5. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci USA*. 2020;117(17):9490-9496.

6. Liu ST, Lin H, Baine I, et al. Convalescent plasma treatment of severe COVID-19: a matched control study. *medRxiv*. 2020;

7. Li L, Zhang W, Hu Y, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial [published online ahead of print 3 June 2020]. *JAMA*. doi:10.1001/jama.2020.10044.

8. COVID-19 Expanded Access Program. Available at: https://www.uscovidplasma.org/. Accessed 17 June 2020.

9. Budhai A, Wu AA, Hall L, et al. How did we rapidly implement a convalescent plasma program? [published online ahead of print 25 May 2020]. *Transfusion*. doi:10.1111/trf.15910.

10. Joyner MJ, Wright RS, Fairweather D, et al. Early safety indicators of COVID-19 convalescent plasma in 5,000 patients. *J Clin Invest*. 2020;140200.

11. Joyner MJ, Bruno KA, Klassen SA, et al. Safety update: COVID-19 convalescent plasma in 20,000 hospitalized patients. *Mayo Clinic Proc*. In press.

DOI 10.1182/blood.2020007638

CLINICAL TRIALS AND OBSERVATIONS

Comment on Hegerova et al, page 759

Convalescent plasma to treat COVID-19

Evan M. Bloch | Johns Hopkins University School of Medicine

In this issue of *Blood*, Hegerova et al report on a study of 20 hospitalized patients with severe or critical coronavirus disease 2019 (COVID-19) who were transfused with convalescent plasma (CP); the authors suggest a favorable, albeit modest, benefit as compared with 20 matched (ie, nontransfused) controls, particularly when transfusion was undertaken within the first 7 days of hospitalization.[1]

CP that has been collected from individuals who have recovered from COVID-19 (ie, COVID-19 convalescent plasma [CCP]) has emerged as a leading treatment of COVID-19. Early studies in China reported benefits of CCP transfusion in patients with COVID-19, including viral clearance, radiological resolution of pulmonary disease, improved oxygenation, and survival.[2,3] This spurred efforts by the US Food and Drug Administration (FDA) and the US blood-banking community to collect and transfuse CCP on an unprecedented scale.[4] To date, over 26 000 patients have been transfused with CCP in the United States alone, primarily through a government-led expanded access program.[5] Data gleaned from this program have shown CCP to be well tolerated, with comparable risk to standard (ie, nonimmune) plasma.[6]

Hegerova et al add to a growing number of observational studies that have reported on the use of CCP to treat COVID-19. The outcomes of the collective studies span dramatic examples of recovery to the absence of demonstrable effect,[2,3,7,8] highlighting the challenges of CP in general. Like many infectious diseases for which CP has been applied, clinical trial data are lacking. CP is typically deployed in times of emergency when the design and execution of randomized clinical trials is most complex. Beyond the administrative, regulatory, and logistical barriers of initiating trials in times of crisis, timing is critical: once epidemics wane, enrollment goals risk going unfulfilled. There is already at least 1 example of this with COVID-19: in a clinical trial in China, critically ill patients with COVID-19 were randomized to CCP with standard therapy vs standard therapy alone.[9] Despite encouraging signals of benefit in earlier—severe rather than life-threatening disease, the trial failed to show a significant difference in clinical improvement 28 days following randomization, its primary outcome. With only 103 of its targeted 200 subjects enrolled, the study was ultimately underpowered.

Currently, we are reliant on observational data of CCP to guide practice.[2,3,7,8] Observational studies have proven invaluable to the CCP initiative but they share a host of methodologic limitations. Some are constrained by small sample sizes and lack of controls. All have selected for severe COVID-19 in which most patients have received other therapies, in addition to CCP, such as steroids, antibiotics, and antivirals, blurring interpretation of the findings. Hegerova et al acknowledged this as a limitation in their study in which one-half of their control group had received remdesivir, an investigational antiviral that could well have masked the differential effect of CCP if indeed one were present. Beyond concomitant therapies, the investigators highlight 2 other elements that have not been standardized across studies: dosage and titering. Dosing of CCP has been gleaned from studies of severe acute respiratory syndrome; the pharmacokinetics of CP in the context of COVID-19 are not well understood. Most studies of COVID-19 have reported use of 1 to 2 U (~200-500 mL) of CCP to treat COVID-19; while practical, this fails to account for differences in the volumes of distribution or acuity of disease. Furthermore, the approaches that have been used to qualify donors and/or the transfused units of CCP have varied enormously, from qualitative assessments of immunoglobulin G to formal viral neutralization assays and associated titers.[4]

Independent of the study design, the patient population that is being targeted for CCP use may be suboptimal. An enduring finding across studies of CP is the need for early intervention.[1,4,7,9] Yet, the overwhelming majority, if not all, CCP to date has been transfused to patients with advanced COVID-19. At inception, the US expanded access program required severe or life-threatening COVID-19 for enrollment.[6] Although the desire to help those who are most sick is intuitive, this is the population for which evidence of benefit from CP is weakest. Late intervention also fails to abrogate the societal burden of disease. In short, intervention likely needs to occur earlier in the disease process.[4]

In conclusion, observational studies and compassionate use programs have been instrumental in the mobilization of CCP to contend with a global health emergency. Although safety has been addressed, efficacy data are critically needed to transition CCP's status from an investigational product to a standard therapy. The latter has practical ramifications, offering a formal mechanism for reimbursement and

thus a durable treatment strategy. Broadly, COVID-19 presents a rare opportunity to study CP. If shown to be effective, CP would offer a scalable model that could be applied both to the current pandemic as well as to future emerging infectious diseases. It could also facilitate development of hyperimmune globulin and vaccine design. Clinical trials are already under way to address the uncertainty of use. Nonetheless, harmonization of efforts is needed along with creative approaches to overcome looming obstacles, such as pairing of trials of similar design and/or metanalysis. We must not be left wondering whether the intervention worked after the pandemic wanes.

Conflict-of-interest disclosure: E.M.B. reports personal fees and nonfinancial support from Terumo BCT and Grifols Diagnostics Solutions outside of the submitted work. ■

REFERENCES

1. Hegerova L, Gooley TA, Sweerus KA, et al. Use of convalescent plasma in hospitalized patients with COVID-19: case series. *Blood*. 2020;136(6):759-762.
2. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. *Proc Natl Acad Sci USA*. 2020; 117(17):9490-9496.
3. Shen C, Wang Z, Zhao F, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA*. 2020;323(16):1582.
4. Bloch EM, Shoham S, Casadevall A, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. *J Clin Invest*. 2020;130(6):2757-2765.
5. Mayo Clinic. COVID-19 expanded access program. https://www.uscovidplasma.org/. Accessed 29 June 2020.
6. Joyner MJ, Bruno KA, Klassen SA, et al. Safety update: COVID-19 convalescent plasma in 20,000 hospitalized patients. Mayo Clin Proc. https://www.uscovidplasma.org/safety-report. Accessed 29 June 2020.
7. Liu STH, Lin H-M, Baine I, et al Convalescent plasma treatment of severe COVID-19: a matched control study. https://www.medrxiv.org/content/10.1101/2020.05.20.20102236v1. Accessed 29 June 2020.
8. Zeng Q-L, Yu Z-J, Gou JJ, et al. Effect of convalescent plasma therapy on viral shedding and survival in COVID-19 patients with coronavirus disease 2019. *J Infect Dis*. 2020;222(1):38-43.
9. Li L, Zhang W, Hu Y, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial. [published online ahead of print 3 June 2020]. *JAMA*. doi: 10.1001/jama.2020.10044.

DOI 10.1182/blood.2020007714

TRANSFUSION MEDICINE

Comment on Berzuini et al, page 766

COVID-19 and the Coombs test

Jeanne E. Hendrickson and Christopher A. Tormey | Yale University School of Medicine

In this issue of *Blood*, Berzuini et al[1] describe immunoglobulin G (IgG) bound to the red blood cells (RBCs) of patients with COVID-19 and associate this bound IgG with increased RBC transfusion requirements. The intrigue behind these observations is not just the high (46%) direct antiglobulin test (DAT) positivity rate, but also the novel finding that eluates (ie, antibodies stripped from the surface of the reactive RBCs) from these DAT-positive patients react not with standard-reagent RBCs but exclusively with RBCs from DAT-negative COVID-19 patients.

Although the association of DAT positivity and anemia is limited by study design, the serologic findings may be teaching us something important about modifications to RBCs that occur in COVID-19. The authors are to be congratulated on the rapid dissemination of these data and on the clever idea of using RBCs from COVID-19 patients as blood bank reagents in addition to standard, commercially available reagent RBCs.

First, a quick overview of the DAT, also known as the direct Coombs test. The DAT is designed to identify IgG or complement (C3) bound to a patient's own RBCs. A DAT positive for IgG could be caused by several things,[2] including autoimmunity, drugs, or intravenous immune globulin, among others, and not all positive DATs are clinically significant. Thus, the significance and specificity of a DAT can be further elucidated by comparing the results of an RBC antibody screen (indirect antiglobulin test that evaluates antibody in the plasma or sera) with the DAT and evaluating the results of the eluate. As Berzuini et al describe, IgG was detected in most of the positive DATs in hospitalized patients with COVID-19, and all of the IgG-positive DATs re-tested using a flow cytometric–based assay were positive for IgG, which helped to exclude nonspecific artifacts. Furthermore, C3d was detected in 12% of the positive DATs, either in combination with IgG or in isolation. The eluates from the IgG-positive DATs were negative against standard-reagent RBCs but uniformly positive with a panel of washed RBCs generated from 5 DAT-negative patients with COVID-19 (see figure). Possible causes for the unique pattern of eluate reactivity observed in patients with COVID-19 are RBC membrane modifications, complement effects, or drug effects.

Could downstream effects of severe acute respiratory syndrome coronavirus 2 (SARs-CoV-2) infection lead to a modified RBC membrane, resulting in the unique IgG binding DAT pattern described? One existing model of RBC membrane modification related to infection involves neuraminidase released by *Streptococcus pneumoniae*, which cleaves terminal N-acetylneuraminic acid (sialic acid) from glycoproteins and glycolipids. This exposes the Thomsen-Friedenreich crypt T antigen and, in effect, converts self RBCs to non-self RBCs. There is a debate regarding the exact role that these non-self RBCs play in the atypical hemolytic uremic syndrome pathophysiology, although impaired complement factor H binding to the desialylated RBC membranes and alternative pathway complement activation are thought to be involved.[3]

How might complement be related to the findings by Berzuini et al? Gao et al[4] have recently described lung biopsy samples from patients with severe COVID-19 disease showing C3-fragment deposition; it is thought that the SARS-CoV-2 nucleocapsid