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Early View

Original research article

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

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Running Title: Convalescent Plasma for severe COVID-19

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Take-Home Message

In this open-label, randomised clinical trial, two infusions of convalescent plasma therapy plus standard of care compared to standard of care did not result in a higher proportion of clinical improvement on day 28 in hospitalized patients with severe COVID-19.

Abstract

Background: The effects of convalescent plasma (CP) therapy hospitalized patients with coronavirus disease 2019 (COVID-19) remain uncertain. This study investigates the effect CP on clinical improvement in these patients.

Methods: This is an investigator-initiated, randomised, parallel arm, open-label, superiority clinical trial. Patients were randomly (1:1) assigned to two infusions of CP plus standard of care (SOC) or SOC alone. The primary outcome was the proportion of patients with clinical improvement 28 days after enrolment.

Results: A total of 160 (80 in each arm) patients (66.3% were critically ill and 33.7%, severe) completed the trial. The median age was 60.5 years (interquartile range [IQR], 48-68), 58.1% were men and the median time from symptom onset to randomization was 10 days (IQR, 8-12). Neutralizing antibodies titres >1:80 were present in 133 (83.1%) patients at baseline. The proportion of patients with clinical improvement on day 28 was 61.3% in the CP+SOC and 65.0% in the SOC group (difference, -3.7%; 95% Confidence Interval [CI], -18.8%-11.3%). The results were similar in the subgroups of severe and critically ill. There was no significant difference between CP+SOC and SOC groups in prespecified secondary outcomes, including 28-day mortality, days alive and free of respiratory support and duration of invasive ventilatory support. Inflammatory and other laboratorial markers values on days 3, 7 and 14 were similar between groups.

Conclusions: CP+SOC did not result in a higher proportion of clinical improvement on at day 28 in hospitalized patients with COVID-19 compared to SOC alone.

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can cause severe illness in a considerable proportion of infected patients leading to severe progressive pneumonia, multiple organ dysfunction and death [1, 2].

Passive immunotherapy using convalescent plasma (CP) collected from COVID-19 recovered patients has been advocated for the treatment of severe cases of this disease [3]. The US Food and Drug Administration issued an emergency use authorization for CP for the treatment of hospitalized patients with COVID-19 based on results of observational studies showing that CP was safe and could be associated with better clinical outcomes [4, 5]. Nevertheless, the two existing randomised clinical trials at the time of authorization [6, 7] and further multicentre randomised clinical trials[8, 9] have failed to demonstrate significant clinical benefit of CP in patients with severe COVID-19. The long duration of disease when intervention occurred and low neutralizing antibody titres in administered plasma may, at least partially, explain the absence of significant improvement in clinical outcomes in intervention groups in two of these trials [6, 7]. Other two larger clinical trials also did not find any benefit of CP on clinical outcomes. However, these studies used anti-SARS-CoV-2 spike IgG as a surrogate for neutralizing antibodies titres, impairing inferences that could be done on the baseline patient status regarding these antibodies and the investigated intervention[8, 9].

Given the heterogeneity regarding CP characteristics, including volume, number of doses and neutralizing antibody titres, as well as distinct levels of pre-existing antibody titres at baseline in both intervention and control groups, further clinical trials with

different administration strategies and distinct populations are necessary to better define the role of this therapy in hospitalized patients with severe COVID-19. In the present randomised clinical trial, we assessed the effect of two doses of 300 mL of CP therapy administered in the first 14 days of symptoms onset on clinical improvement in severe and critically ill COVID-19 patients.

Methods

Study design and oversight

PLACOVID was an investigator-initiated, unicentric, randomised, parallel arm, open-label, superiority clinical trial performed at a single COVID-19 reference hospital from Porto Alegre, Brazil.

This study was approved by the Brazilian National Commission for Research Ethics and the institutional review board of Hospital de Clínicas de Porto Alegre (approval number, 20-0158). Written informed consent was obtained from all study participants or their legal representatives. The trial was overseen by an external and independent data and safety monitoring board (DSMB). The trial protocol and statistical analysis plan are available in Supplementary Material 1. The trial was registered with the number NCT04547660 (https://clinicaltrials.gov/ct2/show/NCT04547660).

Participants

Patients admitted to the hospital were assessed for eligibility if they were 18 or older, had a positive reverse transcriptase polymerase chain reaction (RT-PCR) for SARS-CoV-2 (Supplementary Material 2), had less than 15 days of initial symptoms onset, and had severe respiratory disease, as defined by the presence of at least one of the

following: respiratory rate >30 breaths per minute in room air; oxygen saturation (O2) ≤93% in room air; arterial partial pressure of oxygen (PaO2)/fraction of inspired oxygen (FiO2) ≤ 300; need for supplemental O2 to maintain O2 saturation >95%; need for supplemental O2 by high flow nasal cannula, non-invasive ventilation, or invasive mechanical ventilation. Exclusion criteria were impossibility for any reason to perform the first plasma infusion within 14 days of the onset of symptoms; use of immunosuppressive drugs for other non-COVID-19 underlying diseases in the last 30 days before enrolment; pregnancy; history of serious adverse reactions such as transfusion anaphylaxis; disagreement of attending physician; and participation in other interventional randomised clinical trials.

Plasma donation procedures

A full description of plasma donation selection and procedures is shown in Supplementary Material 2.

Randomization and interventions

Patients were randomly assigned in a 1:1 ratio to receive two infusions 48 hours apart of 300ml of CP plus Standard of Care (SOC) or SOC alone. Randomisation was performed using computer-generated randomization with random block sizes of 2 or 4 and stratified according to the unit of hospitalization on enrolment (medical ward or intensive care unit [ICU]; unit of hospitalization on enrolment was used as a proxy for disease severity). Patients and investigators were unmasked, except interviewers performing follow-up telephone calls, who was unaware of the assigned trial group.

The SOC for COVID-19 was at the discretion of the treating physicians. The use of glucocorticoids, other immunomodulators, antibiotic agents, and antiviral agents was allowed. Remdesivir was not available in Brazil during the trial period.

Clinical and Laboratory data

Definitions of baseline variables assessed in the baseline are presented in the Supplementary Methods. Neutralizing antibodies were determined in all donor plasma units and on patient serum collected on days 0 and 3 (after the second plasma infusion) after enrolment, following previously described protocol[10]. Nasal and oropharyngeal swabs were collected at day 7 after enrolment or at hospital discharge. Blood samples were collected on days 0 (pre-infusion), 3 (post second infusion), 7 and 14 after enrolment in hospitalized patients.

Outcomes and follow-up

The primary outcome was the proportion of patients with clinical improvement 28 days after enrolment. Clinical improvement was defined as hospital discharge or reduction of 2 points in a 6-level ordinal scale. Levels on the scale were defined as follows: a score of 1 indicated not hospitalized; 2, hospitalized and not receiving supplemental oxygen; 3, hospitalized and receiving supplemental oxygen; 4, hospitalized and receiving oxygen supplementation administered by a high-flow nasal cannula or noninvasive ventilation; 5, hospitalized and receiving mechanical ventilation or extracorporeal membrane oxygenation; and 6, death. Prespecified secondary outcomes included RT-PCR for SARS-CoV-2 from nasal and oropharyngeal swab at day 7 from enrolment or hospital discharge (if earlier than 7 days); clinical status assessed using the 6-level ordinal scale and all-cause mortality at days 14 and 28 after enrolment; time to hospital discharge and days alive and free of supplemental oxygen support (non-survivors and patients requiring oxygen support at day 28 were assigned as 0 supplemental oxygen support free-days) within 28 days from enrolment; Sequential Organ Failure Assessment (SOFA) score and National Early Warning Score 2 (NEWS) 2 on day 7 after enrolment; and length of invasive ventilatory support (for those who received

mechanical ventilation). Adverse events were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or 4 adverse up to day 28 after enrolment or hospital discharge. Other prespecified exploratory outcomes were levels of serum inflammatory markers and cytokines, measured on days 3, 7 and 14 after enrolment (Supplementary Material 2).

Patients were followed daily up to day 28 after enrolment or hospital discharge by researchers who were aware of the trial-group assignments. For patients who were transferred to another hospital before day 28, a structured telephone call to the patient or the patient's family was conducted by an interviewer to assess the level on the ordinal scale at day 28.

Sample size calculation and protocol changes

We had originally planned for the trial to include 160 patients considering all-cause mortality within 28 days as the primary outcome and an absolute difference between arms of 20% to achieve a power of 80%, using the formula for two binomial proportions and two-sided tests, as described by Rosner [11]. However, due to the evolving knowledge on COVID-19, the steering committee assumed that a reduction of 20% in mortality would be very unlikely to occur and that estimated proportions for survival and death within 28 days were better suited for clinical response. Therefore, it was decided to submit a protocol amendment on July 27, 2020 (when eight patients had been included in the trial) modifying the primary outcome to clinical improvement on day 28 after enrolment.

In the revised sample size calculation, assuming a proportion of clinical improvement of 60% in the SOC group (Supplementary Material 2), a sample of 160 patients (80 in each arm) was estimated to achieve a power of 80% to detect an absolute difference of 20%

or greater in the proportion of patients with clinical improvement at day 28 with a 2-sided α level of .05. Other modifications are detailed in the study protocol in Supplementary Material 1.

Statistical analysis

Data were primarily analysed according to the intention to treat principle. The proportion of patients with clinical improvement on day 28 and relative risk were assessed using robust Poisson regression. Prespecified subgroups were defined according to the unit of hospitalization (medical ward [considered severe patients] or ICU [considered critically ill patients]) and mechanical ventilation needed on enrolment. Consistency of intervention effects on the primary outcome across these subgroups was assessed by means of interaction tests.

A post-hoc sensitivity analysis was performed for the primary outcome considering a per-protocol population. Secondary outcomes were compared Generalized Linear Models, according to the probability distribution of the outcome, or with the Wilcoxon-Mann Whitney test as appropriate. The potential effect of variables with a P value ≤0.20 at the baseline on the primary outcome was addressed in Poisson regressions models. Also as an exploratory analysis considering the clinical improvement outcome as a reduction of 1 point in the ordinal scale.

One pre-planned interim analysis for efficacy and safety evaluation after 80 patients with complete follow-up was conducted (Supplementary Material 2). The stopping rule for efficacy and safety was a P value<.05. There was no adjustment in the final threshold for statistical significance for sequential analysis.

All analyses were performed using the R software version 4.0.2 (R Core Team). No adjustments for multiplicity were performed. Thus, the results of secondary outcomes

and subgroup analyses should be interpreted as exploratory. A 2-sided P value of less than 0.05 was considered statistically significant.

Results

Patients

From July 15 to December 10, 2020, 496 patients were assessed for eligibility. Of these, 160 were eventually enrolled: 80 in the CP+SOC group, and 80 in the SOC alone group (Figure 1). The follow-up was completed on January 7, 2021. A total of 106 (66.3%) patients were located at the ICU and 54 (33.7%) at the medical ward at randomization. Baseline characteristics are shown in Table 1. The median age was 60.5 years (interquartile range [IQR], 48.0-68.0), 93 (58.1%) were men, and the median time from symptom onset to randomization was 10 days (IQR, 8-12). A total of 133 (83.1%) patients presented neutralizing antibody titres above 1:80 at randomization (median, 1:1280; IQR, 1:320-1:2560). All but 2 (1.2%) patients were receiving glucocorticoids at the time of entry into the trial. The baseline characteristics of the participants enrolled in CP+SOC group and of those enrolled in SOC alone group were similar, except for median neutralizing antibody titres, which were significantly higher in control than in intervention group, and interleukin-6 levels, which significantly higher in intervention thank in control group (Table 1 and Supplementary Material 2).

Interventions

Sixty-two (77.5%) patients received the CP from the same donor, while 15 (18.8%) received the second infusion from a distinct donor. The median neutralizing antibody titres from donors' plasma administered to patients from the intervention group was

1:320 (IQR, 1:160-1:960), which was significantly lower than baseline neutralizing antibody titres of patients previously to the infusion (P <0.001). Only five donors' plasma had neutralizing antibody titres lower than 1:80 (four 1:40 and one 1:20). Other characteristics of CP donors are shown in Supplementary Material 2.

Two patients allocated to CP (1.3%) did not receive any intervention (1 due to the lack of compatible plasma units and 1 patient that died before receiving transfusion) and other two patients (1.3%) did not receive the second plasma infusion. One patient allocated to CP received four additional plasma infusions pending on discretion of the attending physician. One patient allocated to the control group received one unit of CP, also on discretion of the ICU team.

On day 3, there was a significantly higher increase in neutralizing antibody titres in the intervention than in control group (P =0.001) in relation to titres at randomization (day 0) (Figure 2). The median neutralizing antibody neutralizing titres on day 3 was not significantly different between CP and SOC groups (1:5120 [IQR, 1:2560-1:10240] vs 1:2560 [IQR, 1:1920-5120]; P =0.19) (Figure 2).

Primary Outcome

On day 28, there was no significant difference between the CP+SOC group and the SOC alone group in the proportion of patients with clinical improvement (61.3% vs. 65.0%; difference, -3.7% [95% Confidence Interval [CI], -18.8 to 11.3]; Relative Risk [RR], 0.94 [95% CI, 0.74-1.19]; P=0.623) (Table 2). Results for the per-protocol population were similar to those of the main analysis (Supplementary Material 2). In subgroup analyses, tests for interaction were not statistically significant for subgroups defined by the unit of admission, need of mechanical ventilation, age and neutralizing antibody titres at baseline (Supplementary Material 2).

Secondary outcomes

CP+SOC group effects were not significantly different from SOC alone group for 28-day mortality (22.5% vs. 16.3%; difference, 6.2% [95%CI, -7.5%-20.7%; RR, 1.38 [95% CI, 0.73-2.63]; *P*=0.32), proportion of scores on the 6-level ordinal scale on day 28 (*P*=0.64) and median of days alive and free of respiratory support within 28 days (11.0 vs. 7.5; difference, -0.6 days [95%CI, -3.9-2.6]; *P* =0.44). There was no significant difference between groups in other secondary outcomes (Table 2; Supplementary Material 2).

The proportion of patients with a positive RT-PCR for SARS-CoV-2 from nasal and oropharyngeal swab on day 7 or on discharge if earlier was similar CP+SOC and SOC alone groups (76.3% of vs. 74.1%; difference, 2.2% [95%CI, -13.6-17.9]; RR, 1.03 [95%CI, 0.84-1.27 *P*=0.79) (Table 2). There was no statistically significant difference in inflammatory markers and other laboratorial parameters between groups on days 3, 7 and 14 both in all patient's population and in those who had completed the sequence of the three collections of laboratorial (Figure 2; and Supplementary Material 2).

Adverse events

The safety population included 79 patients who received at least one infusion of CP and 81 patients who received only SOC. A total of 52 (65.8%) and 48 (59.3%) of patients presented an adverse effect in CP+SOC and SOC alone groups, respectively (absolute difference 5.0% [95%CI. -10.0% to 20.1%]; RR, 1.08 [95%CI, 0.85-1.38]; *P* =0.51). CTCAE grade 3 or 4 adverse effects were noted in 50 (63.3%) and 44 (54.3%) of patients in intervention and control groups, respectively (absolute difference, 7.5% [95%CI, -7.8%-22.8%]; relative risk, 1.14 [95%CI, 0.88-1.48]; *P*=0.34). A full description of adverse effects is shown in Supplementary Material 2.

Post-hoc analyses

There was no significant difference in no prespecified subgroup analysis by age and neutralizing antibody titres at baseline (Supplementary Material 2). There was no significantly difference between intervention and SOC groups in Poisson regression models including variables with a P value ≤0.20 at the baseline (Table 3). There was also no significantly difference in clinical improvement on day 28, considering this outcomes as an one point reduction in the ordinal scale (61.3% vs. 68.8% in intervention and control groups, respectively; RR, 0.89 [95%CI, 0.71-1.12]; P =0.33).

Discussion

In this randomised clinical trial with severe and critically ill COVID-19 patients, CP therapy administered in the first 14 days of the onset of symptoms plus SOC did not significantly increase the proportion of clinical improvement on day 28 compared with SOC alone. Similar results were found in both critically ill and patients hospitalized at medical ward subgroups. These findings are consistent with previous randomised clinical trials that could not find significant benefit of CP in hospitalized patients with COVID-19 [6-9].

There were also no significant differences in clinical and laboratorial outcomes between intervention and control groups, including 14- and 28-day mortality, clinical status on days 14 and 28 assessed by an ordinal scale, days free of ventilation, days of hospitalizations and SOFA and NEWS2 scores. No difference was observed considering one point reduction in the ordinal scale as clinical improvement.

One strength of our study is that virtually all patients were treated with corticosteroids, mostly dexamethasone, as SOC and other drugs were not used. Additionally, this study was the first to evaluate some laboratory exams in patients' follow-up. The demonstration of absence of difference in these markers are consistent with clinical findings and help to reduce the level of uncertainty on the potential benefit of CP in severe COVID-19. Notably, in contrast to Li et al.[6] we could not find any difference in SARS-CoV-2 RT-PCR positivity rate between groups.

As found in a previous trial [7], most of the patients included in the study have already presented high levels (above 1:80) of neutralizing antibody titres at randomization. These titres were even higher in the SOC group (more than 75% of patients with titres equal or greater than 1:640). Two infusions of 300 ml of CP increased the levels of these antibodies on day 3 in the intervention group. The increase in neutralizing antibody titres from randomisation to day 3 was significantly higher in intervention group, and although the levels were higher in intervention than in control on day 3, this difference was not statistically significant probably because baseline levels in the former group was lower than the later. Nonetheless, this increase seemed to have no impact on both clinical and laboratorial outcomes, as indicated by the absence of any significant difference of inflammatory markers between groups in any point of collection from day 0 to 14. It must be acknowledged that the presence of high levels of neutralizing antibodies titres at randomization favours the null hypothesis, even though the effect on primary outcome was not affected when adjusted for this variable in the Poisson regression model. Furthermore, it is highly relevant from a pragmatic perspective, i.e., increment in antibody response in patients through passive administrations does not seem to be worthy in patients with severe COVID-19.

Notably, patients in the intervention group presented significantly higher levels of interleukine-6 at randomisation. Although interleukin-6 levels, as other variables analysed in Poisson regression models, did not significantly modify the effect of convalescent plasma on the outcome, and we at first attributed is as a casual difference that may be observed even with the randomisation process, we can not fully rule out that patients in intervention arm might be more severely ill. However, as shown in sensitivity analysis, if present, this would not be a disbalance able to affect the main results of the study.

A recently published meta-analysis evaluated the effect of CP on mortality and other clinical outcomes, including preprint publications and a press release of one randomised trial, could not find any significant difference of this strategy from SOC or placebo [12]. Given the heterogeneity of doses, neutralizing antibody titres and time of CP administration, along with the fact that most randomised trials have been prematurely interrupted, as pointed out by the authors, the certainty of the evidence was low to moderate for all-cause mortality and low for other clinical outcomes. We updated that meta-analysis using the same methodology, including data from a preprint publication [9] previously available only as a press release, and PLACOVID trial for mortality. The updated result remains non-significant, with low inconsistency and narrow confidence interval (RR 0.98; CI 95% 0.81-1.19; *P*=0.29; I2=16%) (Supplementary Material 2).

This study has some limitations First, it is an open-label study and data collectors were not blinded to the patients' group assignment. Despite not finding a positive effect of intervention, potential biases associated with this design cannot be completely ruled out. Second, our clinical trial is a single-centre study in a COVID-19 reference tertiary-care university-affiliated hospital, which may impair the generalizability of the findings; however, the overall findings point towards the same direction of previous multicentre

studies. Third, this study is composed mostly by critically ill patients, a group of patients whose potential benefit could be less expected. Nonetheless, similar results were found in both critically ill and patients hospitalized at medical ward. Finally, we were underpowered to evaluate the efficacy in patients with low neutralizing antibody titres. Despite the low number of patients, the exploratory analysis of patients with titre less 1:160 indicates a change in the direction of the effect (Supplementary Material 2). Along with previous studies suggesting a potential benefit with CP [13] or monoclonal antibodies in early periods of mild to moderate COVID-19 [14, 15] patients with severe COVID-19 and low levels of neutralizing antibodies might still be a group of interest for future studies with passive immunotherapy.

In conclusion, in severe or critically ill COVID-19 patients, almost all receiving corticosteroids as SOC, CP+SOC did not result in a higher proportion of clinical improvement on day 28 compared to SOC alone.

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Figures

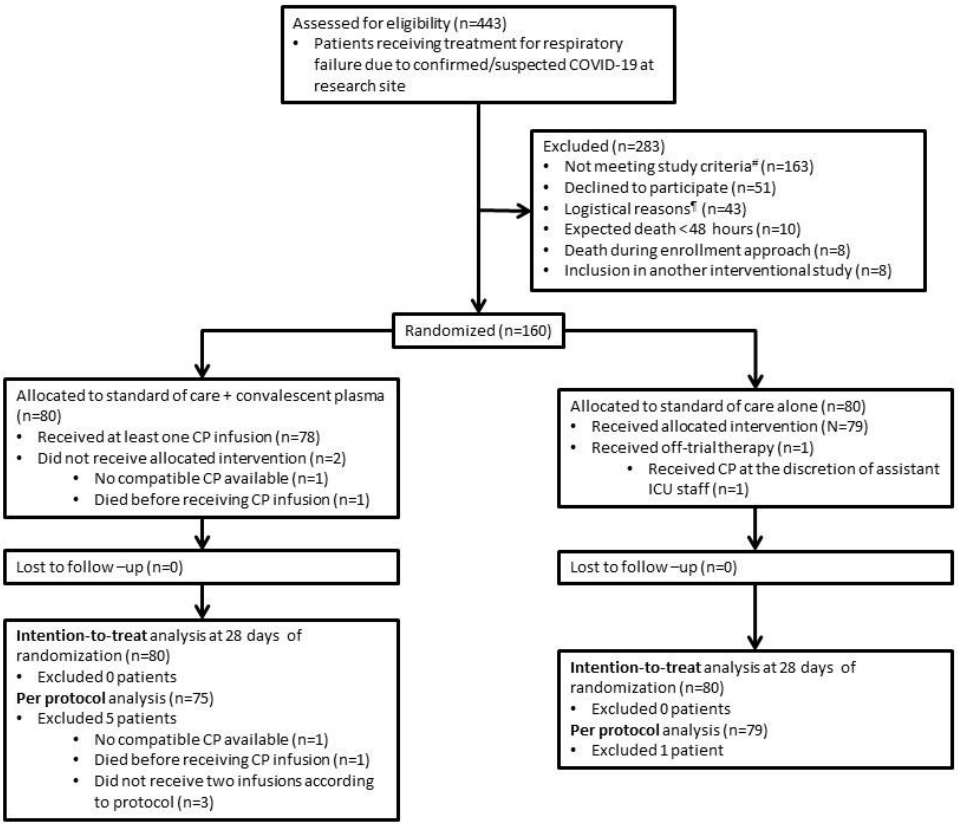

Figure 1. Flow diagram of patients in the clinical trial.

[a] Not meeting inclusion criteria: more than 14 days of symptoms (n=88); negative SARS-CoV-2 RT-PCR (n=38); previous use of immunosuppressants (n=30); no need for oxygen support (n=5); under 18 years old (n=2).

[b] Screened patients were sequentially approached until a maximum of four subjects enrolled daily due to limited capacity from the research team to collect blood samples and infuse convalescent plasma within advocated time interval. Eligible patients exceeding this limit were approached the next day or were excluded from the study if not complying with inclusion criteria anymore.

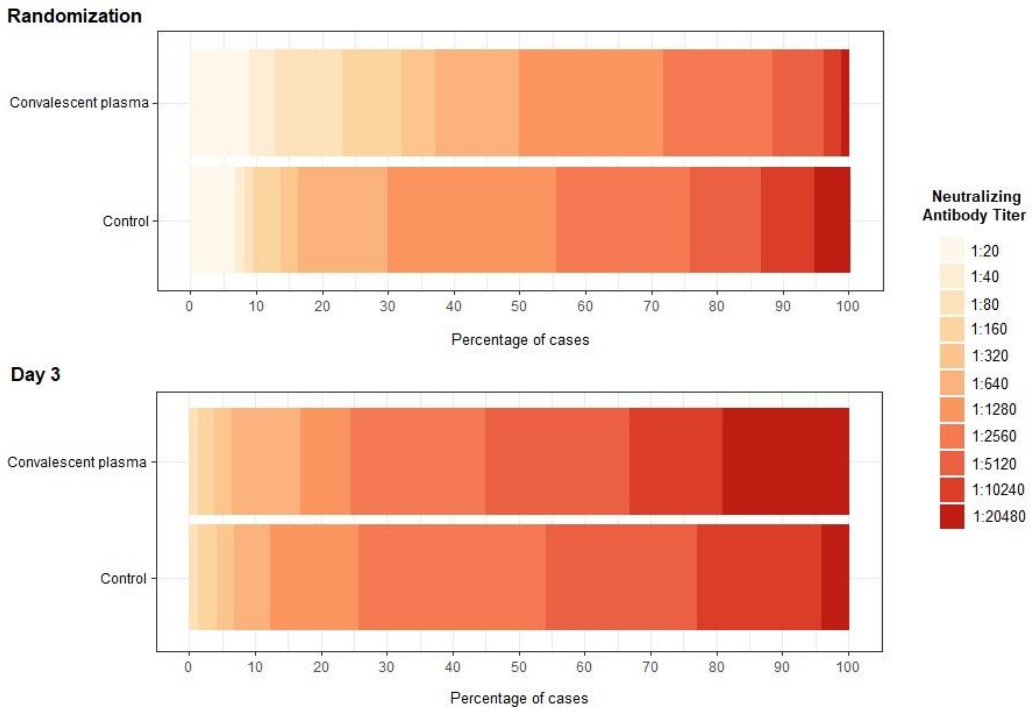

Figure 2. Distribution of neutralizing antibody titres in convalescent plasma and standard of care groups at randomization and on day 3.

Each color indicates the proportion of patients with a given neutralizing antibody titre. Titres of 1:10 or 1:20 were groups in 1:20 category. At randomization, n=80 (convalescent plasma) and n=78 (control); and on day 3, n=78 (convalescent plasma) and n=76 (control).

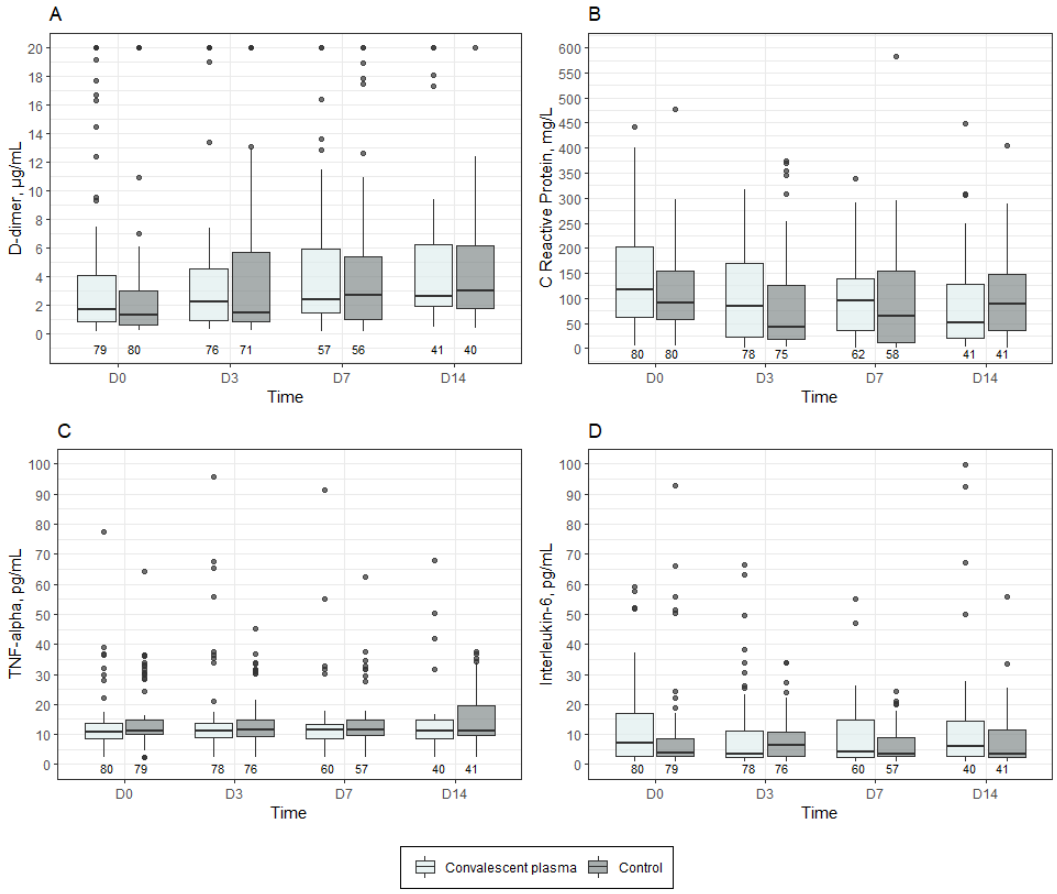

Figure 3. Inflammatory markers at randomization, and on days 3, 7 and 14.

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 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.

TABLE 1 Baseline characteristics of patients.

Characteristics	**Convalescent Plasma (n=80)**	**Control (n=80)**
Gender, male	49 (61.2)	44 (55.0)
Age, years	59.0 (48.0 - 68.5)	62.0 (49.5 - 68.0)
Age category [#]		
< 65 years	55 (68.8)	52 (65.0)
≥65 to <80 years	20 (25.0)	27 (33.8)
≥80 years	5 (6.3)	1 (1.3)
Comorbidities		
Diabetes	34 (42.5)	29 (36.3)
Hypertension	49 (61.3)	49 (61.3)
Cardiovascular Disease	19 (23.8)	16 (20.0)
Chronic Pulmonary Disease	13 (16.3)	9 (11.3)
Obesity	43 (53.8)	38 (47.5)
Randomization location		
Intensive Care Unit	53 (66.3)	53 (66.3)
Medical Ward	27 (33.8)	27 (33.8)
Time from symptom onset to randomization, days	10.0 ± 3.0	9.8 ± 3.2
Time from hospitalization to randomization, days [¶]	3 (2-5)	3 (2-5)
Score on six-level ordinal scale		
2- hospitalization without supplemental oxygen	0 (0)	1 (1.3)
3 - hospitalization plus supplemental oxygen	18 (22.5)	21 (26.3)
4 - hospitalization plus noninvasive ventilation or high-flow supplemental oxygen	28 (35.0)	24 (30.0)
5 - hospitalization plus invasive mechanical ventilation and/or extracorporeal membrane oxygenation	34 (42.5)	34 (42.5)
Vasoactive Drugs	17 (21.3)	14 (17.5)
NEWS 2	7 (6 - 10)	7 (6 - 9)
PaO2 / FiO2[+]	191 (134-246)	167 (100-258)
SOFA[+]	3.5 (2 - 7)	4 (2 - 7.8)

Characteristics	**Convalescent Plasma (n=80)**	**Control (n=80)**
Laboratorial findings at randomization		
Neutralizing Antibody Titre [+,§]	960 (160-2560)	1280 (640-2560)
Neutralizing Antibody Titre ≤ 80[§]	19/80 (23.8)	8/78 (10.3)
White blood cell count, cells $\times 10^3/\mu L$	7.7 (5.2 - 11.7)	8.2 (6.3 - 11.3)
Neutrophil count, cells $\times 10^3/\mu L$ [‡]	6.4 (4.2 - 8.4)	7.1 (4.9 - 9.4)
Lymphocyte count, cells/μL[‡]	0.8 (0.6 - 1.2)	0.8 (0.5 - 1.1)
Platelet count, cells $\times 10^3/\mu L$	224.3 ± 85.2	225.9 ± 81.5
C reactive protein, mg/L[‡]	117.4 (60.9 - 203.2)	90.6 (56.8 - 155.3)
D-dimer, μg/mL [+,‡]	1.7 (0.9 - 4.1)	1.3 (0.7 - 3.1)
Interleukin-6, pg/mL [+,‡]	7.0 (2.9 - 17.3)	3.7 (2.8 - 8.7)
Tumor necrosis factor-alpha, pg/mL[+,‡]	10.9 (8.5-13.6)	11.3 (10-14.9)
Medications at randomization		
Glucocorticoids[‖]	79 (98.8)	79 (98.8)
Antibacterials	73 (91.3)	71 (88.8)

Data are n (%), median (Interquartile Range) or mean ± standard deviation. Abbreviations: PaO2/FiO2, arterial oxygen partial pressure /fractional inspired oxygen; NEWS, National Early Warning Score; SOFA, Sepsis-Related Organ Failure Assessment. [#] P = 0.15. This variable was selected for post hoc analysis by a Poisson regression model to adjust the effect of intervention. [¶] P = 0.11. This variable was selected for post hoc analysis by a Poisson regression model to adjust the effect of intervention. [+]Ten control (12.5%) and 10 convalescent plasma (12.5%) had PaO2/FiO2 estimated from peripheral oxygen saturation /FiO2 ratio adjusted to the positive end-expiratory pressure. One patient (1.3%) in the intervention group and one (1.3%) in the control group did not collect blood samples for neutralizing antibody titre measurement. One (1.3%) patient in the intervention group did not collect D-dimer; one (1.3%) patient in the control group did not collect interleukin-6 and tumor necrosis factor-alpha at randomization. [§] P = 0.002 for median neutralizing antibody titres and P = 0.041 for neutralizing antibody titre ≤1:80. Median neutralizing antibody titres was selected for post hoc analysis by a Poisson regression model to adjust the effect of intervention. [‡] Leukocyte counts and inflammatory markers: P = 0.14 for median white blood cell count; P = 0.17 for median neutrophil count; P = 0.16 for C Reactive Protein; P = 0.11 for D-dimer; P = 0.046 for Interleukine-6; and P = 0.20 for Tumor Necrosis Factor-alpha. These variables were selected for post hoc analysis by a Poisson regression model to adjust the effect of intervention. [‖]Among patients who were treated with corticosteroids, 78 (99.7%) and 77 (97.5%) received dexamethasone in plasma convalescent and control groups, respectively. Other corticosteroid drugs were used in 10 (12.7%) and 16 (20.1%) patients from intervention and control groups, respectively.

Table 2. Primary and Secondary Endpoints.

	Convalescent plasma (n=80)	Control (n=80)	Absolute difference (95%CI)	Relative Risk (95% CI)	*P*
Primary outcome					
Clinical Improvement on day 28	49 (61.3)	52 (65.0)	-3.7% (-18.8% - 11.3%)	0.94 (0.74 - 1.19)	0.623
Secondary Outcomes					
Death on day 14	10 (12.5)	5 (6.3)	6.2% (-3.3% - 16.7%)	2.00 (0.72 - 5.59)	0.186
Death on day 2, No. (%)	18 (22.5)	13 (16.3)	6.2% (-7.5% - 20.5%)	1.38 (0.73 - 2.63)	0.321
Ordinal Scale on day 14	3 (1-5)	3 (1-5)		ND	0.679
1-Discharged	29 (36.3)	30 (37.5)			
2-Hospitalized with no supplemental oxygen	8 (10.0)	5 (6.3)			
3-Hospitalized with low-flow supplemental oxygen	6 (7.5)	8 (10.0)			
4-Hospitalized with high-flow supplemental oxygen and/or noninvasive ventilation	3 (3.8)	3 (3.8)			
5-Hospitalized plus invasive mechanical ventilation and/or extracorporeal membrane oxygenation	24 (30.0)	26 (32.5)			
6-Death	10 (12.5)	5 (6.3)			
Ordinal Scale on day 28	1 (1-5)	1 (1-5)		-	0.644
1-Discharged	44 (55.0)	46 (57.5)			
2-Hospitalized with no supplemental oxygen	2 (2.5)	2 (2.5)			
3-Hospitalized with low-flow supplemental oxygen	4 (5.0)	8 (10.0)			
4-Hospitalized with high-flow supplemental oxygen and/or noninvasive ventilation	0 (0)	1 (1.3)			
5-Hospitalized plus invasive mechanical ventilation and/or extracorporeal membrane oxygenation	12 (15.0)	10 (12.5)			
6-Death	18 (22.5)	13 (16.3)			
Days alive and free of respiratory support, days	11 (0-21)	7.5 (0-22)	-0.63 (-3.91 - 2.66)	ND	0.444
Duration of invasive ventilatory support, days	12 (6.5-16.5) [n=15]	13 (7-21) [n=17]	-1.93 (-7.76 - 3.80)	ND	0.515
Time from randomization to hospital discharge, days	10 (6-15) [n=44]	8 (5-17.8) [n=46]	0.25 (-2.72 - 3.23)	ND	0.869
	Convalescent plasma	**Control (n=80)**	**Absolute difference**	**Relative Risk**	***P***

	(n=80)		(95%CI)	(95% CI)	
Secondary Outcomes					
PaO2/FiO2 ratio on day 7	178.7 (144.6-246.1)	171 (137.8-255.5)	25.2 (-30.3 - 80.8)	ND	0.337
SOFA score on day 7	3.5 (2-7)	4 (2-7.8)	-0.28 (-1.02 - 0.46)	ND	0.463
NEWS2 score on day 7	8 (4.8-11)	8 (4-11)	0.25 (-0.73 - 1.23)	ND	0.617
NEWS2 score on day 14	7.5 (5-10)	9 (7.5-11)	-1.15 (-2.37 - 0.08)	ND	0.067
Positive RT-PCR on day 7	45/59 (76.3)	43/58 (74.1)	2.13% (-13.6% - 17.9%)	1.03 (0.84 - 1.27)	0.789

Data are n (%) or median (Interquartile Range).

Abbreviations: CI, Confidence Interval; ND, not determined; PaO2/FiO2, arterial oxygen partial pressure /fractional inspired oxygen; NEWS, National Early Warning Score; SOFA, Sepsis-Related Organ Failure Assessment; RT-PCR, Reverse Transcriptase Polymerase Chain Reaction.

TABLE 3 Sensitivity analysis by Poisson regression models including variables with a P value ≤0.20 at the baseline.

	Adjusted effect of convalescent plasma on primary outcome	
Variables [#]	**Odds Ratio (95% CI)**	**P value**
Age and sex	0.92 (0.73 - 1.17)	0.51
Neutralizing Antibody Titre	0.97 (0.77 - 1.21)	0.77
Interleukin-6	0.94 (0.75 - 1.18)	0.58
Time from hospitalization to randomization	0.92 (0.73 - 1.16)	0.47
D-dimer	0.94 (0.74 - 1.19)	0.58
C reactive protein	0.98 (0.78 - 1.23)	0.88
White blood cell count	0.90 (0.71 - 1.14)	0.38
Neutrophil count	0.90 (0.71 - 1.14)	0.37
Tumor necrosis factor-alpha	0.89 (0.71 - 1.13)	0.35

Abbreviations: CI, Confidence Interval.

[#] The effect of each variable alone was evaluated in different Poisson regression models including the group allocation, age and sex.

Hospital de Clínicas de Porto Alegre

Hemotherapy Service

Clinical Hematology and Bone Marrow Transplantation Service

Research project

CONVALESCENT PLASMA FOR TREATING SEVERE COVID-19 PATIENTS

Porto Alegre, first version April 2020 - last updated version January 2021

Title

Convalescent plasma for treating severeCOVID-19 patients

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1. Problem characterization

Since December 2019, the world has been facing a pandemic due to coronavirus 2 (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]). SARS-CoV-2 causes coronavirus disease, known as COVID-19.

Currently, there are no specific therapeutic options for treating SARS-CoV-2. No vaccines, monoclonal antibodies or drugs have been scientifically proven to be effective. Thus, the present study aims to investigate the use of convalescent serum, a therapy based on passive antibodies, for treating severe COVID-19 patients.

1.1. Antibody-based therapy

Passive antibody therapy involves administering antibodies against a specific agent to prevent or treat infectious disease caused by that agent. Conversely, active vaccination requires an immune response to occur, which requires time and varies by recipient. Thus, the administration of passive antibodies is a way of providing immediate immunity to susceptible/infected people. These antibodies can be obtained through convalescent serum from the plasma of recovered patients.

The use of this therapeutic modality to treat infectious diseases began around 1890[1] and continued until 1940, when antimicrobials became the first line of treatment for bacterial diseases. Nevertheless, the use of antibody-based therapy has been expanded to include pathologies caused by poisons, toxins and some viral infections (rabies, hepatitis A and B, chickenpox and pneumonia caused by the respiratory syncytial virus)[2].

Antibody-based therapy has evolved since 1975, when a method of producing monoclonal antibodies by immortalized B cells was discovered[3]. It thus became possible for the first time to produce large quantities of immunoglobulins of a predefined specificity and isotope *in vitro*. This technique culminated in the development of several monoclonal antibodies, which are mainly used to treat

neoplasms, rheumatological diseases and prevent rejection after transplants. However, the monoclonal antibody palivizumab has been approved for the prophylaxis of respiratory syncytial virus[4].

Passive antibodies have been used to treat a range of infectious diseases (Table 1)[2]. Unlike monoclonal antibody therapy for malignant diseases, when differentiation between host and tumor antigens is necessary, in infectious diseases recognizing antigens from microorganisms is easier due to their great differences from the host.

Passive antibodies are currently used to treat and prevent diseases caused by the hepatitis B virus, rabies, respiratory syncytial virus, *Clostridium tetani*, *Clostridium botulinum*, vaccinia virus, echovirus, and enterovirus. Because they are fast-acting biological weapons against such pathogens, this has led to the development of similar therapies for *Bacillus anthracis* toxins and Ebola virus[2]. In addition, convalescent serum can also be used in the form of hyperimmune immunoglobulin (H-IVIG), which has been proven to suppress viral load and alter the cytokine profile (IL-6 induces the release of IL-10, which can be important in neutralizing the effects of inflammatory cytokines), as well as mechanisms such as viral inactivation, in addition to dispensing with ABO compatibility. The greatest disadvantage of this type of therapy is the preparation time: convalescent plasma requires approximately 2-6 weeks, while H-IVIG requires approximately 6 months to produce[5-8].

In the 20th century, convalescent serum was used to treat outbreaks of viral diseases such as polio[9], measles[10], rubella[11] and influenza[12]. A retrospective meta-analysis of 8 studies that used convalescent serum to treat 1703 patients during the H1N1 (Spanish flu) epidemic in 1918 suggested that treated patients had lower mortality[13]. It is important to note that, historically, convalescent serum has been used when there was no means of measuring antibody titers, as well as in clinical studies that did not meet current criteria for randomization or blinding[14].

In addition, convalescent serum was used during the 2009-2010 H1N1 influenza virus pandemic in intensive care patients. Obtained through apheresis, it reduced inflammatory response and mortality[15]. During the 2013 Ebola epidemic, treating patients with convalescent whole blood led to increased survival rates[14]. Similar reports have also been published regarding H5N1[16,17] and H7N9[18].

1.2. The advantages of antibody-based therapy

One of the main advantages of antibodies is their high specificity; they do not alter host flora or select resistant microorganisms. Another advantage is their different biological effects, since they are natural products of the immune system and can interact with similar components. In addition to neutralizing toxins and viruses, they can activate the complement system and cause a direct antimicrobial effect, all of which are host independent. They can also stimulate cell cytotoxicity and opsonization. Antibodies also function as immunomodulators, being effective against microorganisms for which they do not mediate a direct biological effect[2].

Antibodies can function in their intact form or through fragments. In the intact molecule, the variable region binds to the antigen, while the constant region determines the biological properties of the immunoglobulin molecule, interacts with cellular receptors, and activates the complement. When working against a toxin or virus, only one fragment is necessary to mediate the binding effect of the variable region to the antigen. Intact molecules are required when efficacy depends on interaction with effector cells to mediate phagocytosis or to direct cell cytotoxicity[2]. The effect of mimicking and actively participating in the immune response against the pathogen is an important advantage of this type of therapy.

1.3. The disadvantages of antibody-based therapy

Its high specificity means that more than one antibody preparation may be required to target microorganisms with antigenic variation[2]. There is also a temporal issue, ie, the therapy is more effective when provided at the beginning of the infection. This makes rapid diagnosis essential. In infectious diseases, the effectiveness of this therapy is known to be reduced after symptom onset [19-21].

The use of convalescent serum also entails risks related to blood transfusions, such as infections due to the transmission of other pathogens, as well as reactions to serum constituents, which can lead to serum sickness. However, the rigorous modern techniques used in hemotherapy centers are designed to reduce these risks.

Administering plasma to individuals with lung disease (such as individuals with severe forms of COVID-19) can lead to complications, such as transfusion-related acute lung injury[22]. The majority of patients with this complication require intensive care and mechanical ventilation. However, critically ill COVID-19 patients may already be under invasive ventilatory support when they receive the transfusion. No data have been published about the combined mortality of transfusion-related acute lung injury and COVID-19.

Antibody-dependent enhancement is a theoretical risk related to the use of convalescent serum. This phenomenon has mainly been described in *in vitro* studies of viral diseases; certain mechanisms have been described in relation to coronaviruses and there is concern that antibodies against one type of coronavirus could enhance another viral chain[23]. However, since convalescent serum against SARS-CoV-2 would be composed of high antibody titers, there is less risk of antibody-dependent enhancement.

Another theoretical risk would be that the administration of passive antibodies could attenuate the body's natural immune response. A study that administered passive antibodies against respiratory syncytial virus prior to vaccination found that humoral immunity was attenuated, although cellular immunity was not altered[24]. This area should also be investigated for SARS-CoV-2 and, if the risk is real, individuals who have received passive antibodies should be vaccinated as soon as possible.

1.4. COVID-19: Dimensions of the problem in Brazil and worldwide

Since its inception in Wuhan, China in late 2019, COVID-19 has globally affected more than 1,900,000 people, causing more than 120,000 deaths. It was declared a pandemic by the World Health

Organization in March 2020. It is known that these numbers are increasing exponentially. By April 14, 2020, 23,430 cases and 1328 deaths had already been confirmed in Brazil [https://saude.gov.br/]. In the state of Rio Grande do Sul, 700 cases and 18 deaths had been reported by April 14, 2020 [http://ti.saude.rs.gov.br/covid19/], while in the city of Porto Alegre, 324 confirmed cases and 7 deaths had occurred by April 13, 2020 [https://sites.google.com/view/coronavirus-cievs-saudepoa/].

Severe Acute Respiratory Syndrome (SARS) is a major complication of patients with severe COVID-19. Because this is a new disease, data still differ regarding its real prevalence in affected populations. In a study of 138 patients, 20% developed SARS after 8 days, and mechanical ventilation was required in 12.3% of the cases[25]. In another study of 201 hospitalized patients in Wuhan, China, 41% developed SARS[26].

Hypoxemia is the primary symptom in cases that require hospitalization, with many patients needing high oxygen flow rates. Specific treatments to fight the virus are still under investigation. The World Health Organization and the Centers for Disease and Control and Prevention do not recommend the use of glucocorticoids, unless there are other specific indications related to the patient's underlying diseases[27,28]. Remdesivir is being evaluated in randomized trials due to its response to SARS-CoV-2 in *in vitro* studies and animal models for other coronaviruses[29-31]. Chloroquine and hydroxychloroquine inhibited SARS-CoV-2 in *in vitro* studies[32], and some small studies have suggested that they have clinical benefits[33-36]. A combination of lopinavir and ritonavir also demonstrated *in vitro* activity against SARS-CoV[37], and tocilizumab is being evaluated in a clinical trial[38.]

It is important to point out that the overall mortality of COVID-19 is 2.3%, although this percentage has shown great variability in different regions. However, patients who require intensive care have a mortality rate of approximately 50%[39].

1.5. Antibody-based therapy for coronavirus

Passive antibody-based therapy has already been used in other coronavirus outbreaks, such as SARS-CoV-1[40]. In the case of SARS-CoV-2, the anticipated mechanism of action would be viral

neutralization. However, other mechanisms may be possible, such as direct antibody-mediated cell toxicity and phagocytosis[14].

Possible sources of antibodies against SARS-CoV-2 include convalescent serum from patients who have recovered from COVID-19, monoclonal antibodies, or preparations from animal hosts. Although the latter two are under development, convalescent serum is the only currently available option. Furthermore, as more infected individuals recover, the potential number of donors increases.

The therapy is based on assessing antibody levels against SARS-CoV-2 in patients who have recovered from COVID-19. If these patients have reasonable levels of antibodies, plasma is collected for infusion for severe cases or even as prophylaxis (Figure 1).

In the 21st century, there have been two coronavirus epidemics associated with high mortality: the SARS-1 epidemic in 2003 and the Middle East Respiratory Syndrome epidemic in 2012. Although SARS-1 was contained, Middle East respiratory syndrome became endemic in the Middle East and was part of a subsequent outbreak in South Korea.

The largest study to have used convalescent serum in these epidemics included 80 patients with SARS-CoV-1 in Hong Kong[41]. Patients treated before D14 had a better prognosis, which was defined as hospital discharge before D22. In addition, patients with positive SARS-1 C-reactive protein and negative antibody titers had a better response to this therapy. Convalescent serum was also used in critically ill patients; 3 SARS patients in Taiwan were treated with 500 mL of convalescent serum[42] and survived, in addition to 3 Middle East respiratory syndrome patients in South Korea[43].

An analysis of 99 convalescent serum samples from recovered SARS patients found that 87 had antibodies, with a geometric mean titer of 1:61[44], which suggests that production decreases over time; thus, collection must be carried out promptly.

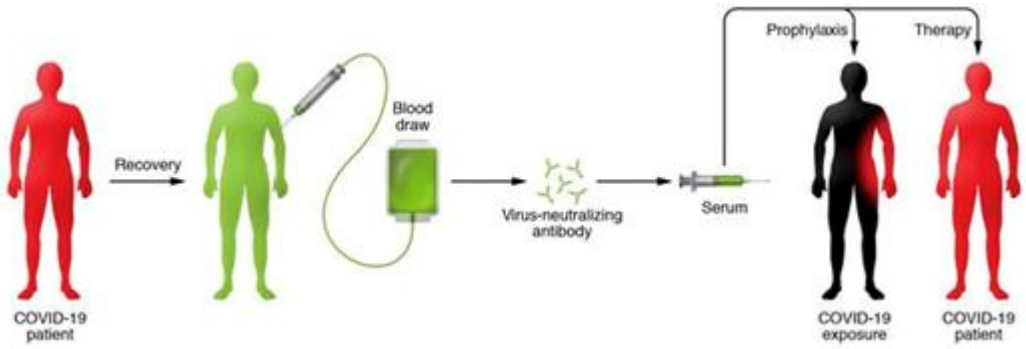
Chinese studies have reported using this therapy to treat critically ill patients with COVID-19[45]. Despite small samples, their results suggest that convalescent serum can reduce viral load and improve risk scores, such as the Sequential Organ Failure Assessment (SOFA) score.

Table 1: Microorganisms the antibody has been used against. Adapted from Casadevall, [E1] 2020[14].

Microorganism	**Human disease**
Bacillus anthracis	Anthrax
Bordetella pertussis	Whooping cough
Clostridium tetani	Tetanus
Clostridium botulinum	Botulism
Cryptococcus neoformans	Cryptococcosis
Cryptosporidium parvum	Cryptosporidiosis
Enterovirus	Gastrointestinal tract disease
Group A streptococcus	Necrotizing fasciitis
Hepatitis B Virus	Hepatitis B
Measles Virus	Measles
Mycobacterium tuberculosis	Tuberculosis
Neisseria meningitidis	Meningitis

Parvovirus	Aplastic anemia
Rabies virus	Rabies
Respiratory syncytial virus	Respiratory syncytial virus disease
Streptococcus pneumoniae	Pneumonia
Varicella-zoster virus	Chickenpox, pneumonia, herpes zoster
Variola major	Smallpox

Figure 1: The use of convalescent sera to contain COVID-19. Adapted from Casadevall, [E2] 2020[14].



2. Justification

The SARS-CoV-2 pandemic and its global implications for health systems and human behavior require emergency measures. Thus, if Brazil and the state of Rio Grande do Sul undergo the aggressive infection scenarios estimated by mathematical models, the health system will be overcrowded with seriously ill patients. The Hospital de Clínicas de Porto Alegre (HCPA), a reference center for humanized, quality health care in many sectors, is part of a network specifically developed to combat SARS-CoV-2. In line with its institutional mission to provide the best available care to patients, during the current crisis we propose using convalescent plasma to treat severe COVID-19 patients. Historically, in adverse situations, health services must prioritize effective and speedy use of the best available evidence. At various times in history, convalescent plasma has been shown to be effective in combating viral diseases, including coronavirus infections, and it is currently being investigated in several world centers. In this context, randomized clinical trials are needed to define the role, if any, of convalescent plasma in the treatment of patients with severe covid-19.

3. Hypothesis

Primary Null Hypothesis

The administration of convalescent plasma in patients with severe covid-19 has no effect of clinical improvement within 28 days compared to standard of care.

Conceptual Hypothesis

The administration of convalescent plasma in patients with severe covid-19 increases the proportion of patients with clinical improvement within 28 days compared to standard of care.

4. Objectives

Main

To evaluate the impact of convalescent plasma therapy on the frequency of clinical improvement within 28 days.

Specifics

1. To assess the impact of convalescent plasma on length of intensive care unit (ICU) stay;
2. To assess the impact of convalescent plasma on the time of mechanical ventilation and ventilatory parameters;
3. To assess the impact of convalescent plasma on SOFA score;
4. To assess the impact of convalescent plasma on length of hospital stay;
5. To evaluate the therapy's association with other clinical variables, including age, gender, time from symptom onset, symptoms presented, and comorbidities;
6. To assess the therapy's association with other complications, such as shock, acute cardiac injury and arrhythmia;
7. To assess the therapy's safety with respect to transfusion complications;
8. To determine neutralizing antibody titre values associated with clinical responses.

5. Methods

5.1. Study design

This investigator-initiated, unicentric, randomized, parallel arm, open-label, superiority clinical trial will evaluate patients severely affected by COVID-19 who receive convalescent plasma as an adjunct therapy during standard of care, as compared to a control group who receive standard of care alone.

5.2. Study population

The convenience sample will include patients aged18 years or older admitted to HCPA with a COVID-19 diagnosis who meet the study eligibility criteria.

5.3. Primary outcome

The primary outcome was the proportion of patients with clinical improvement 28 days after enrollment. Clinical improvement was defined as hospital discharged or reduction of 2 points in a 6-level ordinal scale. Levels on the scale were defined as follows: a score of 1 indicated not hospitalized; 2, hospitalized and not receiving supplemental oxygen; 3, hospitalized and receiving supplemental oxygen; 4, hospitalized and receiving oxygen supplementation administered by a high-flow nasal cannula or noninvasive ventilation; 5, hospitalized and receiving mechanical ventilation or extracorporeal membrane oxygenation; and 6, death.

5.4. Secondary outcomes

The clinical response criteria for convalescent plasma infusion include mortality from any cause by D14 and D28, length of ICU stay, duration of mechanical ventilation and impact on ventilatory parameters, improvement of the PaO2/FiO2 ratio by D7, change in SOFA score by D7, time to symptom resolution in days, change in the 6-point assessment scale (used by Goldman et al.[2]) and proportion of patients classified in each 6-point scale stratum by D14 and D28, change in the NEWS 2 score (UK Royal College of Physicians)[3] by D14 and D28 compared with randomized scores, length of hospital stay, ventilator-free days until D28, complications such as shock, acute cardiac injury, and arrhythmia, in addition to safety outcomes such as transfusion complications.

The laboratory response criteria for convalescent plasma infusion included: measurement of IL-6 and TNF-alpha (both by ELISA), C-reactive protein, troponin, lactate dehydrogenase, activated partial thromboplastin time, prothrombin time, fibrinogen and D-dimers (D0, D3, D7 and D14). On D7 (1 week after plasma transfusion), reverse transcriptase polymerase chain reaction (RT-PCR) assays of nasal and oropharyngeal swab specimens are planned for detectable RNA.

5.5. Donor selection

The donor search will be conducted by telephone contact with patients and collaborators (hospital employees) who underwent RT-PCR assays at HCPA and who meet the study inclusion criteria. There will also be a media campaign to recruit donors using current HCPA Hemotherapy Service recruitment tools.

Eligibility criteria for collecting convalescent plasma from donors

1. Age between 18 to 60 years;

2. Diagnosis of COVID 19 by RT-PCR according to World Health Organization criteria and/or IgG serology confirmed for SARS-CoV-2 by ELISA or chemiluminescence;

3. No symptoms for at least 14 days, preferably less than 40 days from symptom onset;

4. A second negative RT-PCR result for a nasal swab specimen;

5. Hemoglobin >12.5 g/dL for women (preferably nulliparous) and >13.0 g/dL for men;

6. Blood donation only according to Ministry of Health criteria (Portaria da Consolidação 5 28/9/2018 and RDC 34 11/6/2014).

5.6. Convalescent plasma recipients

Inclusion criteria for recipients

1. Age 18 years or older;

2. Diagnosis of SARS-CoV-2 infection by RT-PCR screening of nasal cavity or oropharynx swabs;

3. COVID-19 severe pneumonia defined according to World Health Organization criteria: fever and at least 1 of the following: respiratory rate >30 breaths per minute, acute respiratory failure, oxygen saturation <93%; OR oxygen supplementation by nasal catheter; OR ICU admission for any reason related to COVID-19 infection;

4. Less than 14 days since symptom onset;

5. No history of serious adverse reactions, such as transfusion anaphylaxis;

6. The attending physician's consent.

Exclusion criteria for recipients

1. Inability to perform the first plasma infusion within 14 days of symptom onset;
2. Use of immunosuppressants for any other underlying disease in the last 30 days;
3. Pregnancy.

5.7. Intervention

5.7.1. Description of the therapy

Convalescent plasma will be collected by apheresis. This procedure is performed when collecting only one component of whole blood, resulting in higher yield than the conventional donation process. In this case, the component of interest is the plasma, which contains antibodies against SARS-CoV-2. The collection will be carried out at the HCPA Hemotherapy Service using peripheral access venipuncture, through which the donor's blood will be drawn and subsequently reinfused. Processing is performed with disposable kits in a closed system, which allows the blood components to be separated by centrifugation. Plasmapheresis will be performed with a Spectra Optia leukapheresis system (Terumo BCT, Lakewood, CO, USA) and the respective intermediate density lipoprotein kit (or equivalent). The collected plasma volume will depend on certain donor factors: sex, weight, clinical condition on the day of collection, tolerance to the procedure, and blood count parameters on the day of collection. Maximum of 8 and 9 mL/kg of plasma are intended for women and men, respectively. The collected samples will be stored in appropriate bags in 300 ml aliquots at -20 to -30˚C.

5.7.2. Administration of convalescent plasma

A first 300 mL fresh-frozen plasma will be defrosted according to institutional routine and will be administered on the day of the patient's enrollment in the study (D0) and a second administration of 300 mL of fresh-frozen plasma on D2.The infusion will be performed according to institutional routines. The transfusion will be suspended if there are any adverse reactions and a transfusion reaction investigation will be opened.

5.7.3. Collection of biological material from convalescent individuals

In addition to the mandatory blood donation tests required (serology for hepatitis B, hepatitis C, Chagas disease, human T-cell lymphotropic virus type I/II, syphilis and nucleic acid amplification testing for hepatitis B, hepatitis C and HIV), additional blood samples will be collected from convalescent patients for subsequent measurement of SARS-CoV-2 specific IgG and IgM antibodies.

5.8. Ethical aspects

The ethical aspects of clinical trials are of crucial importance, since they are interventional studies, and patient safety is always a priority. However, it is important to point out the unique situation of proposing a therapy during a pandemic. When the lives of thousands of people are threatened and no specific therapy exists, it may be ethically acceptable to accept greater risks and offer patients interventions whose effectiveness has not been conclusively proven[46]. Similar discussions about experimental therapies occurred during the Ebola epidemic [47-49]. In general, in extreme situations and after discussion with the local community, novel therapies have been approved since no other effective alternatives existed.

To assess the ethical validity of clinical trials in unique situations, the Doctors Without Borders Ethics Council proposed an evaluation structure, which is attached to the project as Questionnaire 2[49].

It is important to point out that both donors and recipients (or their family/guardians) must provide written informed consent prior to the procedure. Donor risks: exposure to a blood bank environment during the COVID-19 pandemic; peripheral venous access (venous puncture), including local hematoma, pain, and leakage; possible adverse effects during donation, including plasmapheresis, although there is usually has a low incidence of serious complications. The rate of adverse events of any type during the procedure ranges from 4 to 5%. Complications are described in Table 2[50].

5.9. Interim Analysis Plan

An interim analysis will be performed when 50% of the patients have been randomized (80 patients, 40 in each group) and complete the 28-day follow-up. The interim analysis will be conducted confidentially by an independent consultant who is blinded to patient allocation. The results of the interim analysis will be reported to the Independent Data and Safety Monitoring Committee (IDSMC).

The criteria for interrupting the experimental treatment (which consists of an infusion of fresh frozen plasma on one or two occasions, depending on the attending physician's judgment) include serious adverse reactions attributed to the transfusion, such as: a) allergic reactions that worsen ventilatory or hemodynamic patterns; b) transfusion-related acute lung injury; c) febrile non-hemolytic reactions that impair proper sepsis management; c) any other serious adverse events as determined by the health care team.

The criteria defined for interrupting the study at the interim analysis are: a statistically significant difference in primary outcome (clinical response) and overall 28-day mortality between the intervention groups, or the occurrence of serious adverse events, whether predicted or not, in the

intervention group, as assessed by the IDSMC. Equivalence between the groups for the previously-mentioned outcomes at the interim analysis will not be considered a reason for interrupting the study.

5.10. Independent Data and the Safety Monitoring Committee

The IDSMC will consist of 5 members who are completely independent (directly or indirectly) of the study, and who may or may not be affiliated with the HCPA, including at least 1 hematologist, 1 intensive care physician, and 1 statistician. The other members will include other health professionals and researchers with recognized experience in clinical studies.

All adverse events observed in enrolled patients of either group will be immediately reported to the IDSMC. Any serious complications, even if not primarily associated with the intervention, will be regularly reported to the IDSMC. The IDSMC's evaluation will occur on a weekly basis (or when treatment interruption for an enrolled patient is required) and will be transmitted to the lead researcher, who will be responsible for implementing its decisions in the research group.

The activities of the IDSMC will include the assessment of adverse effects reported in enrolled patients and determining whether treatment should be interrupted when any relevant criteria are observed. Likewise, the IDSMC will be responsible for determining any adverse events not previously anticipated in the study design, as well as for their clinical investigation and final attribution to the intervention. The IDSMC will also be responsible for the interim analysis, as well as the decision to interrupt the study.

5.11. Plan for monitoring and analyzing adverse events

The monitoring and analysis of adverse events will be carried out by the Research Subcommittee on Safety and Quality, which is also responsible for other interventional studies on COVID-19 at HCPA (statement attached as a supplement).

Table 2. Complications described for apheresis procedures.

Complication	Signs/symptoms	Prevention/Treatment
Hypocalcemia	Perioral or peripheral paresthesia; taste changes; nausea; tremors; spasms; muscle contractions; tetany; convulsions; arrhythmias	Monitoring citrate infusion
Vasovagal and hypovolemic reaction	Pallor; cold skin; hypotension	Using the Trendelenburg position; crystalloid infusion; reinfusing the extracted volume;
Gas embolism	Depends on the involved organ	Checking that there is no air in the equipment lines connected to the patient;

Recipient risks: recipient risks are related to transfusion of the blood component. The plasma to be infused will be subject to current blood component safety legislation. However, despite such care, there is still a risk of transfusion reactions. Transfusion reactions can be divided into immediate and delayed. Table 3 shows the main immediate transfusion reactions, which are of great concern for the present project[50].

Table 3. Transfusion reactions associated with fresh-frozen plasma.

	Immune	Non-immune
Immediate	Febrile non-hemolytic reaction	Circulatory overload associated with transfusion
	Acute immune hemolytic reaction	Bacterial contamination
	Allergic reaction	Hypotension
	Transfusion-related acute lung injury	Non-immune hemolysis
		Metabolic disorders
		Air embolism
		Hypothermia

5.12. Sample Size and Statistical analysis

With a power of 80% and a 95% confidence interval, to find an increase from 60% to 80% in the rate of clinical improvement within 28 days between the control and intervention groups, 160 equally distributed patients (80 per group) will be required.

Depending on the normality analysis, the data will be presented as mean ± SD or as median and percentiles. Comparisons between groups will be made with Student's *t*-test, the Wilcoxon-Mann-Whitney test, ANOVA, or Pearson's chi-square test, as appropriate. Pearson's or Spearman's test will be used to determine correlations between the variables. The primary outcome will be assessed between the groups with the chi-square test. Survival analysis will be performed using the Kaplan Meyer curve. P <0.05 will be considered statistically significant. All statistical analyses will be performed in SPSS, version 21.0 (SPSS Inc., Chicago, IL, USA). The primary endpoint and the main secondary endpoints will be assessed in *a priori*-defined subgroups, namely: patients admitted to the ward vs. ICU at randomization, patients on vs. not on mechanical ventilation at randomization. A summary of the statistical analysis plan for the primary and secondary endpoints is summarized in the Table 4 (below).

Table 4. Statistical Analysis Plan

Outcome	**Description**	**Statistical test**
Clinical improvement by D28	Difference between the control and intervention groups in the frequency of clinically improved patients by D28.	Chi-square
	Time until clinical improvement in both groups. Deaths or hospitalizations on D28 will not be considered.	Log-rank
Deaths at D14 and D28	Difference between the control and intervention groups in the frequency of deaths by D14 and D28.	Chi-square
	Time until death in the control and intervention groups by D28. Living patients on D28 will not be considered.	Log-rank

Clinical condition at D14 and D28	Difference in frequency (proportion) between groups for patients at each clinical stage according to the 6-point assessment scale	Chi-square
NEWS 2 score in relation to baseline on D7 and D14	(1) Comparison of median group scores on D7 and D14 and (2) Change in NEWS 2 score between groups in relation to baseline.	(1) Wilcoxon-Mann-Whitney test (2) Simple linear regression.
Ventilator-free days until D28	Difference in the mean or median (according to the variable's distribution) of the time that participants are alive and ventilator-free until D28. The death rate will be zero.	Student's t-test or the Wilcoxon-Mann-Whitney test
Length of hospital stay	Difference in length of hospital stay between groups. (1) Mean and median among the survivors and (2) time until the outcome between the groups (the entire population) excluding deaths and patients still hospitalized on D28.	(1) Student's t-test or the Wilcoxon-Mann-Whitney test (2) Log-rank
Mechanical ventilation time	Difference in the mean or median (according to the variable's distribution) of the time that patients on ventilation at the time of enrollment remained ventilated. Only survivors will be considered. Patients on mechanical ventilation on D28 will not be considered.	Student's t-test or the Wilcoxon-Mann-Whitney test
Quantitative laboratory tests	(1) Comparison of mean or median scores between groups on D3 and D7 and (2) Change in relation to the baseline between the groups.	(1) Student's t-test or the Wilcoxon-Mann-Whitney test (2) Simple linear regression.
RT-PCR for SARS-CoV-2	Difference in frequency of positive tests on D7 (or earlier if discharged) between the intervention and control groups	Chi-square
Adverse effects	Difference in the frequency of adverse effects between the intervention and control groups.	Chi-square or Fischer's exact test

6. Expected results and impact

The expected result is to achieve the primary outcome: a higher rate of clinical improvement within 28 days in severe COVID-19 patients who receive convalescent plasma. Additionally, the expected improvement of other analysis parameters, especially length of ICU stay, duration of mechanical ventilation and the length of hospital stay, may provide benefits at both an individual and a community level. How this pathology will evolve in the local community is still uncertain, and there is a risk that the existing health resources may be insufficient for the demand. Thus, it is of great interest to develop therapies that can reduce the use of specialized services, such as those offered by HCPA.

7. Chronogram

The study's development and execution schedule is shown in Table 5.

7.1. Flowchart

- Patients with confirmed SARS-CoV-2 infection will be invited to participate in the study through telephone contact if they fulfill the above-mentioned clinical criteria for donors;
- Patients arrive at the HCPA for blood-donor screening; if the screening criteria are met, the above-mentioned specimen collection procedures are performed and plasmapheresis is scheduled;
- The donor returns to the blood bank for plasmapheresis, and a volume to be determined by individual characteristics will be collected;
- The plasma is processed in aliquots and frozen;
- The potential recipient is evaluated according to the above-described inclusion criteria;
- The recipient receives the plasma sample according to the institution's infusion routine;
- The recipient is monitored daily by the research team until hospital discharge;

- Telephone contact is made with the donor 30 days after the donation to check for any delayed signs/symptoms related to the donation.

Table 5. Project schedule

Steps (month/year)	6/2020	7/2020	8/2020	9/2020	10/2020	11/2020	12/2020
Ethics Committee Approval	✓						
Donor collection		✓	✓	✓	✓		
Recipient infusion		✓	✓	✓	✓	✓	✓

Steps (month/year)	1/2021	2/2021	3/2021	4/2021	5/2021
Data analysis	✓	✓	✓		
Article writing				✓	✓

8. Risks and difficulties

The convalescent plasma collection and infusion risks have been described above. The study has some viability issues: the 160 total patients (80 in the intervention group) required to find the estimated outcome is a considerable number and it is not known how long it will take to achieve it. It is possible that both the number of cases and a lack of consent will be barriers.

Another possible difficulty concerns finding available donors. The donation process is completely voluntary, which means that some convalescent patients will not be available. The Hemotherapy Service team is working daily on fundraising strategies to overcome this barrier. These strategies will also be applied to the overall project, and it is hoped that during the pandemic community involvement will be widespread.

8.1. Detailed description of protocol modifications

All modifications in the original protocol were submitted to the Brazilian National Commission for Research Ethics and approved by this commission and the institutional review board of Hospital de Clínicas de Porto Alegre.

The first amendment was submitted to the Brazilian National Commission for Research Ethics on June/20/2020 when the recruitment of patients had not been initiated. It comprised: 1) The Inclusion of a secondary endpoint: time to clinical improvement (days) defined by breathing at room air with oxygen saturation >95% for two consecutive days; 2) The inclusion of dosage of interleukin-6, tumor necrosis factor-a, C reactive protein, troponin, dehydrogenase lactate, prothrombin time, activated partial thromboplastin time, fibrinogen and D-dimers ate days 0, 3, 7 and 14; and 3) The addition in the inclusion criteria the need of a diagnosis of SARS-CoV-2 infection confirmed by a positive RT-PCR. The respective reasons for these modifications were as follows: 1) Clinical improvement was a relevant endpoint that had not been initially registered (the definition of clinical improvement was further

changed to a modified ordinal scale of clinical status as more studies have been published using such outcome); 2) The budget for laboratorial exams was only available after the first protocol submission; and 3) The need of confirmatory RT-PCR as an inclusion criterion was planned but missed in the first protocol submission.

On July/27/2020, when 8 patients had been included, none had finished the 28-day follow-up (outcome blinded to principal investigators and only known to data collectors), a second amendment was submitted to the Brazilian National Commission for Research Ethics. This second amendment included the major modification: Change of the primary outcome from mortality in 30 days to proportion of clinical improvement, as defined by breathing at room air with oxygen saturation >95% for two consecutive days or hospital discharge since there was no need for rehospitalization in the next 7 days, in 28 days. As more trials have been published, it became clear that clinical improvement was a relevant outcome and that a difference in mortality of 20% would not be expected with two plasma infusions. This first definition of “clinical improvement" was thought to be more “objective” than the 2 points improvement in ordinal clinical scale, and it was believed to be more appropriate to an open trial. As more trials become available using this scale as a primary or key secondary outcome, we assume that it would be a more suitable outcome to compare to other studies. The clinical status by the 6-level ordinal scale had already been recorded by data collectors.

Additional modifications were submitted to the committee in this amendment: 1) Specification that comparison of SOFA score would be performed on day 7; 2) Addition of a follow-up nasal and oropharyngeal swab for RT-PCR for SARS-CoV-2 on day 7 after infusion; 3) Addition of recruitment of plasma donors with no positive RT-PCR if they have a positive IgG for SARS-CoV-2 by ELISA or immunoluminescence; and 4) Addition of eligibility criteria of no more than 14 days from the onset of symptoms and exclusion of those not able to receive the plasma during this period. The respective reasons for these modifications were as follows: 1) The time for SOFA assessment had been defined but the specification had been missed in previous versions; 2) The budget for RT-PCR on day 7 in all patients was not previously available; 3) The acceptance of donors with positive IgG serology modification was to increase the number of eligible donors since many patients willing to donate plasma

had clinical symptoms compatible with covid-19 and had not performed or had a negative RT-PCR; and 4) The first inclusion criteria referred to no more than 3 days of hospitalization as an eligibility criterion. This had been shown problematic for two reasons: i) the research team was not prepared to recruit all patients within this short timeframe and many potential candidates with early symptoms would be lost; and ii) Since our hospital is a reference covid-19 hospital in the state of Rio Grande do Sul, many patients had been transferred from another hospital and, although they were within the first days in our hospital, we would have to account for this period of hospitalization in other hospitals and it would undesired workload. Finally, it seemed more plausible that time from onset of symptoms would better define those who would most likely benefit from convalescent plasma.

The trial was registered on clinicaltrials.gov on September/14/2020. At this time, 64 patients had been included and 27 had finished the 28-day follow-up (outcome blinded to principal investigators and only known to data collectors). No interim analysis by the independent committee had been performed. The trial was registered with the modification in the definition of clinical improvement, which was defined by improvement of 2 points from randomization in a 6-point ordinal severity scale (6 points, death; 5 points, hospitalization plus extracorporeal membrane oxygenation or invasive mechanical ventilation; 4 points, hospitalization plus noninvasive ventilation or high-flow supplemental oxygen; 3 points, hospitalization plus supplemental oxygen (not high-flow or noninvasive ventilation); 2 points, hospitalization with no supplemental oxygen; 1 point, hospital discharge). The registration in clinicaltrials.gov after the initiation of the study was due to difficulties and challenges imposed by putting forward an investigator-initiated clinical trial during a pandemic in a highly hit region. The rapidly evolving knowledge regarding convalescent plasma and clinical covid-19 management in general has indicated that some adjustment would be necessary to improve the quality of the trial and comparability with other studies. Some procedures were also depending on an additional budget that has been approved and released as the study was ongoing, as detailed above.

In the registration on clinicaltrials.gov we also included an additional secondary endpoint: days alive and free of respiratory support. This endpoint was judged to be relevant in the assessment of therapeutic interventions for severe covid-19 patients and had not been considered previously.

These modifications as registered in clinicaltrials.gov were communicated to the Brazilian National Commission for Research Ethics on January/05/2021. We also included the evaluation of neutralizing antibodies titers, since the budget for dosing neutralizing antibodies had been approved in the last two weeks of patients enrollment.

Annex 1: Data extraction form.

Confidential

Record ID	_____
Patient's full name	_____
Patient record	_____
Allocation:	○ Control ○ Intervention
Severity	○ Severe (infirmary) ○ Life risk (ICU)
Date of birth	_____
Age	_____
Sex	○ Female ○ Male
Comorbidities	☐ Diabetes ☐ Obesity ☐ Hypertension ☐ Heart disease ☐ Respiratory disease ☐ Vascular disease ☐ Other comorbidities
Which one?	_____
Which one?	_____
Which one?	_____
Day of symptom onset	

	_____
Symptoms	☐ Diarrhea ☐ Fever ☐ Cough ☐ Odynophagia ☐ Myalgia/Arthralgia ☐ Dyspnea ☐ Headache ☐ Other symptoms
Which ones?	_____
Which ones?	_____
Which ones?	_____
Date of study entry (D0)	_____
Date of hospital admission:	_____
ICU admission	○ Yes ○ No
Date of ICU admission:	_____
Mechanical ventilation start date	_____
Extubation date	_____
Diagnostic examination date	_____
ADMISSION LABORATORY EXAMINATIONS	
Hemoglobin	_____ (g/dL)
Total leukocytes	_____ ((x10^3/µL))

_____

Segmented leukocytes

_____
((x10³/µL))

Lymphocytes

_____
((x10³/µL))

Platelets

_____
(x10³/µL)

Total bilirubins

_____
(mg/dL)

GOT

(U/L)

GPT

_____
(U/L)

Urea

_____
(mg/dL)

Creatinine

_____
(mg/dL)

Lactate

_____
(mmol/L)

Gasometry - pH

_____

Gasometry - pO2

_____
(mmHg)

Gasometry - pCO2

(mmHg)

pO2/Fi02

Auxiliary table for calculating PO2/FIO2

[Attachment: Auxiliary table for calculating PO2]

Bilateral ground-glass opacities in the imaging exam (Tomography)

- ○ Yes
- ○ No
- ○ Not performed
- ○ Other

Which ones?

Bilateral interstitial infiltrate in the radiological examination

- ○ Yes
- ○ No
- ○ Other

Which one?

Hospitalization

Complications

- ☐ Shock
- ☐ Cardiac dysfunction/arrhythmia
- ☐ Renal failure with need for hemodialysis
- ☐ Other

Which one?

Which one?

Which one?

Death

- ○ Yes
- ○ No

Cause of death

Date of death

	_____
Date of ICU discharge	_____
Date of hospital discharge	_____
Date of symptom resolution (absence of supplemental O2 and sat >95% for 2 consecutive days)	_____
Use of corticosteroids	○ Yes ○ No
Which one?	_____
Which one?	_____
Which one?	_____
Use of antibiotics	○ Yes ○ No
Which one?	_____
Which one?	_____
Which one?	

Plasma Infusion

Date and time of first plasma infusion	_____
Volume of first infusion	_____
Date and time of second plasma infusion	_____

Volume of second infusion	_____
Transfusion reactions	○ Yes ○ No
Which ones?	_____
Which ones?	_____
Which ones?	_____
Specific laboratory analysis parameters D0	
RT-PCR	○ Detected ○ Not detected ○ Inconclusive
Six-point severity scale D0	○ 6 Death ○ 5 Hospitalization plus extracorporeal membrane oxygenation (ECMO) or invasive mechanical ventilation ○ 4 Hospitalization plus noninvasive ventilation or high-flow supplemental oxygen ○ 3 Hospitalization plus supplemental oxygen (not high-flow or noninvasive ventilation) ○ 2 Hospitalization with no supplemental oxygen ○ 1 Discharge
NEWS2 score:	_____
SOFA scale	_____
C-reactive protein D0	_____

_____
____

_____
____

Troponin D0

(pg/mL)

Lactate dehydrogenase (LDH) D0

(U/L)

D-dimer D0

_____
____
(µg/ml)

Activated prothrombin time D0

_____
____
(seconds)

Prothrombin time D0

_____
____
(seconds)

Fibrinogen D0

_____
____ (mg/dL)

Specific laboratory analysis parameters D3

Six-point severity scale D3

- ○ 6 Death
- ○ 5 Hospitalization plus extracorporeal membrane oxygenation (ECMO) or invasive mechanical ventilation
- ○ 4 Hospitalization plus noninvasive ventilation or high-flow supplemental oxygen
- ○ 3 Hospitalization plus supplemental oxygen (not high-flow or noninvasive ventilation)
- ○ 2 Hospitalization with no supplemental oxygen
- ○ 1 Discharge

NEWS2 score:

_____

C-reactive protein D3

_____
____

_____
____
_____
____

Troponin D3

_____
____
(pg/mL)

LDH D3

_____
____
(U/L)

D-dimer D3

_____
____
(µg/ml)

Activated prothrombin time D3

(seconds)

Prothrombin time D3

(seconds)

Fibrinogen D3

_____
____ (mg/dL)

	_____
	_____
	_____
	_____
Specific laboratory analysis parameters D7	
RT-PCR	○ Detected ○ Not detected ○ Inconclusive
Six-point severity scale D7	○ 6 Death ○ 5 Hospitalization plus extracorporeal membrane oxygenation (ECMO) or invasive mechanical ventilation ○ 4 Hospitalization plus noninvasive ventilation or high-flow supplemental oxygen ○ 3 Hospitalization plus supplemental oxygen (not high-flow or noninvasive ventilation) ○ 2 Hospitalization with no supplemental oxygen ○ 1 Discharge
NEWS2 score:	_____
SOFA scale	_____ _____
C-reactive protein D7	_____ _____
Troponin D7	_____ _____ (pg/mL)
LDH D7	_____ _____ (U/L)
D-dimer D7	_____ _____ (µg/ml)
Activated prothrombin time D7	

_____
____

_____
____
_____
____
(seconds)

Prothrombin time D7

_____
____
(seconds)

Fibrinogen D7

(mg/dL)

pO2/Fi02

Specific laboratory analysis parameters D14

Six-point severity scale D14

- ○ 6 Death
- ○ 5 Hospitalization plus extracorporeal membrane oxygenation (ECMO) or invasive mechanical ventilation
- ○ 4 Hospitalization plus noninvasive ventilation or high-flow supplemental oxygen
- ○ 3 Hospitalization plus supplemental oxygen (not high-flow or noninvasive ventilation)
- ○ 2 Hospitalization with no supplemental oxygen
- ○ 1 Discharge

NEWS2 score:

_____

C-reactive protein D14

_____
____

Troponin D14

_____
____
(pg/mL)

LDH D14

_____
____
(U/L)

	_____ ___
	_____ ___
D-dimer D14	_____ ___ (µg/ml)
Activated prothrombin time D14	_____ ___ (seconds)
Prothrombin time D14	_____ ___ (seconds)
Fibrinogen D14	_____ ___ (mg/dL)

Specific laboratory analysis parameters D28	
Six-point severity scale D28	○ 6 Death ○ 5 Hospitalization plus extracorporeal membrane oxygenation (ECMO) or invasive mechanical ventilation ○ 4 Hospitalization plus noninvasive ventilation or high-flow supplemental oxygen ○ 3 Hospitalization plus supplemental oxygen (not high-flow or noninvasive ventilation) ○ 2 Hospitalization with no supplemental oxygen ○ 1 Discharge
Ventilator-free days until the 28th day	_____
Rehospitalization in up to 7 days:	○ Yes ○ No
Date	_____
Reason	_____

Annex 2: Ethical evaluation questionnaire for projects conducted during times of crisis

(Adapted from the Doctors Without Borders Ethics Committee [48]).

Research and methodology questions

(1.1) What is the main research question and why is it important?

The main research question is: What is the impact of convalescent plasma as a treatment for critically ill COVID-19 patients regarding mechanical ventilation time? This outcome is important because severe COVID-19 patients need mechanical ventilation, practically the only treatment to have shown a benefit, despite not being specific for viruses. In general, these patients require prolonged mechanical ventilation (over 14 days), which overloads ICUs.

a. Why is this question scientifically important? What knowledge gap will it fill? Decreasing mechanical ventilation time in these patients is an essential outcome, since it reduces the length of ICU stay and reduces the complications associated with prolonged mechanical ventilation, ie, increased mortality and length of hospital stay. Since COVID-19 is a new disease, treatment knowledge is still scarce although studies on this subject are filling this knowledge gap.

b. Why is the issue important to the affected community? Brazil, Rio Grande do Sul, Porto Alegre and the HCPA are all dealing with the pandemic. Management strategies are urgently needed for COVID-19 patients currently admitted to the HCPA ICU.

c. Are there other research-related questions? If so, why was this chosen as the main one? Since COVID-19 is a new disease in our population, this study will make a great contribution not only regarding the proposed intervention, but in understanding the clinical behavior of these patients. The main question was chosen according to the study's methodological limitations and reports from other international researchers.

d. What are the potential losses if the research is not conducted? As previously mentioned, the number of suspected and confirmed COVID-19 cases at HCPA is increasing daily. For critically ill patients in need of intensive care, there is no evidence of a specific treatment for COVID-19. This study is a way of using previous knowledge that has been applied in other crisis situations to improve patient outcomes and the hospital's capacity to serve them.

(1.2) How are the proposed methodology and analysis suitable for these questions?

Since the study involves an intervention, a clinical trial is the proposed research tool. However, in the current situation, it is not possible to conduct a clinical trial with a comparative, randomized or blinded group. Therefore, a single-arm clinical trial was chosen.

a. How does the study design provide a better way to answer questions? Since this is an intervention study, the best methodology would be that of a clinical trial. However, since these critically ill COVID-19 patients are hospitalized in ICUs and require specific care with respect to isolation, a blind study cannot be conducted. Proposing a control group would be unethical since there is no established therapy for the disease. Thus, all patients who meet the study inclusion criteria must receive treatment.

b. What review methodology was carried out before the study was submitted? The PubMed database was searched for publications related to convalescent plasma therapy for COVID-19, which are scarce. Media information on studies conducted in other countries was also used.

c. What ethical considerations shaped this methodology? What is the justification for the standard treatment for this condition? There is no standard treatment for COVID-19, although the international scientific community is researching more effective therapeutic possibilities.

(1.3) In what context will the research be carried out? What influenced this study design?

The study will be conducted in the context of the COVID-19 pandemic, a new disease that is infecting people around the world. The context was crucial in developing the research methodology since there is an urgent need to investigate treatments for this pathology.

a. Have the needs of the local community been taken into account? What is the researcher's strategy for engaging the community in the research? The world is engaged in dealing with COVID-19, and HCPA is a reference center for treating patients severely affected by this pathology. The local community trusts HCPA's work. With the emergence of this new challenge, the institution must naturally seek ways to improve patient care.

b. What collaborative research exists in relation to this project? Each location affected by the COVID-19 pandemic is working according to its characteristics and needs to combat it. The present project involves a collaboration between several areas of HCPA, including: the blood bank, laboratories, the ICU, the emergency department, etc.

c. To what extent can partnerships be structured and fair? Each collaborative unit has a well-defined role in conducting the research.

d. How do the researchers intend to increase local research capacity through this project? HCPA employees are engaged in measures to more effectively combat the pandemic at our institution.

e. Was the research submitted to the local ethics committee? It will be submitted to the National Research Ethics Commission.

(1.4) Will the researchers have training and protection?

The researchers are HCPA collaborators. Each one has been trained in their specific area of expertise for the project.

a. Does the research team have expertise in the subject? Since this is a new disease, there is no way to obtain expertise in the subject at the present time.

b. How will the research team's training be carried out? Each area will act according to its previously established training activities.

c. What risks will the team be exposed to? How can they be minimized? The team's main risk is SARS-CoV-2 infection. This will be minimized by using HCPA's pre-established protection measures.

d. Do any team members have conflicts of interest? No.

Respecting and protecting the participants and their communities

(2.1) What are the anticipated benefits and harms? The benefits are a therapy that can decrease mechanical ventilation time, length of hospital stay, and mortality among critically ill COVID-19 patients.

a. In light of the best available evidence and relevant experience, what are the expected harms? The risks of administering convalescent plasma have been described in detail in the research project.

b. What is the possible social damage? The research team will dedicate part of its time to research rather than direct assistance.

c. What is the process for monitoring unknown harm? A team member will be assigned to assess possible harm during the event.

(2.2) What are the plans for obtaining consent?

Donor consent will be obtained after a clear explanation of the risks and benefits of donation. Recipient consent will be obtained through family members/guardians, since, in their clinical situation, the patients cannot provide it.

a. What information will be provided? The reasons for conducting the research, information from the researchers, and details about the risks and benefits of the intervention.

b. Will the information be provided in written and oral form? Yes, by the research team.

(2.3) What is the plan to maintain confidentiality?

Patient data is already subject to the hospital's confidentiality policy. Thus, only active researchers will be able to participate in the data collection and updating process.

(2.4) What is the plan for accessing, storing and distributing the collected biological material?

The HCPA blood bank already has specific policies for plasma collection and storage. These rules will be applied to the present research project.

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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, 5×10 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 $TCID_{50}$/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 −20ºC 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 $MgSO_4$); 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.

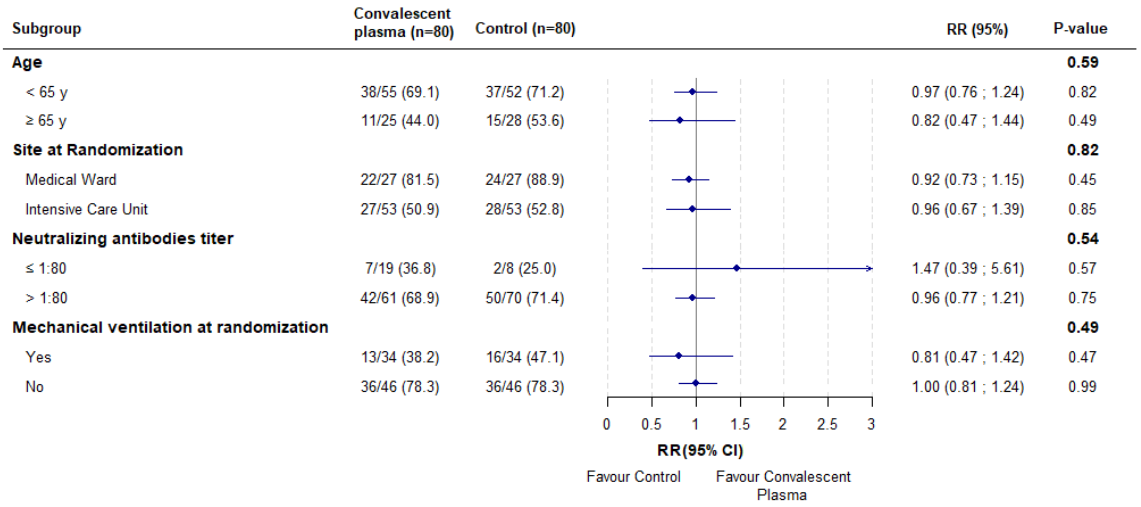
Characteristics of Donors	**n=48**
Male sex	31 (64.9)
Age, years	37 (32.6 - 46.8)
Donor ABO/RhD	
A+	21 (43.8%)
O+	13 (27.1%)
B+	3 (6.3%)
AB+	2 (4.2%)
A-	8 (16.7%)
O-	1 (2.1%)
B-	0
AB-	0
Characteristic of Plasma Donation Procedures	**n=91**
Plasma collection volume, mL	626 (512 - 693)
Procedure duration, 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.

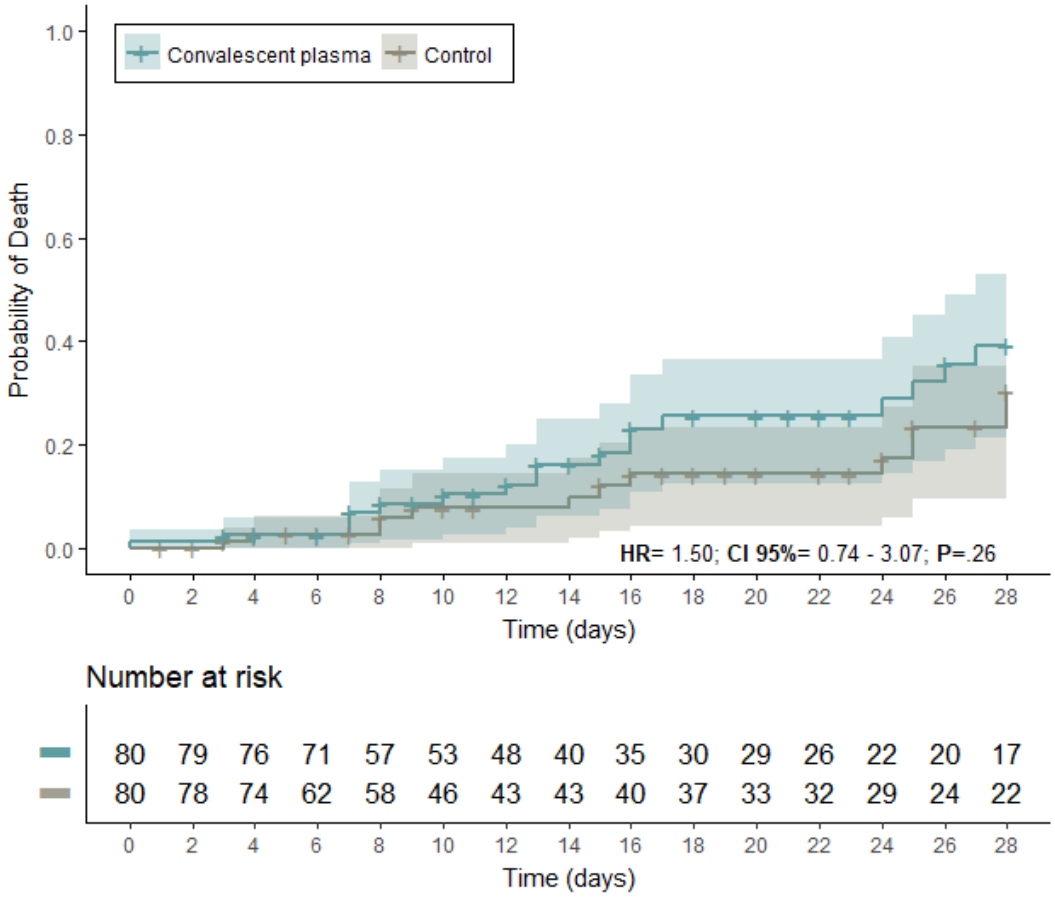
	Convalescent plasma (n=75)	**Control (n=79)**	**Absolute difference (95%CI)**	**Relative Risk (95% CI)**	**p-value**
Primary outcome					
Clinical Improvement on day 28	47 (62.7)	52 (65.8)	-3.1% (-18.4 - 12.1)	0.95 (0.75 - 1.21)	0.683
Secondary Outcomes					
Death on day 14	9 (12.0)	5 (6.3)	5.7% (-3.97 - 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

FIGURE 1 Primary outcome according to subgroups.



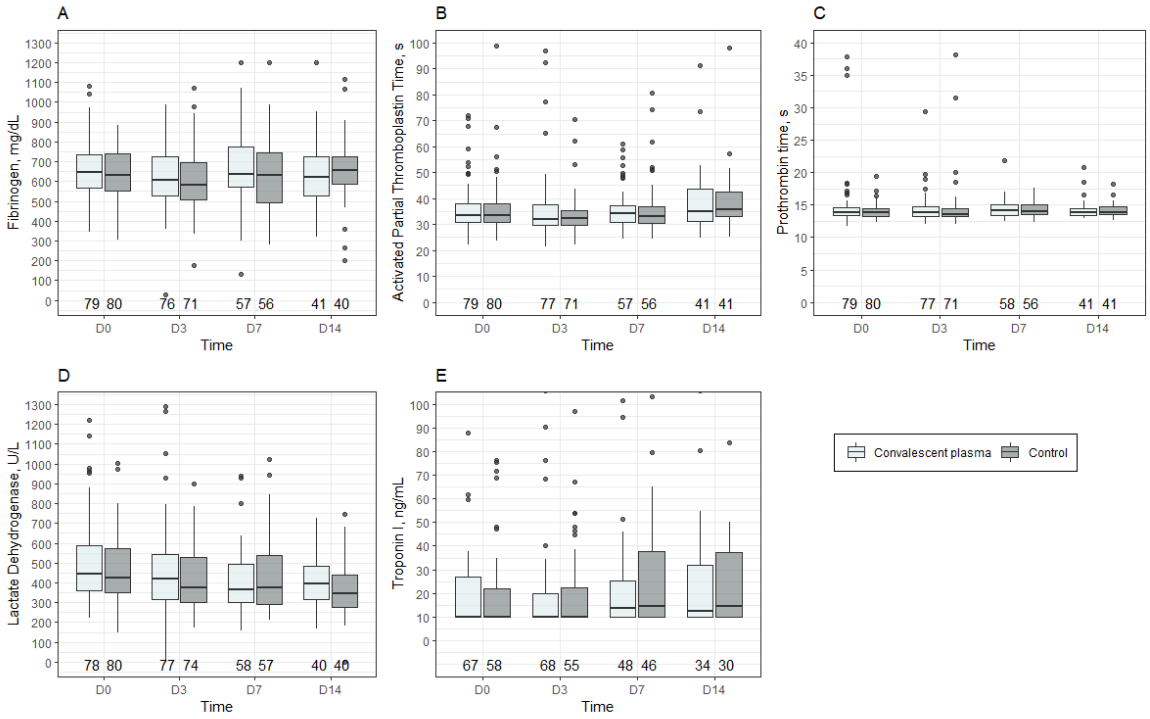
Unit of admission and need of mechanical ventilation were prespecified subgroups. Age and neutralizing antibody titres comprise post hoc analysis.

FIGURE 2 Probability of death in the intervention and control groups.



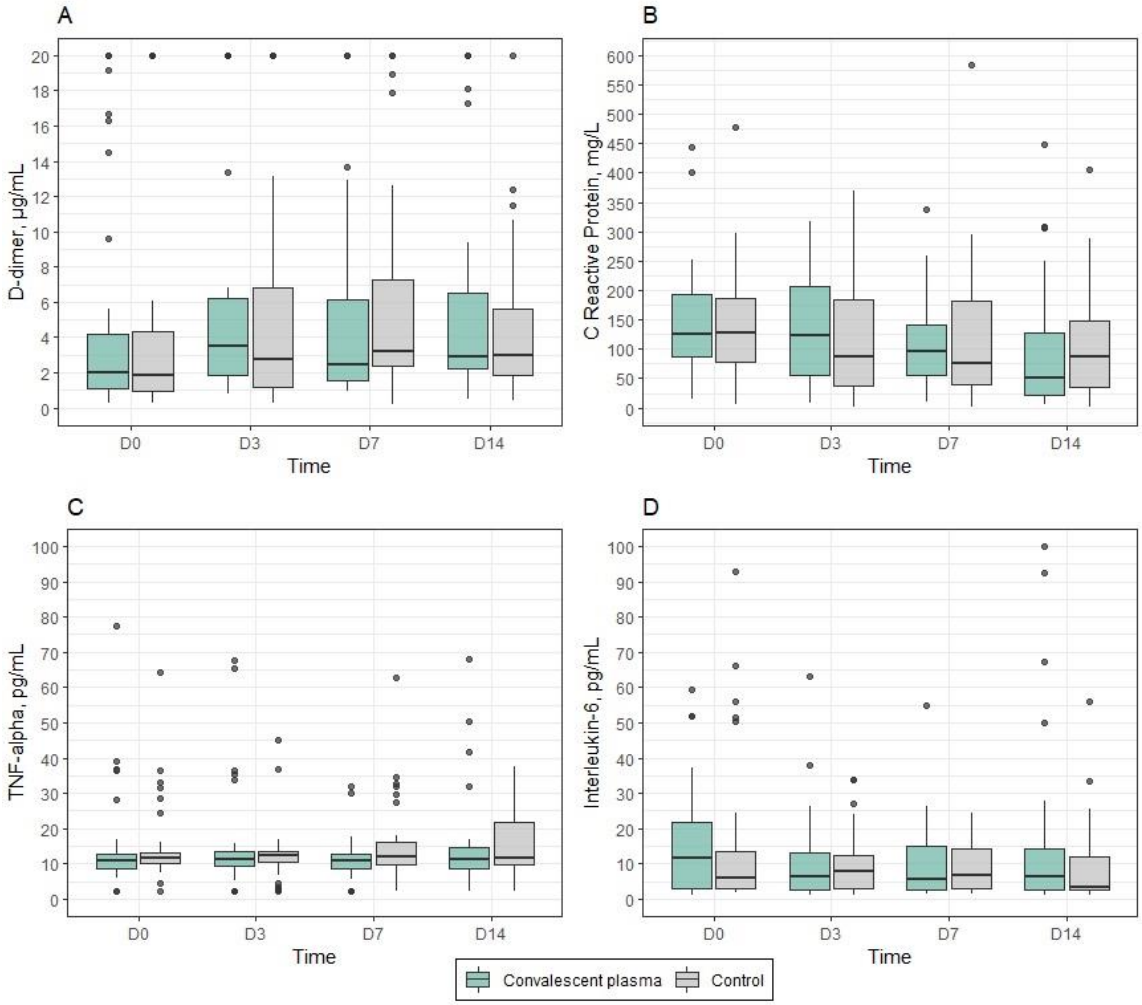
Shadow areas represent the 95% Confidence Interval.

FIGURE 3 Additional laboratorial parameters.



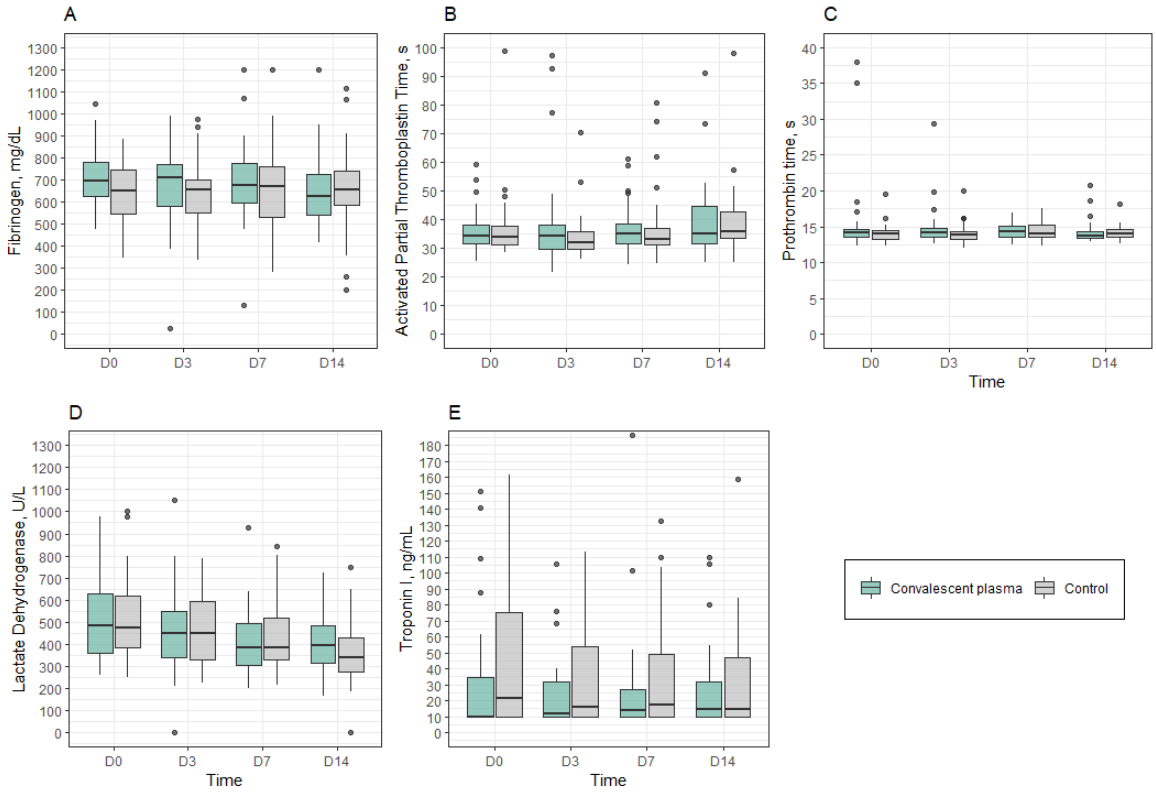
The box plot inner horizontal lines indicate median; boxes, interquartile range (25th and 75th percentiles); whiskers extend to the most extreme observed values with 1.5 times the interquartile range of the nearer quartile, and dots represent observed values outside that range. The numbers of patients 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 [#]		
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

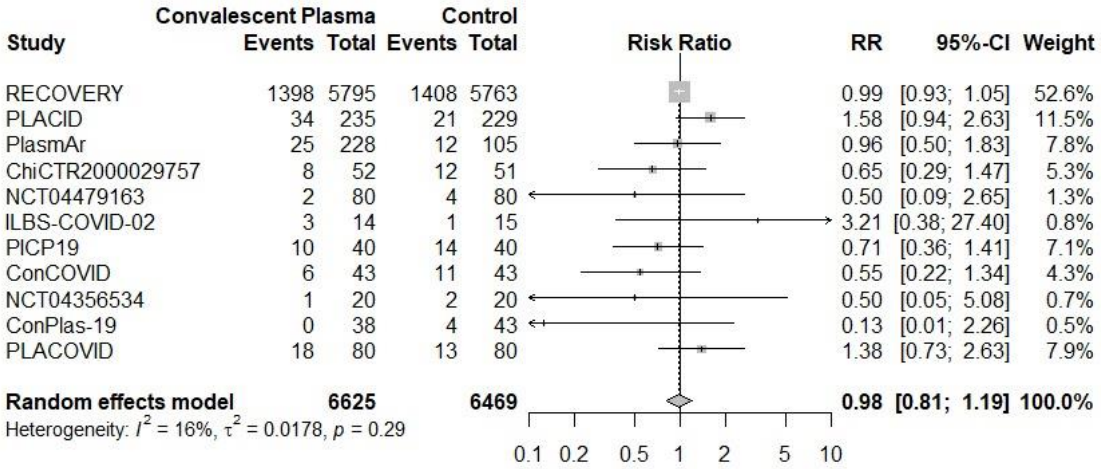
[#] Absolute number of each type of adverse effect.

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 [#]		
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 PLACOVID clinical trial and RECOVERY pre-print results. RR, risk ratio; CI, Confidence Interval.

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