Convalescent plasma to treat coronavirus disease 2019 (COVID-19): considerations for clinical trial design

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KEY IDEAS

- Case series studying convalescent plasma use in the treatment of COVID-19 have been promising, but additional, high-quality studies are needed to determine the efficacy of the treatment when applied for prophylaxis, for early phases of illness, and for severe illness.
- Previous studies of convalescent plasma in treating other viral diseases have identified factors to consider when designing treatment protocols, including timing of administration relative to onset of illness, timing of donation relative to resolution of symptoms, severity of illness of the donor, pretransfusion serology of the recipient, and antibody titers of the donor.
- There are many clinical trials studying treatment of, and prophylaxis against, COVID-19 using convalescent plasma. In addition to clinical trials, the FDA also allows treatment through two other pathways: the "Expanded Access to Convalescent Plasma for the Treatment of Patients with COVID-19" protocol, and emergency investigational new drug applications. The FDA also provides criteria for donation of convalescent plasma.

At present, prevention and supportive care dominate the approach to coronavirus disease 2019 (COVID-19). Treatments directly targeting the virus and the inflammatory response to it remain investigational. Convalescent plasma (CP) is one such therapy. Here we will review the results of studies on CP use for treating other viral diseases, namely severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), influenza, Ebola virus (EBOV), and respiratory syncytial virus (RSV), followed by recent case series on its use for treating COVID-19. We will then summarize Food and Drug Administration (FDA) requirements for administering CP for COVID-19 and review trials being conducted in North America.

USE OF CP FOR OTHER VIRUSES

In the largest study on CP for treatment of SARS, 80 severely ill patients refractory to steroid and antiviral therapy received 200 to 400 mL of CP.[1] The timing of administration was dependent on CP availability. The authors examined which of the recipients experienced a "good outcome," defined by discharge by Day 22 since symptom onset. Discharge requirements were afebrility for 4 days as well as improvement in inflammatory laboratory values and radiographic lung findings. Patients who experienced a good outcome were younger (30.2 $\pm$ 15.1 years vs. 37.9 $\pm$ 12.5 years; $p < 0.001$). They received the plasma earlier in their disease course (Day 11.7 $\pm$ 2.3 vs. Day 16.0 $\pm$ 6.0; $p < 0.001$); put differently, 58.5% of patients receiving CP before Day 14 postonset of illness (dpoi) had a good outcome compared with 15.6% among those receiving it after. Finally, 60% of patients with a good outcome were seronegative for SARS antibody before receiving plasma, compared with only 21% of those with a poor outcome, suggesting that supplying antibodies to patients who are already seropositive is less likely to be effective.

In 2015, a protocol for collecting CP for use in MERS patients was established.[2] The authors recommended screening potential donors from three cohorts: exposed health care workers, recovering patients with known or

ABBREVIATIONS: CP = convalescent plasma; dpoi = day(s) postonset of illness; EBOV = Ebola virus; HIVIG = hyperimmune immunoglobulin; MERS = Middle East respiratory syndrome; RSV = respiratory syncytial virus; SARS = severe acute respiratory syndrome..

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suspected MERS, and household contacts of known MERS patients. The minimal acceptable anti-MERS–specific titer was 160. In 2016, the same authors published on their follow-up experience with the protocol.[3] Although they identified 196 convalescent patients, 17 family members of two patients, and 230 exposed health care workers, only 12 people tested positive for antibody by enzyme-linked immunosorbent assay. The authors postulated that this low positivity rate could be due to a prolonged interval between recovery and plasma collection.

In a small case series of MERS patients,[4] three patients requiring mechanical ventilation were treated with CP. Only one had a serologic response (detectable neutralizing antibodies) after transfusion. This patient was the only one who received plasma with a plaque-reducing neutralization test titer of at least 80. One explanation provided by the authors for the low titer of donor plasma was the relative mild nature of their illnesses compared to other MERS patients, noting that patients with mild cases of MERS without pneumonia had lower seroconversion rates than patients who developed pneumonia.

Convalescent plasma was also investigated in the treatment of EBOV. However, because EBOV is a Biosafety Level 4 pathogen (SARS-CoV-2 is Level 3), and because EBOV outbreaks have mostly occurred in resource-poor settings, it has been difficult to carry out high-quality, randomized controlled trials on this subject. In one study, convalescent whole blood was used instead of CP due to a lack of apheresis collection devices.[5] In another, CP was used, but plasma-neutralizing antibody titers were not tested. Neutralizing titers require culture of live virus, and sufficient biosafety equipment was unavailable.[6] Neutralizing antibodies are antibodies that bind and directly interfere with a virus's replication. Their antiviral function does not depend on host immune cells (e.g., phagocytes), and they can directly inhibit viral growth in culture. This is in contrast to binding antibodies, which bind to their target antigen but cannot prevent a virus from infecting cells independently and do not inhibit viral growth in culture.

In addition to CP, hyperimmune immunoglobulin (HIVIG) has also been used to treat viral illnesses. In a randomized control trial studying H1N1 influenza, mortality was compared between 17 patients receiving HIVIG and a control group of 18 patients receiving IVIG.[7] No survival benefit was noted overall; however, a mortality difference was detected between intervention and control group patients treated within 5 dpoi (0% vs. 40%; $p = 0.04$). The authors noted some advantages of HIVIG over CP: neutralizing antibody titer is higher due to concentration of product, there is no need to match ABO or D blood groups, and the infusion volume is lower, decreasing the risk of fluid overload. However, they also noted that HIVIG takes months to prepare.

Hyperimmune immunoglobulin has also been tested in pediatric patients with RSV. Three studies investigating its use saw no significant difference in clinical endpoints, both in children with congenital diseases and in otherwise healthy children.[8-10] However, two other studies investigating prophylactic use of HIVIG in children with underlying conditions, especially prematurity-associated bronchopulmonary dysplasia, showed improvements in endpoints.[11,12]

USE OF CP FOR COVID-19

Two case series were recently compiled in China examining the therapeutic use of CP in patients with COVID-19.[13,14] Between the two studies, a total of 15 patients were treated; all were severely ill before transfusion and were positive for SARS-CoV-2 by polymerase chain reaction (PCR). Donor plasma-neutralizing antibody titer was tested (≥40 in the five-patient study; ≥640 in the 10-patient study). All plasma was treated with methylene blue photochemistry and stored at 4°C (never frozen). Both studies showed improvement in many respects, including clearance of the virus, decreased need for supplemental oxygen and mechanical ventilation, normalization of laboratory values, and improvement of radiologic pulmonary findings. Of note, in the 10-patient study, recovery was faster in patients receiving CP before 14 dpoi than in those receiving it later.[14] Neither study had a control arm.

These case series are encouraging; however, they have significant limitations and must be followed by additional studies. Both studies targeted severely ill patients, and the patients also had positive neutralizing antibody titers before receiving CP, with some patients in the study by Duan and colleagues[14] having pretransfusion titers of at least 640.[14] The authors did not specify any titer level greater than that, even for posttransfusion neutralizing antibody titer, so it is unclear whether those patients had any increase in their neutralizing antibody titer associated with transfusion.[14] It is therefore difficult to explain how CP may have benefited those patients.

In the smaller study by Shen and colleagues[13] ($n = 5$), the authors provided each patient's neutralizing antibody titer before and after transfusion as well as the change in clinical variables for each individual patient in the days after transfusion. Some parameters suggest that patients with a higher pretransfusion neutralizing antibody titer had a worse clinical course after transfusion than those with lower titers. For example, the patient with the highest pretransfusion neutralizing antibody titer (≥160) had the smallest improvement in PaO_2/FiO_2 (from 276 to 284 mmHg over 12 days), compared with a mean improvement from 193 to 336 mmHg in the other four patients. That patient also required the most days (7) to become afebrile after transfusion, compared with 1 to 3 days in three of the other patients (the last patient was not febrile before receiving CP). Finally, the patient with a pretransfusion

neutralizing antibody titer of 160 was one of only two who remained intubated at the end of the study. The other patient who remained intubated had one of the lowest pretransfusion neutralizing antibody titer levels (≥40); however, that was also the only patient to require extracorporeal membrane oxygenation before transfusion. Changes in other variables, such as C-reactive protein level and sequential organ failure assessment score, are less convincing. Nevertheless, these findings are consistent with the concept that patients earlier in their clinical course when neutralizing antibody titer levels are still low or undetectable may respond better to CP than patients who are farther along in their disease course. Additional study of CP in the early phases of the illness, or even as prophylaxis in patients with exposures, would be helpful.

As of April 11, 2020, the US FDA has established specific requirements for donation of CP. These are detailed in Table 1.[15] Although neutralizing antibody titer testing is encouraged with a recommended cutoff value of 160, it is not required, and several US blood suppliers are not performing neutralizing antibody titers before releasing CP to transfusion services. Studies have not yet established what minimum neutralizing antibody titer is necessary for therapeutic benefit from CP, but the case series above at least demonstrated that some level of neutralizing antibody was present in the donor plasma before transfusion. A key question to consider is what percentage of patients with COVID-19 develop neutralizing antibodies and over what time course. According to one study, which measured neutralizing antibodies in 175 patients infected with the virus, all patients had low neutralizing antibody titers, defined as less than 500, by 10 dpoi. Their neutralizing antibody titers increased from Day 10 to Day 15 and were essentially steady after that.[16] Only 10 of the 175 patients never developed detectable neutralizing titers (<40) and 70% ultimately generated neutralizing titers greater than 500. Low-titer patients were generally younger and disproportionately female. Neutralizing antibody titer was positively correlated with C-reactive protein level but negatively correlated with lymphocyte count. Of note, another study found that detectable anti-SARS-CoV-2 IgG antibodies appear between 6 and 10 days after infection. Interestingly, the IgG appears at approximately the same time as the IgM.[17] These antibodies were detected by enzyme immunoassay and therefore are not necessarily neutralizing antibodies. However, IgG levels correlated strongly with neutralizing antibody titers in that study.

The FDA allows for administration of CP to COVID-19 patients through three pathways.[15] The first is via the "Expanded Access to Convalescent Plasma for the Treatment of Patients with COVID-19" protocol, sponsored by the Mayo Clinic. Patients meeting specific criteria (>18 years of age, with laboratory-confirmed diagnosis requiring admission to an acute care facility with high risk of progression to severe or life-threatening disease) can register to receive a transfusion from a participating center. The second pathway is through a single-patient emergency investigational new drug application, which is set up between a licensed physician and the FDA and does not require a specific center; however, the criteria for eligibility are similar to those for the expanded-access protocol.[15] The third pathway is through clinical trials.

TABLE 1. FDA requirements for donation of convalescent plasma for COVID-19*

Donors must meet all standard donor eligibility requirements for donation of plasma by apheresis, i.e., compliant with the following Code of Federal Regulations:
- 21 CFR 630.10
- 21 CFR 630.15
- 21 CFR 630.30
- 21 CFR 630.40

Donors must have tested positive for SARS-CoV-2 by *either:*
- A diagnostic test (e.g., nasopharyngeal swab) *during* illness
- A serologic test after recovery

Symptoms of infection must have completely resolved by *:*
- 14 days prior to donation

If female, donors must *either:*
- Have never been pregnant
- Been tested negative for HLA antibodies since their most recent pregnancy

Testing for SARS-CoV-2–neutralizing antibody titers is recommended if available, but not required.
- Titers ≥160 are recommended, but if an ABO-compatible unit is unavailable, ≥80 is acceptable
- If titers are not available, consider storing a retention sample for future testing

* Provided that plasma is collected according to the above specifications, blood establishments do not need an IND to collect the plasma; however, an IND will be required to administer it.

CLINICAL TRIALS

A multitude of clinical trials have been started around the world, many of which are taking place in the United States and Canada. The details of some of these North American trials are shown in Table 2; many more trials are being performed internationally. Together, the trials will study use of CP in different patient populations; some will focus on severely ill patients, the same group targeted by the expanded-access program. Others, however, will target hospitalized but not intubated patients. Some studies are not explicitly targeting either; whether they will ultimately lump severely and moderately ill patients together or analyze them separately is not yet clear. At least one study will target high-risk individuals who have been exposed but have not yet tested positive for the virus, nor shown any symptoms. As seen above in work on SARS and in the larger COVID-19 case series, CP may be more efficacious earlier in the disease course. The RSV HIVIG studies, meanwhile, only

TABLE 2. Summary of clinical trials in North America (excludes trials that only study donation)

Sponsor	Control	Participants	Population
Hackensack Meridian Health	None (single arm)	55	Two tracks: -Hospitalized, without mechanical ventilation -Hospitalized, with mechanical ventilation
Primary outcomes: *-First track: mechanical ventilation rate at 7 days from starting treatment* *-Second track: mortality at 30 days*			
Hamilton Health Sciences Corporation	Standard of care	1200	Hospitalized, nonintubated patients
Primary outcomes: Intubation or death in the hospital at 30 days			
Stony Brook University	Standard plasma	500	Hospitalized patients (intubation status unspecified)
Primary outcomes: Ventilator-free days through Day 28 postrandomization (patients who die in this period are assigned 0 days)			
University of Chicago	None (single arm)	10	Severe or immediately life-threatening disease
Primary outcomes: *-Number of consented donors, amount of plasma successfully harvested* *-Number of patients transfused* *-Level of respiratory support (*e.g.*, room air, high flow oxygen, intubation) at 28 days after plasma administration*			
Johns Hopkins University	Standard plasma	150	Asymptomatic, with negative PCR test *and* high risk exposure *and* higher risk for severe illness
Primary outcomes: *-Death* *-Requiring mechanical ventilation and/or in ICU* *-Non-ICU hospitalization, requiring supplemental oxygen* *-Non-ICU hospitalization, not requiring supplemental oxygen* *-Not hospitalized, but with clinical and laboratory evidence of COVID-19 infection* *-Not hospitalized, no clinical evidence of COVID-19 infection, but positive PCR for SARS-CoV-2*			
Baylor Research Institute	Standard of care	115	Hospitalized patients (intubation status unspecified)
Primary outcome: Reduction in ventilation and oxygen support			

showed benefit when used prophylactically. These trials will help establish what the optimal timing of administration is.

The clinical trials do not all have a control arm, and those that do vary in the type of control. Some will administer standard plasma as the control, while others will simply use standard of care. Standard plasma has the advantage of allowing for blinding. However, it carries certain disadvantages as well: it will consume valuable blood product with no expected clinical benefit, it will expose patients to potentially immunogenic antigens that will complicate future transfusions, and it will put patients at risk for transfusion reactions. Furthermore, the use of standard plasma, which carries many of the same risks of CP but none of its benefits, may mask some of the risks of using CP that would be more easily detected if the control was standard of care.

The outcome measures of the various trials vary depending on the specific goals of the individual trials. Some trials are specifically interested in the feasibility of donor identification and plasma collection. Therefore, the endpoints include donors successfully screened, consents signed, and plasma units collected. The outcomes of studies on prophylactic use of CP include incidence of PCR detection of the virus, incidence of symptoms, hospitalization with and without need for oxygen supplementation, intubation, and death after exposure. Studies focused on nonintubated, hospitalized patients set endpoints measuring both improvement (reduction in oxygen requirement) and deterioration (intubation and death). Studies focused on severely ill patients usually include death as an endpoint, as well as decrease in ventilator support. Primary endpoints rarely stray from these choices, but secondary endpoints are much more variable, including length of hospitalization (sometimes specifying ICU stay length or non-ICU stay length), antibody titers at set days posttransfusion, rate of clearance of the virus, impact of donor titer levels on efficacy, need for extracorporeal membrane oxygenation or renal replacement therapy, and even onset of myocarditis. The strong degree of overlap in primary endpoints will facilitate comparison and meta-analysis of the studies, while the variability in secondary endpoints will allow the individual studies to answer more specific questions about the use of CP in COVID-19 treatment.

CONCLUSIONS

CP is under active investigation as a direct treatment for COVID-19 as well as prophylaxis. CP use for SARS-CoV-2 and other viruses has shown promise. Critical factors to consider when designing treatment protocols include timing of administration relative to onset of illness, timing of donation relative to resolution of symptoms, severity of illness of the donor, pretransfusion serology of the recipient, and antibody titers of the donor. Outcome measures should be tailored to the population being studied.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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