8 (6, 12)*	10.16±5.79 10 (6, 12)*	0.644
Patients on mechanical ventilation till 7 days (%)	2 (40)	3 (60)	0.68
Respiratory rate at 48 hours (per min)	25.43±7.1 24 (22–28)*	24.96±3.56 24 (22–28)*	0.409
Respiratory rate at 7 days	23.72±0.7.9 22 (20–24)*	23.24±2.67 24 (22–24)*	0.472
O2 saturation at 48 hours (%)	94±4 95 (93–96)*	93.96±4.37 95 (93–96)*	0.923
O2 saturation at 7 days (%)	95.25±2.75 96 (94–97)*	95.39±2.48 96 (94–97)*	0.636
SOFA score 48 hours	2.64±1.3 2 (2–3)*	2.42±1.05 2 (2–2)*	0.095
SOFA score 7 days	1.81±1.17 2 (1–2)*	2.12±1.45 2 (2–2)*	0.077
PaO2/FiO2 at 48 hours	158.89±62.53 161.55 (114.83–202)*	159.20±74.29 155.55 (100–198)*	0.97
PaO2/FiO2 at 7 days	268.83±142.12 246.29 (144.58–388.57)*	226.98±152.27 173.8 (116.4–308.57)*	0.036
Duration of ICU stay (days)	11.1±7.77 9 (6–14)*	10.91±6.96 9 (6–15)*	0.823
Duration of hospital stay (days)	13.8±7.03 12 (9–16)*	13.82±7.19 13 (9–18)*	0.983
Mortality till 7 days (n) %	25 (54.3%)	21 (45.7%)	0.64
Mortality till 28 days (n) %	42 (53.2%)	37 (46.8%)	0.62

Data are in mean±SD.
*Median (IQR) value.
COPLA, COVID-19 convalescent plasma; FiO2, Fractional inspired oxygen; ICU, Intensive Care Unit; O2 Saturation, Oxygen Saturation; PaO2, Partial pressure of oxygen; SMT, standard medical therapy; SOFA, Sequential Organ Failure Assessment.

mortality was observed within 7 days of randomisation in both the treatment groups, and the majority were discharged within 14 days of randomisation.

The median duration of O_2 therapy was 8 days (IQR=6–12) in COPLA group as compared with 10 days (IQR=6–12) in SMT group, but the difference between both the groups was not significant (p=0.644). Improvement was observed in oxygen saturation and respiration (reduction in the respiratory rate) at 48 hours and 7 days with no significant differences in both the treatment groups, and on further subgroup analysis it was not significantly different with transfusion timings. (online supplemental material 3 as oxygen saturation with time of transfusion). Improvement in PaO_2/FiO_2 ratio was observed at 48 hours and 7 days in both the treatment groups, and it showed a clinically significant improvement at 7 days in the COPLA group as compared with SMT group (p=0.036). Total 51 patients needed mechanical ventilation till 7 days, out of which 27 (52.9%) were in COPLA group while 24 (47.1%) were in the SMT group, and on day 7, 2 patients were on active mechanical ventilation in COPLA group while three patients were in SMT group and rest in both the groups were succumbed. No significant difference in requirement of mechanical ventilation was observed between both the groups till day 7 (p=0.61). The median

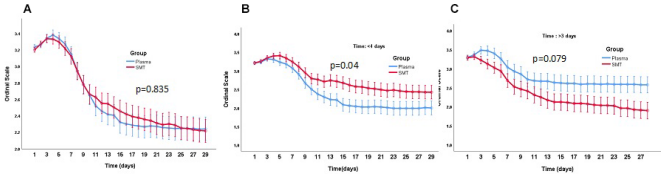

Figure 2 Comparison of ordinal scale in both treatment groups. SMT, standard medical therapy.

Table 3 Proportion of the patients with ordinal scale at different timelines

Ordinal Scale	Ordinal scale at baseline		Ordinal scale at 48 hours		Ordinal scale at 7 days		Ordinal scale at 14 days		Ordinal scale at 28 days	
	Plasma	SMT	Plasma	SMT	Plasma	SMT	Plasma	SMT	Plasma	SMT
1	0	0	0	0	29 (14.5%)	33 (16.5%)	116 (58%)	101 (50.5%)	136 (68%)	132 (66%)
2	0	1 (0.5%)	2 (1%)	4 (2%)	60 (30%)	51 (25.5%)	24 (12%)	27 (13.5%)	11 (5.5%)	13 (6.5%)
3	154 (77%)	158 (79%)	140 (70%)	141 (70.5%)	56 (28%)	56 (28%)	12 (6%)	16 (8%)	6 (3%)	9 (4.5%)
4	45 (22.5%)	41 (20.5%)	51 (25.5%)	47 (23.5%)	28 (14%)	36 (18%)	9 (4.5%)	22 (11%)	5 (2.5%)	9 (5.5%)
5	1 (0.5%)	0	2 (1%)	0	2 (1%)	3 (1.5%)	6 (3%)	4 (2%)	0	0
6	0	0	5 (2.5%)	8 (4%)	25 (12.5%)	21 (10.5%)	33 (16.5%)	30 (15%)	42 (24.3%)	37 (22.8%)

SMT, standard medical therapy.

Figure 3 Progression of antibody titres in both groups in patients with no baseline titres. RBD, receptor-binding domain.

SOFA scores were comparable at 48 hours and day 7, with no significant difference between the groups, as shown in table 2.

Antibody levels

There was a significant increase in S1 RBD IgG antibody titres and neutralising antibody titres in the COPLA group compared with SMT group at 48 hours, 7 days and 14 days post-transfusion, as shown in figure 3 and table 4. On subgroup analysis in COPLA group to see mortality at 7 days and 28 days and change in ordinal scale, we did not find any differences with baseline titres in patients at the time of randomisation and transfusion. On repeated measure analysis, when we considered both initial zero titres and transfusion less than 3 days, we observed a significant reduction in the ordinal scale (p=0.016) related to the timing of transfusion irrespective of baseline titre value as shown in figure 4.

In both the study arms, baseline cytokine levels were comparable. On convalescent plasma transfusion, median post-transfusion IL-1β, IL-6, were reduced at 48 hours and 7 days, and in SMT group, only IL-1β level was reduced at 48 hours and 7 days while IL-6 was increased at 48 hours and then decreased on day 7. The TNF-α level was increased in both the COPLA and SMT groups at 48 hours and 7 days. The cytokine differences did not attain statistical significance in both the groups except the 48 hours TNF-α level, which was higher in the SMT group (p=0.048). Acute-phase reactants, including Serum ferritin, CRP and D-dimer, were assessed at baseline, 48 hours and 7 days. Serum ferritin levels were raised at 48 hours and then decreased below the baseline at 7 days in both groups with no significant difference. The CRP showed a decline at 48 hours and 7 days from the baseline level in both groups, which was not statistically significant. The median D-dimer levels were near the baseline value in the COPLA group while rising in the SMT group after 48 hours and 7 days. The median D-dimer levels were significantly higher in the SMT group at 7 days than in the COPLA group (p=0.02). Levels of cytokine and acute-phase reactants are as shown in table 5. We found a significant increase in D-dimer, IL-6, serum ferritin and CRP levels in the non-survivors compared with the survivors at day 28, as shown in table 6.

Table 4 Changes in viral load and SARS CoV-2 antibody titre

Parameter	COPLA (n=200)	SMT (n=200)	P value
Ct value at baseline	25.27±5.35 25.73 (21.48, 29.62)*	25.52±5.05 25.51 (21.62, 29.43)*	0.631
Ct value at 48 hours	30.36±7.96 30.66 (24.84, 40)*	31.04.±6.65 31.04 (27.25, 35.43)	0.428
Ct value at 7 days	34.31±6.61 40 (29.58, 40)*	34.70±6.20 40 (30.14, 40)*	0.654
Ct value at 14 days	38.53±5.51 40 (40, 40)*	38.9±4.97 40 (40, 40)*	0.51
Ct value at 28 days	40 (40, 40)*	40 (40, 40)*	–
S1 RBD IgG antibodies at 48 hours (median)	80 (80,80)*	40 (0, 80)*	0.001
S1 RBD IgG antibodies (%) at 48 hours (proportion)	198/199 (99.4%)	100/193 (51.8%)	0.001
Neutralising antibodies at 48 hours (median)	80 (80, 80)*	0 (0, 80)*	0.001
Neutralising antibodies (%) at 48 hours (proportion)	196/199 (98.4%)	93/194 (47.9%)	0.001
S1 RBD IgG antibodies (median) at 7 days	640 (80, 640)	80 (80, 640)	0.001
S1 RBD IgG antibodies (%) at 7 days (proportion)	186/186 (100%)	167/174 (95.9%)	0.006
Neutralising antibodies at 7 days (median)	640 (80, 640)	80 (80, 80)	0.001
Neutralising antibodies (%) at 7 days (proportion)	186/186 (100%)	167/176 (94.8%)	0.002
S1 RBD IgG antibodies at 14 days (median)	640 (640, 640)	640 (80, 640)	0.004
S1 RBD IgG antibodies (%) at 14 days (proportion)	82/82 (100%)	82/84 (97.6%)	0.497
Neutralising antibodies at 14 days (median)	640 (80, 640)	80 (80, 640)	0.001
Neutralising antibodies (%) at 14 days (proportion)	78/78 (100%)	83/85 (97.6%)	0.173

*Median (IQR) value.
COPLA, COVID-19 convalescent plasma; Ct, cycle threshold; RBD, receptor-binding domain; SMT, standard medical therapy.

Mortality

Total mortality was 19.75% (79/400) at 28 days.25 (54.3%) died till 7 days, and 42 (53.2%) till 28 days in the COPLA group, while 21 (45.7%) died till 7 days, and 37 (46.8%) till 28 days in the SMT group. No statistically significant difference in mortality was observed between the groups at 7 days (p=0.64) or 28 days (p=0.62). Mortality prediction by Kaplan-Meier survival analysis revealed no significant difference in mortality at day 28 (p=0.537) in both the treatment groups. In COPLA group, subgroup analysis revealed no significant difference in mortality at day 7 (p=0.32) or day 28 (p=0.2) when convalescent plasma was transfused within 3 days of hospital admission, while significantly high mortality was observed at day 7 (p=0.03) and day 28 (p=0.01) when the convalescent plasma was transfused beyond 3 days of admission (figure 5).

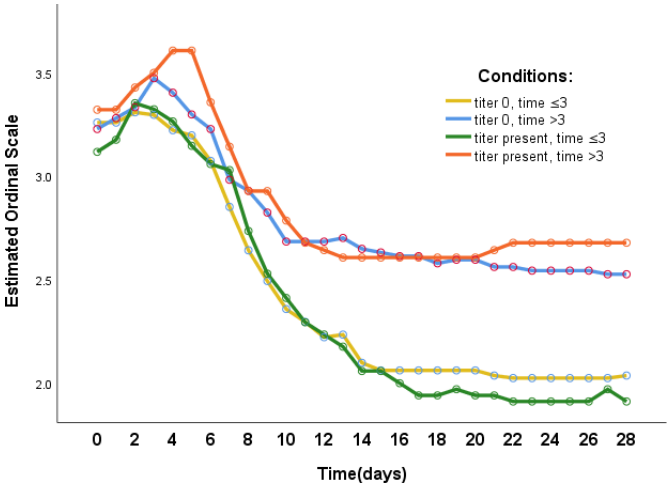

Figure 4 Impact of titres and transfusion timings on ordinal scale.

The median duration of ICU stay was 9 days (IQR=6–14) and hospital stay was 12 days (IQR=9–16) in the COPLA group while the median duration of ICU stay was 9 days (IQR=6–15), and hospital stay was 13 days (IQR=9–18) in the SMT group. However, the difference was not significant (p=0.983). During plasma transfusion, mild allergic reactions (urticarial) were observed in three patients, managed by an antihistaminic with symptomatic relief. No other transfusion reactions, including transfusion-related acute lung injury, were observed in the COPLA group.

DISCUSSION

In this randomised controlled trial, we identified the antiviral effects of convalescent plasma transfusions as add-on therapy to the standard medical treatment in the initial

Table 5 Post-transfusion cytokine and acute phase reactants level

Parameters	COPLA (n=200)	SMT (n=200)	P value
IL-1 at 48 hours (pg/mL)	12.5 (5.01–25.2)*	12.2 (5.22–29.4)*	0.209
IL-1 at 7 days (pg/mL)	13.1 (5.34–29.3)*	13 (5.36–29.8)*	0.598
IL-6 at 48 hours (pg/mL)	21.2 (6.88–68.7)*	27 (10.8–85.9)*	0.209
IL-6 at 7 days (pg/mL)	8.8 (3.18–22.95)*	10.8 (3.67–35.5)*	0.915
TNF-α at 48 hours (pg/mL)	44.77±90.05 15.3 (11.2–29.87)*	77.31±162.07 17.2 (11.7–39.9)*	0.048
TNF-α at 7 days (pg/mL)	50.15±115.42 14.55 (10.21–29.4)*	40.33±83.32 12.95 (9.53–27.8)*	0.449
Serum ferritin at 48 hours (ng/mL)	739±696.98 562 (311.5–922.5)*	640.70±590.86 461 (220–870.5)*	0.227
Serum ferritin at 7 days (ng/mL)	515.19±468.89 383 (167–681)	543.83±502.59 438.5 (200.25–731)*	0.645
D-dimer at 48 hours (mg/L)	1.168±1.56 0.29 (0.13–1.69)*	1.48±1.89 0.58 (0.24–1.8)*	0.3
D-dimer at 7 days (mg/L)	0.78±1.05 0.24 (1.6–1.03)*	1.24±1.5 0.6 (0.22–2.0)*	0.02
CRP at 48 hours (mg/mL)	72.38±67.29 48.6 (22.92–104.25)*	74.26±62.37 56.9 (24.75–109.75)*	0.815
CRP at 7 days (mg/mL)	24.53±38.04 7.74 (2.72–26.6)*	36.78±60.28 9.2 (2.9–36.6)*	0.071

Data are in mean±SD.
*Median (IQR) value.
CRP, C reactive protein; IL-1, Interleukin-1; IL-6, Interleukin-6; TNF-α, Tumour necrosis factor α.

days of SARS COV-2 infection. COVID-19 has affected more than 170 million people, and the tally of death has reached 3.5 million patients and counting in different waves worldwide.[10]

Different treatment modalities

Recently, the initiation of vaccination against COVID-19 in different parts of the world has been encouraging, although these vaccines are of varying efficacy and there is limited access due to high demand. Because of limited access to vaccine and mutant variants of SARS CoV-2, most of the world population is still vulnerable to infection. Even after more than 1 year, no specific treatment has evolved to curtail SARS CoV-2 infection. Dexamethasone has shown some effectiveness in the management of severe cases in the RECOVERY trial by reducing the mortality from COVID-19.[11] Summary recommendation

Table 6 Factors affecting mortality in COVID-19 patients

Parameters	Dead (n=79)	Alive (n=321)	HR D28	P value D28
Age (years)	61.97±10.59	53.93±10.74	1.060 (1.039, 1.082)	0.001
SOFA score	3.56±1.4	2.39±0.82	1.778 (1.548, 2.043)	0.001
Lymphocyte	6.14±4.06	12.69±9.26	0.842 (0.794, 0.893)	0.001
Neutrophil	87.82±6.04	79.65±11.7	1.106 (1.067, 1.146)	0.001
N/L Ratio	21.42±14.11	11.53±12.48	1.023 (1.015, 1.031)	0.001
D-dimer (mg/L)	1.91±1.89	0.81±1.37	1.322 (1.175, 1.488)	0.001
IL-6 (pg/mL)	84.16±140.19	40.38±72.22	1.002 (1.001, 1.004)	0.001
CRP (mg/mL)	130.97±82.26	87.28±73.76	1.005 (1.003, 1.008)	0.001
Serum ferritin (ng/mL)	728.29±515.23	592.14±506.87	1.00 (1.00, 1.001)	0.038

CRP, C reactive protein; IL-6, Interleukin-6; N/L Ratio, Neutrophil lymphocyte Ratio; SOFA, Sequential Organ Failure Assessment.

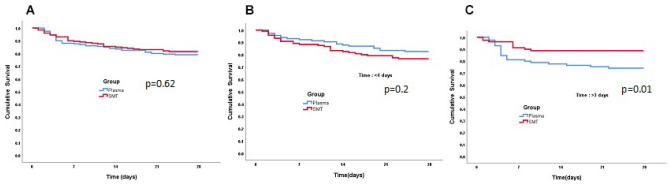

Figure 5 Comparison of mortality in both treatment groups. SMT, standard medical therapy.

of National Institutes of Health has suggested convalescent plasma therapy and SARS-CoV-2immunoglobulin for COVID-19 as under investigational drugs due to insufficient data available on its effects, timing and doses of transfusion on the survival of COVID-19 patients.[12]

Role of convalescent plasma therapy in COVID-19

Convalescent plasma therapy can be given to the SARS-CoV-2 patients in view of expected possible clinical efficiency, immediate availability of a large pool of the donors, relative ease of procuring, storage and transfusion. Convalescent plasma therapy is based on the principle of providing neutralising antibodies against the SARS-CoV-2spike protein to patients with active infection.[6] COPLA transfusion was done in two divided aliquots on consecutive days since patients are already in respiratory distress, and a large single dose of 500 mL might be an aggravating risk factor for the development of transfusion-associated cardiac overload. Further aliquoting of 250 mL was done at the time of collection, so the quality of COPLA was maintained. Due to this approach, we did not encounter any reaction related to cardiac overload or respiratory distress. In our study, both the treatment groups were comparable at baseline in terms of the presence of S1 RBD IgG antibodies and neutralising antibodies. Almost 30% of patients in both groups had antibodies due to the natural course of infection, suggesting the need to determine the baseline antibody titres in recipients before transfusion to use resources judiciously and for favourable outcomes. We found a significant rising trend in antibodies post-transfusion of convalescent plasma compared with SMT, which might be useful to expedite the immune response against the virus. Additionally, we found that patients with no previous antibody titres in COPLA group have shown a reduced proinflammatory cytokine response and acute phase reactants which are strong predictors of mortality in such patients. On the assessment of laboratory parameters, we found elevated baseline levels of D-dimers; IL-6, serum ferritin and CRP as predictors of mortality, similar to the findings reported in a meta-analysis done by Huang *et al*, and Kermali *et al*[13 14]

Further Zhang *et al* and Yao *et al* found that D-dimer levels were elevated in COVID-19 patients correlating with disease severity and an important predictor of in-hospital mortality in severe COVID-19 patients.[15 16] In the current study, we observed no significant difference between both the treatment groups in terms of the time of two-point reduction in the ordinal scale similar to the study done by Salazar *et al*.[17] Similar to the other studies, we did not find any significant difference in the duration of O_2 therapy, ICU stay and hospital stay between both the groups. Even the overall mortality in both the groups till 28 days was comparable, indicating no survival benefits with convalescent plasma transfusion.[18–21]

Impact of timing of COPLA transfusion

When we considered the timing of transfusion from admission to randomisation within 3 days, on repeated measure analysis, we found significant improvement in ordinal scale in the COPLA group patients who were transfused convalescent plasma within 3 days of admission as compared with SMT group. Similarly, Arnold Egloff *et al*, conducted a retrospective study on patients hospitalised with COVID-19 and found that mortality risk was lower with quicker recovery in patients who received convalescent plasma within 3 days of admission as compared with patients who received in more than 3 days.[22] Furthermore, our study highlighted that if convalescent plasma was transfused after 3 days of admission, it significantly worsened the improvement on an ordinal scale. Generally, most of the patients seek a hospital facility after 3–5 days of infection; the window of transfusion of convalescent plasma remains very small, and justifies its use up to 3 days from admission. Our findings justified transfusion of convalescent plasma within 3–7 days from the onset of symptoms or infection of COVID-19 while condemning its use beyond 7 days. It further emphasises that the early transfusion of convalescent plasma can be beneficial, while delayed transfusion can have detrimental effects, strongly supporting the Indian Council of Medical Research (ICMR) guidelines.[23] Further, Libster *et al* reiterated that early transfusion of high-titre convalescent plasma in COVID-19 infection could reduce the disease's progression in elderly patients favouring the findings of our study.[24]Although overall mortality was similar in both the treatment groups, we found a reduction in the mortality on the transfusion of convalescent plasma within 3 days. In this study, we also found that if plasma transfusion after 3 days had no beneficial effect of reducing significantly high mortality, similar to the PLACID trial.[18]

Comparison with other studies

This study analysed all the critical parameters related to treatment with convalescent plasma, which included timing of transfusion, the dose of transfusion, and all clinical and laboratory parameters required for assessing COVID-19 infections. On review of various studies, we found few studies documented improvement in clinical and laboratory parameters. In contrast, other studies had shown neither improvement in the clinical status nor reduced mortality compared with the SMT alone or with the placebo treatment.[4 18–21 25–28]The noteworthy common short-coming observed was that most previous studies included severely affected COVID-19 patients who already had organ involvement or required mechanical

ventilation. Further, baseline neutralising antibody titre was not performed in these studies, and the timing of transfusion was not mentioned, which is crucial to the expected outcome of convalescent plasma therapy. Donor neutralising antibody titre was not performed in many of these studies.[18–24] However, Körper *et al* performed CAPSID trial on severe COVID-19 patients and found that after 21 days, success rate was higher in patients receiving convalescent plasma as compared with standard treatment alone. They performed a subgroup analysis of high titre versus low titre convalescent plasma and concluded that high titre convalescent plasma significantly reduces time to clinical improvement, significant reduction in time to discharge from hospital and better overall survival.[29] Joyner *et al* further categorised convalescent plasma as high, medium and low titre, and found that patients not receiving mechanical ventilation had lower risk of death with transfusion of high titre plasma as compared with transfusion of low titre plasma.[30] Kunze *et al* studied the effect of geographic distribution of donors and found death within 30 days was lower in group receiving near-sourced plasma than in group receiving distant- sourced plasma.[31] This trial showed promising safety with transfusion of convalescent plasma which makes it a considerable treatment option.[4]

In this study, we strictly followed for the duration from screening to randomisation and transfusion within 3 days (primarily within 24 hours), but the duration from admission to transfusion could not be regulated as most of the patients were not fulfilling inclusion criteria on the day of admission. As a result of this, we could find that participants whose admission to randomisation (plasma transfusion) duration was less than 3 days had a better outcome. Although timely use of convalescent plasma has better outcomes, mortality was similar in both the study group, suggesting multifactorial pathophysiology of the disease. One of the reasons postulated was that the precise duration of onset of illness was unknown to most patients and their attendants. Additionally, in this study, we could find that the baseline titre of the patient had no role in patient outcomes in terms of mortality and reduction in ordinal scale with COPLA transfusion. Further applying this concept will reduce the delay in transfusion waiting for baseline titre results and unnecessary costs incurred by patients or the government based on the Indian council of medical research guidelines.[23]Among the benefits is that the collected plasma can be stored in the lyophilised form to overcome avoidable delay in the transfusion and make it readily available for COVID-19 patients for timely transfusion. In addition, it will further avoid delays due to the logistics and availability issues, even in remote areas.

CONCLUSION

Convalescent plasma with adequate antibody titres should be transfused in COVID-19 patients along with standard medical treatment in the initial 3 days of hospitalisation to SARS CoV-2 for better clinical outcomes.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Institute of Liver and Biliary Sciences, New Delhi, Reference number or ID for ethics: IEC/2020/77/MA05. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data (unpublished data in its row form in Microsoft excel sheet) will be available with principal investigator of the study and it will be available for researcher in unidentified data form on reasonable request to principal investigator till 5 years from date of publication. Rest all data relevant to the study are included in the article or uploaded as online supplemental information

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Undertaking by the Principle Investigator

Proposal for **"Efficacy of convalescent plasma therapy in patients with COVID-19: A randomized control trial"**

Name and code number of the project: **COPLA Trial-II/ILBS/2020 dated 24.05.2020**

1. Name, designation, and department of the Project Investigators:

Project Investigator I ILBS:
Dr. Meenu Bajpai, Add. Professor, Transfusion Medicine
Project Investigator II MAMC:
Dr. Suresh Kumar, Medical Director LNJP and HOD Medicine, MAMC
Project Investigator III RGSH:
Dr. Vikas Dogra, Asst. Prof. Pulmonology, RGSH, Delhi
Co-Project Investigators:

1. **Dr. Vandana Saluja, Associate Professor, Critical Care**
2. **Dr. Pratibha Kale, Associate Professor, Microbiology**
3. **Dr. Ashish Maheshwari, Asst. Professor, Transfusion Medicine**
4. **Dr. Amita Gupta, Blood Centre Officer, Transfusion Medicine**
5. **Dr. Ekta Gupta, Professor, Virology**
6. **Dr. Nirupama Trehanpati, Professor, Molecular and Cellular Medicine**
7. **Dr. Shiv Kumar Sarin, Professor, Hepatology**

2. Name and address of any other medical college, hospital, or Institution where parts of the study will be done: Delhi Govt. Hospitals which are designated COVID-19 treating Centres.
3. Number of ongoing project/clinical trials in which you are PI: **None**

a) I confirm that I will initiate the study after obtaining all regulatory clearance
b) I will not implement any deviation from the approved protocol without the prior consent of the sponsor and it will be intimated with the IEC at the earliest.
c) I confirm that the co-investigators and other members of the study team have been informed about their obligations and are qualified to meet them.
d) I will personally supervise the study and ensure that requirements of obtaining informed consent and other ethical requirements under ICMR and national regulatory guidelines are adhered to.
e) I will maintain accurate and complete records of all cases in accordance with GCP provisions and make them available for audit/inspection by IEC, regulatory authorities, sponsors, or their authorized representatives.
f) I confirm that all research/ study-related investigations/ procedures/ treatment/ any other activity will not be charged from the study participants it will be done at no additional charge to the study participant. Only the routine standard of care & treatment will be charged from the study subjects.
g) I will inform the IEC and the sponsors of any unexpected or serious adverse event at the earliest and definitely within seven days of its occurrence.
h) I will maintain the confidentiality of the identity of all participating subjects and assure the security and confidentiality of study data.
i) I and my colleagues will comply with statutory obligations, requirements, and guidelines applicable to such clinical studies.
j) I will inform IEC of the date of starting the study within 2 weeks of initiation of the trial and submit annual progress reports and final report to member secretary, IEC within 4 weeks of the due date.

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Submission of Project by the Project Investigator (PI) to Institute Ethics Committee (IEC)
Project Title: "Efficacy of convalescent plasma therapy in patients with COVID-19: A randomized control trial"

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Section1: Project title

Section 2: Broad Subject
Transfusion Medicine and Pulmonary Medicine

Section 3: Duration
The estimated total duration of the project is 4months.

Section 4: Total Cost & FE Component: None

Section 5: Funding agency to which applying: None

Section 6: Project Category
Investigator-Initiated Randomized Controlled Trial

Section 7A: Collaborating Investigators/Institutions (Indian/International)
Institute of Liver and Biliary Sciences and
Lok Nayak Jai Prakash Hospital (LNJP)
Rajiv Gandhi Super-specialty Hospital (RGSH)

Section 8: Project summary

Currently, no effective treatments are available for the COVID-19. Scientists and Researchers are working on many aspects of treatment options for the development of vaccination and medication to combat this life-threatening problem. Convalescent plasma from recovered COVID-19 patients contains antibodies against COVID-19 which may be beneficial to severely sick COVID-19 patients. We have recently concluded a pilot phase II open-label RCT on the efficacy of convalescent plasma in severe COVID 19 patients in which we have seen encouraging results. We plan to further study the efficacy and safety of convalescent plasma in COVID-19 severely sick patients through an RCT. We will collect up to 500 ml Convalescent Plasma from the COVID-19 recovered persons after 14 days of clinical recovery with two consecutive SARS CoV-2 negative tests by PCR at least 24 hours apart. This plasma will be tested and frozen and stored. On requisition it will be thawed and sent to the treating center. Two doses of 250 ml convalescent plasma each will be transfused on two consecutive days to patients who fit the eligibility criteria (Severely sick COVID-19 patients) and are randomized to the convalescent plasma group along with the standard of care and the other group will receive standard of care alone. Data will be collected to study the benefits and adverse events related to convalescent plasma transfusion.

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Section 9: Subject Keywords
COVID-19, COVID-19 Convalescent Plasma, Donor Plasmapheresis, ARDS, Immunoglobulins, Antibodies

Section 10: State of knowledge

The outbreak of severe acute respiratory syndrome due to COVID-19 (due to SARS-CoV-2 virus), which had originated in Wuhan, China, has become a pandemic involving more than 5million people across the globe. Currently, no specific drug therapy has been found useful with some benefits reported for remidisvir.

The virus causing COVID-19, SARS-CoV-2 is isolatable using VeroE6, Huh7, or human airway epithelial cells. Serological assays are needed for evaluation of the results of vaccine trials and the development of therapeutic antibodies. Among the four coronavirus structural proteins, the spike (S) and the nucleocapsid (N) are the main immunogens. Apart from antiviral treatment, virus-specific neutralizing antibody, which could accelerate virus clearance and prevent entry into target cells, serves as the main mechanism for the restriction and clearance of the viruses by the host. The convalescent plasma of the patients with these neutralizing antibodies can be used to treat patients with COVID-19. To date, no specific treatment has been proven to be effective for SARS-CoV-2 infection. The current evidence-based strategy relies on providing supportive care in mild cases and the need for mechanical ventilation and extracorporeal membrane oxygenation in severe cases. For over a century convalescent plasma has been used as prophylaxis or treatment of infectious diseases with variable success in different parts of the world

The experience of using convalescent plasma or immunoglobulins has been derived by its utility in improving the survival rate of patients with SARS wherein the patients who had no response to intravenous corticosteroids showed improvement. Lower mortality and shorter hospital stay were recorded for these patients. A study on 1775 patients by Cheng et al, the 80 patients who received convalescent plasma had a lower rate of mortality as compared to the overall mortality. They reported encouraging outcomes of the patients with the use of convalescent plasma in the 2003 SARS-CoV pandemic. In this study, they further found that 33 patients who received convalescent plasma transfusion within the two weeks of symptom presentation showed better outcomes. It has also been proven from previous studies that convalescent plasma can limit viral replication. Providing passive antibody therapy by convalescent plasma in COVID-19 could be one of the approaches towards disease mitigation in the absence of definitive therapy. Recovered donor convalescent plasma products demonstrate donor-related variations in antibody titers and specificities against specific infections.

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Convalescent plasma from recovered patients has shown improved survival in a recent study by Shen et al. on 5 critically ill patients with COVID-19. Improvements in Sequential Organ Failure Assessment (SOFA) Scores, PaO_2 /FiO_2, Acute Respiratory Distress Syndrome (ARDS) were seen along with a decrease in viral loads and an increase in protective antibody tires, although this intervention was not performed as a randomized clinical trial.

Dose: In recent reviews of studies on convalescent plasma therapy, the dose of CP has ranged from 200 ml to 600 ml in single or divided doses. This depends on the logistics of collection (The amount allowed in one donation as per the country's regulations). There is presently no consensus on the dose.

Titer: Neutralizing titre is difficult to perform as it requires a live virus culture and a BSL 3 facility. IgG/IgM titers can be done on ELISA (Most studies doing this method). Few studies have shown that IgG titre closely follows the neutralizing titre. The minimum titers vary from study to study (see Table 1)

Minimum Titre Requirements for Convalescent Plasma (ref-22-25)

Author	Country	Neutralizing antibody titer
Duan et al	China	>1:640
FDA recommendations	USA	>1:160 (1:80 acceptable)
ISBT Working party recommendations for convalescent plasma for LIC and MIC	-	1:160 (1:80 acceptable)
Shen et al	China	>1:40
Zhang et al	China	Not determined before transfusion
Joyner et al	USA	Not mentioned

The International Society of Blood Transfusion (ISBT) as well as the US FDA recommends a minimum titer of 80 (> 160 is preferable).

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Risks

Recipient Safety (ref:21)

In a recent report from Mayo Clinic, USA on 5000 convalescent plasma transfusions (CPT) in COVID-19 patients, 36 Serious Adverse Events (SAEs) were reported(< 1% of all transfusions). Of the 15 deaths reported (0.3% of all transfusions) four were judged to be related (possible: 3, definitely: 1) CPT. There were 21 non-death SAEs (see Table 2)

Table 2: Serious Adverse Event (SAE) associated with convalescent plasma transfusion (n=5,000)

Four hour mortality reports	Reported(n=36)	Related(n-25)	Estimated(95% CI)
Mortality	15	4	0.08% (0.03%, 0.21%)
Transfusion associated circulatory overload(TACO)	7	7	0.14% (0.07%, 0.29%)
Transfusion related acute lung injury(TRALI)	11	11	0.22% (0.12%, 0.39%)
Severe allergic transfusion reaction	3	3	0.06% (0.02%, 0.18%)
Seven Day reports	**Reported**		**Estimated(95% CI)**
Mortality	602		14.9% (13.8%, 16.0%)

Donor Safety: The convalescent plasma donation by Plasmapheresis was found to be a safe procedure in the RCT done at ILBS-MAMC. No adverse events were noted during 16 procedures done under this trial. No other studies reported any SAEs during donor plasmapheresis

Section 11: Importance of the proposed project/ Justification for subject area:

COVID-19 is a major pandemic that has spread across all countries with a case-fatality rate varying from 1.2-10% or higher. Currently, there is no definitive therapy recommended for the management, and the results of convalescent plasma transfusion as a supportive therapy appear encouraging. The data from our pilot RCT is encouraging and therefore there is a need for a wider randomized controlled trial that could address the safety and efficacy of CP in the management of patients with severe COVID-19 using a larger sample size.

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If patients are counseled on recovery to donate convalescent plasma and this is frozen and sent to treatment centers, it may be used to transfuse severely sick patients and may improve outcomes.

Section 12: **Review of facilities and expertise** available in the institution

The study will be carried out jointly at ILBS which has a fully functional Transfusion Medicine Department and facilities for collection of convalescent plasma and Delhi Govt. hospitals which are designated as COVID-19 treatment centers and are equipped with modern facilities for patient care and have the infrastructure for carrying out this study.

Section 13: **Study Design**

Aim and Objectives

Primary Objective: To study the 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)

The six-point scale is as follows:

1. *death=6;*
2. *hospital admission for extracorporeal membrane oxygenation or mechanical ventilation=5;*
3. *hospital admission for non-invasive ventilation or high-flow oxygen therapy=4;*
4. *hospital admission for oxygen therapy (but not requiring high-flow or non-invasive ventilation)=3;*
5. *hospital admission but not requiring oxygen therapy=2;*
6. *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.*

Secondary Objectives:

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)

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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 on days 0, 3, 7, 14, 21 & 28 after plasma administration, as long as the patient remains in the hospital.

9. Cytokines and acute phase reactants

10. To study the titers in COVID-19 convalescent plasma donors and correlate with duration of illness, the severity of symptoms, duration of hospital stay, drugs used in therapy, duration between recovery, and donation.

Study Centres:

Institute of Liver and Biliary Sciences
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– Collection and testing of Convalescent Plasma

Treatment Centres

Lok Nayak Jai Prakash Hospital (LNJP)

Rajiv Gandhi Super-speciality Hospital

B) Methodology

Donor Plasmapheresis

COVID-19 recovered patients will be counseled and informed regarding convalescent plasma donation. The contact information of those who agree will be sent to the coordinator at ILBS Blood Centre.

- The prospective donor will be contacted and if willing to come for donation, the donor will be provided conveyance if required, to come to ILBS Blood centre for Plasma Donation
- At ILBS the donor will be counseled and the doctor in-charge will explain the procedure to the Donor.
- The donor will be given a Donor Information Sheet and Informed consent will be taken on the document
- The eligibility for plasma donation will be ascertained through Medical History, Physical Examination, and laboratory tests.

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Donor Eligibility for Plasmapheresis

- Virologically documented (PCR positive by nasopharyngeal swab) who is recovered and free of symptoms for 14 days.
- Has tested negative for SARS CoV 2 on two consecutive tests 24 hrs apart.
- Fulfill all criteria of donor eligibility for donor Plasmapheresis under the Drugs & Cosmetics Act 1940 and Rules 1945, amended 11.03.2020
- Females who have been pregnant may be tested for anti-HLA antibodies and eligible if negative for the same.

The following Donors will be excluded

- Do not fulfill all criteria of donor eligibility for donor Plasmapheresis under
- the Drugs & Cosmetics Act 1940 and Rules 1945, amended 11.03.2020
- Females who have been pregnant and have not been tested for HLA antibodies or are HLA antibody positive if tested and previously transfused donors (to prevent TRALI)
- Donors who have taken steroids during treatment for COVID-19

Donor Selection Process

- A detailed medical history of the donor will be taken and documented
- Physical examination (Height, Weight, Blood Pressure, Temperature, adequate veins for phlebotomy)
- Laboratory Testing: complete Blood count, Testing for hepatitis B virus, hepatitis C virus, HIV, malaria, and syphilis) by serology, blood grouping, and antibody screening. Serum protein will be done in repeat donors (Ref: D&C Act and Rules)
- Serum COVID-19 specific IgG antibody positive (with an IgG titre higher than 80).
- All Results Evaluated & Clinically Correlated

The donor is deemed eligible/non-eligible

Plasmapheresis Procedure

- Determine Volume to be collect-approx. 500 ml
- As per the Drugs & Cosmetics Act 1940 and Rules 1945, amended 11.03.2020
- Start Donor Plasmapheresis Procedure(As per SOP)
- Collect Convalescent Plasma

The convalescent plasma will be properly labeled and frozen at -80^{o} C in a separate Deep Freezer. It will not be issued to other patients who are with non-COVID.

- **Label Detail**s: As per the Drugs & Cosmetics Act 1940 and Rules 1945, amended 11.03.2020.
- **Storage condition-** Below -30^{o}C
- **Shelf life: -** 1 Year

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Other Sources of Convalescent Plasma:

Convalescent Plasma collected during a CP donation drive by Delhi Govt. following all rules and regulations of the Drugs& Cosmetics Act 1940 and Rules 1945, amended 11.03.2020 is stored at ILBS and will be used in this trial.

The tests for Donors:

1. **Real-time PCR for** SARS-CoV-2:
   Nasal swab samples will be taken prior to donation and tested for SARS-CoV-2 by real-time PCR method it the donor does not have two negative reports 24 hrs apart.

Antibody Titers of Convalescent Plasma and Patients Plasma by ELISA

For Donors:

The titre of serum neutralizing antibody which is the spike protein antibody, directed against the SARS-CoV-2 RBD (receptor binding domain) proteins. The titre will be done by IgG ELISA or by Rapid IgG antibody titre. The minimum titre of 80 is needed for the use of convalescent in patients.

For recipients:

1. **Real-time PCR for** SARS-CoV-2:
   Nasal swab samples will be taken prior to transfusion and tested for SARS-CoV-2 by real-time PCR method. The test should be positive for eligibility of the recipient along with clinical criteria for COVID-19.

2. Antibody titre: The serum of each recipient will be obtained and IgG antibody titre by enzyme-linked immune-sorbent assay (ELISA) or Rapid IgG antibody method will be tested one day prior to the convalescent plasma transfusion. Changes of IgG antibody titre before and after convalescent plasma transfusion in patients will be studied. The serum will be stored for neutralizing antibody titers by plaque reduction will be done subject to availability.

Study Population:

Adult patients with severe COVID -19 infections defined as WHO Interim Guidance and the Guideline of Diagnosis and Treatment of COVID-19 of National Health Commission of China (version 5.0) 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 93% in resting state
4. Partial pressure of oxygen (PaO_2)/oxygen concentration (FiO_2) ≤ 300 mmHg
5. Lung infiltrates > 50% within 24 to 48 hours

Study Design:

An open label randomized controlled trial.

The study group will comprise of adult patients with severe COVID -19 as detailed above. Randomization will be done in the ratio of 3:1 in with 150 patients in the treatment arm and 50 patients in the control arm. Allocation concealment will be done by Sequentially Numbered Opaque Sealed Envelopes (SNOSE) method.

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Justification for Unequal Allocation

We want to collect the additional safety profile associated with convalescent plasma group hence enrolling more number of cases in this group.

Sample Size calculation:

The study will be designed as an open label RCT with an aim to enrol 150 patients in the treatment arm and 50 patients in the control arm.

Intervention:

Intervention Arm: Two doses of 250 ml Convalescent plasma from recovered COVID-19 patients + Standard of Care will be given to severely sick COVID-19 patients in the treatment arm

Control Arm: Standard of Care will be given to severely sick COVID-19 patients in the control arm

Details of Standard of Care

The Ministry of Health and Family Welfare has issued detailed guidelines for the management of sCOVID-19 based on varying grades of severity which may be periodically updated. 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. (ref: Guidelines on Clinical Management of COVID-19. MoHFW, GoI.2020.)

Monitoring and Assessment: Daily until clinical improvement

Adverse Effects: Will be documented

Stopping rule: None

(C) The expected outcome of the project: We expect convalescent plasma therapy to be a safe and efficacious therapy based on our pilot RCT. This study will determine if there is clinical improvement /mortality benefits and further elaborate on its safety in patients with severe COVID-19

Section 14: Inclusion and exclusion criteria for the admission of patients in the study

Inclusion criteria

Patients with severe COVID-19 (as described above) 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-

- Patients on ventilator (in last 24 hours)
- Respiratory distress, RR ≥30 beats/min
- Oxygen saturation level less than 93% in resting state

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- Partial pressure of oxygen (PaO_2)/oxygen concentration (FiO_2) ≤ 300 mmHg
- Lung infiltrates > 50% within 24 to 48 hours

Exclusion criteria

- Patient/ family members who do not give consent to participate in the study.
- Patients with age less than 18 years
- Patients presenting with multi-organ failure
- Pregnancy
- Individuals with HIV and Viral Hepatitis and Cancer
- Extremely moribund patients with an expected life expectancy of less than 24 hours
- Hemodynamic instability requiring vasopressors
- Previous history of allergy to plasma
- Cirrhosis
- Severe renal impairment with GFR< 30ml/min or recipients of RRT, peritoneal dialysis
- Patients with uncontrolled diabetes mellitus, hypertension, arrhythmias and unstable Angina

Transfusion of COVID-19 Convalescent Plasma

Dose-250 ml
Frequency – 2 doses on consecutive days
Duration –Start by day 3 of symptom onset in eligible patients

Blood Group Compatibility

ABO identical plasma will be the first choice followed by ABO compatible plasma.

In case compatible plasma is not available reduction of titers will be done using an ABO antibody immune-adsorption column.

Patient Monitoring

All included patients will be randomized to receive either 500 ml of convalescent plasma in two doses of 250 ml on two consecutive days along with the standard of care or standard of care alone

Clinical information of all enrolled patients including symptoms at presentation, time to presentation to the hospital, and development of pulmonary symptoms will be recorded. The details of comorbid diseases as measured by the Charlson index of co-morbidity and Acute Physiology and Chronic Health Evaluation II (APACHE II). Details of cross-sectional imaging, chest-x-ray, bacterial or fungal co-infections, and details of antibiotic treatment will be recorded. Development of complications including shock, acute kidney injury, acute coronary syndrome, myocarditis, acute respiratory distress syndrome, need for mechanical ventilation and nosocomial infection will be recorded. The use of high-flow oxygen, non-invasive, and invasive ventilation will follow standard guidelines and will be recorded. The details of antiviral treatment will be recorded for all enrolled patients.

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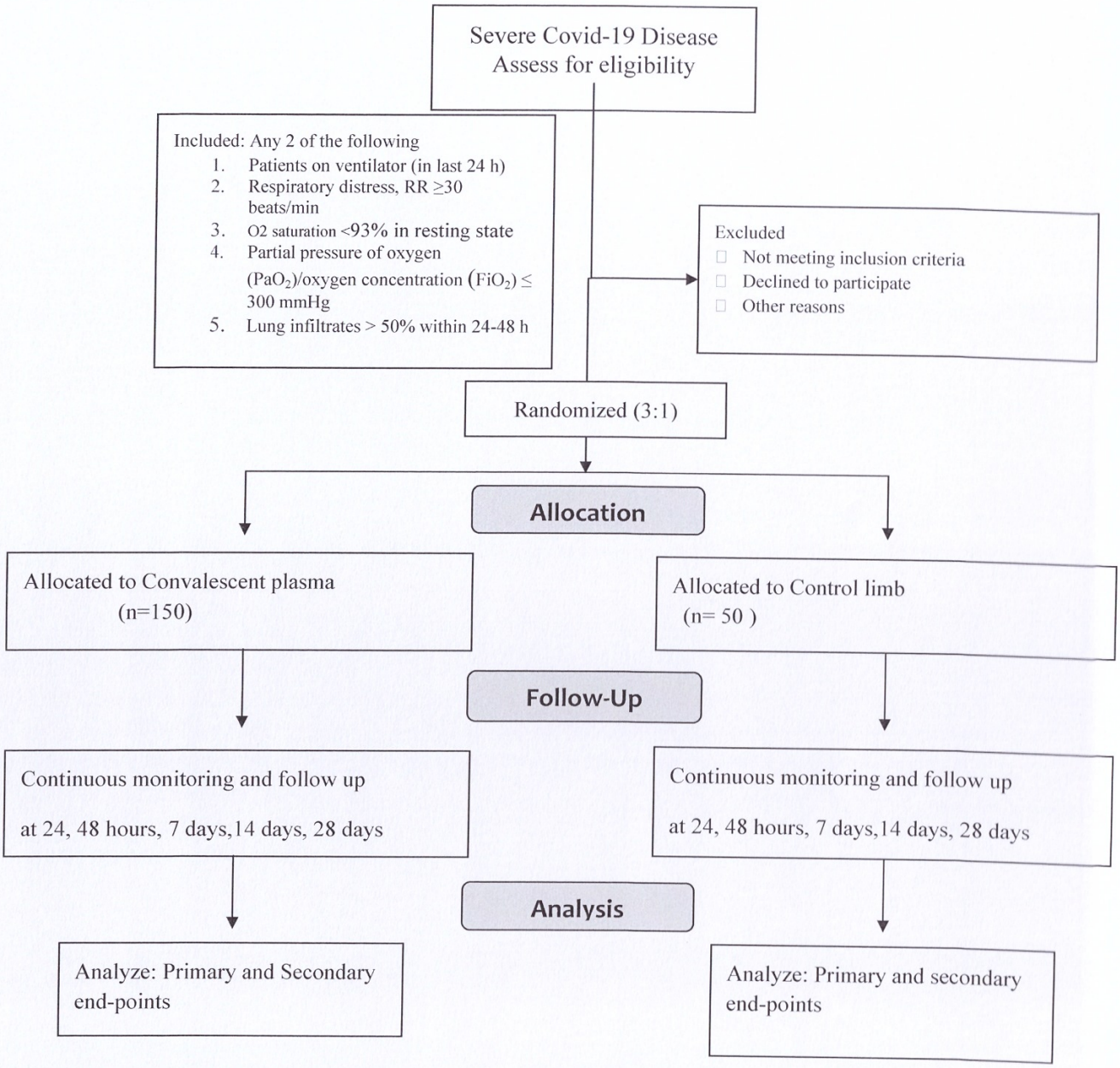
Flow Chart



- All variables shall be expressed in mean (SD) or median (range)
- Variables will be compared by Mann- Whitney U test
- For Categorical variables, we will use Chi-Square or Fisher's test
- Survival analysis will be done using Cox-proportional regression analysis

The actuarial probability of survival shall be calculated by Kaplan- Meier graph and compared by the log-rank test.

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Section 15: Work Plan with the schedule of activities giving milestones

An RCT which will be completed within 4 months

Section 16: Permission from Drug Controller of India for use of drug/vaccine/device in the study, wherever applicable-

ILBS Blood Centre has a license for Plasmapheresis and approval of DCGI

Section 17: Safety of the proposed interventions, drugs, or vaccine to be tested including results of relevant laboratory and animal toxicity and safety trials, and results of studies carried out in humans

Human studies have been done and reported rare SAEs*, it has been approved by CDC under emergency Investigational New Drug Applications (eINDs)

*(Joyner MJ, Wright RS, Fairweather DL, senefeld JW, Bruno KA, klassen SA et al. Early Safety Indicators of COVID-19 Convalescent Plasma in 5,000 Patients. MedRziv. doi.org/10.1101/2020.05.12.20099879)-See review of literature

Section 18: Description of plans to withdraw or withhold standard therapy in the course of research-No

Section 19: For research carrying more than minimal risk, an account of plans to provide medical therapy for such risk or injury –Insurance cover for patients

The donor may have any of the adverse events related to Plasmapheresis donation (similar to healthy apheresis donors) – Detailed in Donor Information Sheet

Patients may have any of the adverse events related to plasma transfusion- Patient Information Sheet

Section 20: Budget Estimates – None

Section 21: Other research projects with investigators -None

Place: New Delhi

Signature & Designation of PI/Co-PI/Collaborator

Date: 24/05/2020.

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Annexures

1. Donor Information and Consent Form
2. Patient Information and Consent Form
3. Plasmapheresis License
4. Plasmapheresis- Donor Selection and Procedure
5. Apheresis Registration Form
6. Additional Donor Information
7. Transfusion Monitoring of Convalescent Plasma
8. Case Report Form
9. IEC approval ILBS
10. Bio-data of Investigators
11. Executive Summary
12. Form CT04
13. PI Undertaking Meenu
14. Reference paper 1
15. Reference paper 2
16. Reference paper 3
17. Standard of Care

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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.
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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.

Table: 1S oxygen saturation with time of transfusion

	time ≤3 days			time >3 days		
	COPLA	SMT	p value	COPLA	SMT	p value
Mean SPo2 at Baseline	85.97	86.06	0.824	85.51	85.98	0.448
Mean SPo2 at 24 hours	93.35	93.53	0.698	92.70	93.91	0.220
Mean SPo2 at 48 hours	94.27	93.73	0.328	93.62	94.30	0.310
Mean SPo2 at 72 hours	94.96	94.90	0.886	93.80	94.54	0.288
Mean SPo2 at 7 days	95.45	95.41	0.904	94.92	95.37	0.379
Mean SPo2 at 10 days	95.28	94.36	0.364	93.89	95.45	0.216
Mean SPo2 at 14 days	95.18	94.88	0.728	153.33	95.44	0.287
Mean SPo2 at 28 days	95.50	96.00	0.737	96.00	95.67	0.899