Post-trial follow-up after a randomized clinical trial of COVID-19 convalescent plasma [version 1; peer review: 2 approved with reservations]

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Abstract

Background: COVID-19 convalescent plasma (CP) proved to be a safe acute intervention, however, the long-term clinical effects of COVID-19 CP are to date unknown. CP might have a prospective negative effect by down-regulating the inflammatory response suppressing antibody formation and promoting autoantibodies against interferons. Our objective was to establish the long-term safety profile of COVID-19 CP and determine if its administration increases the risk for further respiratory infections in older adults.

Methods: All participants included in the intention to treat analysis of a randomized clinical trial evaluating the efficacy of COVID-19 CP in older adults were invited to participate in this post-trial follow-up study. Patients were strictly followed for at least 6 months after randomization. The primary endpoint was the number of patients with clinically confirmed acute respiratory infections (ARIs). Secondary endpoints included all-cause mortality, time to first respiratory infection, SARS-CoV-2 re-infection, adverse events, and persistence of COVID-19 symptoms after initial infection.

Results: 142 patients were included in the study (total retention rate=92.8%). The mean age was 77.2 years (SD=8.6) and the median duration of follow-up was 10.4 months (IQR=1.63), with no differences among groups. 20 patients had a clinically confirmed ARI during the study. No differences were observed between groups in the proportion of ARIs (CP=11/72 and Placebo=9/70, p-value=0.678) and in the probability of ARI-free survival between groups (log-rank test p-value=0.63). No differences emerged when comparing groups regarding secondary endpoints.
Conclusions:
COVID-19 convalescent plasma remains a safe intervention without increasing the risk of acute respiratory infection or other clinical consequences in the long term.

Keywords
convalescent plasma, COVID-19, post-trial, follow-up, safety, elderly.

This article is included in the Coronavirus (COVID-19) collection.
**Introduction**

COVID-19 convalescent plasma (CP) is one of the most studied pharmacological strategies during the pandemic\(^1\). In a recent double-blind, randomized, placebo-controlled trial we showed that early administration of high-titer CP against SARS-CoV-2 to mildly ill infected older adults was a safe therapeutic intervention and reduced the progression to COVID-19 severe respiratory disease (relative risk = 0.52; 95% confidence interval 0.29 to 0.94; p-value = 0.03) and a relative risk reduction of 48%\(^2\).

The most widely accepted theory on how CP acts in SARS-CoV-2 infection is by neutralizing antibodies inhibiting viral entry and amplification, mediating viral clearance, activating complement, enhancing the dependent cellular phagocytosis, and cytotoxicity, and providing anti-inflammatory cytokines\(^3\).

However, CP may also have a long-term negative effect by down-regulating the inflammatory response (a decrease in activated, effector, and memory CD4\(^+\) T cells, a decrease in activated and effector CD8\(^+\) T cells, and a decrease of naïve B cells)\(^3\), suppressing antibody formation\(^4\) and promoting autoantibodies against interferons\(^5\).

COVID-19 CP proved to be a safe intervention with <1% severe adverse events immediately after its administration\(^1\). Nevertheless, the long-term clinical effects of COVID-19 CP are to date understudied.

Our objective was to establish the long-term safety profile of COVID-19 CP and determine if its administration increases the risk for further respiratory infections in older adults.

**Methods**

**Study population**

The study by Libster et al. included 160 mildly symptomatic participants infected with SARS-CoV-2 who were 75 years of age or older, or between 65 and 74 years of age with at least one coexisting comorbidity (hypertension, diabetes, obesity, chronic renal failure, cardiovascular disease, and COPD)\(^4\). Participants were randomized 1:1 to receive 250 ml of convalescent plasma (with an IgG titer > 1:1000 against SARS-CoV-2 spike protein) or 250 ml of placebo (0.9% normal saline) in less than 72 hours since the onset of symptoms. Next, they were strictly followed for 15 days to evaluate the development of severe respiratory disease\(^4\).

All patients included in the intention to treat (ITT) analysis of the original RCT study\(^1\) were invited to participate in a prospective cohort to assess for clinical differences over time among the CP and the placebo groups.

**Ethics and consent**

This follow-up study was part of the original protocol and was approved by the institutional review boards of each participating institution and the state of Buenos Aires (Comité de Ética Central de la Provincia de Buenos Aires, reference number: 2919–2129–2020, approved 15/5/2020; Clínica y Maternidad Suizo Argentina – Comité de Ética en Investigación, reference number: 1912, approved 19/6/2020; Swiss Medical Group, Clínica Olivos, approved 18/6/2020; Sanatorio Finochietto, approved 10/6/2020; Centro Gallego de Buenos Aires, Comité de Ética en Investigación Clínica, reference number: 1651/55/2020, approved 18/6/2020; Sanatorio Sagrado Corazon-OSECAC, approved 19/6/2020).

All participants or their legal guardians signed an informed consent and provided verbal consent during follow-up.

**Study procedure**

At the end of their participation in the RCT, all participants were instructed to communicate with the research team and report any adverse event, the onset of new symptoms, any confirmed diagnosis, and/or any visit to the health system. In addition, previously trained physicians using pre-designed questionnaires (available in section Data availability) phone-called all participants to assess for the variables and outcomes of interest at 3 and 6 months after randomization, and conducted a final contact between 7–10 months for data quality assurance. Data collection occurred between June 2020 and June 2021 in the city of Buenos Aires and Buenos Aires State, Argentina.

The primary endpoint of the follow-up study was the number of patients with clinically confirmed acute respiratory infections (ARIs) defined by the diagnosis of at least one of the following: pneumonia, upper respiratory infection, bronchitis, pharyngitis, or acute otitis media.

Secondary endpoints included all-cause mortality, time to first respiratory infection, SARS-CoV-2 confirmed re-infection, adverse events probably related to investigational product, and persistence of COVID-19 symptoms after initial infection.

**Statistical analysis**

Differences between participants were compared using the Student t-test and Chi-squared test, where appropriate. A p-value <0.05 was considered statistically significant. Time to first respiratory infection was assessed using the Kaplan-Meier method, and the log-rank test to assess for differences among groups (CP and placebo). We conducted a subgroup analysis by modified intention to treat (mITT) as in the original study excluding those randomized to CP but did finally not receive it\(^1\), and by those receiving a higher SARS-CoV-2 S IgG titer in donor plasma (using 1:3200 as a cutoff point). Stata/SE 13 package for IBM-PC (Stata Corp) was used for analysis and figure creation.

**Results**

From the initial RCT that included a total of 160 patients in the ITT analysis (CP=80 and Placebo=80) and 154 in the mITT (CP=76 and Placebo=78), 153 were eligible to participate in the study (6 died during the original study and 1 withdrew consent). 142 patients were included in the follow-up study. 10 patients were lost to follow-up and 1 patient did not want to participate in the follow-up study, resulting in a total retention rate of 92.8% (CP=92.3% vs Placebo=93.3%) (Figure 1).
The mean age of included participants was 77.2 years (SD 8.6), and their median duration of follow-up was 10.4 months (IQR 1.63), with no differences among groups, with 10.3 months (IQR 1.68) and 10.42 months (IQR 1.6) for the CP and placebo groups respectively (t-value (df 140) =-0.35, p-value=0.724). No treatment switching was evidenced during the follow-up time. 89% of the participants (n=127) received a COVID-19 vaccine during the study with a median time to the first dose of 8.8 months (IQR 2.2).

14% of the participants (n=20) had a clinically confirmed ARI during the study. No differences were observed between groups (CP=11/72 and Placebo=9/70, x²=0.17, p-value=0.678). The median time to first ARI was 8.63 months (IQR 7.13) for the CP group and 9.2 months (IQR 7.53) for the placebo. No statistically significant differences were observed in the probability of ARI-free survival between groups (log-rank test p-value=0.63) (Figure 2).

CP and placebo patients did not present any adverse event related or probably related to the investigational products during this follow-up study. No differences emerged when comparing groups regarding total SARS-CoV-2 reinfections (CP=1/72 vs Placebo=1/70, x²=0.0004, p-value=0.984), all-cause mortality (CP=5/72 vs Placebo=2/70, x²=1.27, p-value=0.261), and in the number of patients with persistence of COVID-19 symptoms after the initial infection (CP=34/72 vs Placebo=30/70, x²=0.27, p-value=0.601).

There were no statistically significant differences in the number of participants with ARI when comparing within the mITT subgroup (CP=10/69 and Placebo=9/68, x²=0.045, p-value=0.831) or when comparing those who received SARS-CoV-2 S IgG titer in donor plasma ≥1:3200 vs. placebo (CP=3/34 and Placebo=9/70, x²=0.36, p-value=0.546). All secondary endpoints remained stable with no difference by treatment in the subgroup analyses (data not shown).

**Discussion**

In this post-trial follow-up study of an RCT of COVID-19 convalescent plasma in older adults, CP did not increase the risk for acute respiratory infections in the long-term follow-up. Furthermore, no differences were observed between groups (CP vs placebo) when assessing for time to first acute respiratory infection, adverse events, SARS-CoV-2 reinfections, all-cause mortality, and persistence of COVID-19 symptoms. In addition, all endpoints remained stable showing no difference in the subgroup analyses.

To our knowledge, this is the first post-trial follow-up after an RCT of COVID-19 CP assessing for long-term consequences of its administration. COVID-19 convalescent plasma remains a safe intervention with no clinical consequences in the long term.

A recent randomized clinical trial with control plasma by Sullivan *et al.* supports the hypothesis of our original study that...
COVID-19 CP should be administered early in the course of the disease and to those presenting mild symptoms\(^1\). In this study, COVID-19 CP presented a relative risk reduction of 54% in COVID-19 related hospitalization within 28 days after transfusion, which increased to 79.9% in those with ≤ 5 days of symptoms. However, no differences were found between groups when comparing patients already vaccinated\(^1\).

Our study adds evidence to the long-term safety of COVID-19 CP. This intervention might have now a lesser role as a strategy against SARS-CoV-2, however, our study supports an early and coordinated evaluation of convalescent plasma use in future pandemics.

**Data availability**

**Underlying data**

figshare: Post-trial follow-up after a randomized clinical trial of COVID-19 convalescent plasma - Stata Dataset. https://doi.org/10.6084/m9.figshare.20311152.v1\(^\text{12}\)

This project contains the raw data file.

**Extended data**

figshare: Post-trial follow-up after a randomized clinical trial of COVID-19 convalescent plasma - Stata do-file. https://doi.org/10.6084/m9.figshare.20311155\(^\text{13}\)

This project contains the Stata do-file.

figshare: Codebook. https://doi.org/10.6084/m9.figshare.20349534.v1\(^\text{14}\)

This project contains the database codebook.

figshare: Data collection instruments & questionnaires – English. https://doi.org/10.6084/m9.figshare.20402136.v1\(^\text{15}\)

This project contains the data collection instruments and questionnaires in English.

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**References**

Open Peer Review

Paula Caporal
Hospital Interzonal Especializado en Pediatría "Sor María Ludovica", La Plata, Argentina

This is a follow-up study from an RCT study comparing COVID-19 convalescent plasma (CP) vs. Placebo for mildly symptomatic COVID-19 with severe respiratory disease as the primary endpoint.

The study explores the long-term safety profile of COVID-19 convalescent plasma, comparing the number of patients with clinically confirmed acute respiratory infections (ARIs) during a ten-month follow-up.

CP was widely used as emergency treatment during the pandemic. Therefore, the Libster et al. (2021) study was crucial since it was one of the first to demonstrate the actual benefit of the CP in mild symptomatic COVID-19. However, there was a concern in the scientific community regarding the potential long-term harm (mainly decreasing the antibody and immune response during a second exposure to the agent). In this sense, Dr. Esteban et al.’s work is scientifically relevant and essential for thinking about CP in a future pandemic scenario.

That said, there are some points the authors should take into account:

1. The conclusions should only be made for the period of follow-up (7-10 months).

2. Intention to treat analysis is preferred when efficacy is the primary outcome. However, when the outcome is an adverse effect of a drug or treatment, researchers should seek to capture the drug’s actual effect and perform a per-protocol analysis. In this case, since it is a follow-up of a prior study, the researchers used the same methodology as the first study and conducted a subgroup analysis of what they called "modified intention to treat (mITT)." Suppose the mITT only excluded patients randomized to CP that finally received Placebo but did not exclude patients randomized to Placebo that finally received CP. In that case, this could alter the final result. Therefore, the authors should clarify how this analysis was performed and the reasons for conducting it.

3. A table 1 with the baseline characteristics should be added.
References

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

*Competing Interests*: No competing interests were disclosed.

*Reviewer Expertise*: Public Health, Epidemiology, Clinical Research, Pediatrics, Pediatric Critical Care

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 18 October 2022

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The present study compares the prognosis among survivors of COVID, in regards to the
administration of convalescent plasma. This was done to evaluate long-term negative consequences of this treatment, after a supposedly beneficial effect in the acute phase.

In any prognosis-improving treatments, many patients receive the intervention in order for a few to get the actual benefit. The few who get the benefit are the contrafactuals that would have the event, but had it prevented. The remaining patients paid the price for the benefit of others. So this study is pertinent in the intention to describe the long-term consequences beyond the acute phase, which is the price paid by those unknown patients. It is a relevant study.

However, the issue of safety has nuances that should be observed by the authors:

1. It is conceptually impossible to prove safety, because safety is the absence of harm, and we do not observe absence. So the authors should be more careful to conclude for safety, especially in such a small trial. “Remains safe in the long term” should be conservatively replaced by something like “secondary harm was not observed over an average of 10 months follow-up”.

2. When safety is the primary purpose of a study, a hypothesis test that places the null to the right of zero and uses a one-sided approach will be better in line with the scientific purpose. This is called a non-inferiority analysis, in which a certain increase of risk (based on relevance) would be rejected as the null hypothesis. So you are not proving risk zero, but only saying that risk is not beyond 0+x.

3. For safety, a per-protocol analysis should be the primary, what the authors called modified intention to treat. ITT is biased towards safety.

   It is not clear why the authors chose only one of the potential side effects as the primary outcome. It seems better to have the primary outcome as the composite of all relevant potential side effects.

4. There should be a Table 1 describing baseline characteristics since the remaining cohort is not exactly the same randomized (emigration by death - emigrative selection bias?).

5. Even if Table 1 does not show unbalance, a multivariate adjustment for main confounders by Cox Regression would be welcome.

6. Sensitivity analysis regarding loss of follow-up should be performed.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Public Health, Epidemiology, Cardiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.