

# COVID-19 convalescent plasma therapy decreases inflammatory cytokines: a randomized controlled trial

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**ABSTRACT** Early COVID-19 convalescent plasma (CCP) transfusion to outpatients with COVID-19 decreases progression to hospitalization, but the mechanism of how CCP reduces severity is unknown. Among 882 COVID-19 participants transfused with CCP or control plasma in a randomized controlled trial, 21 cytokines and chemokines were measured using electrochemiluminescence assays. Wilcoxon rank sum tests were used to evaluate the difference between early (transfused within 5 days of symptom onset) CCP vs early control plasma and late (transfused 6–9 days after symptom onset) CCP vs late control plasma at each visit. Linear mixed-effect models were used to assess the difference in the slope of cytokine change. Median cytokine and chemokine levels were similar between the early CCP and early control groups pre-transfusion. At the day 14 visit, only the median IL-6 ( $P = 0.014$ ) and IL-16 ( $P = 0.036$ ) levels were lower in the early CCP group compared to the early control group, but these differences were not statistically significant after correcting for multiple comparisons (requiring  $P < 0.0024$ ). IL-6 levels decreased significantly faster in the early CCP group from screening to the day 14 visit compared to the early control group ( $P < 0.001$ ). No difference was observed in the slope of cytokine change from screening to day 90 between early CCP and early control groups. Late control and late CCP arms showed similar cytokine and chemokine levels through study follow-up. One mechanism by which early CCP transfusion reduces hospitalization may be by decreasing IL-6 levels, as the reduction is associated with better recovery from COVID-19.

**IMPORTANCE** This study examined the role that cytokines may have played in the beneficial outcomes found when outpatient individuals infected with SARS-CoV-2 were transfused with COVID-19 convalescent plasma (CCP) early in their infection. We found that the pro-inflammatory cytokine IL-6 decreased significantly faster in patients treated early with CCP. Participants with COVID-19 treated with CCP later in the infection did not have the same effect. This decrease in IL-6 levels after early CCP treatment suggests a possible role of inflammation in COVID-19 progression. The evidence of IL-6 involvement brings insight into the possible mechanisms involved in CCP treatment mitigating SARS-CoV-2 severity.

**KEYWORDS** COVID-19, COVID-19 serotherapy, convalescent plasma, SARS-CoV-2, cytokines, chemokines, randomized trial

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Inflammation is one of the leading causes of COVID-19 morbidity [e.g., acute respiratory distress syndrome (ARDS)] and mortality (1–8). The innate immune activation and cytokine storm are associated with COVID-19 severity (9, 10). Individuals who have recovered from COVID-19 have persistently increased inflammatory cytokines, and elevated levels of IL-6 are associated with post-COVID conditions (11, 12). In addition, it has been shown that vaccination reduces inflammatory markers in recently infected individuals (2).

COVID-19 convalescent plasma (CCP) has been shown to decrease hospitalization if it contains high titers of antibodies against SARS-CoV-2 and is provided early relative to the diagnosis or onset of symptoms (13–20). However, the mechanism for CCP to reduce severity is unknown (21). This study compared the levels of 21 different cytokines in participants who received CCP or a control pre-pandemic plasma within 9 days of symptom onset as part of a randomized controlled trial (13).

## MATERIALS AND METHODS

### Population

The randomized double-blinded placebo-controlled clinical trial population included participants from the Convalescent Plasma to Limit Coronavirus-Associated Complications (CSSC-004) trial (NCT04373460), as previously described (13). The trial excluded participants who were or planned to be hospitalized, had a history of adverse transfusion reactions, received monoclonal antibodies, or were unable to comply with protocols. We transfused 1181 participants with either CCP or SARS-CoV-2 seronegative control plasma up to 9 days after COVID-19 symptom onset from 23 sites in the United States between June 2020 and October 2021. For this study, we included 882 participants with samples and symptom data collected at screening, day 14, and day 90 visits, as previously described (Fig. S1) (2, 11).

The JHU Institutional Review Board (IRB) and the Human Research Protection Office of the United States Department of Defense approved the trial. The Navajo Nation Human Research Review Board and the Indian Health Service National IRB also approved study activities at the Center for Indigenous Health sites. All participants provided written, informed consent.

### Data collection

Blood samples were collected in ethylenediaminetetraacetic acid tubes; plasma was separated and stored at  $-80^{\circ}\text{C}$  until cytokines were measured (2, 11). Electrochemiluminescence multiplexed sandwich immunoassays were used to quantify the concentration (pg/mL) of 21 different cytokine and chemokine analytes according to the manufacturer's instructions (MesoScale Discovery, Gaithersburg, MD, USA). Samples and calibrators were run in duplicate (2, 11). Standard curves quantifying cytokine/chemokine concentrations were based on calibrators run with each plate. Both runs of the analytes had to be above the plate-specific lower limit of detection for them to be considered detectable. Cytokine values that were below the lower limit of detection or above the higher limit of detection were extrapolated using standard curves. The duplicates were averaged for analysis. Analytes with a coefficient of variation greater than 25 and a signal greater than 2,000 were considered invalid and rerun (2, 11).

### Statistical analysis

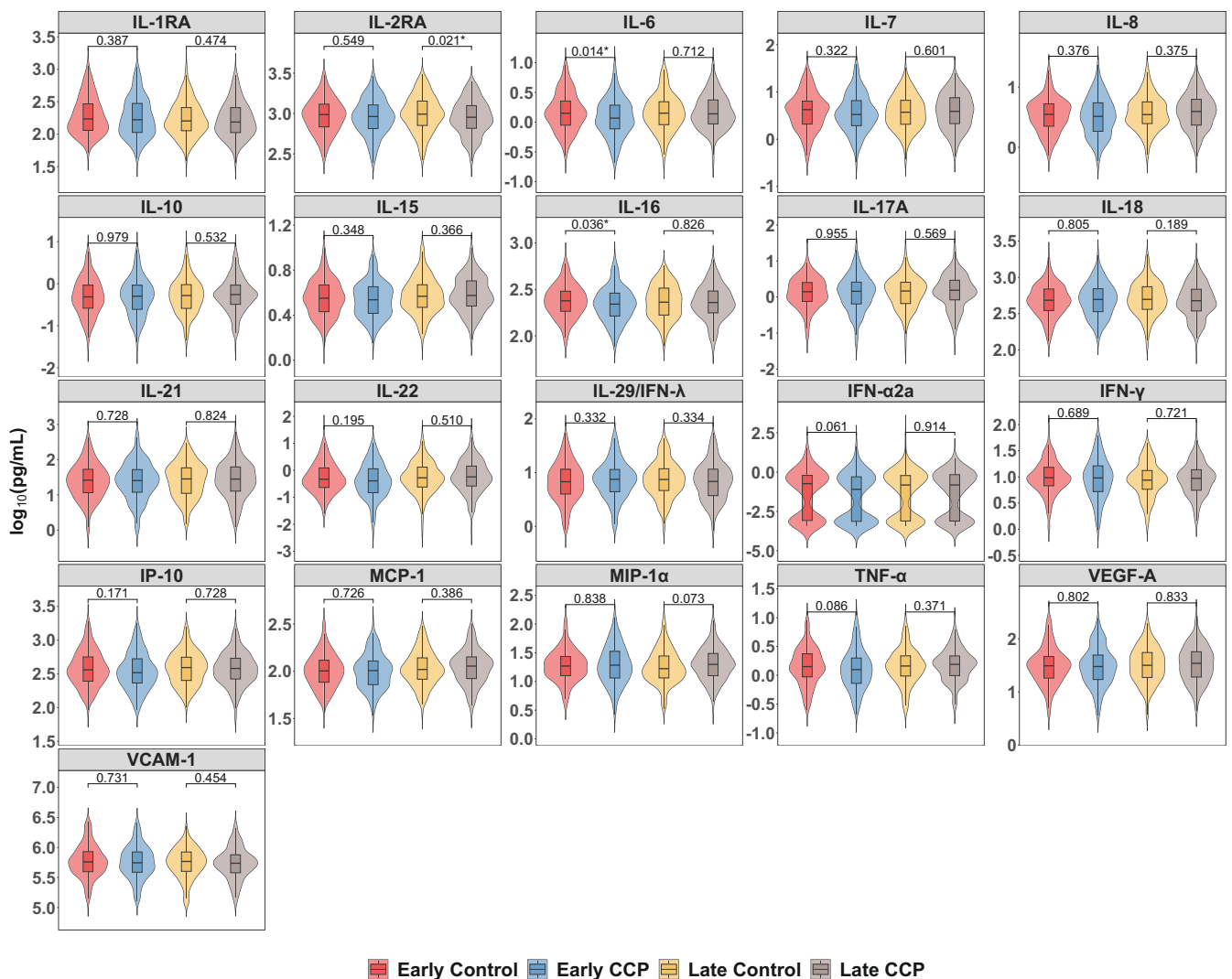
The early CCP and early control plasma groups were defined as transfusions occurring at most 5 days after symptom onset. Late CCP and late control plasma groups were defined as transfusions occurring 6 to 9 days after symptom onset.

Missing cytokine values out of the fit curve range were imputed using a stochastic draw from the extrapolated lower or upper tails of the fitted  $\log_{10}$  normal distribution of available cytokine values, and outliers were excluded as previously described (2, 11).

Cytokine values were  $\log_{10}$  transformed, and comparisons were made between early control vs early CCP and late control vs late CCP. Wilcoxon rank sum tests were used to evaluate the difference in cytokine levels between treatment groups at screening (pre-transfusion), day 14 and day 90. Linear mixed-effect models with an unstructured covariance matrix were used to assess the difference in slope of cytokine change between treatment groups from screening to days 14 and 90, adjusting for age, sex, body mass index (BMI), hypertension, diabetes, vaccine status, and COVID-19 waves. We adjusted for multiple comparisons using Bonferroni correction [requiring two-tailed  $P < 0.0024$  ( $P < 0.05 \div 21$ )]. All analyses were conducted in R 4.2.

**RESULTS**

Among the 882 trial participants, the majority were female (57.4%), and the median age was 43 years, as previously described (Table S1) (2, 11). There were no significant differences between early and late CCP vs early and late control plasma trial arms, respectively, by age, sex, race, ethnicity, BMI, or vaccine status. Among the participants, 197 (22.3%) participants had CCP and 195 (22.1%) had control plasma transfused within 5 days of symptom onset (i.e., early). For participants transfused more than 5 days since symptom onset (i.e., late), 248 (28.1%) had CCP and 242 (27.4%) had control plasma.



**FIG 1** Day 14 cytokine and chemokine levels stratified by treatment group. \* $P < 0.05$ .

Pre-transfusion, the median cytokine and chemokine levels were similar between the early CCP and the early control group. The median IL-2 receptor alpha chain (IL-2RA) levels were lower among the late CCP than the late control group ( $P = 0.008$ ), but the difference was not statistically significant after adjusting for multiple comparisons (requiring  $P < 0.0024$ ) (Fig. S2).

At the day 14 visit, median IL-6 ( $P = 0.014$ ) and IL-16 ( $P = 0.036$ ) levels were lower among the early CCP group compared to the early control group (Fig. 1), but these differences were not statistically significant after adjusting for multiple comparisons. At the day 90 visit, median IL-16 levels of early CCP remained marginally lower than the early control group ( $P = 0.010$ ) (Fig. S3).

From screening to day 14, levels of IL-1RA, IL-6, IL-15, IL-18, IFN- $\alpha$ 2a, and IP-10 decreased faster among the early CCP than the early control group ( $P < 0.05$ ), but only IL-6 levels decreased significantly faster after adjusting for multiple comparisons ( $P < 0.001$ ) (Table 1). No difference was observed in the slope of cytokine change from screening to day 90 between early CCP and early control groups.

Late CCP and late control groups had similar cytokine and chemokine levels through the follow-up periods (Table 1; Fig. S2 and S3).

## DISCUSSION

While the neutralizing antibodies present in CCP are critical, this study offers another potential mechanism by which CCP reduces hospitalization from COVID-19. Overall, patients who received early CCP had lower levels of cytokines and chemokines at day 14 visit than a control group who received early SARS-CoV-2 seronegative plasma. Notably, IL-6 levels declined faster in the early CCP group during the first 2 weeks of follow-up after symptomatic COVID-19 infection.

**TABLE 1** Comparison of the slope of the daily changes of cytokine levels between early control and early CCP

Analyte	Difference in slope of change between early control and early CCP (early CCP minus early control) <sup>c</sup>			
	Screening to day 14		Screening to day 90	
	$\beta$ (95% CI)	<i>P</i> value	$\beta$ (95% CI)	<i>P</i> value
IL-1RA	-0.0058 (-0.0095, -0.0020)	0.003 <sup>a</sup>	-0.0005 (-0.0011, 0.0001)	0.126
IL-2RA	-0.0001 (-0.0021, 0.0019)	0.906	0.0001 (-0.0003, 0.0004)	0.748
IL-6	-0.0100 (-0.0151, -0.0050)	<0.001 <sup>b</sup>	-0.0002 (-0.0011, 0.0007)	0.630
IL-7	-0.0003 (-0.0051, 0.0044)	0.895	-0.0001 (-0.0008, 0.0006)	0.805
IL-8	-0.0010 (-0.0052, 0.0031)	0.631	-0.0001 (-0.0008, 0.0006)	0.780
IL-10	-0.0037 (-0.0102, 0.0029)	0.274	-0.0002 (-0.0014, 0.0010)	0.705
IL-15	-0.0028 (-0.0050, -0.0007)	0.011 <sup>a</sup>	-0.0002 (-0.0006, 0.0001)	0.165
IL-16	-0.0014 (-0.0036, 0.0008)	0.215	-0.0002 (-0.0006, 0.0001)	0.231
IL-17A	-0.0001 (-0.0068, 0.0066)	0.985	-0.0003 (-0.0015, 0.0008)	0.563
IL-18	-0.0030 (-0.0054, -0.0007)	0.012 <sup>a</sup>	-0.0003 (-0.0007, 0.0001)	0.159
IL-21	-0.0023 (-0.0080, 0.0035)	0.438	-0.0001 (-0.0011, 0.0008)	0.750
IL-22	-0.0088 (-0.0208, 0.0032)	0.152	0.0004 (-0.0009, 0.0016)	0.566
IL-29/IFN- $\lambda$	-0.0023 (-0.0073, 0.0028)	0.380	-0.0006 (-0.0014, 0.0002)	0.165
IFN- $\alpha$ 2a	-0.0222 (-0.0441, -0.0003)	0.047 <sup>a</sup>	-0.0009 (-0.0048, 0.0030)	0.645
IFN- $\gamma$	-0.0081 (-0.0163, 0.0002)	0.055	-0.0010 (-0.0024, 0.0005)	0.199
IP-10	-0.0055 (-0.0109, -0.0001)	0.048 <sup>a</sup>	-0.0003 (-0.0014, 0.0008)	0.582
MCP-1	-0.0004 (-0.0030, 0.0022)	0.767	-0.0004 (-0.0008, 0.0000)	0.084
MIP-1 $\alpha$	-0.0030 (-0.0073, 0.0012)	0.160	-0.0002 (-0.0009, 0.0005)	0.610
TNF- $\alpha$	-0.0057 (-0.0115, 0.0000)	0.052	-0.0002 (-0.0013, 0.0008)	0.644
VEGF-A	0.0014 (-0.0029, 0.0057)	0.536	0.0004 (-0.0003, 0.0010)	0.257
VCAM-1	0.0014 (-0.0020, 0.0049)	0.418	0.0001 (-0.0005, 0.0006)	0.802

<sup>a</sup> $P < 0.05$ .

<sup>b</sup> $P < 0.0024$  (statistically significant after adjusting for multiple comparisons using Bonferroni correction).

<sup>c</sup>The difference in slope (95% CI) of the daily changes of log<sub>10</sub> pg/mL values of each analyte during follow-up is presented. Each analyte had a separate mixed-effect model, and all the models were adjusted for age, sex, body mass index, hypertension, diabetes, vaccine status, and COVID-19 waves.

IL-6 levels have been associated with COVID-19 morbidity and mortality due to increased inflammation and cytokine storms (22, 23). IL-6 plays an important role in a cascade of the immune system associated with generating a cytokine storm (22, 24). IL-6 neutralization is effective in reducing COVID-19 mortality (25), and IL-6 has been associated with post-COVID complications (11). Late CCP and late control groups showed similar cytokine levels during all follow-up visits, which further strengthens the importance of the timing of CCP. Given that CCP is an antiviral agent, one possible mechanism for the reduced IL-6 is a reduction in viral burden (26). Observing the significant decrease in the first 2 weeks compared to 3 months can potentially illustrate the role CCP treatment plays in reducing inflammation immediately after infection.

This study has both strengths and limitations. It is one of the largest outpatient studies that measures cytokine and chemokine levels for COVID-19, and data were collected over time, providing a trajectory before and after treatment. The study population is from a randomized controlled trial, which minimizes bias regarding who received CCP treatment or pre-pandemic control plasma. However, despite minimal differences observed between participants receiving late or early CCP or control plasma, there may be unmeasured confounding. Most of the study participants were also enrolled before the availability of vaccines, which may limit inferences about the current stage of the pandemic. In addition, cytokine measurements can be variable, and thus outlier results were excluded. There may be significant variability in cytokine measurements (27), but we used the same assay and lot number to reduce this variation. Finally, only the ability of CCP to decrease cytokines was evaluated, but other mechanisms of action may also be important. Further investigation of the mechanism of clinical benefit is needed.

The role of IL-6 in COVID-19 progression and long-term effects through increased inflammation is well established (11, 22, 28). This study shows that CCP transfusion early after SARS-CoV-2 infection is associated with reduced IL-6 levels.

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#### ADDITIONAL FILES

The following material is available [online](#).

#### Supplemental Material

**Supplemental material (Spectrum03286-23-S0001.docx).** Fig. S1 to S3 and Table S1.

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