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CONVALESCENT PLASMA THERAPY FOR COVID-19: STATE OF THE ART.

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Abbreviations : ADE : antibody-dependent enhancement; CBP : convalescent blood product; COVID-19 : coronavirus disease 2019; CP : convalescent plasma; CWB : convalescent whole blood; ELISA : enzyme-linked immunosorbent assay EVD : Ebolavirus disease; IVIG : intravenous immunoglobulins; MERS : Middle-East respiratory syndrome; PRNT : plaque reduction neutralization test SARS : severe acute respiratory syndrome; TRALI : transfusion-related acute lung injury ; TTI : transfusion-transmitted infection.

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Abstract

Convalescent blood product therapy has been introduced since early 1900s to treat emerging infectious disease based on the evidence that polyclonal neutralizing antibodies can reduce duration of viremia. Recent large outbreaks of viral diseases for whom effective antivirals or vaccines are still lacking has revamped the interest in convalescent plasma as life-saving treatments. This review summarizes historical settings of application, and surveys current technologies for collection, manufacturing, pathogen inactivation, and banking, with a focus on COVID-19.

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Introduction

Emerging viruses rarely provide time to develop vaccines, and prophylactic vaccines are rarely effective in therapeutic setting. Antivirals are currently available only for selected viral families, are often not affordable to developing countries, and their manufacturing is hard to scale up in short times.

Recent viruses with pandemic potential include flaviviruses (e.g. West Nile virus (WNV), dengue virus, Zika virus (1)), chikungunya virus (2), influenzaviruses A, e.g. A(H1N1), A(H5N1) (3), Ebola virus (EBOV) (4), and respiratory betacoronaviruses (SARS-CoV (5), MERS-CoV (6), and SARS-CoV2 (7)).

Transfusion of convalescent blood products (CBP), especially convalescent plasma (CP), are useful against emerging infectious agents if the latter induces neutralizing antibodies (8). CBP are manufactured by collecting whole blood (or plasma) from a donor who has survived a previous infection: donor selection should be based according to neutralizing antibody titer, but in resource-poor settings, ELISA or no selection at all has often been implemented. The donor should preferably live in the same area as the intended recipient(s) to consider mutations of the target viral antigens, even if in areas epidemic for other infectious diseases (e.g. malaria) this could represent a contraindication. Although the recipient is already infected, theoretically transmission of more infectious particles could worsen clinical conditions. For this reason, the right timing of collection is fundamental to ensure no transmission of the pathogen to the recipient. Nevertheless, such concern can be somewhat reduced by treatment with modern pathogen inactivation (PI) techniques.

The main accepted mechanism of action for CBP therapy is clearance of viraemia, which typically happens 10–14 after infection (9). So CBP has been typically administered after early symptoms to maximize efficacy. Concurrent treatments might synergize or antagonize CP efficacy (e.g. polyclonal intravenous immunoglobulins or steroids) (10).

In the setting of respiratory viral infections, secretory IgA, which are the main immunoglobulin isotype on mucosal surfaces, are key players. They are made of 2 IgA molecules (dimers), a joining protein (J chain), and

a secretory component. IgM and IgA are actively transported across epithelia by the polymeric Ig receptor (pIgR) or by neonatal Fc receptor (FcRn), while IgG can passively trasudate into alveolar fluids (11). The lung requires specific antiviral IgG_{2a} for protection in terminal bronchioles and alveoli (12, 13).

Given the emergency related to the COVID-19 pandemic, this review summarized historical settings of application, and surveys current technologies for collection, manufacturing, pathogen inactivation, and banking, of convalescent blood products, with a specific focus on possible applications for COVID-19.

Convalescent plasma and pathogen inactivation

Convalescent whole blood (CWB), in addition to antibodies, provides control of hemorrhagic events, as in Ebolavirus disease, if transfused within 24 h to maintain viable platelets and clotting factors. Nevertheless, convalescent plasma (CP) best fits developed countries standards and settings where antibodies only required. CP should be collected by apheresis in order to ensure larger volumes, more frequent donations, and do not cause unnecessary anemia in the donor.

Technologies to virally reduce plasma (pathogen inactivation)

In several settings donor screening and conventional NAT viral testing (i.e. HIV, HCV and HBV NAT) could not be enough to ensure CP safety. In those scenario, pathogen reduction technologies (PRT) should be used. Several technologies have been approved and are currently marketed.

Solvent/detergent (S/D)-filtered (S/D-F) plasma provides quick > 4 logs inactivation of most enveloped viruses: although the technology was developed and is massively used for large plasma pools, small scale reduction have been reported. The technology relies over 1% tri (n-butyl) phosphate/1% Triton X-45, elimination of solvent and detervent via oil extraction and filtration, and finally sterile filtration (14). Filtration on 75–35 nm hollow fibers could remove large viruses while preserving IgG [48], but has not been implemented yet.

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In recent years photo-inactivation in the presence of a photosensitizer has become the standard for single unit inactivation : licensed technologies include treatment with methylene blue + visible light (15) (Theraflex®), amotosalen (S-59) + ultraviolet A (16) (Intercept®), and riboflavin + ultraviolet B (17) (Mirasol®). These methods do not to affect immunoglobulin activity.

Fatty acids are also an option. In 2002 it was reported that caprylic acid (18) and octanoic acid (19) were as effective as S/D at inactivating enveloped viruses.

Heat-treatment of plasma has been used in the past (20, 21) but goes with the risk of aggregation of immunoglobulins (22, 23).

Pooling

Large-pool products

Pharmaceutical-grade facilities typically pool 100/2500 donors to manufacture S/D-inactivated plasma Intravenous immunoglobulins (IVIGs) are similarly prepared from pools of 2000–4000 L of plasma (or 100-1000 L in the case of hyperimmune IVIG) (24) (25). Such size can be hardly matched from CP donors and facilities rearrangement poses hard GMP issues (25).

Mini-pool fractionation scale (MPFS) into immunoglobulins

In order to be economically sustainable contract fractionation typically requires well over 10 000 liters of plasma per year, and domestic fractionation typically over 100 000–200 000 liters per year in addition to start-up a fractionation facility. A “on the bench” MPFS process (5-10 liters of plasma, i.e. approximately 20 recovered plasma units) using disposable devices and based on caprylic acid precipitation is under development in Egypt since 2003, and has been proven effective at purifying coagulation factors (26) and immunoglobulins (6-fold enrichment) (27). The same disposable bag system has also been combined with S/D reduction (14).

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Lessons from SARS

SARS-specific antibodies are maintained for an average of 2 years, and significant reduction of prevalence and titers occur in the third year (28). IgGs made from CP were experimentally prepared on a small scale to treat patients with SARS (8, 29). Three infected healthcare workers whose condition had progressed severely and who had failed to respond to the available treatment, survived after transfusion with 500 ml CP (confirmed free of residual SARS-CoV by RT-PCR): viral load dropped to zero one day after transfusion (30). SARS-CoV RNA was detected in respiratory samples from one third of patients up to 4 weeks after symptom onset (31). Amotosalen photochemical inactivation of apheresis platelet concentrates demonstrated a >6.2 log10 mean reduction of SARS-CoV (32). Theraflex˚ reduces infectivity of SARS-CoV in plasma (33). Heating at 60°C for 15-30 minutes reduces SARS-CoV from plasma without cells (34), while 60°C for 10 hours is required for plasma products (35). In addition, SARS-CoV was found to be sensitive to S/D, (34, 36).

Lessons from MERS

Antibody responses to MERS persist for less than 1 year and magnitude correlates with the duration of viral RNA detection (but not viral load) in sputum. Mild patients have very low titers, making CP collection challenging in MERS convalescents (37). A study reported that only 2.7% (12 out of 443) exposed cases had a reactive ELISA result, and only 75% of them had reactive microneutralization assay titers (38). CP with a PRNT titre ≥1:80 provide clinical benefit in MERS (39). A case of TRALI following CP transfusion in a patient with MERS was reported (40, 41). MERS-CoV load in plasma was reduced by Theraflex˚ (42), Intercept˚ (43) and Mirasol®(44), and heating at 56°C for 25 minutes (45) : in all cases passaging of the inactivated samples in Vero E6 showed no viral replication even after 7 days of incubation.

Convalescent plasma for COVID-19

As soon as the COVID-19 pandemic appeared (7, 46), several authors suggested CP as a potential therapeutic (47, 48). Of interest, the most critically ill patients show prolonged viremia (strongly correlated with serum IL-6 levels) (49), which leaves room for therapeutic intervention with antivirals and immunoglobulins even in late stages. Viral shedding in survivors can be as long as 37 days (46), mandating SARS-CoV2 RNA screening

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in CP donors. A Chinese pilot study on 10 critically ill patients showed that one dose of 200 mL CP with neutralizing antibody titers > 1:640 resulted in an undetectable viral load (70%), radiological and clinical improvement(50). In another case series from China, 5 patients under mechanical ventilation (4 of 5 with no preexisting medical conditions) received transfusion with CP with a ELISA IgG titer > 1:1000 and a neutralization titer > 40 t day 10-22 after admission. Following plasma transfusion, ARDS resolved in 4 patients at 12 days after transfusion, and 3 patients were weaned from mechanical ventilation within 2 weeks of treatment, the remaining being stable(51). John Hopkins University is currently leading trials in the US of CP with titer > 1:64 for post-exposure prophylaxis (52) and treatment of non-critically ill patients (53).

Appearance of serum IgM and IgA antibody in COVID-19 occurs since day 5 after symptom onset, while IgG is detected since day 14 (54, 55). IgG are universally detected since day 20 (56). Severe female patients generate IgG earlier and higher titers(57). Duration of anti-SARS-CoV2 antibodies in plasma remains unknown, though for other betacoronaviruses immunity typically lasts 6-12 months (58). So a suitable donor could donate 600 ml plasma (equivalent to 3 therapeutic doses) every 14 days for a minimum of 6 months. In contrast to EVD, SARS, and MERS, most COVID-19 patients exhibit few or no symptoms and do not require hospitalization, suggesting that the majority of convalescent donors are best sought after in the general population. Notably, several plasma manufacturers are attempting to develop SARS-CoV2-specific hyperimmune sera, e.g. Takeda's TAK-888 (59) and Kamada's anti-COVID19 IgG (60).

Table 1 lists the ongoing CP trials in COVID-19 patients listed in World Health Organization International Clinical Trial Registry Platform (ICTRP) database. Unfortunately, most trial in Westernized countries (on the contrary of the ones ongoing in China) seem to no have no control arm, which will impair efficacy interpretation.

CP donor recruitment strategies

As previously proofed, donor testing for neutralizing antibodies is mandatory in upstream donor selection. Three approaches are theoretically available to recruit CP donors, everyone having pros and cons. The least

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cost-effective approach is screening the general periodic donor population for presence of anti-SARS-CoV2 antibodies. In endemic areas, this strategy provides many fit donors with the additional benefit of a seroprevalence study in the general population (80% of cases being asymptomatic), but requires a high budget. On the other side of the coin, recruitment of hospital discharged patients is highly cost-effective (patients can be easily tested before discharge and tracked), but patients who have required hospitalization are highly likely to be elderlies with comorbidities, and hence unfit to donate. The intermediate approach is deploying calls to donate to positive cases under home-based quarantine: given the huge numbers, some of them are likely to be periodic donors, and home-based convalescence suggests they are fit enough to donate. Nevertheless, lessons from MERS suggest that patients with mild symptoms could have developed low-titer antibodies (38), making antibody titration even more important in the population-wide and home-based approaches.

CP banking

CP is typically used as a fresh product. Aliquots can be easily achieved with modern PI kits. Banking at temperature below -25˚C (according to EDQM guidelines for ordinary plasma for clinical use (61)) is encouraged in order to translate CP in an off-the-shelf, ready-to-use product. Most regulatory system require that CP is tracked informatically as a blood component different from ordinary plasma for clinical use. The fina validation label should report that the donor has tested negative at PCR for the convalescent disorder and additional microbiological tests, and describe the inactivation method. There is no evidence that a single cycle of freezing and thawing significatively affects quantity or function of immunoglobulins.

Monitoring response to treatment

CP is considered an experimental therapy, and as such phase 3 randomized controlled trials should be encouraged. Despite this recommendation, in emergency settings phase 2 trials are usually started, hampering efficacy analysis. Response in published trials is generally measured clinically or radiologically

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according to target organs. Nevertheless, surrogate endpoints can include antibody titer rise in recipient's plasma and drops in recipient's viral load. Whenever quantitative PCR is not available, cycle threshold (Ct) value increases in qualitative PCR after transfusion could be a proxy for reduced viral load.

Side benefits from CP in COVID-19

Obviously, patients with humoral immune deficiencies can benefit from polyclonal antibodies contained in CP, and patients with hemorrhagic diathesis can benefit from clotting factors. After demonstration that group 0 healthcare workers were less likely to become infected with SARS-CoV (62), a research group proved that anti-A blood group natural isoagglutinins (which can be also found in CP plasma from blood group 0 and B donors) inhibit SARS-CoV entry into competent cells (63) and could opsonize viral particles leading to complement-mediated neutralization (64). Since SARS-CoV2 uses the same receptor as SARS-CoV, anti-A isoagglutinins are expected to have similar effects against SARS-CoV2: accordingly clusters of glycosylation sites exist proximal to the receptor-binding motif of the SARS-CoV (65) and SARS-CoV2 (66) S protein.

A recent publication showed that the odds ratio for acquiring COVID-19 is higher in blood group A than in blood group 0 (67). COVID-19 has more severe clinical presentations and outcome in elderlies and in males : intriguingly, elderly males are known to experience reductions in isoagglutinin titers (68, 69). Studies are hence ongoing to evaluate correlations between isoagglutinin titers and outcome in blood group 0 and B patients. In the meanwhile, while preserving ABO match compatibility, it could be wise to prefer blood group 0 and B donors for CP in COVID-19, and to titre their anti-A isoagglutinins.

Concerns

The main contraindications to CP therapy are allergy to plasma protein or sodium citrate, or selective IgA deficiency (< 70 mg/dl in patients 4 years old or greater). As in many other trial settings, concurrent viral or bacterial infections, thrombosis, poor compliance, short life expectancy (e.g. multiple organ failure), as well as pregnant or breastfeeding women. are also contraindications. Nevertheless, additional concerns apply.

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The first one is the risk for transfusion-transmitted infection (TTI). Modern PI technologies, combined with NAT, reduces the risk for contracting additional TTIs. Most regulatory systems require additional tests (e.g. HAV RNA, HEV RNA, parvovirus B19 DNA) to be performed on CP for additional transfusion safety. CBP obtained from donors in the UK may be problematic for a couple of reasons. Currently CBP obtained from individuals who lived for at least 6 months in the UK during 1980-1996 'mad cow disease (bovine spongiform encephalopathy – BSE)' outbreak may not be acceptable in some countries (70) – or by some individuals. In addition, there is a now a recognized risk of hepatitis E the within UK blood donor population (71), most likely due to the consumption of poorly cooked pork products (72, 73), for which screening has only relatively recently been initiated(74). Although this does not preclude such SARS-CoV-2 convalescent plasma/sera being used therapeutically within the UK, these other risks should be considered during larger clinical trial or individual patient compassionate use. As per the risk of worsening the clinical picture by delivering more viral particles of the targeted virus, it is generally unlikely to worsen the underlying scenario. Respiratory betacoronaviruses produce only a mild and transient viremia. With SARS-CoV, limited replication in lymphocytes(75) leads to significant risk only for recipients of blood products with high concentrations of donor lymphocytes (peripheral blood stem cells, bone marrow, granulocyte concentrates, etc). With SARS-CoV2, viremia has been shown persists only in critically ill patients (49).

The second concern is TRALI, which can be life-threatening in patients who already are suffering from ALI. Male donors are usually preferred in order to avoid the risk of transfusing anti-HLA antibodies from parous women. In the case of COVID-19, where female patients have been shown to have higher IgG levels, this could be detrimental, and anti-HLA antibody screening could be implemented.

Antibody-dependent enhancement (ADE) due to passive or active antibodies facilitating coated virions entry into cells via Fc receptors (76, 77) is also a theoretical concern, but its clinical relevance remains unproven (78).

Conclusions

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Whole blood and plasma may be the first and only options to consider during a pandemic in the meanwhile antivirals and vaccines are tested. They should be prepared under ethical and controlled conditions to ensure optimal safety to both donors and recipients. Randomized controlled trials are important to establish safe modality of treatment and to provide important knowledge for the treatment of future infectious outbreaks.

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Figure 1. Summary of possible convalescent blood products (CBP). Reproduced from ref (79) under STM Permissions Guidelines as of 26 March 2020 (https://www.stm-assoc.org/intellectual-property/permissions/permissions-guidelines/).

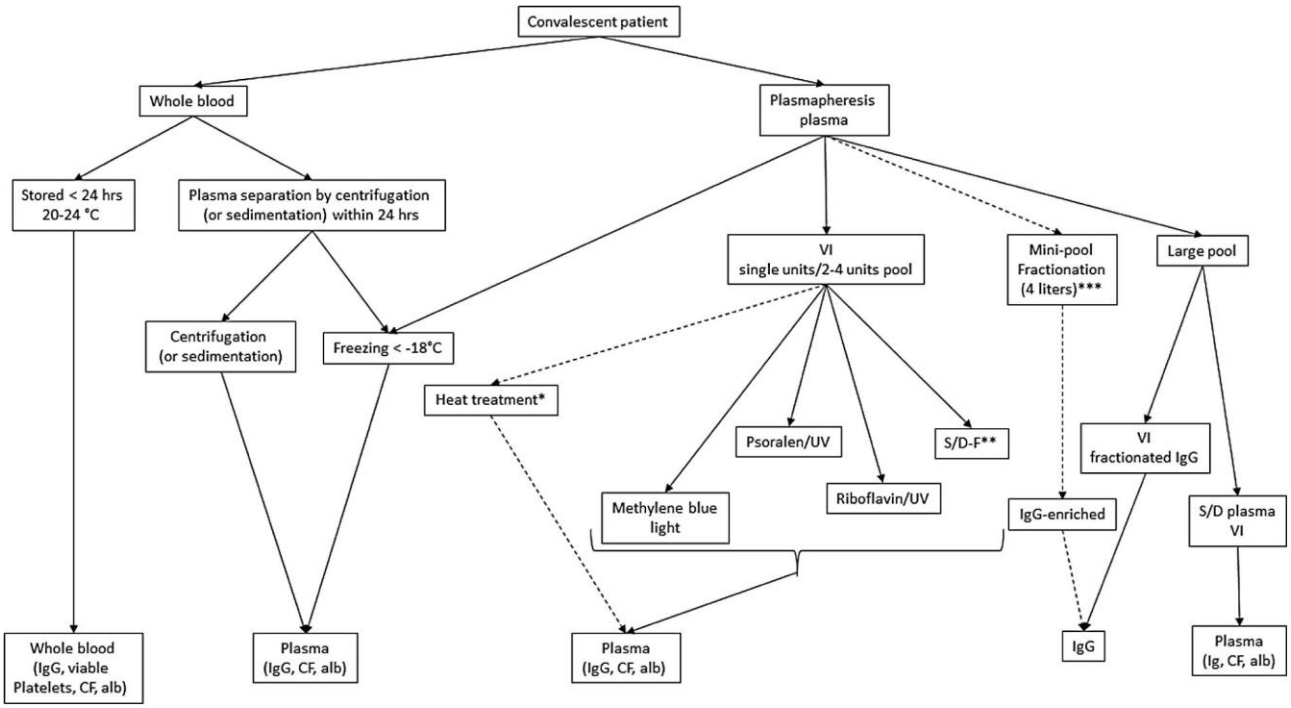

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Table 1. Ongoing interventional clinical trials of convalescent plasma in COVID-19 patients listed in World Health Organization International Clinical Trial Registry Platform (ICTRP) databases (accessed online at https://www.who.int/docs/default-source/coronaviruse/covid-19-trials.xls on April 6, 2020)

Trial number	Title (country)	Study population	Schedule	Donor Titer	Indication
ChiCTR2000029850	Study on convalescent plasma treatment for severe patients with novel coronavirus pneumonia (COVID-19) (China)	Exp:10 Ctr:10	NA	NA	Clinical deterioration despite conventional treatment that required intensive care
ChiCTR2000030179	Experimental study of novel coronavirus pneumonia rehabilitation plasma therapy severe novel coronavirus pneumonia (COVID-19) (China)	Exp:50 Ctr:50	NA	NA	Critically ill patients
ChiCTR2000030046	A single arm trial to evaluate the efficacy and safety of anti-2019-nCoV inactivated convalescent plasma in the treatment of novel coronavirus pneumonia patient (COVID-19) (China)	10	NA	NA	Non critically ill patients

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ChiCTR2000030010	A randomized, double-blind, parallel-controlled, trial to evaluate the efficacy and safety of anti-SARS-CoV-2 virus inactivated plasma in the treatment of severe novel coronavirus pneumonia patients (COVID-19) (China)	Exp:50 Ctr:50	NA	NA	Non critically ill patients
ChiCTR2000030039	Clinical study for infusing convalescent plasma to treat patients with new coronavirus pneumonia (COVID-19) (China)	Exp:30 Ctr:60	2 units of plasma (200/500 mL/24h) *vs* BSC	NA	All patients
ChiCTR2000030627	Study for using the healed novel coronavirus pneumonia (COVID-19) patients plasma in the treatment of severe critical cases (China)	Exp:15 Ctr:15	NA	NA	Severe or critically ill patients
ChiCTR2000029757	Convalescent plasma for the treatment of severe and critical novel coronavirus pneumonia (COVID-19): a prospective randomized controlled trial (China)	Exp:100 Ctr:100	NA	NA	Severe or critically ill patients

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NCT04292340	Anti-SARS-CoV-2 Inactivated Convalescent Plasma in the Treatment of COVID-19 (China)	15	NA	NA	All patients with Covid-19
ChiCTR2000030702	Plasma of the convalescent in the treatment of novel coronavirus pneumonia (COVID-19) common patient: a prospective clinical trial (China)	Exp:25 Ctr:25	NA	NA	Non critically ill patients
ChiCTR2000030929	A randomized, double-blind, parallel-controlled trial to evaluate the efficacy and safety of anti-SARS-CoV-2 virus inactivated plasma in the treatment of severe novel coronavirus pneumonia (COVID-19) (China)	Exp:30 Ctr:30	NA	NA	Non critically ill patients
NCT04321421	Hyperimmune Plasma for Critical Patients With COVID-19 (COV19-PLASMA) (Italy)	49	3 units of plasma (250-300 mL/48h)	NA	Moderate to severe ARDS under mechanical ventilation
NCT04323800	Efficacy and Safety Human Coronavirus Immune Plasma (HCIP) vs. Control (SARS-CoV-2 Non-immune Plasma) Among Adults Exposed to COVID-19 (CSSC-001) (USA)	150	1 unit of plasma (200/250mL)	>1:64	Exposed to the contagion (within 96 hours of enrollment and 120 hours of receipt of plasma)

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NCT04325672	Convalescent Plasma to Limit Coronavirus Associated Complications: An Open Label, Phase 2A Study of High-Titer Anti-SARS-CoV-2 Plasma in Hospitalized Patients With COVID-19 (USA)	20	1-2 units of plasma (300 mL/24h)	>1:64	Severe or critically ill patients
NCT04333251	Evaluating Convalescent Plasma to Decrease Coronavirus Associated Complications. A Phase I Study Comparing the Efficacy and Safety of High-titer Anti-Sars-CoV-2 Plasma vs Best Supportive Care in Hospitalized Patients With Interstitial Pneumonia Due to COVID-19 (USA)	115 Exp: NA Ctr: NA	1-2 units of plasma (250 mL/24h) *vs* BSC	>1:64	All patients with Covid-19
To be assigned	Convalescent Plasma to Treat Coronavirus - Associated Severe Pulmonary Complications: A Feasibility Study Assessing the Safety of Multiple Doses of Anti-SARS-CoV-2 Plasma in Mechanically Ventilated Intubated Patients	30	1-2 units of plasma (200/250 mL/24h)	NA	Critically ill patients

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	with Respiratory Failure due to COVID-19 (CPPulm-001) (USA)				
NCT04332380	Convalescent Plasma for Patients With COVID-19: A Pilot Study (CP-COVID-19) (Colombia)	10	2 units of plasma (250 mL/24h)	NA	Non critically ill patients. 250 ml day 1 + 250 ml day 2
NCT04332835	Convalescent Plasma for Patients With COVID-19: A Randomized, Open Label, Parallel, Controlled Clinical Study (Colombia)	40 Exp: NA Ctr: NA	2 units of plasma (250 mL/24h) *vs* BSC	NA	Non critically ill patients
NCT04327349	Investigating Effect of Convalescent Plasma on COVID-19 Patients Outcome: A Clinical Trial (Iran)	30	NA	NA	Non critically ill patients
NCT04333355	Phase 1 Study to Evaluate the Safety of Convalescent Plasma as an Adjuvant Therapy in Patients With SARS-CoV-2 Infection (Mexico)	20	1-2 units of plasma (250 ml/24h)	NA	Severe or critically ill patients
BSC: best supportive care; NA: not available; Exp: experimental group; Ctr: control group					

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