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Effect of convalescent plasma as complementary treatment in patients with moderate COVID-19 infection

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Abstract

Introduction: South America is one of the regions most affected by the COVID-19 pandemic. Specific and affordable treatments are needed to treat SARS-CoV-2 infection. Evidence regarding the use of convalescent plasma in COVID-19 patients is still limited. We compared the safety and efficacy of COVID-19-convalescent plasma administration as a complement to standard treatment in the early management of patients with moderate SARS-CoV-2 infection.

Methods: We carried out a random double blinded, placebo-controlled trial that compared standard treatment plus convalescent plasma (CP) or plus non-convalescent plasma in the management of COVID-19 patients. The main outcome was survival and secondary endpoints included: length of hospitalisation (LOH), days from treatment to discharge, time to clinical improvement or death within a 28-day period, and adverse reactions to treatment.

Results: Administration of CP with antibodies against SARS-CoV-2 did not affect patient survival, RR = 1.003, 95% CI (0.3938, 2.555). These results led to terminate the RCT prematurely. However, early treatment of COVID-19 patients with CP tended to decrease the LOH while the delay in CP treatment was associated with longer hospitalisation. In addition, delay in CP treatment negatively affected the recovery of the respiratory rate.

Conclusion: Use of CP for the treatment of COVID-19 patients is safe and its early use can decrease the LOH and improve respiratory function. Early administration of antibody-rich CP could contribute to decrease the negative impact of COVID-19 pandemic in patients with impaired immune response.

KEYWORDS
convalescent plasma, COVID-19, Ecuador, passive immunity, SARS-CoV2

1 | INTRODUCTION

The current COVID-19 pandemic that started in China in December 2019 is affecting most countries around the world.[1] The negative socio-economic impact of the pandemic is difficult to estimate due to the magnitude of the problem; however, the major impact of the disease has severely affected low and middle-income countries.[2] At time of writing this manuscript, there are more than 220 million cases and

more than 4.5 million deaths all over the world, with a lethality rate of approximately of 2%. In South America, there are approximately 37 million cases and more than 1.1 million deaths, becoming one of the regions most affected by the pandemic with a lethality rate of 3%.[3]

Currently, there are few specific treatments against SARS-CoV-2 infection, the causative agent of COVID-19.[4] The therapeutic management of COVID-19 targets its clinical presentation that includes severe acute respiratory syndrome, hyperinflammatory state, blood vessels damage, and thrombosis.[5] Accordingly, the following pharmacologic agents are currently in use: inflammatory inhibitors, low molecular weight heparins, antiviral drugs, and hyperimmune immunoglobulins (Igs).[5]

Passive immunity is a therapy used in medicine when there are no specific treatments available for an infectious disease as is the case for SARS-CoV-2 infection.[6,7] The use of convalescent plasma is considered safe with limited adverse events.[8] The World Health Organization and other international agencies have endorsed well-designed studies to test the use of plasma or serum from convalescent patients in severely ill COVID-19 patients.[9–11] Due to the absence of specific treatments for COVID-19, the infusion of plasma of convalescent patients (rich in specific antibodies against SARS-CoV-2) has been evaluated with inconsistent results.[12] Some studies show no beneficial effect while others show reduced progression of the infection and decreased mortality.[13,14] In this randomised double-blind clinical trial, we compared the safety and efficacy of COVID-19-convalescent plasma administration as a complement to standard treatment versus non-immune plasma control added to standard treatment, in the early management of patients with moderate SARS-CoV-2 infection.

2 | MATERIALS AND METHODS

2.1 | Subjects studied

This was random double blinded, non-convalescent plasma (NCP)-placebo controlled clinical trial. A simple randomization scheme was used to allocate participants in the two treatment groups. Patients were recruited from three large hospitals in Quito-Ecuador from May 2020 to January 2021 (when the last patient completed follow-up). Two of these hospitals were designated to specifically treat patients with COVID-19 by the Ecuadorian Ministry of Public health early in the pandemic while the other one was included later on, due to the high demand for acute health care service. Potential study participants were invited to participate and were screened for eligibility within 24 h prior to receiving the study treatments (Figure 1). Standard treatment consisted of symptomatic control and supportive care for COVID-19, mostly based on Ecuadorian COVID-19 treatment guidelines for hospital practice. Supportive common treatments included oxygen administration, antibacterial medication, steroids, other anti-inflammatory drugs, and anti-coagulants.

Patients that met the following criteria were included in this trial: (1) signed informed consent; (2) ≥18 years of age; (3) from both sexes; (4) COVID-19 diagnosis based on: any molecular testing-polymerase chain reaction (RT-PCR), clinical diagnosis or lung imaging tests; (5) patients with impairment of previously normal lung function defined with a SaO_2 <90% at 0.5FiO_2 and/or with an increased O_2 need in the previous 24 h upon admission[6]; Patients with a score of 5–6 on the New Early Warning Scale for COVID-19 patients (NEWS2; see below and Appendix). Patients were not included if they: (1) were pregnant or lactating; (2) had diagnosis of cancer, HIV

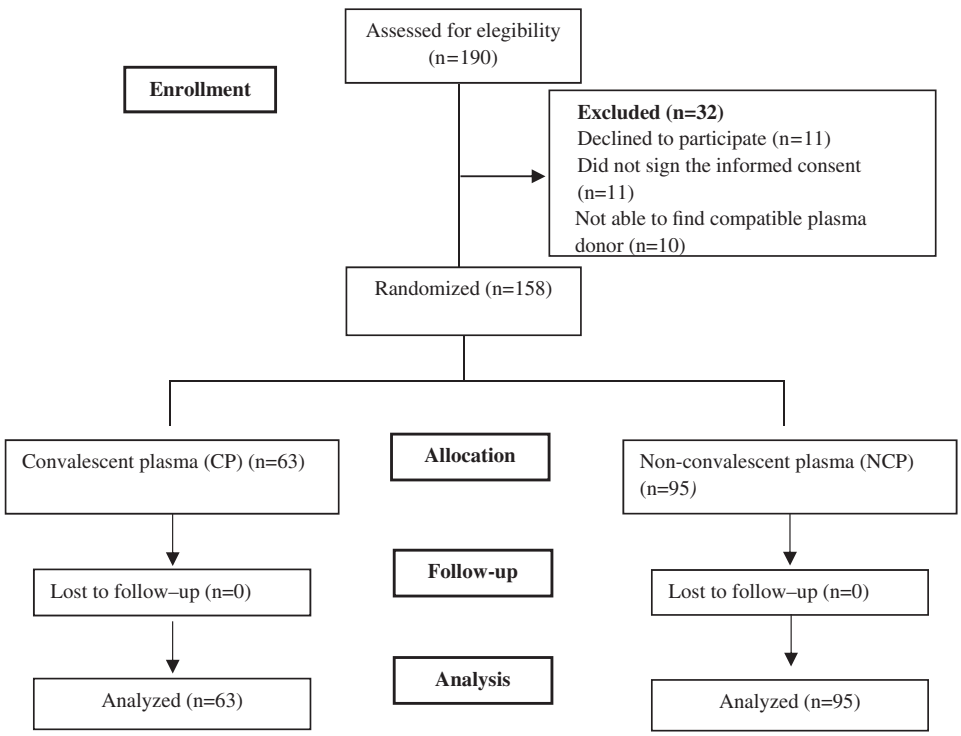

FIGURE 1 Flow chart of study

infection, superimposed systemic infections, liver failure, renal failure, chronic obstructive pulmonary disease, pulmonary fibrosis, and restrictive pulmonary pathologies; (3) had been receiving immunosuppressants for a different condition than SARS-CoV-2 infection; (4) were participating in any other clinical trial; (5) patients with history of previous blood/derivate transfusion.

2.2 | Ethical statement

This study was approved by the ethics committee of Universidad San Francisco de Quito (P2020-025M) and the Ministry of Public Health of Ecuador. Written informed consent was obtained from all study participants or their legal representatives. The study was registered in the International Clinical Trials Registry Platform, ISRCTN85216856; Study record 38 262.

2.3 | Convalescent and non-convalescent plasma transfusion

Plasmas obtained by The Ecuadorian Red Cross (see below) were labelled and coded following the model of the internationally standardised International Society of Blood Transfusion (ISBT) system for the identification and labelling of each blood product.[15] Plasmas were delivered from the Red Cross to the hospitals at −20°C.

Coded plasmas obtained from the Red Cross were randomly assigned to consecutive patients according to the availability of plasma, the chronology of admission of the plasmas to the hospital, and the compatibility of the blood group of available plasmas. Previous to plasma transfusion, ABO typing was performed and only matched plasma was used. Before transfusion, plasmas were thawed in a water bath and were transported from the hospital-blood bank to the patient's room in thermal white boxes without markings together with the hemovigilance sheet where the participant's name was recorded. The blinding of the study was maintained since hospital personnel at the transfusion medicine services, physicians, and the participants were unaware which plasma was convalescent and which plasma was control.

Participating patients received either 5 ml/kg body weight of convalescent or non-convalescent plasma for one occasion. Plasma was administered at approximately 10 ml for the first 15 min, which was then increased to approximately 100 ml/h with close monitoring by medical personnel and members of the research team. Adjustments in the infusion rates were allowed based on the patient's risk for volume overload and tolerance, at the discretion of the treating physicians. No premedication was given before plasma transfusions.

2.4 | Outcome measures

The primary endpoint of the study was survival rate before 28 days since onset of plasma transfusion treatment. The secondary endpoints included length of hospitalisation, days from treatment to discharge, time to clinical improvement or time to death within a 28-day period, and adverse reactions to plasma treatment. In addition, we assessed the number of days from start of symptomatology to admission and the number of days from start of symptomatology to plasma treatment, as important variables for statistical analysis since they could influence the outcomes. Clinical status during hospitalisation was evaluated using the NEWS2 on days 1, 3, 7, and 21. The National Early Warning Score 2 (NEWS2) is an early warning instrument based on six parameters: respiratory rate, oxygen saturation, systolic blood pressure, and heart rate, level of consciousness, temperature and supplemental oxygen dependency.[16] In the pre-COVID-19 era the NEWS2 was used to identify patients with higher risk of poorer clinical outcomes; at the time we started the study, NEWS2 was commonly used to assess patients´ clinical status. Clinical improvement was defined as a reduction on at least two points on the NEWS scale or discharge from the hospital. Patient discharge criteria included body temperature returned to normal for longer than 3 days, respiratory symptoms significantly improved without the need for oxygen support, SaO_2 > 90%. Clinical outcomes were assessed by investigators who were blinded to the treatment groups.

2.5 | Procurement of convalescent plasma

To procure and manage the donated plasma we followed the recommendations of the Scientific Committee on Transfusion Safety of the Spanish Ministry of Health.[17] The Ecuadorian Red Cross, through its communication channels, invited the donation of plasma from convalescent SARS-CoV-2 volunteers. Eligibility of potential convalescent plasma donors was evaluated 12 and 24 h prior the donation using the selection process established in the country regulations for blood banks plus the questions established for COVID-19. Convalescent plasma was obtained from male volunteers from 18 to 65 years of age, without history of previous transfusions, who have fully recovered from SARS-CoV-2 infection. Women were not included to decrease the risk of adverse events in plasma recipients since their plasma could contain antibodies developed during pregnancy against human leukocyte antigens, human neutrophil antigens, and human platelet antigens to avoid transfusion adverse events.[17] Volunteers were negative for SARS-CoV-2 infection by RT-PCR from nasopharyngeal swabs and/or have passed at least 20 days from their diagnosis. Convalescent plasma was obtained by plasmapheresis (TRIMA Accel Therumo) at the Red Cross Blood Center in Quito-Ecuador. Stored plasmas collected in 2018 before the onset of the pandemic from comparable donors were used as controls. Donated plasma was tested for the presence of HIV, hepatitis B and C, Chagas, and syphilis according to Red-Cross standard protocol.

2.6 | Determination of Igs against SARSCoV2

In order to use the CP, the presence of specific Ig against a recombinant protein representing the nucleocapsid (N) antigen of SARS-

CoV-2 were identified by a qualitative method according to the vendor instructions (Roche's Elecsys SARS-CoV-2, Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305). This assay was used because it was the only test available in Ecuador to identify serum with antibodies against SARS-CoV-2 antigens, at the time when the study was carried out.

The presence of antibodies against SARS-CoV-2 antigens was not measured in COVID-19 treated patients.

2.7 | Statistical analysis

To calculate the sample size, the following parameters were assumed: a confidence level of 95%, a power greater than 80%, a fatality rate in the intervention group of 30% and in the non-intervention group of 50%. Since there was no information from previous studies on SARS-CoV-2, this estimate considered the results of passive immunity intervention in Ebola and influenza infections.[18,19] A required sample of 100 subjects was estimated for CP group 1 and 100 subjects for NCP group 2. This research project contemplated three interim statistical analyses with 50, 100, and 150 patients included in the clinical study. Appropriate descriptive statistics were performed for categorical and continuous variables. The relative risk decrease between treatment groups was estimated with a 95% confidence interval. To assess the effect of plasma treatments on the evolution of respiratory rate and oxygen saturation during hospitalisation, we developed a linear model to include the following variables: sex; age; site (hospital) of recruitment; vital status; days from symptomatology to plasma treatment; and days from symptomatology to hospitalisation. We also studied the effect of plasma treatments on length of hospitalisation by building a linear log-Poisson model that controls for the same variables indicated above. To study the effect of plasma treatments on the risk of death we used a linear Cox-proportional-hazards model controlling for the same variables as above; in this model we also controlled by the presence of hypertension, diabetes, and obesity.

The analysis of the study was carried out with the intention of treatment. A p value <0.05 was considered statistically significant. All analyses were performed using R version 4.1.

3 | RESULTS

Considering the results of the intermediate statistical analyses that could not evidence an important advantage in the use of CP over the

TABLE 1 Baseline clinical characteristics, pathological history of the study groups

	Convalescent plasma ($n = 63$; 40.0%)	Non-convalescent plasma ($n = 95$; 60.0%)	p value
Demographic and clinical characteristics			
Age in years mean ± SD	56.3 ± 12.7	55.0 ± 13.3	0.539
Sex, *n* (%)			
Male	42 (66.7%)	65 (68.4%)	0.476
Female	21 (33.3%)	30 (31.6%)	
Clinical history, *n* (%)			
Hypertension	18 (28.6%)	17 (17.9%)	0.083
Diabetes	14 (22.2%)	17 (17.9%)	0.318
Overweight	13 (20.6%)	17 (17.9%)	0.368
Obesity	14 (22.2%)	25 (26.3%)	0.734
Blood group, *n* (%)			
A	11 (17.5%)	19 (20.0%)	0.749
B	1 (1.6%)	3 (3.2%)	
0	51 (81.0%)	73 (76.8%)	
AB	0	0	
Clinical parameters			
Temperature—°C	36.5 ± 0.40	36.6 ± 0.40	0.794
Heart rate—beats/min	80.9 ± 12.4	78.4 ± 12.1	0.218
Respiratory rate—breaths/min	23.0 ± 4.5	23.1 ± 4.1	0.946
SBP—mmHg	123.0 ± 14.0	121.0 ± 14.7	0.384
Oxygen saturation[a]—%	90.8 ± 3.0	91.2 ± 2.7	0.362
Haemoglobin	14.7 ± 1.7	15.5 ± 1.9	0.034
NEWs	6.3 ± 0.90	6.3 ± 0.80	0.718

Abbreviation: SBP, systolic blood pressure.
[a]Patients with oxygen support.

TABLE 2 Survival rate by treatment groups and site of recruitment

	Convalescent plasma ($n = 63$)	Non-convalescent plasma ($n = 95$)	p value
IESS-sur Hospital			
Recovered patients	38/42 (90.5%)	43/50 (86.0%)	0.372
Pablo Arturo Suarez Hospital			
Recovered patients	14/14 (100%)	26/29 (89.7%)	0.296
Hospital General Docente de Calderón			
Recovered patients	4/7 (57.1%)	14/16 (87.5%)	0.142
Total	56/63 (88.9%)	83/95 (87.4%)	0.490

TABLE 3 Specific times from start of symptomatology and length of hospitalisation

	Convalescent plasma ($n = 66$)	Control group ($n = 94$)	p value
Hospitalisation			
Number of days from start of symptomatology to admission	7.5 ± 4.0	7.2 ± 4.2	0.622
Number of days from start of symptomatology to plasma treatment	10.6 ± 4.9	10.4 ± 5.1	0.890
Length of hospitalisation	11.9 ± 7.6	12.7 ± 8.6	0.531
Number of days from start of symptomatology to dead	17.1 ± 8.5	23.3 ± 9.1	0.167
Number of days from hospitalisation to dead	10.3 ± 8.2	15.9 ± 7.5	0.145
Days from treatment-discharge	9.9 ± 5.9	10.4 ± 7.9	0.664

NCP in the survival rate of participating patients, the ethics committee and the research team advised to suspend the trial. Consequently, the study was halted with 79% (158/200) of the calculated sample.

A total of 190 patients were invited to participate, Figure 1. Of these, 32 were excluded because they declined to participate, did not meet the eligibility criteria, did not sign the informed consent, or due to lack of compatible plasma. The 158 patients that were eligible to participate received CP ($n = 63$) or NCP ($n = 95$) and completed the study, Figure 1. By day 3 96.8% ($n = 153$) were still hospitalised, by day 7, 64.6% ($n = 102$), by day 14, 23.4% ($n = 37$) and by day 21, 9% ($n = 14$) patients were monitored until recovery or death. Patients were discharged when their oxygen saturation was greater than 90% and they did not present fever for at least 3 days. There were not serious adverse events associated with plasma treatments.

Table 1 shows the basal sociodemographic characteristics and clinical history of the study population. At base line, treatment groups were similar on age, gender, blood groups and clinical history. In addition, except for haemoglobin concentrations, there were not significant differences in the clinical parameters between treatment groups at baseline, Table 1.

We evaluated the survival rate, the primary endpoint, in the study groups considering the site of recruitment, Table 2. There were no significant differences in the survival rate between treatment groups. However, the survival rate was lower in one of the hospitals that was included to treat patients with COVID-19 late in the pandemic, Table 2; total mortality in the CP treatment group was 11.1% while in the NCP was 12.6%; RR = 1.003, 95% CI (0.3938, 2.555). The hazard ratio in the linear Cox-proportional-hazards model was 1.85, 95% CI (0.49, 6.95). Mortality was more common among men, younger than 65 years ($n = 12/19$) and it was not associated with the presence of diabetes, hypertension or obesity.

Comparison of the time elapsed from the start of symptomatology to hospital admission, to plasma treatment, to death, and from plasma, treatment to discharge did not show significant differences between treatment groups, Table 3. In addition, there were no differences in the length of hospitalisation (LOH) between treatment groups.

3.1 | Evolution of clinical parameters of study groups

Table 4 shows the evolution in key clinical parameters for treatment groups. Clinical development was similar in CP and NCP treatment groups; significant improvements were observed within the first 2 weeks of hospitalisation and most patients were discharged after the first week and before the third week, Table 4. The few patients remaining by day 21 presented a deterioration in their clinical parameters in both treatment groups, Table 4.

To better understand any potential interactions between patients' characteristics and health provision at the different research sites, a linear model that assessed the interactions of sex; age; site (hospital) of recruitment; vital status; days from symptomatology to plasma treatment; and days from symptomatology to hospitalisation was constructed. There was a beneficial association between CP treatment and length of hospitalisation, 0.925, 95% CI (0.843, 1.01), although it

TABLE 4 Evolution of clinical parameters by treatment groups

	Convalescent plasma group				Control group			
	Baseline	3 days	7 days	21 days, $n = 8$	Baseline	3 days	7 days	21 days, $n = 12$
Temperature—°C	36.5 ± 0.37	36.4 ± 0.32[a]	36.4 ± 0.25[a]	36.2 ± 0.6	36.6 ± 0.45	36.5 ± 0.36	36.4 ± 0.3[a,b]	36.4 ± 0.3
Heart rate—beats/min	81.0 ± 12.5	79.4 ± 14.6	75.6 ± 11.8[a]	84.6 ± 16.3	78.6 ± 12.2	76.4 ± 14.4	75.0 ± 14.4[a]	90.3 ± 15.7
Respiratory rate—breaths/min	22.9 ± 4.2	22.1 ± 4.1	20.6 ± 2.9[a,b]	21.4 ± 6.1	23.1 ± 4.2	22.2 ± 4.7[a]	21.0 ± 3.7[a,b]	23.7 ± 8.5
SBP—mmHg	123.4 ± 10.0	122.1 ± 7.9	117.6 ± 17.7	109.8 ± 14.7	120.8 ± 14.6	119.2 ± 14.2	119.1 ± 16.7	111.5 ± 19.1
Oxygen saturation[c]—%	91.0 ± 2.6	90.7 ± 2.6	91.2 ± 3.6	88.6 ± 5.2	91.2 ± 2.7	90.5 ± 4.1	90.7 ± 2.6	90.3 ± 4.1
NEWs	6.3 ± 0.9	6.4 ± 2.4	4.1 ± 2.8[a,b]	6.8 ± 5.0	6.3 ± 0.8	6.1 ± 2.8	4.2 ± 3.2[a,b]	6.8 ± 3.3

[a]Different from baseline.
[b]Different from 3 to 7 days.
[c]Patients with oxygen support.

was not statistically significant. According to this estimation, individuals that received CP treatment reduced their mean hospital stay from 12.4 to 11.5 days compared with regular plasma treatment. In addition, the delay in CP treatment was associated with a longer stay 1.04, 95% CI (1.03, 1.04), and mean stay from 12.4 to 12.9 days. In addition, there was a beneficial non-significant association between CP treatment and the recovery of the respiratory rate, −0.773, 95% CI (−2.48, 0.936). One day delay of CP treatment was associated with a negative recovery of the respiratory rate of 0.29, 95% CI (0.10, 0.47). According to this estimation, individuals that delayed CP treatment had a smaller recovery in their respiratory rate than those that received earlier treatment.

4 | DISCUSSION

Present results showed that administration of CP with antibodies against SARS-CoV-2 to COVID-19 patients with moderate infection did not affect their survival rate compared with infected patients treated with NCP. However, early treatment of COVID-19 patients with CP tended to decrease the LOH while the delay in CP treatment was associated with a longer hospital stay. In addition, delay in CP treatment negatively affected the recovery of the respiratory rate.

Previous reports show different outcomes after CP treatment in COVID-19 patients. In a recent systematic review that included nine RCTs with 12 875 participants, the administration of CP did not reduce mortality up to day 28, 0.98, 95% CI (0.92–1.05) and had little or no impact on clinical outcomes including weaning from invasive clinical ventilation or the need for invasive clinical ventilation.[13] Based on these observations, the authors indicate that CP does not reduce mortality and has little or no impact on measures of clinical improvement in patients with moderate to severe COVID-19 infection. In that review there was not sufficient evidence to determine the effect of CP treatment in patients with mild COVID-19.[13] However, that review did not consider specific antibody concentration against SARS-CoV-2 in CP nor was the time of initiation of passive immunity therapy considered an important variable for measured outcomes.

Similar to the present results, a randomised clinical trial that included 86 hospitalised patients allocated to receive standard of care ($n = 43$) or CP ($n = 43$), reports that CP does not affect patients' survival, clinical course, viral clearance, inflammatory cytokine levels or humoral immune response.[20] However, differently to our results and to other coronavirus studies such as SARS-CoV, CP administration did not affect LOH.[20,21] In that study, most patients presented moderate to severe infection since they required oxygen supplementation or mechanical ventilation similar to the present report; and the majority of CP administered had a dilution factor of 320 in a plaque-reduction neutralisation test (PRNT50). The study was terminated prematurely because the majority of COVID-19 recipients had titers of neutralising SARS-CoV-2 antibodies similar to plasma donors. The authors recommend to study the effect of CP early in the infection before the establishment of the humoral immune response.[20] In another report, early administration of high-antibody titer-CP to patients with mild COVID-19 infection halted the progression of the infection.[22] Thus, in an RCT that included 80 patients in the CP treatment group and 80 patients in the control (0.9% normal saline) the administration of treatments within 72 h after the onset of mild COVID-19 symptoms there was a relative risk reduction for severe respiratory disease of 48%, (RR, 0.52; 95% CI, 0.29–0.94; $p = 0.03$) in the CP group. There were no significant differences in mortality in both treatment groups although the number of events was low, 2/80 versus 4/80.[22] Also, in a large propensity score-matched study in one health care system that included 341 hospitalised patients that received high titer anti-spike protein Igs CP units and 594 not transfused subjects, there was a significant reduction in mortality in the CP group when the treatment was administered within 44 h of hospitalisation and the titer anti-spike protein Igs was ≥1:1350.[23] However, there was not significant statistical differences in mortality between not transfused and high-titer CP treated patients when plasma was administered later than 72 h of hospital admission. In contrast to the report by Salazar et al.,[23] at the time the present study was performed, we did not have means to quantitate the presence of specific anti-SARS-CoV-2 antibodies in the plasma used in the study; we only determined the presence of the antibodies in the plasma sample; however, we instituted passive

immunity therapy early after patient admission (within 72 h). Consequently, timing of CP administration as well as the quality of the plasma evidenced by high specific antibody concentrations could be important determinants for the use of CP in the treatment of COVID-19 patients. Another important variable to consider for the use of CP is the severity of the clinical conditions of plasma recipients in COVID-19. In a recent RCT, the early use of CP with high antibody titers in high-risk outpatients with COVID-19 did not prevent the progression of the infection compared to the placebo group.[24] In addition to passive immunity, there may be other variables such as the clinical history of the patient, host immune response, genetic susceptibility that could have stronger influence on COVID-19 survival.

For SARS-CoV-2 infection, after the initial exposure, an incubation period of 5–6 days has been estimated establishing a peak of viral load within the first week of infection that subsequently declines by 14 days.[25,26] By the second week of infection, specific anti-SARS-CoV-2 antibodies are detected in the majority of infected patients.[26,27] Based on these viral and specific humoral immune response-kinetics in COVID-19, early administration of CP could better contribute in the treatment of the infection, a period of time where the specific immune response mediated by T and B lymphocytes are under development. In our study, the mean number of days from the start of symptoms to plasma treatment was around 10 days. It is possible that an earlier intervention with CP could provide better outcomes in COVID-19 patients.[21,23]

The mechanism of antimicrobial action of antibodies is related to their properties of neutralisation, opsonisation-phagocytosis, and the activation of the complement system. Measurements of antibodies with defined specificities to SARS-CoV-2 antigens in CP could contribute to determine the best plasma in the treatment of COVID-19 patients. Further, this information could be useful to improve donor selection and to evaluate convalescent plasma before its use. In our study, we were not able to neither quantitate specific anti-SARS-CoV-2 anti-bodies nor establish their functionality because we did not have the means for these analyses. It is possible that the CP used in our study did not have high concentration of neutralising antibodies that precluded us to observe better outcomes in the study population. It will be important to assess the immunological quality of CP before its administration to COVID-19 patients.

We acknowledge limitations to the present study, we did not reach the estimated sample size because the ethics committee for research in human beings advised its early termination due to: "The initial clinical uncertainty that justified the performance of the present study is not maintained according to the emerging literature on the subject" and because at the time of the interim analysis there were no statistical differences in the "days of hospitalization, survival, mortality and clinical course" endpoints. A recent statement from the WHO Therapeutics and COVID-19: living guideline further recommends against the use of CP for the treatment of COVID-19 in the regular clinical practice.[11] Another limitation to note is that the Roche's Elecsys SARS-CoV-2 test identifies presence of specific Ig against a recombinant protein representing the nucleocapsid (N) antigen of SARS-CoV-2. However, the presence of anti-nucleocapsid antigen has been shown to positively correlate with the presence of neutralising antibodies in neutralisation assays.[28,29] This could indicate that the CP used in the present study had neutralising antibodies. Although, the Roche's Elecsys SARS-CoV-2 test is not recommended for screening of donated blood, it was the only test available in Ecuador to identify serum with antibodies against SARS-CoV-2 antigens at the time the study was conducted. As indicated, we did not quantitate specific anti-SARS-CoV-2 antibodies to provide plasmas with the highest antibody concentrations for the treatment of participating patients. Only clinical parameters through the NEWS2 were used to assess the clinical condition of the patients at admission, follow up, and discharge because we did not have access to hospital laboratory data. Survival rate was markedly different among the three recruitment sites, it is possible that limited experience in the management of COVID-19 patients in one of the hospitals could have affected the outcome of this study.

One important strength of the present study is the use of NCP as control, most RCTs compare plasma against conventional treatment or to saline infusions (0.9% normal saline). Administration of IgG, the most abundant immunoglobulin in plasma from healthy donors, suppresses immune inflammation including in patients infected with SARS-CoV-2.[30,31] It is possible that the anti-inflammatory properties of NCP could have also favoured the treatment of participating COVID-19 patients in the present study. Similar clinical outcomes have been observed in an RCT that used NCP as control, where plasma administration improved the clinical condition of CP or NCP treated patients in a similar proportion.[32] In that study, there were not significant differences in mortality in both treatment groups, similar to our study. Compared with other RCT that have not used control plasma, present mortality rate in the control group was lower[20] 12.6% versus 26 and 24%.[12] However, in a recent RCT that included 150 patients with severe infection that received CP and 73 that received NCP as control, the mortality rate was lower in the CP treatment group (19/150 [12.6%] versus 18/73 [24.6%], OR 0.44, 95% CI 0.22–0.91, $p = 0.034$) and there was not improvement in the clinical state.[33] The severity of COVID-19 in this last study was greater than in the current RCT. In addition, the similar clinical outcomes in the CP and NCP treatment groups indicated that CP did not increase the risk of antibody-dependent enhancement for recipient patients, further supporting the safety of plasma use.[34] However, a recent report indicates that the therapeutic effect of CP is modulated by its antibody content.[35] Since we did not measure specifically the type of antibodies in the CP it is not possible to evaluate the effect of neutralising, antibody-dependent cellular cytotoxicity, or full transmembrane spike protein-antibodies in the present study. According to the authors, the abundance of IgG against the full transmembrane spike protein increased unfavourable outcomes whereas the presence of neutralisation and antibody-dependent cellular cytotoxicity Igs reduced the potential negative effect of plasma.[35] It will be important to characterise the antibody functionality present in CP before its use.

In conclusion, CP use for the treatment of COVID-19 patients is safe and its early use can decrease the length of hospital staying and improve respiratory function. Early administration of neutralising-antibody-rich CP could be useful in specific clinical conditions in patients

with limited immune response. A common methodology to measure anti-SARS-CoV-2 antibodies will be helpful to compare outcomes from different studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

Data presented in this study can be found in the following link: https://github.com/notblank/plasma_covid.

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SUPPORTING INFORMATION

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