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ORIGINAL RESEARCH

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Experiences in the use of multiple doses of convalescent plasma in critically ill patients with COVID-19: An early phase 1 descriptive study

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Abstract

Background: At the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, transfusion of coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) emerged as a potential therapeutic strategy to help patients severely afflicted by COVID-19. The efficacy of CCP has been controversial as it depends on many variables pertaining to the plasma donor and the patient with COVID-19, for example, time of convalescence or symptoms onset. This feasibility and descriptive study aimed to assess the safety of multiple doses of CCP in mechanically ventilated, intubated patients with respiratory failure due to COVID-19.

Methods: A cohort of 30 patients all experiencing severe respiratory failure and undergoing invasive mechanical ventilation in an intensive care unit, received up to

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five doses of 300–600 mL of CCP on alternate days (0, 2, 4, 6, and 8) until extubation, futility, or death.

Results: Nineteen patients received five doses, seven received four, and four received two or three doses. At 28-day follow-up mark, 57% of patients recovered and were sent home, and the long-term mortality rate was 27%. Ten severe adverse events reported in the study were unrelated to CCP transfusion. Independent of the number of transfused doses, most patients had detectable levels of total and neutralizing antibodies in plasma.

Conclusion: This study suggests that transfusion of multiple doses of CCP is safe. This strategy may represent a viable option for future studies, given the potential benefit of CCP transfusions during the early stages of infection in unvaccinated populations and in settings where monoclonal antibodies or antivirals are contraindicated or unavailable.

KEYWORDS

convalescent plasma, COVID-19, critically ill, neutralizing antibodies, SARS-CoV-2

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has led to an unprecedented surge in research on diagnosis and treatment methodologies.[1] Since the onset of the pandemic in March 2020, evidence-based treatment for all types of patients with COVID-19 has evolved rapidly. Efficacy of many antiviral therapies remains undetermined, with several still in the authorization process in many countries.[2,3] Evidence suggests that passive immunotherapy may not benefit all patients with COVID-19,[4,5] particularly those with moderate and severe illness. Clinical trials with passive immunotherapy have failed to demonstrate a reduction in mortality or improvement in clinical outcomes, such as use of mechanical ventilation or length of hospital stay, among others.[6–9] However, there is potential for certain patient groups—early transfused outpatients, seronegative, not vaccinated, or immunocompromised—to benefit from a reduced risk of COVID-19 progression through the administration of COVID-19 convalescent plasma (CCP).[10–12]

Despite CCP being described as an affordable and readily available therapeutic resource, further clarification is warranted in preparation for future pandemic events.[13] There is a need to improve trial designs and outcome measurement methods.[14,15] Furthermore, there remains a lack of clear definition regarding the optimal and safe CCP dose, the optimal timing for the start of the transfusion, and the efficacy of CCP and monoclonal-antibody therapy in light of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants.[4]

Highlights

- Transfusion of multiple doses (up to 5) of 300–600 mL of convalescent plasma from COVID-19-recovered patients is deemed safe, as it does not induce more severe effects than a single dose.
- Regardless of the number of transfused doses and the titer of neutralizing antibodies, most transfused patients had detectable levels of total and neutralizing antibodies in their plasma.
- Future studies are needed to determine if multiple transfusion doses are more efficient in preventing disease severity than a single dose.

2 | RESEARCH ISSUE AND MAIN OBJECTIVE

The Panamanian government implemented strategies to broaden treatment options for COVID-19. At the start of the pandemic in 2020, the Panamanian Society of Hematologists collaborated by initiating a descriptive early phase 1 study to show the safety of multiple doses of CP in critically ill patients. It sought to delineate the clinical outcomes of these patients and detect any severe effects related to treatment. It is noteworthy that any transfusion of plasma carries inherent risks, including virus transmission (like human immunodeficiency virus [HIV], hepatitis B virus [HBV], hepatitis C virus [HCV], and human T-lymphotropic virus [HTLV], allergic reactions, anaphylaxis, febrile reaction, transfusion-associated acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), and hemolysis due to the administration of an incompatible ABO plasma unit.[16–18]

This study aimed to assess the safety of multiple-dose administration of CCP in patients with COVID-19 who were mechanically ventilated, owing to respiratory failure. Adverse events, such as fever, rash, transfusion-related infections, TRALI, and TACO, were analyzed during the post-transfusion period of each administered CCP unit. Other recorded outcomes were ventilator-free days and mechanical ventilator parameters, intensive care unit (ICU) time,

in-hospital mortality, and long-term (28 and 60 days) mortality. In addition, the research team retrospectively analyzed donor and recipient antibody levels to investigate the possible association between the recipient's neutralizing immune response in the recipient and their safety and survival outcomes.

3 | METHODS

3.1 | Patient cohort and bioethical considerations

This study was conducted from June 1 to October 14, 2020 at three hospitals in Panama City: Complejo Hospitalario Metropolitano Dr. Arnulfo Arias Madrid (CHMAAM) from the Social Security System, the Hospital Santo Tomas, which is the main public hospital, and Hospital Pacifica Salud, which is a private hospital. This study was approved by the National Bioethics Committee (EC-CNBI-2020-04-56), and all authors assume full responsibility for the study procedures. The clinical research team obtained written informed consent from each participant enrolled in the study or from a legally authorized representative; it included an explanation regarding the study and the potential benefits and risks of using CCP as a therapy for critically ill patients.

3.2 | Inclusion and exclusion criteria of CCP recipients

The inclusion criteria were as follows: patients aged ≥18 years, those with severe COVID-19 respiratory symptoms who were hospitalized in the ICU with invasive mechanical ventilation; and those with a positive laboratory diagnosis of SARS-CoV-2, using a reverse-transcription polymerase chain reaction (RT-PCR) test, with a nasopharyngeal swab sample. The exclusion criterion was patients that were contraindicated for transfusion owing to severe volume overload; history of anaphylaxis to blood products; severe multiorgan failure; hemodynamic instability; other documented uncontrolled infections; severe disseminated intravascular coagulation requiring factor replacement, fresh frozen plasma, or cryoprecipitate; dialysis; active intracranial bleeding; or previous clinically significant myocardial ischemia.

3.3 | Data collection and statistical analysis

Clinical and laboratory data (excluding antibody measures) were extracted from the patient's medical records using a case report form designed for this study. We recorded demographics, pre-existing medical conditions, COVID-19 symptoms, infection routes, and time course details. Patients were followed-up daily from the day before transfusion until death, extubation, or 27 days post first transfusion, whichever came first; next, the team made follow-up calls on Days 28 and 60 from the day of the first transfusion.

The research team monitored the following criteria for each patient during transfusion: daily vital signs, including daily oxygen saturation SpO_2 and daily ventilator requirements, such as the fraction of inspired oxygen (FiO_2), positive end expiratory pressure, and respiratory rate. Before and after each transfusion, the researchers recorded the level of oxygen support and clinical outcomes (7-point scale) to monitor the clinical status of transfused patients. Moreover, the appearance of new medical conditions, adverse events, and potential toxicities from CCP transfusion were monitored and documented at every 48 h-intervals (up until Day 8).

Data collection and management for this study were performed using ClinCapture, (V2.1.0). All categorical variables were described as frequencies or percentages, and continuous variables were summarized with mean and standard deviation or median and the interquartile range (IQR). Categorical variables were analyzed with the Pearson Chi-square or Fisher's exact test. The level of significance was set at $p < 0.05$. Data were analyzed using IBM SPSS Statistics (V26; IBM Corp.; 2019).

3.4 | Selection of COVID-19 CCP donors

Those individuals who recovered from COVID-19 and after recovery tested negative for SARS-CoV-2 PCR test at least 14 days prior were determined to be CCP donors. To recruit donors and achieve a sufficient CCP supply for the study, the team used a combination of mass advertising and personal interviews with potential donors. All donors provided written informed consent for donation (permit EC-CNBI-2020-04-44 for the first group of donors and EC-CNBI-2020-04-56 for multiple donations), and samples were frozen and stored for further analysis.

Basic strategies were adopted to ensure the safety of donors and patients in the study. Initially, only male donors and female donors with no pregnancy history were accepted to minimize the possibility of TRALI. However, when the anti-human leukocyte antigen (HLA) screening test was available, female donors with a pregnancy history were also allowed to donate CCP.[18] Eligible donors were evaluated for final screening at the CHMAAM Blood Bank to confirm their health status at the time of donation and to ensure that they had no current infectious disease. Each donation was screened for transfusion-transmitted infections (serology for HIV, HBV, HCV, HTLV-I/II, *Trypanosoma cruzi*, *Treponema pallidum* and nucleic acid testing methodology for HIV, HBV, and HCV). Additional safety measures such as a sterilization of the donor's skin before establishing an intravenous access and blood diversion of the initial blood draw were carefully executed to prevent bacterial contamination.

An average of 600–700 mL of CCP was extracted using Haemonetics, Trima, or Optia spectra apheresis under the supervision of the medical staff and the investigators. CCP units were inactivated using the psoralen method,[19] which destroys any possible viral infection that may not have been detected during screening. CCP was then frozen in 200–250 mL aliquots with their respective labels and stored at −70°C in a dedicated freezer. The entire CCP

extraction process adhered to all the quality controls required for other blood components. During this study, the Panamanian Ministry of Health issued an emergency use authorization for CCP based on similar approval from the FDA and the use of CCP in treating other pathologies.

3.5 | CCP administration

Hospitalized patients received open-label screened CCP. Single or double plasma units (weight-based < or >90 kg) were administered on Days 0, 2, 4, 6, and 8 or until extubation or futility (if occurred before Day 8) as determined by the ICU team. Treating clinicians could omit transfusion at their discretion (e.g., TRALI events are 100% donor-dependent and do not prohibit future transfusions).[20] All transfusions were ABO compatible and administered within 60 min after thawing. For each transfusion and each day between transfusions, the researchers monitored vital signs, ventilator status, concomitant medications, and adverse event.

3.6 | Laboratory assays for SARS-CoV-2

Each patient underwent testing for detection of SARS-CoV-2 RNA in nasopharyngeal swabs using viral RNA extraction followed by real-time RT-PCR before every plasma administration as described previously.[21] Serum samples of donors and recipient patients were analyzed retrospectively for the presence of anti-SARS COV-2 total IgG by ELISA, using SARS-CoV-2 recombinant antigen from spike glycoprotein (S protein) and Nucleocapsid (N protein) (CE-certified Vircell COVID-19 ELISA IgG; Vircell Spain SLU) according to the manufacturer's recommendation. To detect neutralizing antibodies against SARS-CoV-2 and to determine their titer in the plasma of donors and recipients, all samples were tested retrospectively by performing a plaque reduction neutralization test (PRNT) using 800 plaque-forming units (PFU) of the SARS-CoV-2 virus original variant B.1. in Vero cells (ATCC-C1008), with a 1/2 serial dilution of the plasma, starting with 1/10 dilution. This was performed in a BSL-3 laboratory as previously described.[22] $PRNT_{80}$ titer was calculated as "the highest dilution of each sample capable of neutralizing the virus by inhibiting the formation of 80% of viral plaque units."

4 | RESULTS

4.1 | Demographic and clinical description of the CCP recipients during hospitalization

A total of 30 patients were enrolled in this study from June 5 to August 16, 2020. Most patients were male (67%), and the median age was 45.5 years (IQR = 18). A significant proportion of patients, 83% (25/30), had at least one or more comorbidities. Obesity was the most common chronic condition in 83% (25/30), followed by diabetes mellitus and hypertension, both found in 40% (12/30) of the patients. One patient had Down's syndrome, and one was in the second trimester of pregnancy. Five patients had no reported comorbidities. Most patients (73%, 22/30) had an O-positive blood type. Regarding exposure to SARS-CoV-2, 30% (9/30) of study participants suspected exposure at work, 23% (7/30) at home, 7% (2/30) from their social network, and 40% (12/30) were unsure about the route of exposure (Table 1).

The median time from symptoms onset to hospitalization was 7 days (IQR = 5 days) (Table 1). On the day of enrollment, every patient tested positive SARS-CoV-2 RT-PCR test, and most (87%, 26/30) had an initial Sequential Organ Failure Assessment (SOFA) score of ≤6.

The most common COVID-19 symptoms were shortness of breath, fever, and cough (Table 1). Table 2 presents the laboratory results. Initial blood cell counts showed that most patients (29/30) had a white blood cell count >10,000/μL, while all patients had absolute lymphocyte counts of <40% and neutrophil counts of >70% before the first transfusion. Furthermore, 90% participants had an initial glucose level of >110 mg/dL. The most commonly used medications were azithromycin (in 25/30 patients), corticosteroids (28/30), heparin (29/30), and immunoglobulin (16/30) (Table 3). The median time from symptoms onset to the first plasma transfusion was 11 days (IQR = 4 days); for ICU hospitalization, 16.5 days (IQR = 29); for post-transfusion length of hospital stay until recovery or death, 13 days (IQR = 51); and the median total hospital stay, 21.5 days (IQR = 54) (Table 3).

4.2 | Clinical outcomes and safety profile of CCP transfusion

Evaluation of the clinical status of transfused patients and the level of oxygen support needed (Figure 1A and Supporting Information S1: Figure 1) revealed that by Day 9 after the first CCP transfusion, the percentage of extubated patients increased, with 43% (13/30) of participants no longer requiring mechanical ventilation, and only 3.3% (1/30) died owing to the disease.

In this early phase 1 trial, safety was the primary clinical endpoint. Although the trial included a transfusion of five doses of CCP, each transfusion and dose were dependent on extubation or medical condition of the patient. Among 12 critically ill patients, the dose of CCP was reduced from two units (600 mL) to one unit (300 mL), per the ICU physician's discretion. Nineteen patients received all five doses, seven received four, and four received only two or three doses. Of the patients receiving less than five doses, extubation was the primary reason (10 patients), while one (1/30) died before receiving the proposed scheme. At Day 28 of follow-up, 57% (17/30) of all patients had recovered and were discharged home, and 26% (8/30) had died, whereas the majority stayed in ICU (Figure 1A). After 60 days of follow-up, the reported long-term mortality was 27%.

To evaluate if there were differences between the clinical outcomes depending on the onset of symptoms to the first CP

TABLE 1 Characteristic of the convalescent plasma recipient sample cohort ($n = 30$).

Variable		
Age, in years, median (IQR)	45.5	18
Sex, *n* (%)		
Male	20	67%
Female	10	33%
Ethnicity, *n* (%)		
White	12	40%
Afro-descendant	5	17%
Indigenous	3	10%
Other (mestizo)	10	33%
Body mass index, $\bar{x}$ (SD)	32.3	8.6
Categories	n	%
Normal	5	17%
Overweight	11	37%
Obesity class I	5	17%
Obesity class II	3	10%
Obesity class III	6	20%
Comorbidities		
Overweight	25	83%
Diabetes mellitus	12	40%
Hypertension	12	40%
Asthma	4	13%
Hypothyroidism	3	10%
Cancer	1	3%
Type of exposure to SARS-CoV-2		
At work	9	30%
At home	7	23%
From social network	2	7%
Unknown	12	40%
Sequential organ failure assessment (SOFA) score		
0-6	26	87%
≥7	4	13%
Median time from symptoms onset (hospitalization in days; median, IQR)	7	5
Blood type		
O positive	22	73%
Presenting symptoms		
Shortness of breath	30	100%
Fever	27	90%
Cough	29	97%
Fatigue	11	37%
Headache	6	20%
Nasal congestion	5	17%
Sore throat	5	17%
Vomiting	4	13%
Diarrhea	5	17%
Imaging		
Chest radiography	30	100%

Abbreviations: IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 2 Laboratory data at the time of enrollment.

Laboratory test, $n = 30$		
Glucose	$\bar{x}$ (SD)	189.80 (83.72)
Glucose > 110 mg/dL	*n* (%)	27 (90)
Creatinine	$\bar{x}$ (SD)	0.93 (0.64)
Aspartate aminotransferase *n* (%)		
Female > 35 U/L		7 (70)
Male > 50 U/L		13 (65)
Alanine aminotransferase *n* (%)		
Female > 35 U/L		8 (80)
Male > 50 U/L		13 (65)
Complete blood count		
White blood cell/mm^3	Median (range)	11,3500 (5,100–17,100)
White blood cells > 10,000/μL	*n* (%)	29 (97)
Neutrophils > 70%	*n* (%)	30 (100)
Lymphocytes < 40%	*n* (%)	30 (100)
Red blood cells/mm^3 > 3.5×10^6/μL	*n* (%)	30 (100)

transfusion (≤10 days or >10 days), the outcome was analyzed according to the oxygen support status (intubated or extubated) over time (post-transfusion days), and the p values were not statistically significant for any group (Figure 1B).

No adverse events attributed to plasma transfusion occurred in the first 24 h after transfusion. Six moderate and one mild adverse event were recorded during the first 8 days of active transfusion; these included mild sinus bradycardia in one patient and hypotension, fever, gram-positive bacteremia, yeast in tracheal secretions, and leg hematoma in five patients. Ten serious adverse events were detected during the entire follow-up period: six occurred in the first 8 days of

TABLE 3 Time intervals between symptoms onset, hospitalization, transfusion, and final outcome including the drug treatment of convalescent plasma recipients during the hospitalization period.

Patient no.	Time A	Time B	Drug treatments	Time C	Time D	Time E
1	15	1	AZI, INM, COR, HEP	15	14	13
2	16	6	AZI, INM, COR, HEP	24	24	10
3	12	3	AZI, INM, COR, HEP	56	33	43
4	8	1	AZI, INM, COR, HEP	20	16	8
5	15	2	AZI, COR, HEP	29	17	19
6	7	2	AZI, INM, COR, HEP, VIT C, CEF	11	6	2
7	10	2	AZI, COR, HEP	20	11	9
8	12	1	AZI, INM, COR, HEP	16	12	6
9	11	1	AZI, INM, COR, HEP	15	8	11
10	11	2	TOC, COR, HEP, IVER, VIT C, TIA	37	17	28
11	11	1	TOC, INM, HEP TEP, VITC, TIA	17	17	16
12	7	1	TOC, AZI, COR, HEP, TIA	12	10	7
13	14	1	AZI, INM, COR, HEP	18	15	14
14	8	2	TOC, AZI, COR, HEP TEP	28	11	18
15	10	2	AZI, INM, COR, HEP TEP, IVER	59	35	49
16	12	2	AZI, COR, HEP TEP, CEF	23	20	12
17	14	2	INM, COR, HEP TEP	31	26	24
18	14	4	TOC, AZI, INM, COR, HEP	32	25	19
19	16	4	AZI, COR, HEP, TIA, VIT C	17	12	11
20	11	2	TOC, COR, HEP TEP	16	11	12
21	14	2	COR, HEP TEP	55	28	40
22	9	2	AZI, INM, COR, HEP TEP, VIT C, TIA, ZN, IVER	15	15	13
23	10	3	TOC, AZI, INM, HEP TEP, TAZ, IVER	19	17	8
24	12	2	AZI, INM, COR, HEP TEP	26	21	15
25	7	3	HCQ, AZI, COR, HEP TEP	65	35	53
26	11	2	COR INT, HEP, REM	15	9	7
27	17	3	AZI, COR, HEP TEP	24	14	12
28	15	3	AZI, INM, COR, HEP TEP	27	19	18
29	12	2	AZI, COR, HEP	34	27	25
30	10	2	AZI, COR	19	15	9
Median (IQR)	11.5 (4)	2 (1)		21.5 (15.3)	16.5 (13)	13 (11)

Note: Time A, symptom onset to transfusion (days); Time B, admission to ICU to transfusion (days); Time C, length of hospital stay (days); Time D, length of ICU stay (days); Time E, post-transf. length of hospital stay/death (days).

Abbreviations: AZI, azithromycin; CEF, ceftriaxone; COR, corticosteroids; HCQ, hydroxychloroquine; HEP TEP, therapeutic heparin; HEP, prophylactic heparin; ICU, intensive care unit; INM, immunoglobulin; IQR, interquartile range; IVER, ivermectin; REM, remdesivir; TAZ, tazobactam; TIA, thiamine; TOC, tocilizumab; VIT C, vitamin C; ZN, zinc.

follow-up during active transfusion and four occurred in 10–27 days of follow-up. Of these ten serious adverse events, two patients had sepsis secondary to bacteremia (*Achromobacter xylosoxidans*, *Staphylococcus epidermidis*, *Stenotrophomona maltophilia*) and survived. The other eight patients died owing to the following reasons: multiorgan failure ($n = 3$), multiorgan failure and intractable pneumothorax ($n = 1$), septic shock ($n = 2$), sepsis plus intractable pneumothorax ($n = 1$), and respiratory failure ($n = 1$).

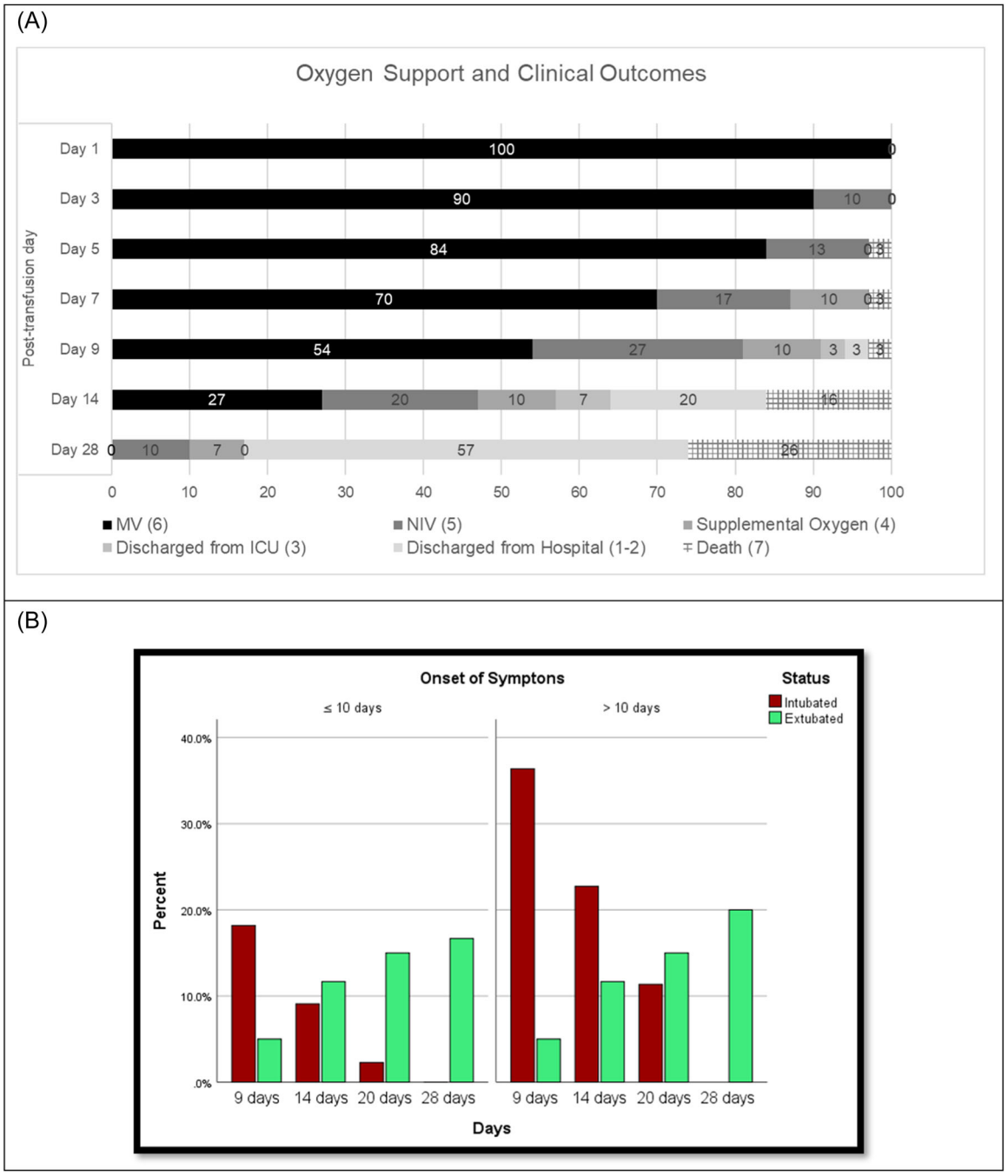

FIGURE 1 Evaluation of clinical status and outcomes and levels of oxygen support in patients that received coronavirus disease 2019 (COVID-19) convalescent plasma transfusions. (A) Graph showing the percentage of patients in terms of level of oxygen support needed and 7-points scales for outcome (scores are in parentheses) for each post-transfusion evaluation (*Y* axis). MV, mechanical ventilation; NIV, noninvasive ventilation. (B) Onset of symptoms to the first convalescent plasma transfusion (≤10 days or >10 days) according to the oxygen support status (intubated or extubated) over time (post-transfusion days). The *p* values (Fisher's exact test) for the comparison between patients intubated versus extubated by Days 9, 14, and 20 were as follows: $p = 0.49$, $p = 0.28$, and $p = 0.19$. By Day 28 all alive patients were extubated.

4.3 | Presence of neutralizing antibodies and clinical outcome

Upon establishing the safety of multiple doses of CCP transfusions, it was considered crucial to investigate whether CCP transfusions were associated with developing neutralizing antibodies in recipient patients. At the time of this study, it was determined that a COVID-19 convalescent patient who demonstrated symptoms resolution for with 14 days or more and tested negative for SARS-CoV-2 PCR test, could be a potential CCP donor. For each of the five CCP dose transfusions, plasma samples from donors and recipients were frozen at the moment of usage. Transfusion was done without

prior knowledge of the serological data, and total anti-SARS-CoV-2 IgG and neutralizing activity were tested from these frozen samples months after transfusion. The serological analysis of the stored samples revealed that at the time of each transfusion, 90%–100% of donors were positive for anti-SARS-CoV-2 IgG (Figure 2A and Supporting Information S1: Table 2). At Day 0 (first transfusion), detectable levels of IgG were found in 90% (27/30) of the CCP plasma units used; however, 80% (24/30) of the recipients also had detectable levels of IgG, with no significant difference between recipients and the units transfused. Anti-SARS-CoV-2 IgG was detected in all recipients at Day 4 post-transfusion. The neutralizing activity analysis of the CCP units used for the first transfusion showed that 43% (13/30) had high neutralizing activity ($PRNT_{80}$ titer ≥1:160), 37% (11/30) had a weak neutralizing activity ($PRNT_{80}$ between 1:20 and 1:80), while 20% (6/30) had no detectable neutralizing activity (Figure 2B and Supporting Information S1: Table 2).

The number of "mean days from symptom onset to donation" were similar between donors (CCP units) with high neutralizing activity and those with low or no neutralizing activity (Supporting Information S1: Table 1). Although there was no significant difference in IgG detection between donors and recipients, almost 86% (26/30) of recipients already had antibodies with neutralizing activity, and 53% (16/30) had strong neutralizing activity at the moment of the first transfusion. The percentage of recipients that had neutralizing antibodies increased over time, as expected, reaching 96% (29/30) on Day 4 after two transfusions (83%, 25/30, with strong neutralizing activity). However, the percentage of donors having neutralizing antibodies. However, by Day 8 of the study, only 21% (4/19) of donors had strong neutralizing titers and around 63% (12/19) had low neutralization titers (Figure 2B and Supporting Information S1: Table 2). This prompted a comparison between the presence of IgG versus the neutralizing activity determined by $PRNT_{80}$ in recipients before the first transfusion and at Day 8. At Day 0, only three recipients had no neutralizing antibodies, even if most of them had detectable levels of IgG (Supporting Information S1: Figure 2). On Day 8, all recipients had detectable levels of IgG, with most exhibiting a high neutralization titer. Only two had low neutralization activity. It appeared that there was no direct correlation between the IgG ELISA optical density measurement and the neutralization titer calculated by $PRNT_{80}$. By the end of the study, four of 16 patients with high neutralization antibodies at the beginning had died (28%), and four of 14 patients with low or no neutralization antibodies had died (25%), showing no statistical differences.

5 | DISCUSSION

The primary endpoint of this trial was to assess the safety of multiple transfusions of CCP in critically ill patients with COVID-19 and to evaluate allergic reactions, such as TACO or TRALI, during the post-transfusion period of each administered CCP unit. Although we proposed the administration of >2 doses of CCP, no adverse reactions related to volume overload were noted, which is a sign of tolerability and safety. To the best of our knowledge, this is one of the few studies where a volume of ≥300 mL of CCP (up to five doses in 8 days) was administered to critically ill patients with COVID-19,[11,23–25] and no adverse events related to the CCP

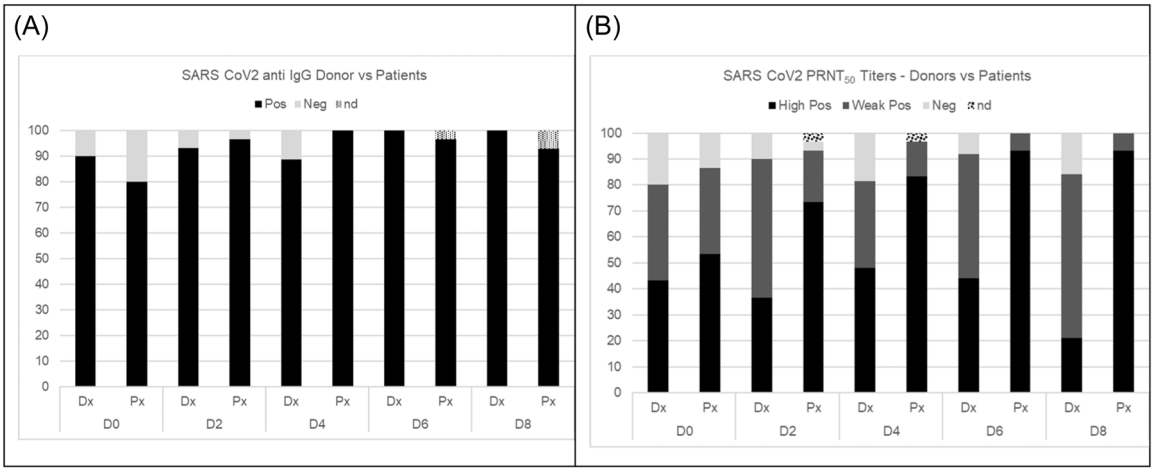

FIGURE 2 Detection of total immunoglobulin G (IgG) and neutralizing antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in donors and recipients for each transfusion. The titers were measured every 2 days before each of the five transfusions (D0, transfusion 1; D2, transfusion 2; D4, transfusion 3; D6, transfusion 4; D8, transfusion 5). Thus, antibodies were measured in the recipients over time. Owing to a limited supply of plasma units from each donor, the donors were not always the same, and there was no longitudinal tracking of the initial donors' antibody responses. Instead, we independently measured the state of the antibodies in the convalescent plasma (CCP) units from various donors for each donation. (A) Levels of total anti-SARS-CoV-2 IgG in donor CCP units and in recipients (*O.D., optical density; Pos, O.D*. > 6.0; Neg, O.D. < 6.0; nd, no data); Dx, donor; Px, recipient. (B) Levels of neutralization antibodies against SARS-CoV-2 in in donor CCP units and in recipients. Plaque-reduction neutralization test (PRNT) titers results are divided as follows: negative, 0; low positive, <1:160; high positive, ≥1:160; nd, no data.

transfusion were recorded. These findings contribute valuable information regarding therapeutic dose for overweight patients during a pandemic, suggesting that consecutive transfusions of CCP units can be safely administered, similar to studies in patients with moderate to severe COVID-19.[26]

In our study, various safety measures were implemented that were likely the drivers preventing adverse events. For example, the hematologist set up different strategies such as the slow transfusion drip rates, pre-transfusion prescription of acetaminophen and diphenhydramine to the patients, and the careful selection of donors with characteristics such as being male or female without previous pregnancies or testing negative for anti-HLA antibodies. Another strategy involved reducing the dose of plasma in consultation with the treating physicians when necessary. In patients with mechanical ventilation, the absence of adverse events solely related to the transfusions complements the evidence regarding the safety of this treatment strategy.

In our study, the research team concluded that the adverse events observed were unlikely to be associated with CCP transfusion owing to the following reasons: (i) the timing of each adverse event exceeded the window period for plasma transfusion-related adverse events; and (ii) all CCP units were processed and stored at −70°C to prevent bacterial proliferation, suggesting that even if patients experienced bacteremia, bacterial contamination from the administered plasma could be ruled out.[27] Moreover, the standard practice of our blood bank involves weekly quality control checks to monitor bacterial growth in every blood product. All the CCP units used for this study were thawed following the same sterile procedure and transfused within 60 min, a practice demonstrated to be safe for preserving sterility.[28]

Although this study was not designed to demonstrate efficacy, the difference in the proportion of extubated versus intubated patients depending on the time of transfusion after symptom onset suggests a trend towards better outcomes when transfusions were initiated earlier in the disease course. This is congruent with other reports that also observed that after Day 9 of symptoms onset, most patients already had potent neutralizing antibodies against SARS-CoV-2 before CCP transfusion, with titers comparable to that of donors,[10,12,24] and that these titers increased or stayed stable during the first month of follow up. Other studies showed the effectiveness of CCP in early interventions (<7 days of symptoms onset) when viral particles were still present in the recipient's blood that can be neutralized by the antibodies from the transfused plasma.[29] The analysis of neutralization titers was performed in recipients; however, most recipients had antibodies before transfusion, which was one of the reasons why efficacy could not be concluded from this study. Future studies to determine the efficacy of CCP transfusion and of multiple transfusions should be done early when the recipients are still in the viremic phase with no personal neutralizing antibodies. Moreover, donors should have more than 14 days of recovery, as in our study; however, the titer of neutralizing antibodies should be measured before performing the transfusion. Newer studies have shown stronger neutralizing antibody titers 15–30 days after SARS-CoV-2 infection, which declines over time.[30] As our study was not conclusive on the impact of CCP on outcomes in patients with COVID-19 having high titers of IgG antibodies, more studies are needed to determine if these patients should be administered with CCP and if this treatment impacts positively or not their clinical outcomes. Additionally, the CCP units used for transfusion should have high neutralizing antibody titers and be used early in the course of the disease.

We found that 93% (28/30) of participants received corticosteroids; thus, it is possible that some of the improvement in the outcomes was related to the effect of this medication. Recent publications have stated the potential overlapping benefits of CCP and corticosteroids.[31]

Despite the advent of new interventions such as vaccines, COVID-19 continues to pose a significant public health threat, particularly with the increasing circulation of SARS-CoV-2 variants in every country. To date, it appears that CCP is more accessible than antiviral drugs and monoclonal antibodies, especially in developing countries.[32] CCP is usually the first antibody therapy available for critical situations like outbreaks of novel viruses.[33] Nevertheless, because of the risks associated with transfusion and the controversial efficacy, future clinical studies with a high number of participants and state-of-the-art design and evaluation are needed to determine if CCP treatment is beneficial for critically ill patients with COVID-19 in settings where vaccination is not available and specific treatments are not fully accessible.

6 | LIMITATIONS

Our study had certain limitations. First, the low number of participants, and second, the lack of knowledge regarding the anti-SARS-CoV-2 IgG and neutralizing antibodies titers in patients before transfusion and for each CCP before being released for clinical use. In multiple cases, patients already had elevated levels of antibodies before being transfused, and some donors had low levels of IgG. However, the current scenario is different, as antibody tests are now widely accessible. There are anti-RBD IgG antibody measurements available, which correlate well with neutralizing antibody levels.[31] Third, we acknowledge that as a non-randomized, convenience sample was included, selection bias regarding the type of patients enrolled and its relationship with the incidence of transfusion-related adverse events may have been incorporated. Fourth, extracting data from clinical records was not an easy task, given the high number of cases and the paucity of information recorded per case at the start of the pandemic. Nevertheless, this study holds significant value as it presents data on the safety of multiple doses of CCP transfusion in hospitalized, critically ill patients. Thus, this study served as a model in 2020 that was completed with the international data obtained at that time, for national guidelines regarding the use of CCP while

keeping security measures in check and extending it for compassionate use for the general population.

7 | CONCLUSION

This study confirmed, in a small group of patients, the safety profile of multiple CCP transfusions in critically ill patients with COVID-19, even if it did not demonstrate a benefit on disease outcome. This study may provide evidence to support future clinical trials on the efficacy of multiple transfusions in the early course of the infection in patients who cannot receive vaccinations, antivirals, or monoclonal antibody treatments (such as pregnant females or immunosuppressed patients) or in potential cases where an emergent variant may evade the response of current vaccines preparations but not that of the neutralizing antibodies in CCP, obtained from survivors of this putative variant.

AUTHOR CONTRIBUTIONS

Ricardo Aguilar: Conceptualization; investigation; supervision; writing—original draft. **Sandra López-Vergès**: Conceptualization; investigation; supervision; writing—original draft. **Anarellys Quintana**: Investigation; resources; writing—review and editing. **Johanna Morris**: Investigation; writing—review and editing. **Lineth Lopez**: Investigation; writing—review and editing. **Ana Cooke**: Investigation; writing—review and editing. **Dimas Quiel**: Investigation; writing—review and editing. **Natalie Buitron**: Investigation; writing—review and editing. **Yaseikiry Pérez**: Investigation; resources; writing—review and editing. **Lesbia Lobo**: Investigation; writing—review and editing. **Maura Ballesteros**: Investigation; writing—review and editing. **Yaneth Pitti**: Investigation; writing—review and editing. **Yamilka Diaz**: Investigation; writing—review and editing. **Lisseth Saenz**: Investigation; writing—review and editing. **Danilo Franco**: Investigation; writing—review and editing. **Daniel Castillo**: Investigation; writing—review and editing. **Elimelec Valdespino**: Investigation; writing—review and editing. **Isabel Blanco**: Project administration; writing—review and editing. **Emilio Romero**: Formal analysis; writing—review and editing. **Alcibiades Villarreal**: Methodology; resources; writing—original draft. **Idalina Cubilla-Batista**: Conceptualization; formal analysis; software; writing—original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information of this article.

ETHICS STATEMENT

This study was approved by the Comité Nacional de la Investigación de Panama (CNBI) by letter No. EC-CNBI-2020-04-56.

TRANSPARENCY STATEMENT

The lead authors Alcibiades Villarreal and Idalina Cubilla-Batista affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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REFERENCES

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis*. 2020:20:533-534. https://doi.org/10.1016/S1473-3099(20)30120-1
2. Food Drug Administration. Remdesivir (Veklury) [package insert]. Food and Drug Administration; 2020. Accessed June 7, 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/214787Orig1s000lbl.pdf
3. National Institutes of Health. Antiviral Therapy. COVID-19 Treatment Guidelines. 2022. Accessed June 7, 2022. https://www.covid19treatmentguidelines.nih.gov/therapies/antiviral-therapy/
4. Piechotta V, Iannizzi C, Chai KL, et al. Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review. *Cochrane Database Syst Rev*. 2021;5(5):013600. https://doi.org/10.1002/14651858.CD013600.pub4
5. Abani O, Abbas A, Abbas F, et al. Casirivimab and imdevimab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet*. 2022;399(10325):665-676. https://doi.org/10.1016/S0140-6736(22)00163-5
6. Janiaud P, Axfors C, Schmitt AM, et al. Association of convalescent plasma treatment with clinical outcomes in patients with COVID-19: a systematic review and meta-analysis. *JAMA*. 2021;325(12):1185-1195. https://doi.org/10.1001/jama.2021.2747
7. Simonovich VA, Burgos Pratx LD, Scibona P, et al. A randomized trial of convalescent plasma in Covid-19 severe pneumonia. *N Engl J Med*. 2021;384(7):619-629. https://doi.org/10.1056/NEJMoa2031304
8. Alemany A, Millat-Martinez P, Corbacho-Monné M, et al. High-titre methylene blue-treated convalescent plasma as an early treatment for outpatients with COVID-19: a randomised, placebo-controlled trial. *Lancet Respir Med*. 2022;10(3):278-288. https://doi.org/10.1016/S2213-2600(21)00545-2
9. Estcourt LJ, Turgeon AF, McQuilten ZK, et al. Effect of convalescent plasma on organ support-free days in critically ill patients with COVID-19: a randomized clinical trial. *JAMA*. 2021;326(17):1690-1702. https://doi.org/10.1001/jama.2021.18178

10. Sullivan DJ, Gebo KA, Shoham S, et al. Early outpatient treatment for Covid-19 with convalescent plasma. *N Engl J Med*. 2022;386(18):1700-1711. https://doi.org/10.1056/NEJMoa2119657
11. Bajpai M, Maheshwari A, Dogra V, et al. Efficacy of convalescent plasma therapy in the patient with COVID-19: a randomised control trial (COPLA-II trial). *BMJ Open*. 2022;12(4):e055189. https://doi.org/10.1136/bmjopen-2021-055189
12. Libster R, Pérez Marc G, Wappner D, et al. Early high-titer plasma therapy to prevent severe Covid-19 in older adults. *N Engl J Med*. 2021;384(7):610-618. https://doi.org/10.1056/NEJMoa2033700
13. Estcourt L, Callum J. Convalescent plasma for Covid-19—making sense of the inconsistencies. *N Engl J Med*. 2022;386(18):1753-1754. https://doi.org/10.1056/NEJMe2204332
14. Sarkar S, Soni KD, Khanna P. Convalescent plasma is a clutch at straws in COVID-19 management! A systematic review and meta-analysis. *J Med Virol*. 2021;93(2):1111-1118. https://doi.org/10.1002/jmv.26408
15. Vegivinti CTR, Pederson JM, Saravu K, et al. Efficacy of convalescent plasma therapy for COVID-19: a systematic review and meta-analysis. *J Clin Apheresis*. 2021;36:470-482 https://doi.org/10.1002/jca.21881
16. Garraud O, Sut C, Haddad A, et al. Transfusion-associated hazards: a revisit of their presentation. *Trans Clin Biol*. 2018;25(2):118-135. https://doi.org/10.1016/j.tracli.2018.03.002
17. Raval JS, Griggs JR, Fleg A. Blood product transfusion in adults: indications, adverse reactions, and modifications. *Am Fam Physician*. 2020;102(1):30-38. https://pubmed.ncbi.nlm.nih.gov/32603068/
18. Roubinian N. TACO and TRALI: biology, risk factors, and prevention strategies. *Hematology*. 2018;2018(1):585-594. https://doi.org/10.1182/asheducation-2018.1.585
19. Schneider K, Wronka-Edwards L, Leggett-Embrey M, et al. Psoralen inactivation of viruses: a process for the safe manipulation of viral antigen and nucleic acid. *Viruses*. 2015;7(11):5875-5888. https://doi.org/10.3390/v7112912
20. Semple JW, Rebetz J, Kapur R. Transfusion-associated circulatory overload and transfusion-related acute lung injury. *Blood*. 2019;133(17):1840-1853. https://doi.org/10.1182/blood-2018-10-860809
21. Franco D, Gonzalez C, Abrego LE, et al. Early transmission dynamics, spread, and genomic characterization of SARS-CoV-2 in Panama. *Emerg Infect Dis*. 2021;27(2):612-615. https://doi.org/10.3201/eid2702.203767
22. Hesse S, Nuñez H, Salazar JR, et al. Case report: first confirmed case of coinfection of SARS-CoV-2 with choclo orthohantavirus. *Front Trop Dis*. 2021;2:769330. https://doi.org/10.3389/fitd.2021.769330
23. AlQahtani, M., Abdulrahman, A., Almadani, A., Alali, S. Y., Al Zamrooni, A. M., Hejab, A. H., et al. Randomized controlled trial of convalescent plasma therapy against standard therapy in patients with severe COVID-19 disease. *Scientific reports*, 2021;11(1):9927. https://doi.org/10.1038/s41598-021-89444-5
24. Gharbharan A, Jordans CCE, GeurtsvanKessel C, et al. Effects of potent neutralizing antibodies from convalescent plasma in patients hospitalized for severe SARS-CoV-2 infection. *Nat Commun*. 2021;12(1):3189. https://doi.org/10.1038/s41467-021-23469-2
25. Balcells ME, Rojas L, Le Corre N, et al. Early versus deferred anti-SARS-CoV-2 convalescent plasma in patients admitted for COVID-19: a randomized phase II clinical trial. *PLoS Med*. 2021;18(3):1003415. https://doi.org/10.1371/journal.pmed.1003415
26. Iannizzi C, Chai KL, Piechotta V, et al. Convalescent plasma for people with COVID-19: a living systematic review. *Cochrane Database Syst Rev*. 2023;5(5):013600. https://doi.org/10.1002/14651858.CD013600.pub6
27. Ramirez-Arcos S, Howell A, Bearne J, et al. Challenging the 30-min rule for thawed plasma. *Vox Sang*. 2022;117(3):328-336. https://doi.org/10.1111/vox.13189
28. Draman R, Yousuf R, Abdul Aziz S, Ding CH, Zainol S, Leong CF. A preliminary study on coagulation parameters and sterility of thawed refrozen fresh frozen plasma. *Ind J Hematol Blood Trans*. 2020;36(1):112-116. https://doi.org/10.1007/s12288-019-01171-0
29. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci*. 2020;117(17):9490-9496. https://doi.org/10.1073/pnas.2004168117
30. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbiol*. 2020;5(12):1598-1607. https://doi.org/10.1038/s41564-020-00813-8
31. Focosi D, Franchini M, Pirofski L, et al. COVID-19 convalescent plasma and clinical trials: understanding conflicting outcomes. *Clin Microbiol Rev*. 2022;35:e00200. https://doi.org/10.1128/cmr.00200-21
32. Joyner MJ, Carter RE, Fairweather D, Wright RS. Convalescent plasma and COVID-19: time for a second—second look? *Transfus Med*. 2023;33(1):16-20. https://doi.org/10.1111/tme.12915
33. Dolscheid-Pommerich R, Bartok E, Renn M, et al. Correlation between a quantitative anti-SARS-CoV-2 IgG ELISA and neutralization activity. *J Med Virol*. 2022;94(1):388-392. https://doi.org/10.1002/jmv.27287

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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