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Hospital-Based Donor Recruitment and Predonation Serologic Testing for COVID-19 Convalescent Plasma

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ABSTRACT

Objectives: Serologic testing for antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in potential donors of coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) may not be performed until after blood donation. A hospital-based recruitment program for CCP may be an efficient way to identify potential donors prospectively

Methods: Patients who recovered from known or suspected COVID-19 were identified and recruited through medical record searches and public appeals in March and April 2020. Participants were screened with a modified donor history questionnaire and, if eligible, were asked for consent and tested for SARS-CoV-2 antibodies (IgG and IgM). Participants positive for SARS-CoV-2 IgG were referred for CCP collection.

Results: Of 179 patients screened, 128 completed serologic testing and 89 were referred for CCP donation. IgG antibodies to SARS-CoV-2 were detected in 23 of 51 participants with suspected COVID-19 and 66 of 77 participants with self-reported COVID-19 confirmed by polymerase chain reaction (PCR). The anti-SARS-CoV-2 IgG level met the US Food and Drug Administration criteria for “high-titer” CCP in 39% of participants confirmed by PCR, as measured by the Ortho VITROS IgG assay. A wide range of SARS-CoV-2 IgG levels were observed.

Conclusions: A hospital-based CCP donor recruitment program can prospectively identify potential CCP donors. Variability in SARS-CoV-2 IgG levels has implications for the selection of CCP units for transfusion.

Key Points

- Hospital-based recruitment may be an efficient way to prospectively identify donors of convalescent plasma.
- Hospital-based recruitment can address the challenges of retrospective testing associated with plasma donation at blood donation centers.
- Variability in IgG level in convalescent plasma has implications for selection of COVID-19 convalescent plasma units for transfusion.

Coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) is being studied in multiple clinical trials to treat patients with COVID-19.¹⁻³ However, recruitment of CCP donors and collection of CCP units initially lagged behind demand for this product.⁴ In normal times, blood centers try to exclude donors with specific infectious disease histories. For CCP donor recruitment, in contrast, blood centers have the challenge of identifying donors with a history of confirmed SARS-CoV-2 infection. Hospitals can assist with the recruitment CCP donors by contacting patients who have recovered from COVID-19 and referring them to blood centers.^{5,6}

According to guidance from the US Food and Drug Administration (FDA), CCP donors must meet all donor eligibility requirements for allogeneic blood donation. In addition, they must have evidence of prior infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) either by a positive polymerase chain reaction (PCR) test at the time of illness or a positive serologic test after recovery if prior diagnostic testing was not performed. Donors must be asymptomatic for at least 14 days at the time of donation. In earlier versions of the FDA guidance, a nasopharyngeal swab that tested negative by

PCR for SARS-CoV-2 and/or a 28-day symptom-free period was required before blood donation.⁶

In this study, we describe a 2-center, hospital-based CCP donor recruitment program run in coordination with Vitalant. We screened study participants for eligibility for allogeneic blood donation and measured levels of anti-SARS-CoV-2 IgG and IgM antibodies. Participants who were positive for anti-SARS-CoV-2 IgG were referred for plasma donation. As part of this program, Vitalant agreed to send units from the first collection of referred CCP donors back to the referring hospital. These hospital-directed units are being used to support ongoing clinical trials.

Materials and Methods

Donor Recruitment

Potential donors were recruited in April and May 2020 through medical record searches and public appeals. Medical records of patients with PCR- or serology-confirmed COVID-19 were identified at University of California, San Francisco (UCSF) Health and Zuckerberg San Francisco General Hospital and screened to exclude individuals who would not be eligible for allogeneic blood donation. Exclusion criteria included known disqualifying infections, medical conditions and medications, or continued hospitalization. Potential donors were contacted by email and offered the opportunity to volunteer for the study (Supplemental Figure 1; all supplemental material can be found at *American Journal of Clinical Pathology* online). Donors were also recruited by public appeal. This appeal included information posted on the health system website about how to participate in the study, interviews about the study granted by the study's principal investigator to local media and university media outlets, and paper flyers promoting the study that were distributed to COVID-19 clinics and other COVID-19 research study sites (Supplemental Figure 2). All materials were developed by the study team. Recruitment materials for the study were made available to clinicians treating patients with COVID-19 and to contact tracers at the San Francisco Department of Public Health, as well as to other investigators recruiting participants for other COVID-19-related studies. The method by which participants heard about the study was not systematically recorded. All potential donor information was recorded and stored exclusively in Research Electronic Data Capture (REDCap, v9.5.25), a secure and Health Insurance Portability and Accountability Act-compliant web-based system for building and managing surveys and databases.

Donor Screening

Participants were asked to provide the location, date, and method of testing for their COVID-19 diagnosis, if any, and answered a secure online version of a modified donor history questionnaire (DHQ) (Supplemental Figure 3). Briefly, the modified DHQ consisted of yes/no questions developed by the local blood donation center with additional follow-up questions requesting information (eg, travel history), as appropriate. An automated scoring algorithm assigned participants as (1) donor eligible without follow-up, (2) physician consult needed, or (3) donor ineligible. Clearly ineligible donors were screened out, whereas those reporting answers that required follow-up were contacted by a study physician for clarification. Participants without a history of COVID-19 confirmed by a laboratory test were screened with the DHQ if they reported close contact with a known case and/or typical COVID-19 symptoms. Participants who were found eligible after the DHQ and who were judged likely to have been infected with SARS-CoV-2 were deemed to have passed the screen and were asked for and gave consent and referred for SARS-CoV-2 antibody testing.

Donor Testing

Blood was collected by venipuncture, and serum was tested for SARS-CoV-2 IgG and IgM using a Pylon 3D Automated Immunoassay System (ET Healthcare).⁷ This assay measures antibodies to the virus spike protein receptor binding domain, as described previously.⁸ The assay result is expressed in relative fluorescence units (RFU). PCR testing of nasopharyngeal swab samples for SARS-CoV-2 was performed if a participant was 14 to 27 days after their last symptoms. PCR was performed with the Abbott RealTime SARS-CoV-2 assay on an Abbott m2000 RealTime system. Participants with a SARS-CoV-2 IgG level above the positive cutoff of 50 RFU were referred for donation by plasmapheresis at the local blood donation center. Participant samples were also tested with the Ortho VITROS anti-SARS-CoV-2 IgG assay, performed according to the manufacturer recommendation.

Analysis

Categorical variables were compared using the 2-tailed χ^2 test. Continuous variables were compared using the Mann-Whitney test. Linear regression models were constructed for IgG and IgM levels vs days since last symptoms and age. All data analysis was performed using Prism (v8.4.2; GraphPad Software).

Institutional Review Board Approval

This study received UCSF institutional review board approval on April 17, 2020 (No. 20-30637).

Results

An overview of the study process is provided in **Figure 1**. The participants who underwent screening represented a mix of participants recruited by email and those who volunteered in response to the public appeal. A total of 24 recruitment emails were sent to patients, and 18 of those respondents underwent screening. The remainder of participants likely volunteered in response to public appeals. Of 179 participants screened, 133 passed the screen, 128 were tested for SARS-CoV-2 antibodies, and 89 were referred for plasma donation. Among those screened, 44 of 179 participants (24.6%) failed screening. Of participants who failed screening, 34 failed based on the DHQ and 10 had insufficient evidence of previous COVID-19. The most common reason for DHQ failure was travel history to areas with elevated risk of variant Creutzfeldt-Jakob disease and men who reported recent sex with another man, as outlined by pre-COVID-19 FDA guidance for blood donation. Most participants screened negative for travel to malaria endemic regions. Five participants who passed the screen did not set up a testing appointment.

Characteristics of the study population that were tested are presented in **Table 1**. Participants were not referred for donation if they tested negative for anti-SARS-CoV-2 IgG. No significant differences were found between participants who were referred for SARS-CoV-2 plasma donation and those not referred when comparing sex, days

since symptom resolution, participant age, or symptom severity. Among participants who were 14 to 27 days past their last symptoms, 32 were tested by PCR for SARS-CoV-2 on nasopharyngeal swab specimens, consistent with previous reports.⁸ Eight of 32 tested positive and were not referred for donation until they were 28 days past their last symptoms. The hospital received 86 CCP units and then stopped accepting additional units because of lack of storage space. Details of how many units were collected from referred donors were not shared by the blood center.

Anti-SARS-CoV-2 IgG was detected in 89 of 128 (69.5%) tested individuals, including 66 of 77 (85.7%) who were confirmed by PCR and 23 of 51 (45.1%) with suspected cases **Table 2**. IgM was detected in 48 of 128 (37.5%) tested individuals: 35 of 77 (45.5%) were PCR confirmed, and 13 of 51 (25.5%) had suspected cases **Figure 2**. Anti-SARS-CoV-2 IgG levels were significantly higher among participants who were confirmed by PCR (median, 184 RFU; range, 10-1,764 RFU) compared with the COVID-19 suspected but PCR-unconfirmed group (median, 35 RFU; range, 5-2,520 RFU; $P < .0001$, Mann-Whitney test). There was no significant difference in IgG levels between participants who were PCR confirmed and unconfirmed if the values below the cutoff (50 RFU) were excluded in this comparison.

The cutoff value for a positive result in the serologic assay was set at 50 RFU for both IgM and IgG, which is 4 SD above the mean of pre-COVID-era plasma controls, and resulted in 100% specificity during assay validation.⁹ IgG values were plotted against days since last symptoms among patients confirmed by PCR **Figure 3A**. We included only those confirmed by PCR to minimize confounding from participants who may

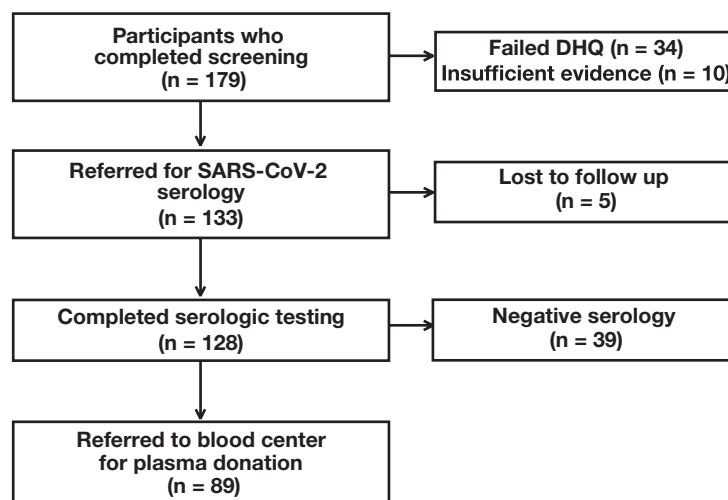


Figure 1 Schematic of the study process. DHQ, donor history questionnaire; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

never have been infected. The IgM level was plotted against days since last symptoms among individuals in the same group **Figure 3B**. There was no correlation between anti-SARS-CoV-2 IgG or IgM levels and days since last illness.

Anti-SARS-CoV-2 IgG and IgM values were plotted against the ages of participants among PCR-confirmed cases **Figure 3C** and **Figure 3D**. A significant positive correlation was found between age and anti-SARS-CoV-2 IgG and IgM levels.

There was no significant difference in anti-SARS-CoV-2 IgG or IgM antibody levels between male and female participants. Those who were hospitalized for COVID-19 had higher levels of anti-SARS-CoV-2 IgG than participants who were diagnosed by PCR but not hospitalized (median, 742 vs 171 RFU; $P = .029$). There was no difference in IgM levels between these groups; however, this analysis is limited by the low number of previously hospitalized patients in this study ($n = 9$).

We tested all participant serum samples for anti-SARS-CoV-2 IgG level using the Ortho VITROS IgG assay recommended by the FDA for labeled “high titer” CCP.¹⁰ There was a linear correlation between the IgG levels detected by the VITROS assay and the ET Healthcare Pylon assay at low and intermediate antibody levels **Figure 4A**. We observed that the linear range of the VITROS assay is limited above an index (S/C) of 20. Among participants with PCR confirmed COVID-19, 39% had a high titer IgG level (≥ 12 S/C), per FDA guidelines **Figure 4B**.

Discussion

Hospitals and public health authorities are uniquely positioned to aid in the recruitment of CCP donors through direct appeals to patients who have known positive test results. In this study, we prescreened potential

Table 1
Participant Demographics

| | All Tested | Referred for Plasma Donation | Not Referred for Donation ^a | P Value (Referred vs Not Referred) |
|--|------------|------------------------------|--|------------------------------------|
| Total | 128 | 89 | 39 | |
| Age, median (IQR), y | 40 (31-56) | 44 (32-57) | 34 (31-49) | NS |
| Minimum-maximum | 18-80 | 18-80 | 18-77 | — |
| Female sex, No. (%) | 68 (53.1) | 48 (53.9) | 20 (51.3) | NS |
| Days since last symptoms, ^b No. (IQR) | 38 (26-46) | 36 (24-42) | 46 (39-67.5) | < .0001 |
| Minimum-maximum | 5-103 | 7-75 | 9-103 | — |
| Mode of diagnosis, No. (%) | | | | |
| PCR | 77 (60.2) | 66 (74.1) | 11 (28.2) | |
| Prior serology | 17 (13.3) | 7 (7.9) | 10 (25.6) | NA |
| Reported exposure | 34 (26.5) | 16 (18.0) | 18 (46.2) | |
| Severity, No. (%) | | | | |
| Asymptomatic | 5 (4) | 2 (2.3) | 3 (7.7) | NA |
| Symptomatic, outpatient | 114 (89) | 78 (87.6) | 36 (92.3) | |
| Hospitalized | 9 (7) | 9 (10.1) | 0 (0) | |

IQR, interquartile range; NA, not assessed; NS, not significant; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aParticipants were not referred for donation if anti-SARS-CoV-2 IgG was negative.

^bFor the “all tested” group, the days since last symptoms was calculated by day of blood draw minus last day of symptoms, excluding asymptomatic individuals. For the “referred” and “not referred” groups, this calculation was by day of referral minus last day of symptoms. The P value is for comparison of the referred vs not referred groups.

Table 2
Anti-SARS-CoV-2 Serology Results for All Participants

| | All | PCR Positive | Suspected | P Value |
|----------------------|------------------|--------------|----------------|----------------------|
| IgG positive (%) | 89/128 (69.5) | 66/77 (85.7) | 23/51 (45.1) | < .0001 ^a |
| IgM positive (%) | 48/128 (37.5) | 35/77 (45.5) | 13/51 (25.5) | .0224 ^a |
| IgG RFU median (IQR) | 144 (37.5- 448) | 184 (89-599) | 35 (7-238.5) | < .0001 ^b |
| Minimum-maximum | 5- 2,520 | 10-1,764 | 5-2,520 | |
| IgM RFU median (IQR) | 38 (25.5- 71.25) | 42 (28-84) | 29.5 (18-50.5) | .0024 ^b |
| Minimum-maximum | 14-2,334 | 14-2,334 | 14-189 | |

IQR, interquartile range; PCR, polymerase chain reaction; RFU, relative fluorescence units; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aBy χ^2 test for PCR positive vs suspected SARS-CoV-2 infection.

^bBy Mann-Whitney test for PCR positive vs suspected SARS-CoV-2 infection.

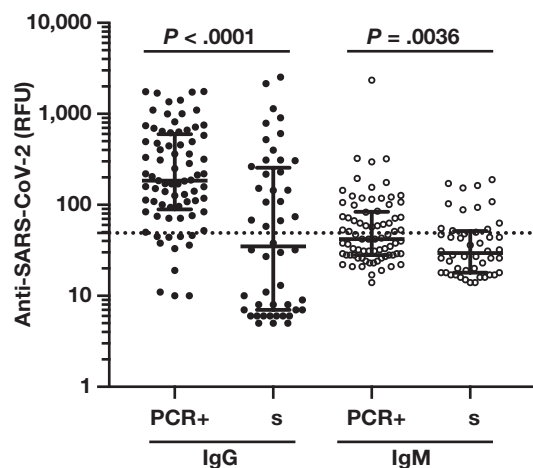


Figure 2 Anti-SARS-CoV-2 IgG and IgM levels among participants with PCR-diagnosed (PCR+) COVID-19 ($n = 77$) and suspected (indicated with “s”) COVID-19 based on history or serology ($n = 51$). IgG and IgM levels are reported in RFU. Dotted line represents RFU cutoff for seropositivity in this assay (50 RFU). COVID-19, coronavirus disease 2019; PCR, polymerase chain reaction; RFU, relative fluorescence units; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

participants with a modified DHQ and a SARS-CoV-2 serologic test to maximize potential successful donation of CCP. In the first months of the COVID-19 pandemic, testing by PCR was limited to patients with the most severe symptoms. Consequently, we included participants in our study with exposure to known cases and/or typical symptoms of COVID-19 but who did not qualify to be tested. Although we did not systematically examine the effectiveness of various recruitment methods, a large proportion of participants responded to the public appeal. Rates of anti-SARS-CoV-2 seropositivity were higher in the PCR-positive group than in the suspected but untested group (85.7% vs 45.1% IgG positive). Given these results, potential donors with known exposure to a patient with COVID-19 or with a history of typical symptoms but no prior testing should not be excluded from potential CCP donation. We did not test study participants for hemoglobin level, infectious disease markers, or anti-HLA antibodies. We have been informed by our partner blood donation center that some referred donors from this study were deferred at the donor center or the collected units were found to be positive for infectious disease markers or anti-HLA antibodies. However, details of deferrals were not shared or reported systematically to us and thus could not be included in our analysis. Because many CCP donors are likely to be first-time blood donors or patients with comorbidities that predispose them to worse COVID-19 symptoms, deferrals may be more likely in this population.

An advantage of this study is that we directly contacted patients who had been diagnosed with COVID-19 to recruit them to donate plasma. We were able to access demographic and health information for COVID-19 patients through hospital electronic medical records. However, as the number of different testing options increased, and test results were increasingly unlinked from any existing medical records, the task of identifying and contacting specific patients became more difficult. The San Francisco Department of Public Health, for example, allowed contact tracers to distribute study recruitment material but declined to give contact information of patients with COVID-19 to our study team. Recruitment efforts for CCP donors targeting potential donors with a known COVID-19 diagnosis at the level of county or state health departments may be helpful to increase the donor pool. Although this study was done with institutional review board approval, an approval to use patient health information for CCP donor recruitment may not be necessary, according to guidance from the US Department of Health and Human Services.¹¹

Anti-SARS-CoV-2 IgG levels in 11 of the 77 PCR-confirmed cases fell below the assay cutoff, raising the possibility that 1 in 7 infected individuals did not produce an IgG response. To address this question, we examined the effect of lowering the cutoff RFU. The percentage of donors who produced anti-SARS-CoV2 IgG at a sufficient level to be called “positive” by our assay was determined by the cutoff value of 50 RFU. This cutoff was set to avoid detection of any false positives and may result in false-negative results in cases with a low quantity of antibodies. If a cutoff of 30 RFU were used, an additional 8 PCR-confirmed cases would be considered IgG positive, resulting in an IgG positive rate of 74 of 77 (96%). Anti-SARS-CoV-2 IgG levels of 10 pre-COVID-19 plasma samples that we tested all fell below 10 RFU, which would argue in favor of counting the 8 that fell above 30 but below 50 RFU as positive. Consequently, a cutoff of 50 RFU is likely not ideal for assessing presence or absence of the antibody response to SARS-CoV-2.

Given the observed variability in SARS-CoV-2 IgG levels in the participants in this study, a “positive” serology result may not translate into a clinically relevant dose of antibodies in a plasma unit. We have observed up to a 50-fold difference in SARS-CoV-2 IgG levels between different CCP units that are confirmed positive for anti-SARS-CoV-2 IgG. This variability in anti-SARS-CoV-2 antibodies has been described by other groups, but its cause is not clear.^{12,13} We note that 39% of participants who were diagnosed by PCR had an antibody level considered high titer by FDA criteria; therefore, this “high titer” designation encompasses a wide range of antibody levels. One limitation of this study is that we did not determine neutralizing antibody titers,

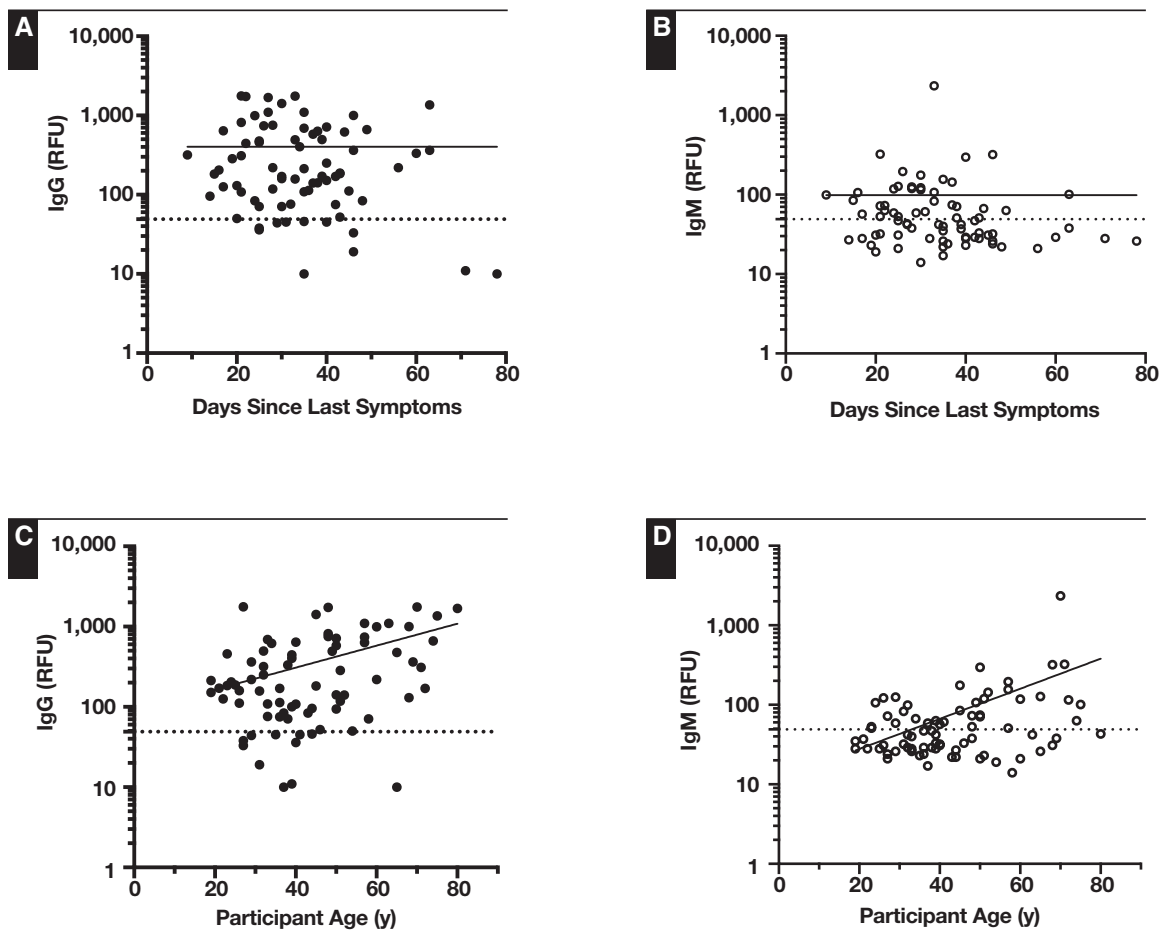


Figure 3 Among participants with confirmed coronavirus disease 2019 levels of IgG (A) and IgM (B) by days since last symptoms and levels of IgG (C) and IgM (D) by age (in years) of participants. A, $y = 10^{(-0.004x + 2.735)}$; $R^2 = 0.0105$. B, $y = 10^{(-0.003x + 2.091)}$; $R^2 = 0.00128$. C, $y = 10^{(0.0137x + 1.942)}$; $R^2 = 0.1968$; $P < .0001$. D, $y = 10^{(0.019x + 1.066)}$; $R^2 = 0.0862$; $P = .0095$. RFU, relative fluorescence units.

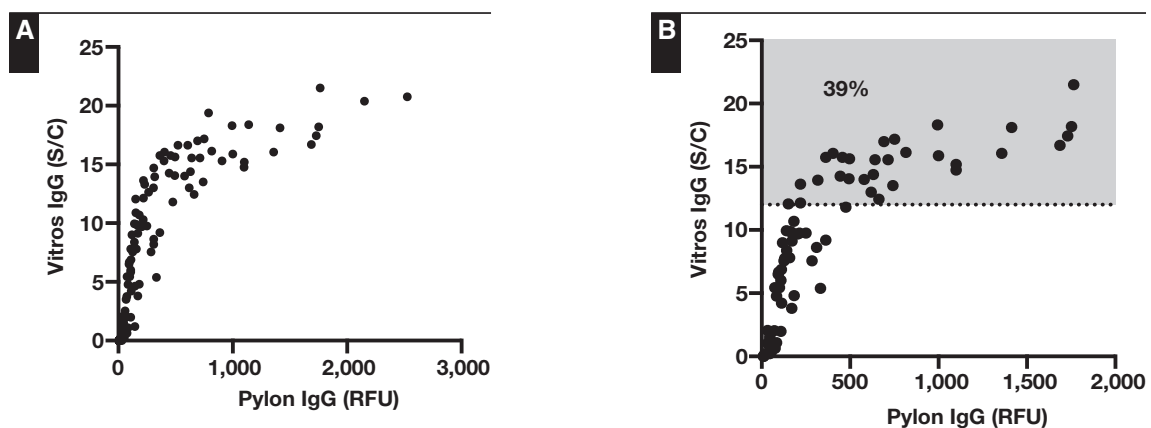


Figure 4 Anti-SARS-CoV-2 IgG by 2 different assays among all tested participants (A) or among all participants positive for SARS-CoV-2 by polymerase chain reaction (B) referred for plasma collection. Results from the ET Healthcare Pylon assay and the Ortho VITROS IgG assay are shown. The US Food and Drug Administration “high titer” cutoff of IgG level (≥ 12 S/C) on the Ortho VITROS assay is indicated with a dotted line. RFU, relative fluorescence units; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S/C, index.

anti-SARS-CoV-2 IgA levels, immunoglobulin subtypes, or antibody affinities. Other groups have shown a correlation between antibody levels to the spike protein receptor binding domain and neutralization titer.^{12,13} Our partner blood donation center did not provide information to correlate the donors with the units that were later obtained by the hospital; therefore, changes in antibody level over time could not be determined. Further work to characterize the functional heterogeneity of antibodies in different CCP donors is important to guide clinical use of CCP.

Given the variability in anti-SARS-CoV-2 IgG levels, the selection of specific units of CCP with higher antibody levels and neutralization titers may be required for clinical efficacy. Prospective testing of segments from CCP units in hospital blood bank inventories or labeling of units with antibody levels by blood suppliers could help with this selection. This approach would give patients a higher potential dose of antibodies with lower volume and donor exposure. However, requiring higher anti-SARS-CoV-2 antibody levels in CCP units may restrict CCP supply and may be costly for blood centers if many collected units fall below a designated cutoff.

Another consideration for using CCP is the amount of anti-SARS-CoV-2 antibody that a patient with COVID-19 has already made. In 16 ICU patients who were COVID-19 positive at our institution, we found anti-SARS-CoV-2 IgG levels at a median of 3,595 RFU (range, 2,086-4,009 RFU).⁹ These levels are far higher than levels measured in our CCP donors (median, 144 RFU; range, 5-2,520). Therefore, the clinical utility of transfusion of CCP in ICU patients with high baseline antibody levels must be considered carefully. This concern about the relative level of antibodies in the patient vs the CCP unit has led to suspension of one randomized controlled trial of CCP.³ Additional work is needed to elucidate the optimal timing of treatment with convalescent plasma.

Finally, knowing the SARS-CoV-2 antibody status of a potential blood donor before blood donation has advantages. Donors can be scheduled for apheresis plasma collection rather than whole blood collection, which increases the amount of plasma collected and decreases the postdonation deferral period. Donors who are curious about their SARS-CoV-2 antibody status will have this information before donation and thus will be more likely to be truthful on the DHQ at the time of donation. Finally, donors with higher levels of SARS-CoV-2 antibodies can be specifically recruited to ensure that only the most potent CCP products are distributed.

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