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EDITORIAL

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The need for and challenges of comparing SARS-CoV-2 antibody assays

Since its appearance in Wuhan, China, in late December 2019, the novel coronavirus SARS-CoV-2 has rapidly spread, causing over 1.2 million deaths worldwide as of early November 2020.[1] Currently, no targeted therapy is available for the associated disease COVID-19, but several options are being investigated, including COVID-19 convalescent plasma (CCP). Its use relies on the principle of passive immunity.

One of the largest experiences comes from the Mayo Clinic expanded access program (EAP) in the United States.[2] The goal was to increase CCP availability for adults in the early phase of the pandemic. Starting in March 2020, convalescent donors were qualified by PCR-based evidence of SARS-CoV-2 infection and a sufficient recovery time interval; antibody testing was not widely available. Several different antibody assays have since obtained regulatory approval in the United States.[3] Retrospective analysis of the EAP data showed that CPP transfusion was safe. Higher CCP antibody levels were associated with improved mortality when a population subset (3082 patients) was analysed. The safety data and suggestion of a dose-response contributed to the Food and Drug Administration issuing an emergency use authorization (EUA) for CCP on August 23, 2020. Of note, an expert panel did comment on limitations from the EAP.[4] In the European Union (EU), CPP treatments have occurred predominantly in randomised clinical trials except for specific compassionate use programmes.[5]

The challenge of determining antibody levels stems from the complexity of antibody responses. For instance, in some patients, the T-cell response may dominate explaining why some recovered donors have few antibodies.[6] Antibodies that prevent viral entry into host cells are called neutralising antibodies and are considered the most effective. The gold standard method for determination of neutralising antibodies is by plaque reduction (PRNT) in infected host cell cultures but requires live SARS-CoV-2 virus. These assays must be performed in high containment biosafety level 3 (BSL 3) laboratories. Alternative assays using pseudovirus still require BSL 2 facilities. Despite being deployed in several EU countries for selecting CCP donors in ongoing trials, such assays are not widely available nor easy to scale up, especially if future needs increase. In contrast, immunoassays are more accessible and are compatible with BSL 1 laboratories but vary in their sensitivity for anti-SARS-CoV-2 immunoglobulins (IgG and/or IgM). Immunoassays that directly detect inhibition of (recombinant) SARS-CoV-2 proteins and host cell receptors are commercially available (eg, AcroBiosystems, Newark, DE) or under development and good correlation with virus neutralisation is suggested.[7,8]

Most clinical trials in the EU arbitrarily define a bottom threshold for including CCP based on neutralising antibody titre measurements in live virus assays. For instance, a threshold of 1:320 means that only CCP that inhibits 50% of SARS-CoV-2 viral activity at a 1:320 dilution in vitro will be included in trials. This threshold may however differ by trial design and donor availability because no robust scientific evidence is available to rationally justify a strict cut-off for the neutralising antibody titre. In addition, titres vary depending on the assay performance and a precise correlation with clinical efficacy is not proven. Depending on the assay used and the clinical protocol being followed, each programme can establish its own policy.[9] In the United States, the CCP EUA specifically calls for convalescent plasma to be assayed for antibodies using the Ortho Vitros IgG SARS-CoV-2.[10] Because many blood collection centres have already implemented other assays, it is very difficult logistically for them to change platforms. Furthermore, reliance on a single assay poses risks in case of supply chain constraints and critical reagent shortages. Therefore, correlations must be established between the various immunoassays and neutralising tests so the EUA can be amended to include other assays.

Harvala and colleagues described a comparison between a live virus (micro)neutralisation assay, a pseudovirus reporter neutralisation (RVPN) assay, and four different enzyme-linked immunoassays (ELISAs) targeting the SARS-CoV-2 spike protein.[11] The goal was to determine optimal immunoassay cut-off values corresponding to adequate neutralising antibody levels. A neutralising titre threshold of 1:100 was selected arbitrarily.[9] In this study, 43% of samples from 52 recovered donors in April 2020 exceeded 1:100, which has implications for the availability of convalescent plasma given that physicians will favour transfusing higher titre units. Blood centres need to encourage high titre donors to return but must also increase recruitment because antibody levels decrease over time.[12]

All ELISAs detected antibodies and the strongest correlation occurred with the EUROimmun IgG. Selecting a signal to cut-off of 9.1 successfully excluded 26 samples below the 1:100 neutralising antibody threshold. Lower signal to cut-offs increased the risk for false positives, that is, the possibility that the CCP contains insufficient neutralising antibodies. However, the EUROimmun reading of 9.1 only identified 65% of donors above the threshold,[11] illustrating the delicate balance between accepting CCP units with low neutralising antibodies and discarding units with sufficiently high levels. Of note, the positive and negative predictive values depend on seroprevalence, which can widely vary between locations,[13] and

whether convalescent donors are identified by population screening or must provide proof of past infection.

These findings mirror other recent reports.[14,15] Luchsinger and colleagues found that most of their CCP samples had modest antibody levels and that commercially available tests have varying accuracy in predicting neutralising antibody activity. Goodhue and colleagues suggested a two-step testing scheme in which samples below an immunoassay threshold undergo reflex neutralising antibody testing. Thus, CCP is qualified if either the immunoassay or neutralising antibody threshold is met.

To support wider use of CCP, equivalent antibody levels must be established between different assays. However, reports have shown that correlation is complicated by differences in donor responses and binding vs neutralising assays. Novel quick and reliable immunoassays that directly measure the presence of neutralising antibodies in a wide dynamic range need to be developed.

CONFLICT OF INTEREST

The authors declare no competing interests.

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REFERENCES

1. Johns Hopkins University. (2020). Johns Hopkins University & Medicine: Coronavirus Resource Center.
2. Joyner MJ, Senefeld JW, Klassen SA, et al. Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: initial three-month experience. *medRxiv*. 2020. https://doi.org/10.1101/2020.08.12.20169359.
3. FDA (2020). Emergency Use Authorization Medical Devices 2020.
4. Pau AK, Aberg J, Baker J, et al. Convalescent plasma for the treatment of COVID-19: perspectives of the National Institutes of Health COVID-19 treatment guidelines panel. *Ann Intern Med*. 2020; M20-6448. https://doi.org/10.7326/M20-6448.
5. Hueso T, Pouderoux C, Péré H, et al. Convalescent plasma therapy for B-cell depleted patients with protracted COVID-19 disease. *Blood*. 2020;136(20):2290-2295.
6. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell*. 2020;183:158.e14-168.e14.
7. Bosnjak B, Stein SC, Willenzon S, et al. Low serum neutralizing anti-SARS-CoV-2 S antibody levels in mildly affected COVID-19 convalescent patients revealed by two different detection methods. *Cell Mol Immunol*. 2020. https://doi.org/10.1038/s41423-020-00573-9.
8. Schöler L, Le-Trilling VTK, Eilbrecht M, et al. A novel in-cell ELISA assay allows rapid and automated quantification 1 of SARS-CoV-2 to analyse neutralizing antibodies and antiviral compounds. *Front Immunol*, Accessed October 9, 2020. 2020; 11:1-11. https://doi.org/10.3389/fimmu.2020.573526.
9. EU. *An EU Programme of COVID-19 Convalescent Plasma Collection and Transfusion: Guidance on Collection, Testing, Processing, Storage, Distribution and Monitored Use*. Brussels: European Commission, Directorate-General For Health And Food Safety; 2020, 2020.
10. FDA (2020). Convalescent Plasma COVID-19 Letter of Authorization 2020.
11. Harvala H, Robb ML, Watkins N, et al. Convalescent plasma therapy for the treatment of patients with COVID-19: assessment of methods available for antibody detection and their correlation with neutralising antibody levels. *medRxiv*. 2020. https://doi.org/10.1101/2020.05.20.20091694.
12. Perreault J, Tremblay T, Fournier M-J, et al. Waning of SARS-CoV-2 RBD antibodies in longitudinal convalescent plasma samples within four months after symptom onset. *Blood*. 2020;136(22):2588-2591. https://doi.org/10.1182/blood.2020008367.
13. Anand S, Montez-Rath M, Han J, et al. Prevalence of SARS-CoV-2 antibodies in a large nationwide sample of patients on dialysis in the USA: a cross-sectional study. *Lancet*. 2020;396:1335-1344.
14. Luchsinger LL, Ransegnola B, Jin DK, et al. Serological assays estimate highly variable SARS-CoV-2 neutralizing antibody activity in recovered COVID19 patients. *J Clin Microbiol*. 2020;58(12):e02005-20. https://doi.org/10.1128/JCM.02005-20.
15. Meyer EG, Simmons G, Gannett M, et al. Qualification of COVID-19 convalescent plasma donations by SARS-CoV-2 antibody testing utilizing a wide-dynamic range antigen-Sandwich S1-antigen immunoassay and reflex neutralizing antibody testing. *Transfusion*. 2020;60: 278A-279A.