

Characteristics of High-Titer Convalescent Plasma and Antibody Dynamics After Administration in Patients With Severe Coronavirus Disease 2019

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We characterized the antibody composition of coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) and the immunologic responses of hospitalized COVID-19 patients after receiving CCP or nonimmune fresh frozen plasma. Despite selection of CCP with significantly higher total immunoglobulin G than recipients, neutralizing antibody levels did not differ between donor plasma and CCP recipients.

Keywords. antibody avidity; convalescent plasma; COVID-19; neutralizing antibody; SARS-CoV-2.

Convalescent plasma has been used to treat coronavirus disease 2019 (COVID-19) and may hasten recovery through passive transfer of antibodies that neutralize severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), enhancement of antibody-dependent cytotoxicity, and other immunomodulation factors. The US Food and Drug Administration (FDA) requires a high immunoglobulin (Ig)G titer for COVID-19 convalescent plasma (CCP) [1]. COVID-19 convalescent plasma efficacy may be influenced not only by antibody quantity but also neutralizing activity, binding avidity, diversity of response, and

antibody isotypes, which are not well characterized in CCP [2]. It is notable that antibody avidity has been associated with survival in a small cohort [3] and has been proposed as an additional criterion for plasma donor selection [4]. Although antibody dynamics have been characterized in patients with COVID-19 infection, little is known about the spectrum of antibody response after CCP receipt [3, 5], including the potential for impaired humoral response, as has been reported after respiratory syncytial virus (RSV) passive immunization [6]. This analysis aims to characterize the antibody composition of CCP and antibody responses of COVID-19-infected individuals after CCP receipt.

METHODS

We characterized antibodies from CCP units and CCP recipients enrolled in a randomized, double-blinded, placebo-controlled trial evaluating the efficacy and safety of CCP in hospitalized, hypoxic, nonventilated adults with confirmed COVID-19 (ClinicalTrials.gov Identifier NCT04421404). Fresh frozen plasma (FFP) served as a control because of plasma's potential impact on the coagulation cascade in COVID-19. Participants enrolled in June through October 2020 at 2 hospitals in San Francisco, California were randomized 1:1 to receive 1 unit of convalescent plasma or nonimmune FFP (200–250 mL). We selected CCP with the highest anti-SARS-CoV-2 IgG antibody levels available. All units met FDA high titer criteria by VITROS Anti-SARS-CoV-2 IgG assay (Ortho Clinical Diagnostics) [1]. Control FFP was collected before December 31, 2019 or confirmed negative for anti-SARS-CoV-2 antibodies.

Six antibody assays were measured from each unit of donor plasma and recipients at study day 1 (before plasma administration) and from recipients on day 29. The VITROS Anti-SARS-CoV-2 IgG, SARS-CoV-2 Reporter Viral Particle Neutralization (Vitalant Research Institute), and SCoV-2 Detect IgA ELISA (InBios International, Inc.) assays targeted the spike protein. The Pylon COVID-19 IgG/IgM assay (ET Health) targeted the spike protein receptor binding domain (RBD) and the nucleocapsid protein [7]. An internal IgG avidity assay targeted the RBD [8]. Nasopharyngeal swabs obtained on study days 1, 2, 5, and 8 were tested for SARS-CoV-2 viral load using quantitative polymerase chain reaction (PCR) (Integrated DNA Technologies) and reported as cycle thresholds (CTs).

Antibody results are reported as medians and interquartile ranges (IQRs). Antibody levels and PCR CTs were compared between groups using Mann-Whitney non-parametric *t* tests and between time points using paired Wilcoxon rank-sum tests. *P* < .05 was considered statistically significant. Results from the primary endpoint will be reported through the COMPILER consortium [9].

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Patient Consent Statement

Participants provided written consent for study inclusion. This study was approved by the Advarra Institutional Review Board and FDA under Investigational New Drug (IND) 21774.

RESULTS

In this study, we enrolled 34 study participants: 16 received CCP and 18 received nonimmune FFP. The investigators and study monitoring committee closed the study before completing target enrollment due to declining COVID-19 cases in San Francisco in October 2020. Baseline characteristics were similar between the 2 groups (Table 1). Median symptom duration before transfusion was 8 days among CCP recipients and 9 days among FFP recipients. A total of 8.8% of participants were transfused within 3 days of symptoms and 41.2% within 7 days. A total of 94.1% received remdesivir and 20.6% received dexamethasone per local treatment guidelines.

All 6 antibody assays were available on 100% of plasma units, 82.4% of recipients on day 1, and 58.8% of recipients on day 29. Among participants with baseline Vitros IgG results, IgG reactivity was detected in 19 of 28 (67.9%) before transfusion. There were no significant differences for any of the 6 assays between participants who reported ≤ 7 days of symptoms and those with > 7 days (Supplementary Figure). Donor plasma median IgG was significantly higher than recipient baseline IgG: 1025.0 relative fluorescence units (RFU) (IQR, 766.0–1465.0) vs 38.0 RFU (IQR, 5.8–161.0), $P < .05$ by Pylon IgG and 16.63 signal-to-cutoff ratio (S/CO) (15.75–20.78) vs 3.42 S/CO (0.43–11.78), $P < .05$ by Vitros IgG (Figure 1A). Median IgG avidity was also higher in donor plasma than recipients at baseline: 69.0% (47.8–82.8) versus 0.0% (0.0–47.0), $P < .05$. Conversely, the median neutralizing antibody level did not differ between donor plasma and recipients: 553.6 inhibitory concentration of 50% (IC_{50}) (358.0–809.6) vs 1105.0 IC_{50} (1518.0–2292.0), $P = .47$. It is notable that recipient neutralizing antibody values varied substantially, ranging from 0.0 to 11358.2 IC_{50} .

In CCP recipients, median IgG and avidity levels significantly increased from day 1 to day 29, but neutralizing antibody, IgA, and IgM levels did not (Figure 1B). Median Pylon IgG was 21.5 RFU (IQR 4.3–138.8) at baseline compared to 1679.0 RFU (1006.0–2291.0), $P < .05$ at day 29, and median Vitros IgG increased from 2.51 S/CO (0.08–8.08) to 21.66 S/CO (20.58–23.30), $P < .05$. Avidity increased from 0.0% (0.0–42.3) to 67.5% (55.5–79.5), $P < .05$. Median neutralizing antibody level was 295.5 IC_{50} (123.0–1288.0) at baseline compared to 1518 IC_{50} (919.3–3955), $P = .56$ at day 29. However, none of the median antibody levels differed significantly between CCP recipients and FFP recipients at day 29.

Quantitative PCR was completed in 32 of 34 (94.1%) recipients. Two FFP recipients and 1 CCP recipient had undetectable viral loads at baseline, and the median CT was 24.0 (20.4–27.9)

Table 1. Baseline Demographics and Clinical Characteristics of Study Participants

Characteristics	Convalescent Plasma Group (n = 16)	FFP (Control) Group (n = 18)
Sex, N (%)		
Female	10 (63%)	9 (50%)
Male	6 (38%)	9 (50%)
Age, median years (IQR)	52 (40–64)	62 (49–74)
Ethnicity, N (%)		
Hispanic or Latino	12 (75%)	11 (65%)
Not Hispanic or Latino	4 (25%)	6 (35%)
Race, N (%)		
American Indian/Alaska Native	0 (0%)	1 (6%)
Asian	2 (13%)	2 (11%)
Black or African American	1 (6%)	1 (6%)
Unknown/ Not Reported	10 (63%)	9 (50%)
White	3 (19%)	5 (28%)
Coexisting Medical Conditions, N (%)		
Obesity	3 (19%)	4 (22%)
Diabetes	6 (38%)	4 (22%)
Hypertension	4 (25%)	7 (39%)
CHF and CAD	2 (13%)	2 (11%)
Chronic lung disease or asthma	2 (13%)	3 (17%)
Cancer	1 (6%)	2 (11%)
Pregnancy	2 (13%)	1 (6%)
Days of Symptoms Before Transfusion, Median (IQR)	8 (5–9)	9 (5–10)
Timing of Plasma Administration, N (%)		
Within 3 days of symptom onset	2 (13%)	1 (6%)
Between 3 and 7 days of symptom onset	4 (25%)	7 (39%)
Greater than 7 days since symptom onset	10 (63%)	10 (56%)
WHO 8-Point Ordinal Scale Score, N (%)		
3 (hospitalized, no O ₂)	2 (13%)	0 (0%)
4 (hospitalized, low-flow supplemental O ₂)	10 (63%)	16 (89%)
5 (hospitalized, high-flow supplemental O ₂)	4 (25%)	2 (11%)
Therapeutics		
Remdesivir	16 (100%)	16 (89%)
Dexamethasone	4 (25%)	3 (17%)
Baseline IgG reactivity (Ortho Vitros IgG)		
Nonreactive (S/CO <1)	6 (38%)	3 (17%)
Reactive (S/CO ≥ 1)	8 (50%)	11 (61%)
Missing baseline Ortho Vitros IgG data	2 (13%)	4 (22%)
Baseline IgG value, median S/CO (IQR)	2.5 (0.1–8.1)	7.5 (1.8–16.7)

Abbreviations: CAD, coronary artery disease; CHF, congestive heart failure; IgG, immunoglobulin G; FFP, nonimmune fresh frozen plasma; IQR, interquartile range; S/CO, signal-to-cutoff ratio; WHO, World Health Organization.

in the remaining participants. Polymerase chain reaction CT did not correlate with symptom duration (Pearson $r = 0.013$; 95% confidence interval, -0.36 to 0.38); see Figure 1C. There were no significant differences between groups in the proportion with undetectable virus or the median CT values at days 2, 5, and 8 (Figure 1D). There was a nonsignificant trend towards lower viral load at days 2 and 5 in the FFP recipients.

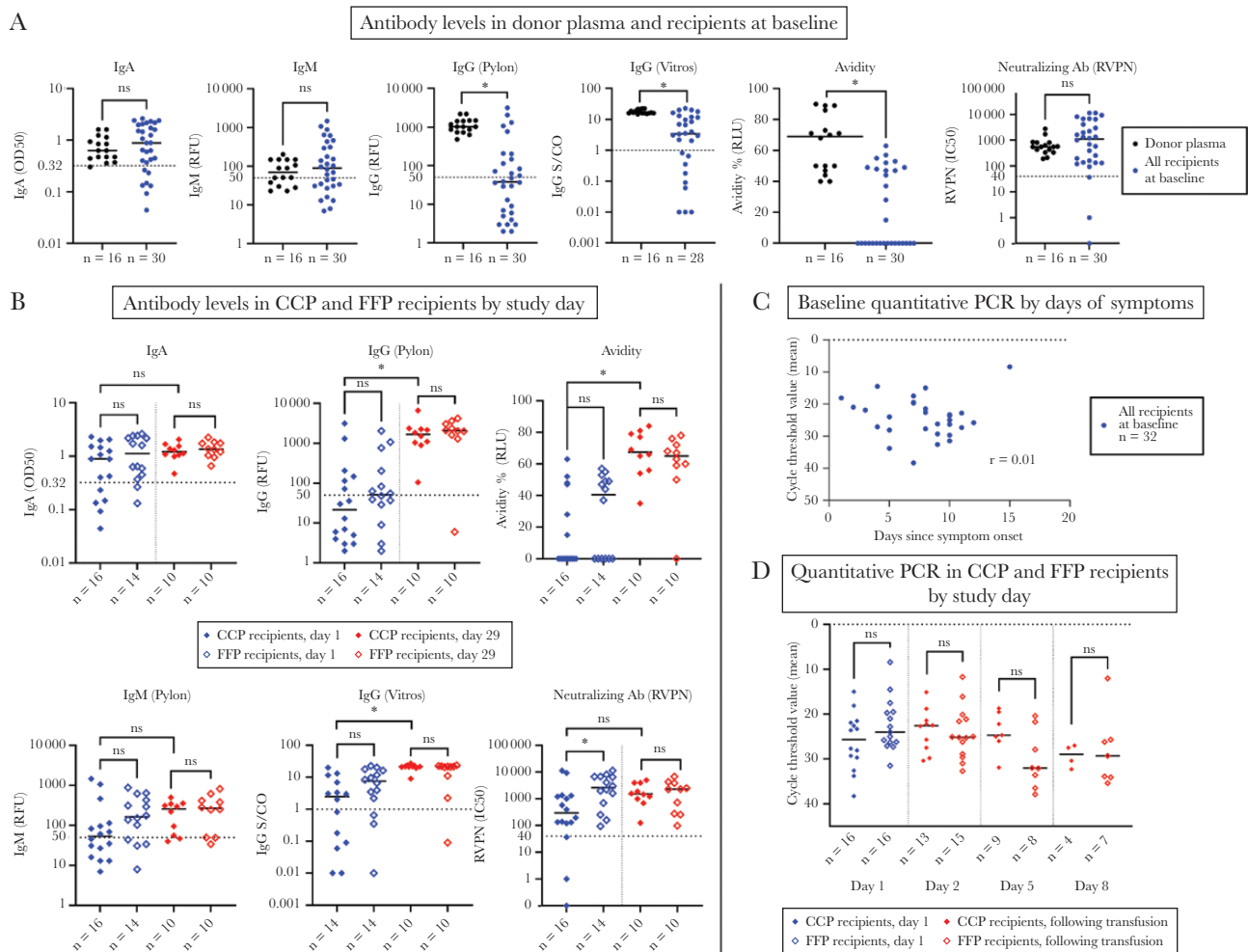


Figure 1. Antibody (Ab) composition of high titer coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) and antibody and viral load dynamics in COVID-19 patients who received CCP or nonimmune fresh frozen plasma (FFP). (A) Immunoglobulin (Ig)A, IgM, IgG, avidity, and neutralizing antibody levels in donor plasma and study participants at baseline (before transfusion). (B) Immunoglobulin A, IgM, IgG, avidity, and neutralizing antibody levels at day 1 and day 29 stratified by CCP and FFP groups. (C) Baseline severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) quantitative polymerase chain reaction (PCR) cycle threshold by days since symptom onset ($r = 0.01$). (D) Median SARS-CoV-2 quantitative PCR cycle threshold in CCP recipients compared with FFP recipients at day 1 (before transfusion), day 2, day 5, and day 8. Dotted horizontal lines on each graph denote positivity cutoff for each assay (there is no cutoff for the avidity assay). * = statistically significant, $P < .05$. r = Pearson correlation coefficient. IC_{50} , inhibitory concentration of 50%; ns, not significant; OD_{50} , optical density 50; RFU, relative fluorescent units; RLU, relative light unit; RVPN, reporter viral particle neutralization; S/CO, signal-to-cutoff ratio.

DISCUSSION

Studies suggest that CCP is most beneficial when administered to high-risk patients early in infection [10]. We present additional evidence that CCP specifically selected for higher IgG levels did not inhibit the humoral immune response to SARS-CoV-2, supporting the reassuring safety profile of CCP [11]. Compared to antibodies present in plasma recipients, the spectrum of antibodies in high-titer CCP differed in type and activity. The robust IgG and avidity levels exceeded existing antibody levels in recipients, suggesting that high-titer IgG was an appropriate surrogate for high avidity. There were no differences between IgA, IgM, and neutralizing antibody levels in CCP units and recipients at baseline. Although units of CCP were specifically selected to ensure higher IgG levels, they did

not provide neutralizing antibody levels consistently higher than those of recipients.

Neutralizing activity may decline in the months after infection; thus, plasma collected later in recovery may have lower neutralizing antibody levels despite persistently elevated IgG [12, 13]. Donor time from infection to plasma collection was unavailable, but neutralizing antibodies may have waned if significant time elapsed between COVID-19 recovery and CCP donation. In addition, many participants enrolled later in their disease course, already had detectable neutralizing antibody levels, and were unlikely to benefit from passive antibody transfer. Neutralizing antibody assays are not standardized and the clinical implications of lower concentrations are unclear. The range of neutralizing antibodies highlights the heterogeneity of

CCP and the difficulty of standardizing based on a single element like IgG titer.

In participants who received CCP, there was a significant increase in IgG and avidity levels from day 1 to day 29 that did not differ from the antibody increases in the FFP group. More importantly, we found no evidence that CCP impaired participants' native humoral response, as has been postulated based on RSV convalescent plasma experience [6]. Coronavirus disease 2019 convalescent plasma did not produce a more robust humoral response to COVID-19, nor did it reduce viral load in the nasopharynx more than FFP. Indeed, there was a trend towards a steeper decline in SARS-CoV-2 viral load in the FFP arm. The lack of correlation between symptom duration and viral load may reflect heterogeneous viral shedding and also highlights the subjectivity of patient-reported symptom onset.

Strengths of our study include an extensive analysis of antibody isotypes and functional activity to characterize both the composition of high-titer CCP and the recipient immune response before and after CCP or control FFP infusion. This study was limited by the small sample of hospitalized, hypoxic patients. Most participants were beyond 72 hours of symptom onset, the period when studies suggest that CCP is most likely to be beneficial [10]. Antibody testing was not available for all participants at each timepoint. Antibody dynamics between study days 1 and 29 may have been missed. Finally, data on the timing of CCP donation compared to initial COVID-19 infection were unavailable.

CONCLUSIONS

In summary, CCP specifically selected for higher IgG levels did not promote or inhibit the humoral immune response to SARS-CoV-2 by day 29. These findings may be relevant to the settings in which CCP may have a role for COVID-19 treatment, such as within 72 hours in high-risk elderly patients [10] and in those with impaired humoral immunity, or for COVID-19 use as passive immunization. Future studies of CCP could consider selection of plasma with high neutralizing antibodies in addition to overall high IgG, which correlated with higher avidity, to maximize activity. The contribution of neutralizing activity to the clinical efficacy of convalescent plasma merits further evaluation.

Supplementary Data

Supplementary materials are available at *Open Forum 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.

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