SARS-CoV-2 Antibody Avidity Responses in COVID-19 Patients and Convalescent Plasma Donors

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Background. Convalescent plasma therapy is a leading treatment for conferring temporary immunity to COVID-19–susceptible individuals or for use as post-exposure prophylaxis. However, not all recovered patients develop adequate antibody titers for donation and the relationship between avidity and neutralizing titers is currently not well understood.

Methods. SARS-CoV-2 anti-spike and anti-nucleocapsid IgG titers and avidity were measured in a longitudinal cohort of COVID-19 hospitalized patients (n = 16 individuals) and a cross-sectional sample of convalescent plasma donors (n = 130). Epidemiologic correlates of avidity were examined in donors by linear regression. The association of avidity and a high neutralizing titer (NT) were also assessed in donors using modified Poisson regression.

Results. Antibody avidity increased over duration of infection and remained elevated. In convalescent plasma donors, higher levels of anti-spike avidity were associated with older age, male sex, and hospitalization. Higher NTs had a stronger positive correlation with anti-spike IgG avidity (Spearman ρ = 0.386; P < .001) than with anti-nucleocapsid IgG avidity (Spearman ρ = 0.211; P = .026). Increasing levels of anti-spike IgG avidity were associated with high NT (≥160) (adjusted prevalence ratio = 1.58 [95% confidence interval = 1.19–2.12]), independent of age, sex, and hospitalization.

Conclusions. SARS-CoV-2 antibody avidity correlated with duration of infection and higher neutralizing titers, suggesting a potential alternative screening parameter for identifying optimal convalescent plasma donors.

Keywords. SARS-CoV-2; avidity; anti-spike; anti-nucleocapsid; convalescent plasma.
potent antiviral receptor binding domain specific antibodies were found in all COVID-19 convalescent plasma donors despite low titers of neutralizing antibodies [17]. While the overall antibody titer is most likely an important factor in determining the viral neutralization potential of a given convalescent plasma donor, multiple other factors likely play a role. These include antibody binding avidity, diversity of response, and amounts of differing antibody classes. While antibody avidity, combined strength of the antibody-antigen complex, can have substantial impact on the humoral immune response, there are limited data on temporal dynamics and correlates of SARS-CoV-2 antibody avidity responses, and whether a stronger avidity response is associated with higher neutralizing titers. An association between antibody avidity and neutralizing antibody titer may also help to identify optimal convalescent plasma donors.

The aim of this study was to characterize SARS-CoV-2 immunoglobulin G (IgG) antibody titers and avidity responses in acute and convalescent patients, and compare their association with neutralizing antibody titers.

METHODS

Study Sample

Longitudinal COVID-19 Sample Patients

All patients had confirmed SARS-CoV-2 infection via a positive RNA nasopharyngeal swab and a known date of symptom onset. Discarded blood serum samples were collected from specimens sent for clinical testing of patients over the duration of their inpatient stay. Samples were selected from individuals with multiple time points available after observed seroconversion. In total 16 distinct hospitalized patients at the Johns Hopkins Hospital contributed 84 serum samples with a median of 5 samples per patient (range, 2–8; interquartile range [IQR], 4.5–6.0).

Convalescent Plasma Donors

Recovered COVID-19 patients were contacted by study personnel to determine interest in donating convalescent plasma. All subjects had confirmed infection of SARS-CoV-2 determined by a positive reverse transcription polymerase chain reaction (RT-PCR) of a nasopharyngeal swab. All potential donors had to be at least 18 years old and meet criteria for blood donation (ie, never been diagnosed with human immunodeficiency virus [HIV], not pregnant, etc.). Potential donors had 25 mL of whole blood collected. Blood was separated into plasma and peripheral blood mononuclear cells within 12 hours of collection, and plasma samples were aliquoted and stored at −80°C. All donors provided informed written consent.

Ethics Statement

Studies of both cohorts were approved by The Johns Hopkins University School of Medicine Institutional Review Board.
urea solution, samples DC_{50} were truncated at 8. DC_{50} calculations were performed using AAT Bioquest IC50 calculator using 4 parameter logistic regression model [21].

Statistical Analysis
Descriptive statistics were used to characterize both study populations: the longitudinal cohort of hospitalized COVID-19 patients and the cross-sectional sample of COVID-19 convalescent plasma donors. Percentages were calculated for categorical variables and continuous variables were described with medians and corresponding interquartile ranges (IQR). The primary study outcomes included anti-spike IgG avidity and anti-nucleocapsid IgG avidity as measured by the Euroimmun and EDI assays, respectively. Longitudinal trajectories in IgG titers and avidity outcomes were visualized by spaghetti plots and examined descriptively by time since symptom onset in the cohort of hospitalized COVID-19 patients. The remaining statistical analyses were conducted among the sample of convalescent plasma donors.

The distribution of anti-spike IgG avidity and anti-nucleocapsid IgG avidity was examined by epidemiologic characteristics, including sex, age, race/ethnicity, and hospitalization status (ie, COVID-19 illness severity). Differences between groups were calculated by Wilcoxon rank-sum tests or Kruskal-Wallis tests, as appropriate. Correlations between age and avidity were assessed by Pearson correlation coefficient. Ordinary least-squares linear regression was used to examine the association of age, sex, and hospitalization status with avidity DC_{50} values. Multivariable models included age, sex, and hospitalization status regardless of statistical significance, as they have previously been shown to be important for other IgG antibody responses in this study population [19]. The multivariable analysis also included adjustment for time since symptom onset. Subgroup analyses were performed stratified by sex using similar methodology. The primary analysis included avidity DC_{50} values for individuals who were considered indeterminate for a given IgG ELISA based on the manufacturer’s cutoff. Thus, sensitivity analyses were performed that excluded donors who had an indeterminate sample.

As a secondary analysis, evaluations of whether anti-spike IgG titers and avidity as well as anti-nucleocapsid IgG titers and avidity correlated with NT AUC values. Data were visualized with nonparametric locally weighted scatterplot smoothing (LOWESS) curves and correlations were assessed with Spearman rank correlation coefficients. To determine whether IgG titers and avidity are associated with elevated neutralizing antibodies, modified Poisson regression with robust variance was used to estimate prevalence ratios (PR) and corresponding 95% confidence intervals (CI) of an NT AUC value ≥160. Multivariable analyses were performed including the serologic biomarker of interest, age, sex, hospitalization status, and time since symptom onset. This analysis was also repeated using an NT AUC value ≥40 as the outcome of interest.

Sensitivity analyses were also performed including days since initial positive PCR diagnosis in multivariable models as opposed to self-reported days symptom onset; time since initial positive PCR-positive diagnosis, which was confirmed with medical documentation, may be a more reliable indicator of duration of infection than a self-reported measure.

RESULTS
Characteristics of the Study Population
There were 16 hospitalized COVID-19 patients in the longitudinal cohort who contributed an average of 5 samples per person (n = 84 total samples). Plasma samples were collected from patients at a median of 13 days (range, 4–35; IQR, 10–16) after symptom onset (Table 1). Using the manufacturers’ cutoffs, 76% (n = 64) of the samples were considered seropositive for anti-spike IgG by the Euroimmun assay and 89% (n = 75) were considered seropositive for anti-nucleocapsid IgG using the EDI assay. Of note, 19 (23%) samples were seronegative for anti-spike IgG and 7 (8%) samples were seronegative for anti-nucleocapsid IgG. However, all specimens collected after 14 days since symptom onset were considered to be seropositive by the Euroimmun assay and the EDI assay (Supplementary Table 1). IgG avidity was measured in seropositive and indeterminate specimens; anti-spike IgG avidity was measured in 65 samples (16 patients) and anti-nucleocapsid IgG avidity was measured in 76 samples (16 patients).

There were 130 participants in the cross-sectional sample of convalescent plasma donors; the median age was 42 years (IQR, 29–55), 46% (n = 60) were female, and 75% (n = 98) were non-Hispanic white (Table 1). Plasma samples were collected from donors a median of 49 days (IQR, 43–55) after symptom onset. Only 11 (8%) donors were previously hospitalized during the course of their infection, although 2 donors were missing data on hospitalization status. Using the manufacturers’ cutoffs, 90% (n = 117) of donors were considered seropositive for anti-spike IgG by the Euroimmun assay, while 81% (n = 105) of donors were considered seropositive for anti-nucleocapsid IgG using the EDI assay. There were some samples that were considered indeterminate for anti-spike IgG serostatus (5% [n = 7]) and anti-nucleocapsid IgG serostatus (7% [n = 9]); plasma from 4 donors were indeterminate by both assays. A greater proportion of donors were seronegative for anti-nucleocapsid IgG (12% [n = 16]) than for anti-spike IgG (4% [n = 5]). Characteristics of donors were similar by sex; however, males were more likely than females to be non-Hispanic Asian (17% vs 3%) and seropositive for anti-spike IgG (96% vs 83%). Prevalence of a high neutralizing titer AUC value greater than 160 was 25% (32/126) overall, 21% (12/58) in females, and 29% (20/68) in males.

Longitudinal Trajectories in SARS-CoV-2 IgG Titers and Avidity Responses
Longitudinal trajectories in SARS-CoV-2 IgG titers and avidity responses were examined by time since symptom onset among...
Table 1. Characteristics of Participants Overall and by Sex

| Characteristics                                      | No. of Samples (%) (n = 84 Samples; N = 16 Participants) |
|------------------------------------------------------|----------------------------------------------------------|
| **Longitudinal Cohort**                              |                                                          |
| Days post symptom onset (IQR)                        | 13 (10–16)                                               |
| Euroimmun anti-spike IgG                             |                                                          |
| Seropositive                                         | 64 (76)                                                  |
| Indeterminate                                        | 1 (1)                                                    |
| Seronegative                                         | 19 (23)                                                   |
| EDI anti-nucleocapsid IgG                            |                                                          |
| Seropositive                                         | 75 (89)                                                   |
| Indeterminate                                        | 2 (2)                                                     |
| Seronegative                                         | 7 (8)                                                     |
| **Convalescent Plasma Cohort**                       |                                                          |
| No. of participants (%)                              |                                                          |
| Overall (n = 130)                                    |                                                          |
| Male (n = 60)                                        |                                                          |
| Female (n = 70)                                      |                                                          |
| Age group, y                                         |                                                          |
| 18–29                                                | 34 (26)                                                   |
| 30–39                                                | 23 (18)                                                   |
| 40–49                                                | 28 (22)                                                   |
| 50–59                                                | 23 (18)                                                   |
| >60                                                  | 22 (17)                                                   |
| Race/ethnicity                                       |                                                          |
| Non-Hispanic white                                  | 98 (75)                                                   |
| Hispanic                                             | 5 (4)                                                     |
| Non-Hispanic Asian                                  | 12 (11)                                                   |
| Other/multiracial/unknown                           | 9 (7)                                                     |
| Median days post symptom onset (IQR)*                | 49 (43–55)                                                |
| Hospitalization status severity                      |                                                          |
| No                                                   | 117 (90)                                                  |
| Yes                                                  | 11 (8)                                                    |
| Missing                                              | 2 (2)                                                     |
| Euroimmun anti-spike IgG                             |                                                          |
| Seropositive                                         | 117 (90)                                                  |
| Indeterminate                                        | 7 (6)                                                     |
| Seronegative                                         | 5 (4)                                                     |
| Missing                                              | 1 (1)                                                     |
| EDI anti-nucleocapsid IgG                            |                                                          |
| Seropositive                                         | 105 (81)                                                  |
| Indeterminate                                        | 9 (7)                                                     |
| Seronegative                                         | 16 (12)                                                   |

Abbreviations: EDI, Epitope Diagnostics Inc.; IgG, immunoglobulin G; IQR, interquartile range.

Data were missing for 1 participant.

The distribution of anti-spike IgG avidity was examined by sex, age, race/ethnicity, and hospitalization status among convalescent plasma donors (Figure 2). The median avidity value was 4.12 (IQR, 3.52–4.53) among females and 4.27 (IQR, 3.85–4.68) among males (P = .084; Figure 2A). Age was positively correlated with anti-spike IgG avidity among males (r = 0.316; P = .008) but not among females (r = 0.022; P = .872; Figure 2B). There was no significant difference in anti-spike IgG avidity by race/ethnicity (P = .588; Figure 2C). Donors who were hospitalized during the course of their infection had stronger anti-spike IgG avidity than donors who were not hospitalized (P < .001; Figure 2D). After adjustment for age, hospitalization, and time since symptom onset, males had on average greater anti-spike IgG avidity than females but this association was not statistically significant (βadj = .248 [95% CI, −.014 to .510]; Table 2). In multivariable analyses, hospitalization was a consistent predictor of stronger anti-spike IgG avidity overall, among females and males. In contrast, age was significantly associated with anti-spike IgG avidity among males (age per 10 years, βadj = .150 [95% CI, .032–.267]) but not among females (βadj = .018 [95% CI, −.114 to .150]).

The distribution of anti-nucleocapsid IgG avidity was also examined by sex, age, race/ethnicity, and hospitalization status (Figure 3), with similar findings as anti-spike IgG avidity, except sex did not modify the correlation between age and anti-nucleocapsid IgG avidity (Figure 3B). In multivariable analysis, age was associated with anti-nucleocapsid IgG avidity in the overall model (age per 10 years, βadj = .229), as was hospitalization status (βadj = .565 [95% CI, .016–1.113]; Table 2). In multivariable analyses, age was associated with anti-nucleocapsid IgG avidity among males (age per 10 years, βadj = .385–4.68) among males (βadj = .150 [95% CI, .032–.267]), but not among females (βadj = .018 [95% CI, −.114 to .150]).

In sensitivity analyses excluding indeterminate samples, effect estimates were slightly attenuated but inferences remained similar (Supplementary Tables 2 and 3). Inferences were also similar for time since diagnosis when included in the multivariable models as opposed to time from symptom onset (Supplementary Tables 4 and 5).

Epidemiologic Correlates of SARS-CoV-2 IgG Avidity Among Convalescent Plasma Donors

There was a strong positive correlation between anti-spike IgG titers and anti-spike IgG avidity (Spearman ρ = 0.541; P < .001); however, there was no significant correlation between anti-nucleocapsid IgG titers and anti-nucleocapsid IgG avidity (Spearman ρ = 0.165; P = .078; Supplementary Figure 2). Both anti-spike IgG titers and anti-spike IgG avidity were positively correlated with neutralizing antibody titer AUC values (Spearman ρ = 0.772 and 0.386, respectively; P < .001 for both comparisons; Figure 4A and 4B). Higher levels of anti-spike IgG titers (PR = 1.57 [95% CI, 1.42–1.74]) and higher levels of anti-spike IgG avidity (PR = 1.80 [95% CI, 1.45–2.22]) were significantly associated with a higher prevalence of a neutralizing...
antibody titer AUC value > 160 (Table 3). These associations were also significant after adjustment for age, sex, hospitalization status, and time from symptom onset in multivariable analysis. Similar results were obtained when days since diagnosis was incorporated into the model rather than time from symptom onset (Supplementary Table 6).

While there was a strong positive correlation between anti-nucleocapsid IgG titers and neutralizing antibody titer AUC values (Spearman ρ = 0.685; P < .001; Figure 4C), the correlation between anti-nucleocapsid IgG avidity and neutralizing antibody titer AUC values was weak (Spearman ρ = 0.211; P = .026) and nonlinear (Figure 4D). Anti-nucleocapsid IgG titers were strongly associated with a higher prevalence of a neutralizing antibody titer AUC value ≥160 in univariable, as well as multivariable analysis (Table 3). Anti-nucleocapsid IgG avidity did not have a statistically significant association with neutralizing antibody titer AUC value ≥160 (adjusted PR = 1.57 [95% CI, 0.40–6.18]). Associations were attenuated when considering a neutralizing antibody titer AUC value ≥40 but remained in the same direction of association.

**DISCUSSION**

SARS-CoV-2 remains a critical health threat. Better understanding of antibody development is necessary. While antibody response is used to evaluate both the early and late stages of immune responses, the relationship between antibody titers and avidity has yet to be established in COVID-19 [22, 23]. Antibody avidity testing has been used to aid in diagnosis of recent infections, including Epstein-Barr virus, HIV, West Nile Virus, and other SARS CoV infections [24–27]. As some recovered patients do not develop high antibody titers following infection, not everyone is an ideal candidate for plasma donation and those who may find it burdensome to return to the clinic following titer testing to donate plasma. Thus, based on these data that anti-spike IgG avidity is better correlated with neutralizing titers, testing antibody
avidity has potential use as another screening parameter to identify optimal convalescent plasma donors. However, further research is needed to examine the potential benefit of this strategy.

As was expected, antibody avidity and titers were low during initial infection, but increased as infection progressed. In previous SARS outbreaks, low antibody avidity was observed early in infection and increased within the first month of symptom onset [22, 23]. Additionally, high IgG avidity was observed 3 weeks following symptom onset in recovered COVID-19 individuals [28]. Consistent with those findings, anti-spike and anti-nucleocapsid IgG avidity increased with antibody titers within the first month of symptom onset and remained elevated following viral clearance. Of the 2 major immunogenic proteins, nucleocapsid proteins are more abundantly expressed, which could help to explain why titers for anti-nucleocapsid IgG appears earlier than anti-spike IgG [22, 29]. Avidity for both antibodies appears to peak around 3 weeks following symptom onset, which corresponds to previous reports of IgG seroconversion in COVID-19 patients occurring between 8 and 21 days following symptom onset [30, 31].

Male bias has been documented in COVID-19 patients, with males having increased disease severity and mortality compared to females [32–34]. Additionally, increased risk for severity of disease is associated with advancing age, even though males and females both have the same susceptibility risk [33, 35]. In the convalescent plasma cohort, antibody avidity was increased in older males compared to younger males. However, no statistically significant differences in anti-spike avidity between males and females was observed, which may potentially be due to lack of power, particularly for anti-spike avidity. All participants donating convalescent plasma had recovered from COVID-19, and even though males tend to have a higher mortality rate than females, those parameters were not established in these studies.

Avidity was significantly higher among hospitalized patients compared to those who were not hospitalized. Those hospitalized may have had higher viral loads and/or increased exposure,
exacerbating symptoms, increasing immune response and antibody production to combat increased viral loads. Moreover, anti-spike avidity was correlated with higher neutralizing antibody titers. Neutralizing antibodies prevent viral replication by binding to viral proteins and blocking the interaction with cellular receptors or cell surface attachment to inhibit host membrane fusion [36]. There was a correlation between anti-nucleocapsid titers and neutralizing antibody AUC, but this may reflect the overall anti-SARS-CoV-2 antibody levels, as anti-nucleocapsid antibodies are not believed to have neutralizing activity. However, nucleocapsid antibodies may be necessary for viral clearance via antibody-dependent cellular cytotoxicity, antibody-dependent cell-mediated phagocytosis, and/or complement activation [33, 35].

Asymptomatic individuals have lower antibody titers as compared to symptomatic patients and those individuals who recover from COVID-19 without becoming hospitalized typically do not have high neutralizing antibody activity [36]. In these studies, persons infected with SARS-CoV-2 who were symptomatic but not hospitalized had lower antibody avidity and 4%–12% had no detectable antibody titers. Those with low titers and avidity may have either robust innate immune or adaptive T-cell responses or strongly binding antibodies early during exposure that results in viral clearance, and it will be important to examine these additional aspects of the anti-COVID immune response moving forward. The mechanisms behind why some individuals do not reach detectable antibody titers following viral clearance needs further investigation.

This study had limitations. Antibody titers were analyzed using 2 different methods, and although increasing values of AU and/or ODn may indicate increase in antibody titers, these values are proxies for quantitative measures. Plateaus observed in both assays may be due, in part, to a small dynamic range of the assay, and as a result antibodies do not appear to continue to rise. Only hospitalized patients were assessed in the longitudinal study and

| Table 2. Epidemiologic Correlates of Anti-spike and Anti-nucleocapsid SARS-CoV-2 IgG Avidity (DC₅₀) Among Potential COVID-19 Convalescent Plasma Donors |
|-----------------------------|-----------------------------|
| Epidemiologic Characteristic | Outcome | Univariable Models | Multivariable Models |
| | | β (95% CI) | P | β (95% CI) | P |
| **Euroimmun anti-spike IgG avidity DC₅₀** | | | | |
| Overall population | Age, per 10 y | .096 (.002 to .191) | .046 | .091 (.004 to .178) | .040 |
| | Male sex | .213 (.075 to .500) | .146 | .248 (.014 to .510) | .063 |
| | Hospitalization | 1.257 (.799 to 1.715) | < .001 | 1.258 (.800 to 1.717) | < .001 |
| | Time post symptom onset, per 5 d | .047 (.031 to .124) | .234 | .010 (.0061 to .080) | .790 |
| Female population | Age, per 10 y | .012 (.134 to .158) | .872 | .018 (.114 to .150) | .783 |
| | Hospitalization | 1.327 (.693 to 1.962) | < .001 | 1.224 (.571 to 1.877) | < .001 |
| | Time post symptom onset, per 5 d | .122 (.011 to .232) | .031 | .075 (.031 to .181) | .161 |
| **EDI antinucleocapsid IgG avidity DC₅₀** | | | | |
| Overall population | Age, per 10 y | .169 (.045 to .293) | .008 | .150 (.032 to .267) | .013 |
| | Male sex | .213 (.075 to .500) | .146 | .248 (.014 to .510) | .063 |
| | Hospitalization | 1.244 (.574 to 1.915) | < .001 | 1.180 (.519 to 1.840) | .001 |
| | Time post symptom onset, per 5 d | .020 (.129 to .089) | .715 | 4.044 (2.980 to 5.108) | .348 |
| Female population | Age, per 10 y | .142 (.000 to .284) | .558 | .040 (.361 to .280) | .804 |
| | Hospitalization | .352 (.316 to .102) | .295 | .357 (.327 to .1041) | .299 |
| | Time post symptom onset, per 5 d | .028 (.087 to .142) | .627 | .019 (.092 to .131) | .731 |
| Male population | Age, per 10 y | .138 (.024 to .301) | .094 | .119 (.043 to .281) | .146 |
| | Hospitalization | .889 (.016 to 1.761) | .046 | .796 (.093 to 1.685) | .078 |
| | Time post symptom onset, per 5 d | .035 (.103 to .174) | .612 | .012 (.125 to .148) | .865 |

Ordinary least-squares linear regression models were used to examine associations with anti-spike and anti-nucleocapsid IgG avidity DC₅₀ values. The multivariable regression models included all other covariates shown. Separate models were constructed for each outcome and subgroup analyses were performed stratified by sex. Values bolded indicate statistical significance (P < .05).

Abbreviations: CI, confidence interval; COVID-19, coronavirus disease 2019; DC₅₀, 50% dissociation constant; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Table 3. Associations Between SARS-CoV-2 IgG Antibody Responses and Elevated SARS-CoV-2 Neutralizing Antibody Titers

| Serologic Biomarker | Outcome | Univariable Models | Multivariable Models |
|---------------------|---------|--------------------|---------------------|
|                     |         | PR (95% CI)        | PValue              |
| **Neutralizing Antibody Titer AUC value ≥ 160** |         |                    |                     |
| Anti-spike IgG titer, AU | 1.57 (1.42–1.74) | < .001 | 1.61 (1.43–1.81) | < .001 |
| Anti-spike IgG avidity, DC50 | 1.80 (1.45–2.22) | < .001 | 1.58 (1.19–2.12) | .002 |
| log2 anti-nucleocapsid IgG titer, ODn | 7.02 (3.64–13.56) | < .001 | 10.43 (4.65–23.41) | < .001 |
| log2 anti-nucleocapsid IgG avidity, DC50 | 2.80 (.73–10.75) | .133 | 1.57 (.40–6.18) | .516 |
| **Neutralizing Antibody Titer AUC value ≥ 40** |         |                    |                     |
| Anti-spike IgG titer, AU | 1.24 (1.17–1.31) | < .001 | 1.24 (1.15–1.33) | < .001 |
| Anti-spike IgG avidity, DC50 | 1.31 (1.16–1.48) | < .001 | 1.18 (0.99–1.41) | .064 |
| log2 anti-nucleocapsid IgG titer, ODn | 2.01 (1.56–2.49) | < .001 | 2.25 (1.66–3.04) | < .001 |
| log2 anti-nucleocapsid IgG avidity, DC50 | 1.67 (.84–3.32) | .140 | 1.42 (.77–2.64) | .264 |

Prevalence ratios of a neutralizing titer AUC value ≥160 and ≥40 were estimated from Poisson regression models with robust standard errors. A different model was used for each serologic biomarker shown. Multivariable models were used to estimate adjusted prevalence ratios which included adjustment for age, sex, hospitalization, and time from symptom onset. Values bolded indicate statistical significance (P < .05).

Abbreviations: aPR, adjusted prevalence ratio; AU, arbitrary unit; AUC, area under curve; CI, confidence interval; DC50, 50% dissociation constant; ODn, normalized optical density; PR, prevalence ratio.
the antibody titers or avidity findings may not be generalizable to those who have mild or subclinical disease. Longitudinal samples did not have demographic characteristics collected, so avidity cannot be attributed to sex or age differences in that cohort. In the convalescent plasma donors, less than 10% were hospitalized and only 1 time point was collected per individual.

Additionally, the usage of modified ELISA avidity assays have not been approved by the FDA for convalescent plasma qualification. However, alternative antibody assays are under evaluation for convalescent plasma testing [37]. Although this study did not analyze longitudinal convalescent plasma, it should be further investigated as reports of declining antibody titers are observed 2–3 months following infection [38]. This would help to identify the optimal window of plasma donation. Furthermore, future studies should explore convalescent plasma in relation to ABO blood groups [39, 40].

The use of avidity in conjunction with other serologic testing may be useful in establishing parameters for optimal convalescent plasma donor screening. Increase in avidity following viral clearance is a good indication that strong antibodies and/or neutralizing antibodies are still present. Those with higher anti-spike antibody avidity also have higher neutralizing titers and can aid in identification of those who establish robust adaptive immune response to infection.

**Supplementary Data**

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.
Notes

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