Serial Convalescent Plasma Infusions for the Initial COVID-19 Infections in the Appalachian Region of West Virginia

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Abstract

Purpose: The rapid spread of SARS-CoV-2, the virus that is responsible for causing COVID-19, has presented the medical community with another example of when convalescent plasma (CP) is still used today. The ability to standardize CP at the onset of a pandemic is unlikely to exist in a reliable and uniformly reproducible way. We hypothesized that CP of unknown strength given in a serial manner will promote health and reduce mortality in those infected with COVID-19.

Methods: Participants were given up to 8 CP-units depending on their condition upon entry into the study and their response.

Results: 102 out of 117 participants were given CP. The earlier a participant received CP correlated with survival (p = 0.0004). The number of CP-units given, throughout all the clinical severities, was not significant with outcomes, p = 0.3947. A higher number of CP-units given to the severe/critical participants (without biological immunosuppressants or restrictive lung disease) did correlate with survival p = 0.0116 (2.8 vs. 2 units). Lower platelets on admission correlated with mortality. Platelet levels increase correlated with CP infusions p < 0.0001.

Conclusion: This study supports the serial use of CP of unknown strength based on clinical response for those infected with COVID-19. The use of 3–4 units of CP was found to be statistically significant for survival for severe and critical participants without restrictive lung disease and chronic biological immunosuppression. Increased platelet levels after CP infusions supports that CP is promoting overall health regardless of outcomes.

Keywords
convalescent plasma, COVID-19, pediatric, adult, SARS-coV-2, coronavirus

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Introduction

The use of convalescent plasma to aid in the fight against infections dates back over 100 years.\textsuperscript{1,2} It’s use in general practice has been replaced by immunoglobulin immunotherapy. There still, however, exists a special niche for convalescent plasma in specific situations when there are a paucity of treatments available for a rare or novel infection.\textsuperscript{3} The rapid spread of SARS-CoV-2, the virus that is responsible for causing COVID-19, has presented the medical community with another example of these specific situations.\textsuperscript{4} As before, the questions of how much, potency, when, how often to give and safety of convalescent plasma have been raised.\textsuperscript{3} Upon the onset of novel infections, the ability to standardize the effectiveness of convalescent plasma is unlikely to exist in a reliable and uniformly reproducible way.\textsuperscript{5} Likewise, the ability to determine viral loads is also not clinically available. With these fundamental academic limitations, the appropriate dosage is remanded to “more” with safety and availability being the natural dosing cap. We hypothesized that convalescent plasma of unknown strength given in a serial manner that is titrated to clinical response will help to promote health and reduce mortality in those inflicted with COVID-19.

Methods

This study was approved by the institutional review board (IRB) at West Virginia University (WVU) School of Medicine # 2004965705, the United States Food and Drug Administration (FDA) Investigational New Drug (IND) # 20060 and registered on ClinicalTrials.gov # NCT04376034. Consent was obtained in writing or oral when written was not possible secondary to COVID-19 hospital policies with contact precautions and visitation. Plasma enriched in antibodies from those that have recovered from COVID-19 was transfused into those infected with SARS-CoV-2 in a compassionate care manner. The study took place during the initial onset of COVID-19 in the United States between late March of 2020 to December of 2020 and prior to reports of COVID-19 variants. Inpatient participants above the age of 28 days of life with COVID-19 were eligible to enroll. Convalescent plasma (CP) treatment exclusion criteria consisted of historical knowledge of selective IgA deficiency that has not been found to be absent of anti-IgA antibodies or on comfort care measures only. Main outcome of survival was defined at discharged home-, short- or long-term recovery facility and no readmission after 30 days. Additional primary outcomes were safety, time from identifying CP donors willing to donate and time from CP infusion eligibility upon entering study to CP infusion.

For the purpose of this study, dyspnea was defined as any shortness of breath that required greater than 2 Liters/minute of nasal canula oxygen to relieve symptoms for more than 4 h. To be eligible to receive CP, one had to have at least one of the following:\textsuperscript{7}

1. Dyspnea as defined above.
2. Respiratory frequency of \( \geq 30/\text{minute} \)
3. Blood oxygen saturation of \( \leq 93\% \)
4. Partial pressure of arterial oxygen to fraction of inspired oxygen ratio <200
5. Lung infiltrates > 50% within 24 to 48 h of admission
6. Respiratory failure, septic shock, and/or multi organ dysfunction or failure.

There was no control or placebo group, but intention to treat was to be used as a pseudo-control arm. Intention to treat was defined as: enrolled and meeting criteria for CP infusions but secondary to shortages the participant did not receive CP. Study recruitment was based on consult from the primary care teams. The study also took place across 5 different hospitals in West Virginia, 4 of the hospitals are within the WVU Medicine health system in West Virginia.

There was only one arm to the study with 3 entry points that served as a treatment continuum to determine initial CP unit dosing based on the criteria in table 1.

Once enrolled the timing of when the participant would have been eligible was retrospectively investigated in addition to if they were eligible to receive CP at enrollment. If not eligible at enrollment, they were prospectively monitored in the event that their status progressed and became eligible. For the purpose of statistical analysis only the term \textit{critical} was reserved for a participant on mechanical ventilation and/or ECMO.

The mild severity group was monitored for progression and was given CP if their symptoms worsened (Table 1). Moderate severity with concern for rapid progression received a loading dose of 1 CP unit. Severe and critical severity received a loading dose of 2 CP units either given in 1 day or 1 unit per day for two consecutive days. Over 90% of the those receiving 2 units were given 1 unit per day for 2 days discussed further below. Once the full protocol was approved each participant was eligible for an additional 1 CP unit every 3 days for failure to improve or worsening illness with a general cap of 8 CP units. The initial emergency protocol was capped at 2 units. Nineteen of the 117 participants were enrolled under the emergency protocol and only 15 met criteria for CP infusion. The decision to give more CP for the other 98 participants in the full protocol was defaulted to the primary care team’s discretion. Cases were routinely discussed with the primary care team prior to infusion of additional CP. They were given the guidance to give more CP if the participant was failing to improve significantly based on routine clinical judgement. Example, going from HFNC at 60L to HFNC at 40L would not be considered significant improvement and an additional unit of CP was advised. However, going from HFNC to LFNC would be considered significant improvement and the additional unit of CP was...
Table 1. Initial Convalescent Plasma Dosing.

Mild: 1 plus 2 or 3. No CP, obtain type and screen in case of progression
1) Responding to conventional treatments, or absence of sustained progression of illness for greater than 4 h based on routine clinical judgement
2) On room air
3) On Low flow nasal cannula oxygen = ≥2L with no shortness of breath or higher for less than 4 h at a time with no sob

Moderate: 1 and 2 must be met. Convalescent Plasma 1 Unit
1) SOB on more than LFNC as specified under “Mild”, but not on Mechanical Ventilation or High Flow Nasal Canula
2) Not responding to conventional treatments, acute worsening of condition for greater than 4 h based on routine clinical judgement

Severe and critical: 1 plus 2 or 3; or 1 plus 4 mechanical ventilation and/or clinical judgement; or 5 alone.

Convalescent Plasma 2 Units
1) Dyspnea as defined above
2) Respiratory frequency ≥ 30/min*
3) Blood oxygen saturation ≤ 93%
   a. partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300
   b. lung infiltrates > 50% within 24 to 48 h
4) Mechanical Ventilation or High Flow Nasal Canula
5) Life-threatening disease is defined as:
   a. respiratory failure
   b. septic shock, and/or
   c. multiple organ dysfunction or failure

HFNC was defined as at least 6L/minute of nasal canula oxygen. All pediatric patients were to receive 10 mL/kg (1 dose) if moderate with concern for rapid progression if up to 1 Unit; 10 mL/kg up to 2 units if severe or critical.

*Respiratory frequency ≥50/min in pediatric patients under 1 years old.

not advised. Should the participant be found to be clinically worsening without an identifiable reason an additional unit of CP was advised (example of an identifiable reason, pneumothorax was discovered or bacterial sepsis and thought to be the main or sole reason for worsening health status).

It is important to note that West Virginia, as a State, does not have a single entity that has the FDA registration or licensure and the equipment on site required to collect plasma for the purpose of plasma transfusion. To overcome this obstacle, 20 units of CP was purchased to act as a critical reserve for the study. This critical reserve of CP allowed for a reduction in delays in procuring CP from the American Red Cross once ordered. As such our intention to treat arm is limited to an n of 2 and was not used for statistical analysis. Convalescent plasma did not come with segments for testing. It was not possible prior to transfusion to select high titered CP units in preference even once testing became available or in a research lab setting. To mitigate the potential of giving only low titered and lower in neutralization ability plasma to one recipient and high titered CP to another, when a participant was to receive at least 2 units of CP, the 2 units were not from the same donor in nearly all cases. In over 90% of the cases no more than 1 unit of CP was given in a calendar day during the initial loading doses regardless of the participants severity. Rather if loading with 2 units, 1 unit was given sequentially on two consecutive days with at least 12–18 h separating the two doses.

A participant’s course was tracked with respect to dates of onset of symptoms, diagnosis, admission, meeting CP criteria, days to transfusion, length of hospital stay, mortality and serial routine laboratory results when available. Hospital stay complications as well as pre-existing health conditions were explored. Various treatment modalities were track such as medications used, respiratory support, procedural interventions, the number of CP units given and the timing of the CP given.

Student t-test, Mann-Whitney, ANOVA was done using Statistical analyses were performed using Prism 9 (GraphPad, San Diego, CA) with a confidence level of 95%. Unpaired Student t-test was used for determining statistical significance of ages, BMI, Platelet levels. Mann-Whitney was used for determining statistical significance of pre-existing health co-morbidity health conditions, number CP units and survival, and medications given and survival. ANOVA was used for determining statistical significance of age groups and survival. Given the low numbers of certain age groups, those 40–49 and under were grouped for the ANOVA as were age groups 80 and 90.

Results

One hundred and seventeen participants consented for CP (Table 2). Of the 117 participants, one was a child. Fifty-eight females were enrolled and 59 males. Ages ranged from 15 to 95 years of age. The average age for both females and males was 65 years old and the difference in age was not statistically significant. The overall survival in the study was 77% or 23% mortality. Decades 7 and 8 in age was not statistically significant. ANOVA was used for determining statistical significance of ages, BMI, Platelet levels. Mann-Whitney was used for determining statistical significance of pre-existing health conditions, number CP units and survival, and medications given and survival. ANOVA was done using statistical analysis for the age groups was statistically significant for age groups 70 and above (p = 0.0028).

Co-morbid pre-existing health conditions and correlation with mortality was significant for (Table 2): Thyroid disease (p = 0.0018), COPD/Emphysema (p = 0.0190), and restrictive lung disease (p = 0.0024). However, diabetes mellitus, CHF, CKD, hypertension, hyperlipidemia, CAD, and BMI were not significant. There were eight individuals that had no pre-existing health conditions and all survived, but it was not statistically significant.

Fifteen participants did not receive CP, 11 of which did not meet criteria of CP during their hospitalization. Two were not given CP secondary to shortages and 2 recovered to the point of no longer being eligible to receive CP just prior to the CP’s arrival. Of the 11 that did not qualify for CP, 100% survived to discharge and were not readmitted.
within 30 days. The two that did not receive CP secondary to shortages were at the beginning of the study and one succumbed to COVID19 (69 year old female, critical severity) and one survived (63 year old male, severe severity). The two that recovered prior to CP infusion were both moderate in severity.

Platelet levels on arrival to the hospital were found to statistically correlate with outcomes $p = 0.0030$ (Figure 1a). Average platelet levels were $228 \pm 111$ for those that survived and succumbed respectively. Lymphocyte levels on arrival to the hospital were not found to be statistically significant with survival. Platelet levels prior to each CP unit and after each CP unit were found to statistically increase the number of platelets in a paired two tailed student t-test $p < 0.0001$ (Figure 1b). The lymphocyte and platelet ratios with neutrophil levels on arrival to the hospital were not statistically significant ($p = 0.5145$ and $0.4124$ respectively) for correlating to final outcomes.

Thirty-eight did not meet criteria for CP within 24 h of admission with 33 of them recovering for an overall survival rate of 87%. Twenty six of the 38 participants did eventually meet criteria for CP. The 5 participants that passed away were given CP within 24 h of entering the study. Each of the 5 that did not survive were listed as severe upon entering the study. The 5 that did not survive are discussed further below.

Of the 102 participants that were given CP, the earlier they received CP correlated with statistical significance on survival. This was true for both the days of admission and days of meeting CP study criteria ($p = 0.0079$, 0.0004 respectively), the latter shown in Figure 2a. Days from first COVID-19 symptom or diagnosis to CP transfusion was not statistically significant with outcomes ($P = 0.6756$ and 0.1714 respectively). Two hundred and fifty-one CP units were transfused throughout the study. The mean number of units per person was $2.4 \pm 1.3$. The number of CP units for those that survived and succumbed respectively. Lymphocyte levels on arrival to the hospital were not found to be statistically significant with survival. Platelet levels prior to each CP unit and after each CP unit were found to statistically increase the number of platelets in a paired two tailed student t-test $p < 0.0001$ (Figure 1b). The lymphocyte and platelet ratios with neutrophil levels on arrival to the hospital were not statistically significant ($p = 0.5145$ and 0.4124 respectively) for correlating to final outcomes.

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| Table 2. Participant Characteristics and Co-Morbid Preexisting Health Conditions. |
|-----------------------------|-----------------------------|-----------------------------|
| n | (Recovered/Mortality) | n | (Recovered/Mortality) |
| Thyroid Disease | 18/14 | 0.0018 | CHF | 11/7 | 0.1245 |
| Diabetes Mellitus | 43/13 | >0.9999 | HTN | 65/23 | 0.8240 |
| COPD/Emphysema | 16/11 | 0.0190 | HLD | 53/17 | 0.2105 |
| Restrictive Lung Disease | 0/4 | 0.0024 | CAD | 26/10 | 0.4784 |
| CKD | 19/8 | 0.4352 | BMI | 36.4/35.6 | 0.7519 |

1) ANOVA of age groups 40 and under, 50, 60, 70, 80 and up. * marks significance
2) Total n of recovered/mortality respectively = 90, 27
3) unpaired student t-test on BMI recovered verse Mortality standard deviation is 10.54 and 11.12 respectively. Abbreviations: COPD = chronic obstructive pulmonary disease, CKD = chronic kidney disease, CHF = congestive heart failure (chronic), HTN = hypertension, HLD = hyperlipidemia, CAD = coronary heart disease, BMI = body mass index.

Figure 1. Platelet level association and correlations with outcomes and cp infusions.

Figure 1a. (Left): platelet levels mean with sd. b (right): Platelet levels mean with SD. Pre = platelet levels prior to CP infusion ($257 \pm 111$). Post = platelet levels after CP infusion ($258 \pm 116$).
infused per person ranged from 1 to 8. The number of CP units regardless of clinical severity was not found to be significant with survival among the total 102 participants, p = 0.3947. The number of days of admission among those that received CP was not statistically significant with final outcomes, p = 0.0699 (20.7 days of admission for those that survived to discharge and 28.7 days for those that did not survive).

The number of CP units given to the subgroups severe and critical at the time of CP infusion was not found to be statistically significant with a p = 0.1076 (Figure 2b). When controlling for the participants on biological immunosuppressants or with restrictive lung disease within the severe and critical groups, survival was statistically significant with a p = 0.0116 (Figure 2c). The mean CP units for the survival group was 2.8 +/- 1.4 units, with 4 units at the 75% percentile and 2 units at the 25% percentile. The mean CP units for the mortality group was 2 unit +/- 0.9987, with the 75% percentile at 3 units and 2 units at the 25% percentile. Statistical significance was also found when examining the number of CP units given among only the critical subgroup alone without biological immunosuppressants or restrictive lung disease p = 0.0096 (with inclusion of immunosuppressed and restrictive lung disease p = 0.2159). Among the total critical cohort, those that received CP after intubation, the number of CP units did correlate with survival (p = 0.0007) seen in Figure 2d. The mean CP units for the survival group was 3.1 units +/- 1.6, with 4 units at the 75% percentile and 2 units at the 25% percentile. The mean CP units for the mortality group was 1.4 unit +/- 0.7, with the 75% percentile at 2 units and 1 unit at the 25% percentile. Those that received CP prior to intubation did not correlate with survival (p = 0.5173).

With consideration of the other medications the participants treated with Remdesivir and Tocilizumab were found to associate with mortality, p = 0.0456 and p = 0.0272 respectively (Table 3). All other medication were not statistically significant with respect to final outcomes. Medications provided that were tracked include: dexamethasone (p = 0.5378), methylprednisolone (p = 0.2362), hydroxychloroquine (0.6206), Ivermectin (p = 0.1238) and antibiotics (p-value ranged from 0.4352 to >0.9999).

Acute kidney injury (AKI) and acute heart failure were shown to be statistically significant for mortality, p = 0.0026 and 0.0249 respectively (Table 3). Sepsis (p = 0.0524) along with encephalopathy (p = 0.5378,) deep vein thrombosis (p = 0.3333), pulmonary embolism (p >0.9999), and pneumothorax (p = 0.1238) were not found to be statistically significant with outcomes (Table 3).

**Discussion**

From a clinical perspective the ability to answer questions such as CP’s potency from unit to unit, and neutralizing ability is not available. However, convalescent plasma is unique among treatment options in medicine as it has in many ways matured more as a standardized process than it has as a standardized medical treatment product. The use of combined donor plasma pools that are highly refined into immunoglobulin immunotherapy such as: IVIG, SCIG, and IMIG products helps to mitigate variance from batch to batch. Monoclonal and polyclonal (high titered antigen specific) antibodies have made standardization of immunoglobulin immunotherapy even more reliable as each batch is near identical. It is for these reasons that this study advocated for the use of CP as an intermediate treatment option for COVID19 and by way of a compassionate care approach until modalities of superior product standardization were available and prior to the onset of variant strains.

West Virginia as a State does not have the ability to collect plasma for the purpose of transfusion; a fact that was realized early on in the pandemic. The potential to use whole blood collection does exist, but the population density of WV made this option less realistic for sustainability. This made the ability to collect, test and select CP units higher in titer strength and stronger viral neutralization ability impossible.
prior to thawing the CP for infusion. The reality of the limitations created the studies approach with the recommendations of serial CP unit infusions based on clinical status/response. The serial use of CP in this way is unique to this study as was in the rural Appalachian setting, defining of dyspnea and the use of a critical reserve of CP to decrease time to transfusion once eligible.

Serological levels on presenting to the emergency department just before admission were chosen for examination of associations with outcomes secondary to the variable treatments provided throughout the study and at different hospital locations. This point in time served as a natural and universal cross-sectional point of reference prior to any treatments. Lower platelet levels, but not necessarily thrombocytopenia, was associated with mortality.9 This is likely secondary to greater viral suppression of platelets. The reciprocal trend for platelet levels increasing after CP infusions was also significant and provides indirect observational evidence that CP infusions were able to promote health to some degree regardless of outcomes. A similar trend was not observed in another study by Erkurt et al, however in this study platelet levels prior to CP infusions and 1 week after CP infusion was compared.10

Lymphocytes on presentation, which can also be suppressed by infections did not correlate with mortality in this study. Nor did the platelet to neutrophil levels or lymphocytes to neutrophil levels on admission in this study. Platelet and lymphocyte ratios to neutrophils have been reported to be a predictor of outcomes in other studies, but not necessarily from admission values.10,11

Without titration of titers and neutralization strengths, the results of this study matched other studies in the same time period of the pandemic that were able to analyze the CP prior to infusion. In this study if CP was given within 3 days of admission it was found to be statistically significant for survival.12-14 With dyspnea being defined in this study it was found that survival was statistically significant if given within 3 days of meeting CP infusion. This suggests that a strict cut off from days of admission could be relaxed in some cases. Duration of time from symptom onset to CP transfusion was insignificant. This insignificance could be secondary to the variable accuracy of the first symptom reporting, types of pre-existing health conditions and potentially initial viral load exposure and route of infection. The reasons for being tested for COVID-19 varied in timing secondary to known exposures without symptoms or severity of symptoms regardless of known exposures and may account for the lack of statistical significance with outcomes.

This study agreed with other studies that showed 1 or 2 units of CP was not statistically significant with long term survival overall.15-17 This was particularly true when looking at those with moderate severity.15 However, in the severe and critical cohort survival was found to be statistically significant with more than 2 units of CP. More impressive is that this was still statistically significant when only examining those that were in a critical state prior to CP infusion. Most studies on CP have been limited to 1–2 units, one study showed statistical significance when up to 4 units were given.18 In this study, 2 units were given on day 1 and if there were no signs of improvement 2 more units were given the next day. The choice to wait 3 days prior to additional CP in our study was secondary to Li et al study showing that after 3 days 87% of those that received CP had no detectable virus in the serum and CP stewardship.19 Ideally, had CP been more plentiful faster redosing of CP may have been beneficial. Although, the use of CP has been shown to be safe, the use of 1 unit of CP per day in this study was preferred for additional safety measures with the volume loads as well as CP stewardship.20,21

The restrictive lung disease cohort included pneumoconiosis (n = 2) and asbestosis (n = 2). The former is an increased risk to the West Virginia population with the mining industry and history in the older patient populations. The decision to control for them both in sub-analysis was secondary to the 100% fatality seen and the uniqueness of the aliments compared to the rest of the study population. The mortality associated with restrictive lung disease has already been reported.22 However, none of the 4 were intubated prior to CP infusion and 1 was DNR/DNI.

The two that were on biological immunosuppressant (Rituximab and Secukinumab) in the critical cohort, as well as the entire study, received CP prior to intubation. It is possible that given the active immunosuppression, the severity of inflammation and by extension, their clinical symptoms were attenuated and delayed treatment. In both cases each survived the initial COVID-19 infection and recovering. However, one was unable to come off ECMO after 122 days of hospital admission and the other had a mechanical ventilation malfunction, acute PE and the care plan was transitioned to comfort measures only 28 days after admission. Controlling for this cohort in statistical analysis is from the unique immunosuppression that both medications created

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**Table 3. Medication and Acute Health Complications Correlation with Mortality.**

| Medications          | p-value | Acute Health Complications | p-value |
|----------------------|---------|-----------------------------|---------|
| Remdesivir           | 0.0456  | AKI                         | 0.0026  |
| Tocilizumab          | 0.0272  | AHF                         | 0.0249  |
| Dexamethasone        | 0.5378  | Sepsis                      | 0.0524  |
| Methylprednisolone   | 0.2362  | Encephalopathy              | 0.5378  |
| Hydroxychloroquine   | 0.6206  | DVT                         | 0.3333  |
| Ivermectin           | 0.1238  | PE                          | >0.9999 |
| Antibiotics          | 0.4352  | Pneumothorax                | 0.1238  |

Mann-Whitney with 95% confidence level. Abbreviations: AKI = Acute kidney injury, AHF = acute heart failure, DVT = deep venous thrombosis, PE = pulmonary embolism.
compared to the other participants that may have led to a delay in giving CP. Additionally, those with immunocompromised systems have been reported to have de novo mutations of the SARS-CoV-2 that escape CP during treatments within the same infectious period.\textsuperscript{23}

The elimination of those on Rituximab, Seckinumab and with restrictive lung disease did not affect the statistical significance on survival for those in critical status prior to CP infusion. In this cohort, an average of 3 CP units with the 75\% percentile of 4 units correlated with survival compared to 1.4 units with the 75\% percentile of 2 units in those that eventually succumbed to COVID-19. The high mortality rate of those on biological immunosuppressants prior to COVID-19 and those with restrictive lung disease advocates for faster use of CP and potentially monoclonal or polyclonal antibodies at lower clinical symptom severity.

The overall survival rate of 87\% for those that did not meet criteria for CP on enrollment into the study was not lower than the overall survival rate of the study. This suggests that waiting to give CP until dyspnea requires >2L NC oxygen support to reveal symptoms is reasonable in most cases. Among the 5 that did not survive, one had idiopathic thrombocytopenia and passed from complications of a chest tube insertion for a pneumothorax, another notably had asbestosis (see above), the third had a partial lobectomy from lung cancer. The remaining 2 participants succumbed to septic shock from bacterial infections. Of those that never met criteria for CP none were readmitted within 30 days after discharge for COVID-19 related reasons.

The initial critical reserve of 20 units formally satisfied the goal of identifying donors. However, the initial intent to identify and eventually be able to collect plasma was not possible in WV. The 20 units and the manner of CP use in this study afforded the ability to provide CP to 5 different hospitals in WV within 24 h of CP request throughout the entire study in over 95\% of the cases. Lastly the primary outcome of safety was in agreement with other reports previously mentioned.\textsuperscript{20} Serious adverse events within 24 h attributed to CP infusions in this study were less than 2\% per CP unit infusions.

**Conclusion**

This study supports the use of convalescent plasma of unknown strength in a serial manner based on clinical responses for those infected with COVID-19. Particularly as a temporary or intermediate stop gap treatment option as more standardizable medications are being developed. Earlier use of CP with respect to either days of admission or days from requiring >2L NC oxygen support correlated to overall survival (within 3 days). Lower platelet levels on admission were associated with higher mortality. Indirect observational evidence of increased platelet levels after CP infusions supports that CP is improving overall health regardless of outcomes. The use of 3 to 4 units of CP was found to be statistically significant for survival for severe (high flow nasal canal or greater) and critical (mechanical ventilation/ECMO) participants without restrictive lung disease and chronic biological immunosuppression. Those with restrictive lung disease or on biological immunosuppressants may benefit from earlier treatments regardless of clinical disposition given their 100\% mortality rate in this study.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| CP           | Convalescent Plasma |
| NC           | Nasal canula |
| HFNC         | High flow nasal canula |
| LFNC         | Low flow nasal canula |
| ECMO         | Extracorporeal membrane oxygenation |
| IVIG         | Intravenous immunoglobulin IgG |
| SCIG         | Subcutaneous immunoglobulin IgG |
| IMIG         | Intramuscular immunoglobulin IgG |

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**Authors’ Contributions**

Brian P. Peppers, DO, PhD is the principle investigator and (1) made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; (2) drafted the article and reviewed it critically for important intellectual content; (3) given final approval of the version to be published; and (4) agrees to be accountable for all aspects of the work related to its accuracy or integrity.

Aaron Shmookler, MD, Johnathan Stanley, DO, Lisa Giblin Sutton, PharmD, Peter L. Perrotta, MD, Theodore Kieffer, MD, and David Skoner, MD have each (1) made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafted the article or reviewed it critically for important intellectual content; (3) given final approval of the version to be published; and (4) agrees to be accountable for all aspects of the work related to its accuracy or integrity.

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**Availability of Data and Material**

Data will be made available in accordance with Clinical Trial Registration. It will also be made upon request by interested parties to the extent that it can be made available without violating local, State and Federal Law within the United States of America.
Consent to Participate
Consent was obtained in writing or oral when written was not possible secondary to COVID-19 hospital policies with contact precautions and visitation

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Approval
This study was approved by the institutional review board (IRB) at West Virginia University (WVU) School of Medicine # 2004965705, the United States Food and Drug Administration (FDA) Investigational New Drug (IND) # 20060

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References
1. Flexner S, Lewis PA. Experimental poliomyelitis in monkey-seighth note: further contributions to the subjects of immunization and serum therapy. J Am Med Assoc. 1910;55(8):662–663. doi:10.1001/jama.1910.04330080028014
2. McGuire LW, Redden WR. The use of convalescent human serum in influenza pneumonia-a preliminary report. Am J Public Health (N Y). 1918;8(10):741–744. doi:10.2105/ajph.8.10.741
3. Delamou A, Haba NY, Mari-Saez A, et al. Organizing the donation of convalescent plasma for a therapeutic clinical trial on ebola virus disease: the experience in guinea. Am J Trop Med Hyg. 2016;95(3):647–653. doi:10.4269/ajtmh.15-0890
4. Wu Z, McGooan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. JAMA. 2020;323(13):1239–1242. doi:10.1001/jama.2020.2648
5. Shen C, Wang Z, Zhao F, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA. 2020;323(16):1582–1589. doi:10.1001/jama.2020.4783
6. Chen L, Xiong J, Bao L, Shi Y. Convalescent plasma as a potential therapy for COVID-19. Lancet Infect Dis. 2020;20(4):398–400. doi:10.1016/S1473-3099(20)30141-9
7. Kakuturu J, McCluskey C, Casey F.L., Cieck S., Hayanga J.A. Extracorporeal membrane oxygenation to treat a 15-year-old patient with severe coronavirus disease 2019 (COVID-19) respiratory failure. JTCVS techniques. 2021;Jun(7):265–266. doi:10.1016/j.jtcts.2021.03.012
8. Madariaga ML, Schrantz S, Jansen MO, et al. Integrated COVID-19 Convalescent Plasma Treatment and Antibody Research Program at a Single Academic Medical Center (5/7/2020). Available at https://dx.doi.org/10.2139/ssrn.3605131
9. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in wuhan, China: a retrospective cohort study. Lancet (London, England). 2020;395(10229):1054–1062. doi:10.1016/S0140-6736(20)30566-3
10. Erkurt MA, Sarici A, Berber I, Kuku I, Kaya E, Özgül M. Life-saving effect of convalescent plasma treatment in COVID-19 disease: clinical trial from eastern anatolia. Transfus Apher Sci Off J World Apher Assoc Off J Eur Soc Haemapheresis. 2020;59(5):102867. doi:10.1016/j.transci.2020.102867
11. Chan AS, Rout A. Use of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in COVID-19. J Clin Med Res. 2020;12(7):448–453. doi:10.14740/jocmr4240
12. Klassen SA, Senefeld JW, Johnson PW, et al. The Effect of Convalescent Plasma Therapy on Mortality Among Patients With COVID-19: Systematic Review and Meta-analysis. Mayo Clin Proc. 2021 May;96(5):1262–1275. doi:10.1016/j.mayocp.2021.02.008
13. Ibrahim D, Dulipsingh L, Zapatka L, et al. Factors associated with good patient outcomes following convalescent plasma in COVID-19: a prospective phase II clinical trial. Infect Dis Ther. 2020 Dec;9:913–926. doi:10.1007/s40121-020-00341-2
14. Shenoy AG, Hettinger AZ, Fernandez SJ, Blumenthal J, Baez V. Early mortality benefit with COVID-19 convalescent plasma: a matched control study. Br J Haematol. 2021;192(4):706–713. doi:10.1111/bjh.17272
15. Agarwal A, Mukherjee A, Kumar G, Chatterjee P, Bhatnagar T, Malhotra P. Convalescent plasma in the management of moderate COVID-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID trial). BMJ. 2020 Oct 22;371:30566-3. doi:10.1136/bmj.m30566. Erratum in: BMJ. 2020 Nov 6;371:m4232. PMID: 33093056; PMCID: PMC7578662.
16. Omrani AS, Zaqout A, Baiou A, et al. Convalescent plasma for the treatment of patients with severe coronavirus disease 2019: a preliminary report. J Med Virol. 2021;93(4):706–713. doi:10.1002/jmv.26537
17. Rogers R, Shehadeh F, Mlyona EK, et al. Convalescent plasma for patients with severe COVID-19: a matched cohort study. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2021 Jul 1;73(1):e208–e214. doi:10.1093/cid/ciaa1548.
18. Abolghasemi H, Esghi P, Cheraghali AM, et al. Clinical efficacy of convalescent plasma for treatment of COVID-19 infections: results of a multicenter clinical study. Transfus Apher Sci Off J World Apher Assoc Off J Eur Soc Haemapheresis. 2020;59(5):102875. doi:10.1016/j.transci.2020.102875
19. Li L, Zhang W, Hu Y, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial. JAMA. 2020;324(5):460–470. doi:10.1001/jama.2020.10044
20. Joyner MJ, Wright RS, Fairweather D, et al. Early safety indicators of COVID-19 convalescent plasma in 5000 patients. J Clin Invest. 2020;130(9):4791–4797. doi:10.1172/JCI140200
21. Alsharidah S, Ayed M, Ameen RM, et al. COVID-19 convalescent plasma treatment of moderate and severe cases of SARS-CoV-2 infection: a multicenter interventional study. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis*. 2021 Feb;103:439–446. doi:10.1016/j.ijid.2020.11.198

22. Sahu KK, Mishra AK, Martin K, Chastain I. COVID-19 and restrictive lung disease: a deadly combo to trip off the fine balance. *Monaldi Arch Chest Dis = Arch Monaldi per le Mal del Torace*. 2020 Jun 29;90(2):395–397. doi:10.4081/monaldi.2020.1346

23. Kemp SA, Collier DA, Datir R, et al. Neutralising antibodies in spike mediated SARS-CoV-2 adaptation. *medRxiv [Preprint]*. 2020 Dec 29:2020.12.05.20241927. doi:10.1101/2020.12.05.20241927