Convalescent Plasma for Severe COVID-19 in Hospitalized Patients: An Open-Label, Randomised Clinical Trial

Supplementary Results

Plasma donation selection and procedures

Potential plasma donors were procured both among healthcare workers from the institution and candidates from the community. Eligible donors were required to be male or nulliparous females, between 18 and 60 years of age, asymptomatic from COVID-19 for at least 14 days and have a negative oropharyngeal/nasal swab RT-PCR for SARS-CoV-2 on the day of plasma donation, as well as fulfil all regulatory requirements for blood donation. During the donor qualification process, complete blood count, ABO and RhD typing, and serological tests were performed both for bloodborne diseases (conventional blood donation tests) and for SARS-CoV-2 IgG. Only candidates with a positive results (according to chemiluminescent assay criteria) for SARS-CoV-2 IgG were qualified for donation.

Selected convalescent plasma (CP) were collected from male or nulliparous females, aged between 18 and 60 years old, with previous COVID-19 confirmed either by RT-PCR or positive IgG serological test. Donors were required to be asymptomatic for at least 14 days before plasma donation and comply with all regional conventional regulatory requisites for blood donation. An additional IgG anti-SARS-CoV-2 nucleocapsid test (Abbott Laboratories) was performed in all candidates to confirm serological status at the moment of donation. All donors were required to sign an informed consent acknowledging the research nature of their participation.
Plasma donation

Convalescent plasma was collected from previously qualified donors through apheresis procedure on a Fenwal Amicus Separator (Fenwal, Lake Zurich, IL) with a modified single-needle plateletpheresis protocol. Standard anticoagulant ACD-A solution was used during procedures. For each plasma donation, a target of 600-700 ml of convalescent plasma was set up. Individual donors were allowed to donate additional units with a minimum interval of 14 days between procedures.

Definition of baseline variables

The presence of diabetes, cardiovascular disease (coronary heart disease, cerebrovascular disease, and peripheral vascular disease), and chronic pulmonary disease (chronic obstructive pulmonary disease and asthma) were accounted as registered in medical records. Obesity was defined as a body mass index equal to or higher than 30.

PaO2/FiO2 ratio is the ratio of arterial oxygen partial pressure (PaO2 in mmHg) to fraction of inspired oxygen. Arterial blood gases were ordered at the discretion of patients’ medical teams and were not performed in all patients. When PaO2 was not available, this ratio was substituted by the peripheral oxygen saturation (SaO2)/FiO2 ratio adjusted to the positive end-expiratory pressure (PEEP), as previously reported.[1] This later ratio was also imputed for the calculation of SOFA score when PaO2 was not available. Bilirubin was measured only if deemed medically necessary. When no bilirubin was available and there was no record of previous hepatic disease, we imputed zero points for the liver component of the score.[2]

Vasoactive drugs at baseline were either noradrenaline or vasopressin.
Neutralizing antibodies and other laboratory procedures

Neutralizing antibodies (nAbs) were determined in all plasma bags and on serum collected on the day of enrolment (day 0) and 3 days after CCP transfusions. The titration of nAbs was performed using the cytopathic effect-based virus neutralization test (CPE-based VNT) with SARS-CoV-2/human/BRA/SP02cc/2020 strain virus (GenBank access number: MT350282.1) [3], following a previously described protocol.[4] Briefly, 5×10⁵ Vero cells/mL (ATCC CCL-81) were seeded 24 hours before the infection in a 96-well plate. Serum samples were, initially, inactivated for 30 min at 56°C. We used 11 dilutions (two-fold) of each serum (1:20 to 1:20,480). Subsequently, serum was mixed vol/vol with 1000 TCID₅₀/mL of the virus and pre-incubated at 37°C for 1 hour to allow virus neutralization. Then, the serum plus virus mixture was transferred onto the confluent cell monolayer and incubated for 3 days at 37°C, under 5% CO₂. After 72 hours, the plates were analysed directly under transmitted-light bright-field microscopy (Olympus Co., Tokyo, Japan). The virus neutralization titre referred to VNT100 is described as the highest dilution of serum that neutralized virus growth (absence of cytopathic effect). All the procedures related to CPE-VNT were performed in a biosafety level 3 laboratory, at the Institute of Biomedical Sciences – University of São Paulo, in accordance with WHO recommendations.[5]

Blood samples were drawn on days 0 (day of randomization; before infusion in the intervention group), 3 (after infusion of the second plasma bag in the intervention group), 7, and 14. A total of 5 mL whole blood with K2 EDTA (dipotassium ethylenediaminetetraacetic acid - Vacuette®, Greiner Bio-One, Kremsmünster, Austria) was immediately centrifuged, separated, and plasma stored at −20°C until analysis.

The following laboratory markers were evaluated on days 3, 7, and 14 after randomization: lactate dehydrogenase, troponin I, C-reactive protein, D-dimers, fibrinogen, prothrombin
time, activated partial LDH was measured by a pyruvate kinase assay kit in an Abbott Alinity

C series analyzer. High-sensitivity troponin I was determined by chemiluminescent

microparticle immunoassay (CMIA) in an Abbott Alinity i series analyzer. C-reactive protein

was measured by a particle-enhanced turbidimetric inhibitor immunoassay in Abbott Alinity

C. D-dimers were analysed by optical reaction in a Siemens Sysmex CS2500 system.

Coagulation parameters (prothrombin time, activated partial thromboplastin time and

fibrinogen) were determined in a Stago STA R Max analyzer.

Human IL-6 ELISA Kit (Invitrogen, Thermo Scientific, Vienna, Austria) and Human TNF-

alpha ELISA Kit (Invitrogen, Thermo Scientific, Vienna, Austria) were used to measure IL-6

and TNF-alpha, respectively. The tests were performed as per the manufacturer’s

instructions. Provided standards (low and high control) were used to monitor the assay. In

brief, 50 mL of plasma were added to each well with same volume of assay buffer (1x) for

IL-6 and Sample Dilution solution for TNF-alpha; then, 50 mL of biotin conjugate (1:10 fold)

was pipetted; the plate was incubated at room temperature (18-25°C) for 2 hours in a shaker

set at 400 rpm; the strips were washed and then added to wells containing 100 mL of diluted

(1:10 fold) Streptavidin-HRP; the plate was incubated at room temperature for 1 hour in the

same conditions and washed; 100 mL of TMB Substrate Solution was pipetted into all wells

and the plate was again incubated for about 10 minutes at room temperature; 100 mL of Stop

Solution was added; and the absorbance of each microwell was read on a spectrophotometer

at 450 nm. The minimum limit of detection of the analyte of interest was 0.92 pg/mL for IL-6

and 2.3 pg/mL for TNF-alpha.

The RT-qPCR assay for SARS-CoV-2 was performed as previously described, based on

CDC guidelines.[6] Primers for nucleocapsid regions 1 and 2 (N1 and N2) and human

ribonuclease P gene were used for viral detection and internal control, respectively. Reactions
were performed in a Superscript III one step RT-qPCR system (Thermo Fisher Scientific Inc, USA). The master mix was composed of 5 µL of 2X reaction buffer (0.4 mM of each dNTP and 6 mM MgSO₄); 0.2 µL of SuperScript™III RT/Platinum™ Taq Mix; 0.2 µL of ROX (1:10); 0.75 µL of combined primers/probes mix of nCOV1 (N1 primer) or nCOV2 (N2 primer) or RP (2019-nCoV RUO Kit, Integrated DNA Technologies Inc, USA); and 4 µL of RNA. The cycling reaction was performed at 50°C for 30 min for reverse transcription, followed by 95°C for 2 min and 45 cycles of 95°C for 15 s and 55 °C for 35s in a QuantStudio® 3 system (Applied Biosystems, USA). Three different results were considered: “negative” when neither N1 nor N2 targets amplified; “positive” when both N1 and N2 amplified; and “inconclusive” when only one target (N1 or N2) amplified.

Sample Size

The estimated proportion of patients exhibiting clinical improvement on day 28 was based on the findings of Li et al.,[7] in which this rate was 43.1 % in the control group. We expected a higher proportion of patients presenting 2 points of improvement on the ordinal scale, because the median time from the onset of symptoms to randomization in that trial was 30 days for the control group; thus, we presumed that patients would be more likely to exhibit a 2-point improvement on the scale if followed from an earlier moment after the onset of symptoms. For the same reason, we expected that earlier administration and the second plasma bag could potentially result in a higher effect on clinical improvement rate at day 28 than that of the 8.8% difference observed by Li et al.[7]
Supplementary Results

TABLE 1 Additional laboratory findings of patients at randomization.

| Characteristics                        | Convalescent Plasma (n=80) | Control (n=80) |
|----------------------------------------|----------------------------|----------------|
| Serum creatinine, mg/dL                | 1.1 (0.8 - 1.6)            | 1.0 (0.8 - 1.5) |
| Lactate dehydrogenase, U/L\(^#\)       | 444.5 (358.0 - 590.0)      | 427.0 (348.5 - 578.0) |
| Troponin I, ng/L                       | 10.0 (9.9 - 18.5)          | 10.0 (9.9 - 11.6) |
| Fibrinogen, mg/dL\(^¶\)               | 659.9 ± 133.6              | 629.8 ± 139.5 |
| Prothrombin time, seconds\(^‡\)        | 13.7 (13.0 - 14.5)         | 13.4 (12.6 - 14.2) |
| Activated partial thromboplastin time, seconds\(^b\) | 33.5 (31.0 - 38.1)        | 33.5 (31.0 - 38.1) |

Data are median (Interquartile Range) or mean ± standard deviation.

\(^#\) Two (2.5%) patients in the intervention group did not have lactate dehydrogenase collected at randomization.

\(^¶\) One (1.3%) patient in the intervention group did not have fibrinogen, prothrombin time, or activated partial thromboplastin time measured.
**Convalescent Plasma Donors**

A total of 48 plasma donors have performed 91 apheresis donations. Characteristics from convalescent plasma donors and procedure parameters can be found in eTable1. Thirty (62.5%) of these donors performed two or more plasma donations during trial. The median neutralizing antibody titres from donors’ plasma administered to patients from the intervention group was 1:320 (IQR, 160 to 1:960). Only five donors’ plasma eventually had neutralizing antibody titres lower than 1:80 (four 1:40 and one 1:20).
TABLE 2 Characteristics from convalescent plasma donors and procedure parameters.

| Characteristics of Donors | n=48 |
|---------------------------|------|
| Male sex                  | 31 (64.9) |
| Age, years                | 37 (32.6 - 46.8) |

| Donor ABO/RhD | |
|---------------|------|
| A+            | 21 (43.8%) |
| O+            | 13 (27.1%) |
| B+            | 3 (6.3%) |
| AB+           | 2 (4.2%) |
| A-            | 8 (16.7%) |
| O-            | 1 (2.1%) |
| B-            | 0 |
| AB-           | 0 |

| Characteristic of Plasma Donation Procedures | n=91 |
|-----------------------------------------------|------|
| Plasma collection volume, mL                  | 626 (512 - 693) |
| Procedure duration, minutes                   | 62 (57 - 73) |
| Apheresis serious adverse events              | 0 |
| Need for more than one venipuncture           | 2/91 (2.2) |
| Fluid replacement, mL                         | 300 (300-350) |

Data are n (%) or median (Interquartile Range).
TABLE 3 Primary outcome, 14- and 28-day mortality in the per-protocol population.

|                                | Convalescent plasma (n=75) | Control (n=79) | Absolute difference (95% CI) | Relative Risk (95% CI) | p-value |
|--------------------------------|-----------------------------|---------------|------------------------------|------------------------|---------|
| **Primary outcome**            |                             |               |                              |                        |         |
| Clinical Improvement on day 28 | 47 (62.7)                  | 52 (65.8)     | -3.1% (-18.4 - 12.1)         | 0.95 (0.75 - 1.21)     | 0.683   |
| **Secondary Outcomes**         |                             |               |                              |                        |         |
| Death on day 14                 | 9 (12.0)                    | 5 (6.3)       | 5.7% (-3.97 - 16.3)          | 1.90 (0.67 - 5.40)     | 0.231   |
| Death on day 28                 | 16 (21.3)                   | 13 (16.5)     | 4.8% (-9.02 - 19.3)          | 1.30 (0.67 - 2.51)     | 0.441   |
Unit of admission and need of mechanical ventilation were prespecified subgroups. Age and neutralizing antibody titres comprise post hoc analysis.

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**FIGURE 1 Primary outcome according to subgroups.**

| Subgroup                        | Convalescent (plasma, n=80) | Control (n=80) | RR (95%) | P-value |
|---------------------------------|-----------------------------|----------------|----------|---------|
| **Age**                         |                             |                |          |         |
| < 65 y                          | 38/55 (69.1)                | 37/52 (71.2)   | 0.97 (0.76; 1.24) | 0.82    |
| > 65 y                          | 11/25 (44.0)                | 15/28 (53.6)   | 0.82 (0.47; 1.44) | 0.49    |
| **Site at Randomization**       |                             |                |          |         |
| Medical Ward                    | 22/27 (81.5)                | 24/27 (88.9)   | 0.92 (0.73; 1.15) | 0.45    |
| Intensive Care Unit             | 27/53 (50.9)                | 28/53 (52.8)   | 0.96 (0.67; 1.39) | 0.86    |
| **Neutralizing antibodies titer**|                             |                |          |         |
| ≤ 1.80                          | 7/19 (36.8)                 | 2/8 (25.0)     | 1.47 (0.39; 5.61) | 0.57    |
| > 1.80                          | 42/61 (69.9)                | 50/70 (71.4)   | 0.96 (0.77; 1.21) | 0.75    |
| **Mechanical ventilation at randomization** |             |                |          |         |
| Yes                             | 13/34 (38.2)                | 16/34 (47.1)   | 0.81 (0.47; 1.42) | 0.47    |
| No                              | 36/46 (78.3)                | 36/46 (78.3)   | 1.00 (0.81; 1.24) | 0.99    |
FIGURE 2 Probability of death in the intervention and control groups.

Shadow areas represent the 95% Confidence Interval.
FIGURE 3 Additional laboratorial parameters.

The box plot inner horizontal lines indicate median; boxes, interquartile range (25th and 75th percentiles); whiskers extend to the most extreme observed values with 1.5 times the interquartile range of the nearer quartile, and dots represent observed values outside that range. The numbers of patients evaluated at each time point in both convalescent plasma and control groups are at the bottom of the figure. Abbreviation: D, day.
FIGURE 4 Inflammatory markers in patients who completed the three collection times (day 3, day 7 and day 14).

The box plot inner horizontal lines indicate median; boxes, interquartile range (25th and 75th percentiles); whiskers extend to the most extreme observed values with 1.5 times the interquartile range of the nearer quartile, and dots represent observed values outside that range. The number of patients who had completed all collections times on days 0, 3, 7 and 14, in convalescent plasma and control groups, for D-dimers were 39 and 38, respectively. For C reactive Protein, the numbers in convalescent plasma and control groups were 40 and 41, respectively. For TNF-alpha, the numbers in convalescent plasma and control groups 40 and 40, respectively. For interleukine-6, the numbers in convalescent plasma and control groups were 40 and 40, respectively.

Abbreviation: D, day.
FIGURE 5 Additional laboratorial parameters in patients who completed the three collection times (day 3, day 7 and day 14).

The box plot inner horizontal lines indicate median; boxes, interquartile range (25th and 75th percentiles); whiskers extend to the most extreme observed values with 1.5 times the interquartile range of the nearer quartile, and dots represent observed values outside that range. The number of patients who had completed all collections times on days 0, 3, 7 and 14, in convalescent plasma and control groups, for fibrinogen were 39 and 39, respectively. For activated partial thromboplastin time, the numbers in convalescent plasma and control groups were 39 and 39, respectively. For prothrombin time, the numbers in convalescent plasma and control groups were 40 and 38, respectively. For lactate dehydrogenase, the numbers in convalescent plasma and control groups were 40 and 39, respectively. For troponin I, the numbers in convalescent plasma and control groups were 30 and 21.

Abbreviation: D, day.
### TABLE 4 Number per patient and description of grade 1 or 2 adverse effects.

| Number of adverse events | ≥1 infusion of convalescent plasma (n=79) | Standard of care alone (n=81) |
|--------------------------|------------------------------------------|-------------------------------|
| None                     | 27 (34.2)                                | 33 (40.7)                     |
| 1                        | 16 (20.3)                                | 8 (9.9)                       |
| 2                        | 14 (17.7)                                | 9 (11.1)                      |
| 3                        | 7 (8.9)                                  | 11 (13.6)                     |
| 4+                       | 15 (19.0)                                | 20 (24.7)                     |

**Type of adverse events**

| Type of adverse events | ≥1 infusion of convalescent plasma (n=79) | Standard of care alone (n=81) |
|------------------------|------------------------------------------|-------------------------------|
| Allergic Reaction      | 2                                        | 4                             |
| Cardiovascular         | 14                                       | 7                             |
| Cerebrovascular        | 1                                        | 1                             |
| Fluid and Electrolyte Disturbances | 23                         | 25                             |
| Hematologic            | 23                                       | 21                            |
| Infectious             | 47                                       | 52                            |
| Metabolic              | 21                                       | 32                            |
| Thromboembolic         | 10                                       | 10                            |
| Other                  | 3                                        | 8                             |

* Absolute number of each type of adverse effect.
### TABLE 5 Number per patient and description of grade 3 or 4 adverse effects.

| Number of adverse events | ≥1 infusion of convalescent plasma (n=79) | Standard of care alone (n=81) |
|-------------------------|------------------------------------------|-------------------------------|
| None                    | 29 (36.7)                                | 37 (45.7)                     |
| 1                       | 20 (25.3)                                | 12 (14.8)                     |
| 2                       | 13 (16.5)                                | 10 (12.3)                     |
| 3                       | 9 (11.4)                                 | 13 (16)                       |
| 4+                      | 8 (10.1)                                 | 9 (11.1)                      |

### Type of adverse events *

| Type of adverse events | ≥1 infusion of convalescent plasma (n=79) | Standard of care alone (n=81) |
|------------------------|------------------------------------------|-------------------------------|
| Cardiovascular         | 13                                       | 6                             |
| Cerebrovascular        | 0                                        | 1                             |
| Fluid and Electrolyte Disturbances | 14                                      | 9                             |
| Hematologic            | 16                                       | 31                            |
| Infectious             | 46                                       | 48                            |
| Metabolic              | 17                                       | 27                            |
| Thromboembolic         | 7                                        | 7                             |
| Other                  | 2                                        | 2                             |

*Absolute number of each type of adverse effect.
FIGURE 6 Association of convalescent plasma with all-cause mortality.

Updated meta-analysis with PLACOID clinical trial and RECOVERY pre-print results. RR, risk ratio; CI, Confidence Interval.
References

1. Pandharipande PP, Shintani AK, Hagerman HE, St Jacques PJ, Rice TW, Sanders NW, Ware LB, Bernard GR, Ely EW. Derivation and validation of Spo2/Fio2 ratio to impute for Pao2/Fio2 ratio in the respiratory component of the Sequential Organ Failure Assessment score. *Crit Care Med* 2009: 37(4): 1317-1321.

2. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996: 22(7): 707-710.

3. Wendel S, Kutner JM, Machado R, Fontão-Wendel R, Bub C, Fachini R, Yokoyama A, Candelaria G, Sakashita A, Achkar R, Hamerschlak N, Scuracchio P, Amaral M, Dal Ben M, Araujo D, Soares C, Camargo A, Kallás E, Durigon E, Reis LF, Rizzo LV. Screening for SARS-CoV-2 antibodies in convalescent plasma in Brazil: Preliminary lessons from a voluntary convalescent donor program. *Transfusion* 2020: 60(12): 2938-2951.

4. Araujo DB, Machado RRG, Amgarten DE, Malta FM, de Araujo GG, Monteiro CO, Candido ED, Soares CP, de Menezes FG, Pires ACC, Santana RAF, Viana AO, Dorlass E, Thomazelli L, Ferreira LCS, Botosso VF, Carvalho CRG, Oliveira DBL, Pinho JRR, Durigon EL. SARS-CoV-2 isolation from the first reported patients in Brazil and establishment of a coordinated task network. *Mem Inst Oswaldo Cruz* 2020: 115: e200342.

5. (WHO). WHO. Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV). 2020.

6. Volpato F, Lima-Morales D, Wink PL, Willig J, de-Paris F, Ashton-Prolla P, Barth AL. Pooling of samples to optimize SARS-CoV-2 diagnosis by RT-qPCR: comparative analysis of two protocols. *Eur J Clin Microbiol Infect Dis* 2020: 1-4.

7. Li L, Zhang W, Hu Y, Tong X, Zheng S, Yang J, Kong Y, Ren L, Wei Q, Mei H, Hu C, Tao C, Yang R, Wang J, Yu Y, Guo Y, Wu X, Xu Z, Zeng L, Xiong N, Chen L, Wang J, Man N, Liu Y, Xu H, Deng E, Zhang X, Li C, Wang C, Su S, Zhang L, Wang J, Wu Y, Liu Z. Effect of Convalescent Plasma Therapy on Time to Clinical Improvement in Patients With Severe and Life-threatening COVID-19: A Randomized Clinical Trial. *Jama* 2020: 324(5): 460-470.