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Convalescent plasma treatment for COVID-19: tempering expectations with the influenza experience

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Abstract:

The COVID-19 pandemic caused by the zoonotic coronavirus, SARS-CoV-2 has swept the world in 5 months. A proportion of cases develop severe respiratory tract infections progressing to acute respiratory distress syndrome and a diverse set of complications involving different organ systems. Faced with a lack of coronavirus-specific antiviral drugs and vaccines, hundreds of clinical trials have been undertaken to evaluate repurposed drugs. Convalescent plasma from recovered patients is an attractive option because antibodies can have direct or indirect antiviral activity and immunotherapy works well in principle, in animal models and in anecdotal reports. However, the benefits of convalescent plasma treatment can only be clearly established through carefully designed randomised clinical trials. The experience from investigations of convalescent plasma products for severe influenza offers a cautionary tale. Despite promising pilot studies, large multicentre randomised controlled trials failed to show a benefit of convalescent plasma or hyperimmune intravenous globulin for the treatment of severe influenza A virus infection. These studies provide important lessons that should inform the planning of adequately powered randomised controlled trials to evaluate the promise of convalescent plasma therapy in COVID-19 patients.

Active immunization refers to the induction of protective immunity in a host using a vaccine, while passive immunization refers to the transfer of antibodies to a non-immune person to confer immunity, providing temporary protection from disease. The history of passive immunization began with the work in 1890 of Behring and Kitasato, who succeeded in protecting a rabbit from tetanus by injecting it with serum taken from an immunized animal (reviewed in [1]). This knowledge was applied to protect humans from life-threatening, toxin-mediated bacterial infections including diphtheria and tetanus and subsequently, with varying success in several viral infections including hepatitis A and B, Ebola, SARS and influenza. The rationale for immunotherapy in viral infections is that antibodies can neutralize virus infectivity directly or through Fc-mediated functions including complement activation, antibody dependent cell cytotoxicity or phagocytosis [2, 3]. During the 1918 Spanish Flu pandemic, convalescent blood products were used to treat patients with pneumonia. A meta-analysis of 8 studies of 1703 patients showed that treated patients experienced reduced risk of death, with an overall crude case-fatality rate of 16% (54 of 366) vs 37% (452 of 1219) among controls [4].

Products used for passive immunization can be derived from animals e.g. horses or transchromosomic cattle, or humans e.g. convalescent plasma, immune globulin, hyperimmune globulin and monoclonal antibodies [5]. Convalescent plasma is obtained by apheresis of blood from a recovered patient (donor) and is administered to a recipient. Immune globulin (also called gamma globulin) is prepared from pooled plasma obtained from thousands of people representing a diverse antibody repertoire and is administered by intramuscular or intravenous routes. The latter, intravenous immune globulin, called IVIG is used to treat immune deficiencies, autoimmune disorders and diverse conditions such as idiopathic thrombocytopenic purpura and multiple sclerosis. Hyperimmune globulin is prepared from the plasma of donors known to have high antibody levels against specific pathogens. Hyperimmune globulin against rabies, varicella-zoster and hepatitis B are administered for post-exposure prophylaxis. The advantages of hyperimmune IVIG (hIVIG) over convalescent plasma are (i) neutralising antibody titres in hIVIG are higher, (ii) blood group matching is not required, (iii) the volume of the infusion is smaller, (iv) the antibody concentration can be adjusted during manufacture. However, production of convalescent plasma takes weeks while manufacture of hIVIG can take months [6]. Technological advances in rapid generation of human monoclonal antibodies derived by immortalizing memory B cells from convalescent patients [7], phage display libraries from healthy subjects [8, 9] or from transgenic (humanized) mice [10] have made it possible to use monoclonal antibodies against defined epitopes instead of plasma for passive immunization. Several monoclonal antibodies that were shown to be effective in animal models of SARS and influenza [7-13] have progressed to clinical trials [5].

A review and meta-analysis of 27 studies of convalescent plasma in severe acute respiratory illness (SARI), including SARS, avian influenza, H1N1pdm09 and the 1918 influenza pandemic concluded that convalescent plasma could have an impact on reducing mortality [14]. A meta-analysis that included SARI caused by a range of viruses showed a statistically significant 75% reduction in the odds of death among patients treated with convalescent plasma or serum. Some studies suggested that early initiation of treatment may be of critical importance in reducing mortality [4, 6, 15]. Serious adverse events were not reported and evidence of reduced need for critical care resources or duration of hospital stay were limited. However, the authors who undertook the systematic analysis emphasized the paucity of high-quality studies. Observational studies that lacked control groups were at high risk of bias. Furthermore, appropriate statistical or methodological approaches to control bias and confounding were used infrequently and numerous sources of clinical and methodological heterogeneity were identified [14].

Randomised controlled trials of passive immunization for severe influenza

Clinical trials have been conducted using convalescent plasma and hIVIG to treat patients with severe influenza and the information from these studies is highly relevant to the current discussion about COVID-19.

Immune plasma was evaluated in multicentre phase 2 and 3 studies organised by the US National Institutes of Health. The phase 2 study was an open-label randomised study of immune plasma for severe influenza conducted in 29 US medical centres [16]; 49 participants each were assigned randomly to receive 2 units of ABO compatible immune plasma (225-350 ml/unit and 8 ml/kg paediatric equivalent) in addition to standard care or standard care alone. The geometric mean haemagglutination inhibition (HI) titres of the plasma preparations were 1:259 for influenza A(H1N1) and 1:158 for A(H3N2) (range 1:80-1:1280) and 1:101 for influenza B (range 1:80-1:640). Hospitalised children or adults with laboratory confirmed influenza A(H1N1) or A(H3N2) or B infection with hypoxia (room air oxygen saturation <93%) or tachypnoea were eligible for enrolment. The primary efficacy endpoint of normalisation of respiratory rate and oxygenation by day 28 was seen in 67% of plasma recipients compared with 53% of the standard care group (p=0.069); the hazard ratio of plasma versus standard of care was 1.71 (95% CI 0.96-3.06). Although the study did not show clear benefit in the primary outcome, a significant improvement in clinical status at day 7 (p=0.02) was detected using an ordinal scale of clinical status by level of care [17]. A numerical decrease in mortality (p=0.093) was observed in the plasma group though there was no measurable

effect on decreasing the symptoms of influenza illness. Differences in several secondary outcomes were suggestive of efficacy including numerically fewer days in hospital (median 6 days in the plasma group versus 11 days in the standard care alone group, p=0.13), fewer participants with ICU admissions (57% vs 69% p=0.097) and fewer days on mechanical ventilation (median 0 vs 3 days, p=0.52) in the plasma group. For the primary and secondary endpoints, the benefit of plasma was mainly in participants who were enrolled within 4 days of symptoms. Six patients died during the study, one (2%) in the plasma group and five (10%) in the standard care group (intention to treat analysis p=0.093; hazard ratio (HR) 0.19, 95% CI 0.0.2-1.65). Participants in the plasma group had a better disposition after hospital discharge (p=0.029). Serious adverse events related to underlying influenza, its complications and comorbid conditions were reported in 20% of plasma recipients vs 38% of the standard care group (p=0.041) [16].

Based on the results from the phase 2 study, a randomised, double-blind multicentre phase 3 study of high-titre immune plasma for hospitalised patients with severe influenza A was conducted in 41 large US medical centres [18]. The high-titre plasma units had an HI titre≥1:80 (median HI titre 1:160-1:320 for H1N1 and 1:160-1:640 for H3N2) and the control group received low-titre plasma (HI titre ≤1:10). The primary outcome measure was clinical status at day 7. Participants were randomised 2:1 to receive high (n=92) or low (n=48) titre plasma. The participant's median age was 60.5 years (IQR 45-69). Participants reported illness a median of 3 days prior to enrolment and 78% had received antiviral drugs before they were randomised to treatment groups. At the start of the study, 43% of the study participants were in intensive care and 71% of the 78 who were not in ICU required supplemental oxygen. On day 7, 55% of the recipients of high-titre plasma had been discharged from hospital compared to 47% of the group who received low-titre plasma; the proportional odds ratio was 1.22 (95% CI 0.65-2.29, p=0.54). Serious adverse events occurred in 35% of the high-titre group and 32% of the low-titre group. Acute respiratory distress syndrome (ARDS), allergic transfusion reactions and respiratory distress were the most commonly reported adverse events. Ten deaths were reported during the study; six (7%) in the group that received high-titre plasma and four (9%) in the group that received low-titre plasma. Worsening of ARDS was the most commonly reported cause of death. The study was terminated when independent efficacy analysis showed low likelihood to detect an effect of high-titre plasma even if full accrual of 150 participants was achieved [16]. The definitive conclusion from this prospective study, that patients with severe influenza A did not benefit significantly from receiving high-titre plasma instead of non-immune plasma [18], was contrary to what was expected from previous anecdotal reports and observational studies as discussed earlier in this review [14].

Randomised clinical trials were also conducted with anti-influenza hIVIG prepared from pooled plasma from convalescent patients [6] and healthy volunteers who were recently vaccinated against seasonal or pandemic influenza viruses [19]. The first was a small study in five hospitals in Hong Kong that compared hIVIG with a neutralising titre of 1:640, given to 17 patients vs IVIG with a neutralising titre ≤1:20, given to 18 patients, for treatment of severe H1N1pdm09 influenza. There was no difference in mortality, length of ICU or hospital stay between the groups. However, in a subgroup analysis limited to 22 patients who received immunotherapy within 5 days of onset of symptoms, hIVIG reduced viral load and mortality (0 of 12 in the hIVIG group vs 4 of 10 in the IVIG group). Conversely, in patients treated >5 days after symptom onset, all of the patients in the hIVIG group and none of the IVIG group died [6].

A large international randomised double-blind, placebo-controlled trial was conducted in 329 adults hospitalized with influenza A or B infection enrolled at 34 clinical sites over 5 influenza seasons [19]. Patients were randomised 1:1 to receive 0.25 mg/kg hIVIG up to a maximum of 24.75 g, dissolved in saline to a volume of 500 ml vs saline as placebo, in addition to standard care. The hIVIG product was manufactured annually using high-titre anti-influenza plasma collected from immune volunteers and was required to have substantial activity against contemporary influenza strains. Including participants of a pilot study [20], 168 patients received hIVIG and 161 placebo; 24% had A(H1N1), 44% A(H3N2) and 27% influenza B infection. Patients were enrolled a median of 3 days (range 2-5) since symptom onset and 95% in both groups were prescribed the anti-influenza drug, oseltamivir. The primary endpoint was a six-category ordinal outcome of clinical status on day 7, ranging from death to discharge with full resumption of activity. The adjusted odds ratio (OR) of hIVIG vs placebo for the primary endpoint was 1.25 (95% CI 0.79-1.97, p=0.33). In subgroup analyses, the OR for patients with influenza A was 0.94 (95% CI 0.55-1.59) and the lack of efficacy was evident for both H1N1 and H3N2 subtypes. In contrast, the OR was 3.19 (95% CI 1.21-8.42) for patients with influenza B. Through the 28-day follow-up, a similar number of hIVIG and placebo recipients died, had serious adverse events or grade 3 or 4 adverse events. A composite of these three outcomes was seen in 30% of hIVIG and placebo groups each (hazard ratio 1.06, p=0.79). Viral load followed a similar pattern as the primary outcome and a rise in HI antibody titres following the infusion did not predict clinical or virologic efficacy [19].

Taken together, these multicentre, prospective randomised controlled trials failed to show a benefit of plasma or hIVIG immunotherapy for patients with severe influenza A [6, 18, 19]. However, a recurring observation was that treatment with plasma products within 4 (or 5) days of symptom onset was more effective than later treatment [4, 6, 16].

What is the experience with passive immunization in COVID-19?

There are two case reports, a case-control and a retrospective observational study on the use of convalescent plasma in COVID-19 [21-24] (Table 1) and on July 20, 2020 there were 126 registered clinical trials, of which 65 were recruiting, listed on clinicaltrials.gov. The convalescent plasma used in these studies were collected from local COVID-19 patients who had recovered and was administered soon after collection to COVID-19 patients with severe illness. The recipients were all treated with a variety of antiviral drugs and most had also received steroids (intravenous methylprednisolone). Ahn and colleagues reported their experience with two patients with ARDS. IgG antibodies to SARS-CoV-2 were detected by ELISA in donor plasma and it was administered in 2 doses of 250 ml 12 hours apart, but antibody titres in the recipients were not reported [21]. Shen and colleagues reported a case study of five critically ill patients. Convalescent plasma from five donors with neutralisation titres 80, 120, 240, 240 and 480, respectively was administered and pre-transfusion ELISA titres in recipients increased from 1800 - 48,600 to 5400 - 145,800 and neutralising antibody titres increased from 40-160 to 80-320 following transfusion [23].

Duan and colleagues treated 10 patients with severe COVID-19 disease with convalescent plasma collected from donors who had neutralising antibody titres > 1:640 [22]. Neutralising antibody titres increased in 5 patients within 1-2 days post-transfusion, while titres did not change in 4 patients after transfusion, and 1 patient's serum was not measured. A historical control group of 10 patients who received treatment in the same hospitals were matched to cases by age, gender, and severity of disease but neutralising antibody titres, repeated viral RNA measurements and other data over time were not reported from the control patients (Table 1) [22]. Zeng and colleagues extracted data on epidemiology, demographics, management (clinical and laboratory), and outcome on 21 patients admitted to the ICU for COVID-19, of which 6 received convalescent plasma [24]. The antibody titres in the donor plasma or recipients were not measured. All of the plasma treated patients were PCR negative within 3 days of treatment. However, 5 of the 6 plasma treated patients died, as did 14 of 15 in the control group (Table 1) [24]. The case reports and retrospective studies provide anecdotal evidence of the safety and potential virologic or clinical benefits of convalescent plasma but the absence of a control group is a significant limitation of these studies.

The first prospective, randomised clinical trial of convalescent plasma in patients with severe or life-threatening COVID-19 was reported from China [25]. This open-label study conducted in 7 medical centres in Wuhan enrolled 103 of a planned 200 patients over a 6-week period but was terminated when the outbreak was contained. Forty five patients with severe (respiratory distress and/or hypoxemia) and 58 patients with life-threatening (shock, organ failure or requiring

mechanical ventilation) COVID-19 were randomised 1:1 to receive standard treatment with or without convalescent plasma; randomization was stratified by disease severity, so 23 and 22 patients with severe disease were allocated to treatment and control groups, respectively and 29 patients each with life-threatening disease were allocated to the treatment and control groups. Fresh-frozen plasma with a spike protein receptor binding domain ELISA IgG antibody titre ≥1:640 was administered at a dose of 4-13 ml/kg in a volume of 200-300 ml. Standard treatment was not defined by the study protocol and included symptomatic treatment, antivirals, antibiotics, steroids and Chinese herbal medicine. The primary endpoint was time to clinical improvement within 28 days; this was defined as discharge or a reduction by 2 points on the following 6-point disease severity scale: death (6), hospitalization with extracorporeal membrane oxygenation (ECMO) or mechanical ventilation (5), hospitalization with non-invasive ventilation or high-flow oxygen (4), hospitalization plus supplemental oxygen (3), hospitalization without supplemental oxygen (2), hospital discharge (1). Clinical outcomes were assessed by an investigator who was blinded to the allocation of study groups. The median time from symptom onset to randomisation was 30 days (IQR 20-39 days) in the all patients and was 33 days for those with severe disease and 26 days for patients with life-threatening disease. Few patients were randomised within 14 days of symptom onset (5 with severe disease and 3 with life-threatening disease). No significant difference in clinical improvement within 28 days was reported (primary outcome): 51.9% of the convalescent plasma recipients vs 43.1% in the control group, HR 1.40 (95% CI 0.79-2.49), p=0.26. Because the test for interaction by disease severity was not statistically significant, the analysis of the subgroup of patients with severe or life-threatening illness cannot be interpreted as different [25]. There was also no significant difference in the secondary outcomes, including 28-day mortality and time from randomisation to death or discharge. However, nasopharyngeal swab PCR converted from positive to negative in association with the receipt of convalescent plasma. The authors discussed the limitations of their study including that it may have been underpowered by the small sample size following early termination, late initiation of plasma treatment, the open-label design, physician dependent decisions on clinical management and standard treatment, the potential that 28-day follow-up may be too short to see a clinical benefit and the absence of a control plasma product for the control group [25]. In an accompanying editorial, Casadevall et al. point out that this study highlights the importance of clinical improvement as a primary endpoint and provides valuable information on the magnitude of effects that might be expected. This can guide sample size calculations for future studies of convalescent plasma in COVID-19 patients, perhaps in combination with the antiviral drug, Remdesivir [26].

Considerations for future investigations of convalescent plasma for COVID-19

The theoretical reasons for the likely efficacy of passive immunization, the urgent need felt by clinicians around the world for effective treatment options for COVID-19 and the hope offered by anecdotal and retrospective studies must be balanced against the disappointing collective experience of lack of efficacy in the randomised controlled trials of convalescent plasma and hIVIG in severe influenza and COVID-19. Important lessons from these studies, outlined in Box 1, can inform the planning of adequately powered randomised controlled trials in COVID-19 patients. Several influenza studies suggested that the efficacy of plasma/hIVIG was greater if given within 4 or 5 days of the onset of symptoms[4, 6, 15, 16]. The fact that the incubation period and clinical course of COVID-19 are longer than those of influenza may translate to a longer window of opportunity for intervention in this disease.

A SARS-CoV-1 study in a macaque model showed that anti-spike IgG antibodies induced by active or passive immunization with a modified vaccinia virus vector expressing the SARS-CoV-1 spike protein enhanced acute lung injury following SARS-CoV-1 challenge by skewing macrophage responses [27]. If a similar phenomenon were to occur with SARS-CoV-2, convalescent plasma treatment could cause pulmonary immunopathology and increase the severity of COVID-19 disease under certain circumstances.

Two challenging issues related to the optimal use of convalescent plasma are deciding which patients are most likely to benefit from administration of plasma and how to control for potential confounders such as the phase of illness and other treatments. The premise that convalescent plasma therapy will provide neutralizing antibodies to suppress viral replication suggests that COVID-19 patients who have serum antibody levels that are already detectable on admission may not benefit from administration of convalescent plasma. It is possible, and perhaps likely, that the antiviral activity of immune plasma cannot alter the clinical course of illness in patients who are already severely ill and that it would be more effective if given to patients who are not yet severely ill but are at risk for worsening of clinical disease. Selecting such patients requires a biomarker or predictor of disease severity. With the unprecedented quality and pace of global clinical research in COVID-19, the identification of such biomarkers may be within reach.

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Table 1. A summary of the case reports and observational studies of convalescent plasma for treatment of COVID-19

Type of study	n	Pre-transfusion clinical status	Days (d) since onset of illness	Clinical improvement	Outcome	Reference
Case report	2	ARDS, mechanical ventilation	Patient 1: 10d Patient 2: 6d	Fever, reduction in supplemental Oxygen demand; Over 9-10d, Chest x-ray and C-reactive Protein levels improved; PCR Ct[a] increased	Both off mechanical ventilation; one patient discharged	[21]
Case report	5	ARDS or severe pneumonia with rapid progression and high viral load despite antiviral drugs; PaO_2/FiO_2[b]<300; mechanical ventilation	19-22 d in 4; 10 d in 1 patient	Fever decreased in 3 d; SOFA[c] score and oxygenation improved in 12d, Chest CT[d] improved in 3d; PCR Ct increased	4 weaned from mechanical ventilation or ECMO[e]; 3 discharged; 2 remained hospitalised.	[23]
Case control; historical control matched by age, gender, and disease severity	10	Severe pneumonia	median 16.5 d (IQR 11-19.3 d)	All symptoms disappeared or improved in all, in 1-3 d; Chest CT improved in all; PCR- by d2-6 in the 7 patients who were PCR positive	Cases: 4 of 8 had reduced need for oxygen or ventilatory support; 3 discharged and 7 much improved. Controls: 3 deaths, 6 stabilised and 1 improved	[22]
Retrospective, observational	6 cases; 15 controls	Critically ill; all on high flow nasal cannula; 5 of 6 cases and 12 of 15 controls on mechanical ventilation; 4 of 6 cases and 12 of 15 controls on ECMO	Median 21.5 d (17.8-23)		5 of 6 cases died though they were PCR- at death and 14 of 15 controls died; 3 of them were PCR- at death; time to death longer in cases (p=0.029)	[24]

[a]Ct: cycle threshold; [b]PaO2/FiO2: partial pressure of oxygen in arterial blood/inhaled oxygen concentration; [c]SOFA: sequential organ failure assessment; [d]CT: computerised tomography; [e]ECMO: extra-corporeal membrane oxygenation

Box 1. Key considerations for passive immunization studies for COVID-19

Study design: randomised controlled trials, blinding is important, ideally, the control group should receive non-immune plasma, stratify by disease severity and timing of treatment, other treatment including antiviral drugs and steroid use can be confounding factors. FDA guidance emphasise clinical outcomes including signs and symptoms, duration of hospitalisation, time to normalisation of vital signs and oxygenation, requirement for supplemental oxygenation or assisted ventilation and mortality but the variability of these parameters in severely ill patients requires a large sample size.

The product: the magnitude and affinity of SARS-CoV-2 specific antibody should be pre-defined, though it should be noted there is yet no standardized method for quantification of SARS-CoV-2 – specific neutralizing antibodies; influenza studies suggest that antibody affinity may be important; timing: convalescent plasma can be available in weeks while manufacture of hIVIG can take months; dose: should ideally be adjusted according to the neutralization titre and /or body weight of the patient though the studies in influenza have demonstrated the complexity of pharmacokinetics and pharmacodynamic analysis of infused plasma in infected patients.

Timing of administration: subgroup analyses in several influenza studies showed evidence of efficacy if plasma/hIVIG was given early (4 or 5 days after onset of symptoms).

Outcome measures: Studies in severe influenza and COVID-19 support ordinal clinical scoring rather than mortality, with categories that consider both improvement and deterioration in health status; virologic evidence should be a secondary outcome.