Journal of Translational Autoimmunity 3 (2020) 100069

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.journals.elsevier.com/journal-of-translational-autoimmunity/

Why will it never be known if convalescent plasma is effective for COVID-19

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ARTICLE INFO

Keywords:
Coronavirus
COVID-19
SARS-Cov-2
Convalescent plasma
Clinical trials
Randomized

ABSTRACT

High expectations have been set around convalescent plasma (CP) for the treatment of COVID-19. However, none of the randomized controlled trials (RCTs) conducted so far have reached their primary endpoints. Herein we report that RCTs of CP disclose a high methodological variability in inclusion criteria, outcomes, appropriate selection of donors, dosage, concentration of neutralizing antibodies and times of transfusion. Therefore, at this time there is insufficient evidence to recommend for or against the use of CP as a treatment for COVID-19.

1. Introduction

The current pandemic has challenged health systems given the uncontrolled spread and high mortality in critically ill patients with COVID-19. During the last months, several clinical trials have been conducted to discover new treatments that may reduce the burden of the disease. However, none of these studies have reached the expected primary endpoint. Only systemic corticosteroids have been associated with lower 28-day all-cause mortality [1].

Convalescent plasma (CP) emerged as potential treatment for COVID-19 at the beginning of the pandemic. This is a strategy of passive immunization that has been used in prevention and management of infectious diseases since early 20th century [2]. The CP is obtained using apheresis in survivors with prior infections caused by pathogens of interest and that developed antibodies against the causal agent of disease. The major target is to neutralize the pathogen for its eradication [3]. Given its rapid obtaining, CP has been considered as an emergency intervention in several pandemics, including the Spanish flu, SARS-CoV, West Nile virus, and more recently, Ebola virus [4–9].

Several studies have shown the potential efficacy of CP in COVID-19. A recent meta-analysis of randomized and matched-control studies, showed a reduction of 57% of mortality in COVID-19 patients treated with CP [10]. However, some concerns arise around this analysis. First, quality of evidence across the studies was not evaluated, and pooled estimation of effect came from the mixture of randomized and case-control studies. This approach may produce biased estimations of the effects and may not reflect the true efficacy of CP in COVID-19.

In addition, high methodological heterogeneity has been observed in most of the studies on CP. Inclusion criteria, outcomes, dosage, and concentration of neutralizing antibodies (NAbs), are some factors that may influence the efficacy of this therapy and hinder the pooling of evidence in this topic. Herein, we analyzed randomized controlled trials (RCTs), their risk of bias, comparability and the potential confounding factors that may disturb conclusions on this treatment.

2. Methods

A systematic review of the literature about RCTs in COVID-19 was done following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [11]. MEDLINE, EMBASE, and LILACS were systematically searched for published and unpublished studies. Additional manual searches of the references cited in the articles were done. The search included articles up to October 21, 2020. No restrictions were placed on study period or sample size. Only those articles written in Spanish or English were considered. Other information sources such as personal communications, author's repositories and preprint servers were included. Title and CP text terms in combination with "COVID-19", and "Randomized" were used.

Risk of bias analysis included the following: random sequence generation, allocation concealment, blinding participants and personnel, blinding of outcome assessment, selective reporting and incomplete outcome data. Risk of bias was conducted for mortality and clinical improvement outcomes. Furthermore, the Cochrane GRADE approach was used to assess quality of evidence [12]. Studies were graded from

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https://doi.org/10.1016/j.jtauto.2020.100069
Received 22 October 2020; Accepted 2 November 2020

Table 1
General characteristics of randomized controlled trials in convalescent plasma COVID-19.

Study	Number of patients	Severity of disease intervention group[a]	Severity of disease control group[a]	Dosage	Antibodies concentration	Primary outcomes	Secondary outcomes	Standard of care	Results on selected endpoints	Risk of Bias[b]
Li et al. [13]	103	Total 52 14 in WHO-7 21 in WHO-5 15 in WHO-4 2 in WHO-3	Total 51 11 in WHO-7 23 in WHO-5 15 in WHO-4 1 in WHO-3 1 excluded	4–13 mL/kg Unknown schedule of administration	Unknow exactly concentration of NAbs. Authors argued for an approximately concentration of 1/40. IgG ≥1/640 (IgG ELISA assay in-house)	Time to clinical improvement within 28 days (discharged alive) or reduction of 2 points on WHO-6-point scale	28-day mortality, time to hospital discharge and clearance of viral PCR results within 72 h	Antivirals, antibiotics, steroids, human immunoglobulin, Chinese herbal medicines, or interferon	Clinical improvement: HR 1.40 (95% CI, 0.79–2.49; P = 0.26) Mortality: OR 0.65 (95% CI, 0.29–1.46; P = 0.30) Viral load: OR 11.39 (95% CI, 3.91–33.18; P < 0.001)	Unclear
Gharbharan et al. [14]	86	Total 43 7 in WHO-3 31 in WHO-4 or 5 8 in WHO-6 or 7	Total 43 1 in WHO-3 34 in WHO-4 or 5 5 in WHO-6 or 7	300 mL single dose. Subjects without improvement of clinical status or persistently positive RT-PCR for SARS-CoV-2 receive an additional dose in five days	Unknow exactly concentration of NAbs. Authors argued for an approximately concentration of 1/80. IgG ≥1/640 (Wantai Biological ®)	60-day mortality	Time to hospital discharge and improvement in 2 points on WHO-8-point scale	Chloroquine, Azithromycin, Lopinavir/Ritonavir, Tocilizumab, or Anakinra	Clinical improvement: OR 1.30 (95% CI, 0.52–3.32) Mortality: OR 0.95 (95% CI, 0.20–4.67, P = 0.95) Time to discharge: HR 0.88 (95% CI, 0.49–1.60, P = 0.68)	Unclear
Balcells et al. [15]	58	Total 21 CALL score > 9	Total 24 CALL score > 9	200 mL in two doses separated by 24 h	Nabs 1/160 IgG titers ≥ 1/400 (ELISA Euroimmun®)	Requirement of mechanical ventilation, hospitalization for >14 days and death	30-day mortality, requirement of MCV, days of MCV, total days of HFNC requirement, total days oxygen requirement, total days of intensive and/or intermediate care requirement, Total days of hospital stay, and SOFA score at days 3 and 7	Steroids, Tocilizumab, Hydroxychloroquine, Lopinavir/Ritonavir, Thromboprophylaxis, or Heparin	Clinical improvement: OR 0.51 (95% CI, 0.13–2.05, P = 0.55) Mortality: OR 3.04 (95% CI, 0.54–17.2, P = 0.25) Mechanical ventilation: OR 2.98 (95% CI, 0.41–21.57, P = 0.25)	High
Avendaño-Solà et al. [16]	81	Total 38 10 in WHO-3 28 in WHO-4	Total 43 13 in WHO-3 30 in WHO-4	250–300 mL single dose	NAbs ≥ 1/109 IgG ratio ≥ 1.1 (ELISA Euroimmun®) unknown titer of antibodies.	Proportion of patients in categories 5, 6 or 7 in the WHO-7 points scale at day 15	Mortality at days 15 and 29, Improvement in one point in the WHO scale, Duration in hospital stay, days without oxygen requirement, and days without MCV requirement	Hydroxychloroquine, Lopinavir/Ritonavir; Azithromycin, Remdesivir, Steroids, Tocilizumab, or Heparin.	Clinical improvement: P = 0.55. Mortality: P = 0.06	High
Agarwal et al. [17]	464	Total 235	Total 229	200 mL in two doses separated by 24 h	NAbs ≥ 1/20	Progression to severe disease and mortality at day 28	Clinical improvement at day 7, change in Fio2%, days on MCV, clearance of viral PCR results at day 3 and 7, improvement in WHO ordinal	Hydroxychloroquine, Remdesivir, Lopinavir/Ritonavir, Oseltamivir, broad spectrum antibiotics, steroids, Tocilizumab, Heparin,	Progression to severe disease: OR: 1.09 (95% CI, 0.67–1.77) Mortality:	High

(*continued on next page*)

Table 1 (*continued*)

Study	Number of patients	Severity of disease intervention group[a]	Severity of disease control group[a]	Dosage	Antibodies concentration	Primary outcomes	Secondary outcomes	Standard of care	Results on selected endpoints	Risk of Bias[b]
							scale and requirement of vasopressor support	Azithromycin, or Intravenous immunoglobulin.	OR 1.06 (95% CI, 0.61–1.83)	

[a] WHO severity scale ranging from 1 (discharge) to 8 (death).

[b] Overall bias analysis for main outcomes (Mortality and clinical improvement). NAbs: Neutralizing antibodies; WHO: World Health Organization; RT-PCR: reverse transcription polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; MCV: Mechanical ventilation; HFNC: high flow nasal canula; OR: odds ratio; HR: hazard ratio.; ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G.

very low quality to high quality in a 4-tiered system. All studies for this review were RCTs and started the evaluation as high quality of evidence. A narrative summary of evidence was performed.

3. Participants, treatment and outcomes

Five articles were obtained from the systematic review [13–17]. General characteristics of studies are shown in Table 1. Clinical features varied across studies and represented different stages of disease. Three of 5 five studies included critically ill patients, as well as non-severe COVID-19 cases [13,14,16]. In addition, two studies did not mention severity of disease and just reported a positive RT-PCR SARS-CoV-2 test [15,17]. This represents the first aspect to discuss in the comparability and reproducibility of RCTs.

Although the pathogenesis of COVID-19 is still not completely understood, four overlapping and escalating phases have been proposed to explain the clinical course of the disease [18]. First, there is a viral phase that may well be asymptomatic or mild in the majority, perhaps 80% of patients. In the remaining 20% of cases, the disease may become severe and/or critical. In most patients of this latter group, a hyper-responsiveness of the immune system is characteristic. A third phase corresponds to a state of hypercoagulability. Finally, in the fourth stage organ injury and failure occur [18].

Severity of disease at inclusion is pivotal and may influence outcomes in unknown ways. At this respect, all RCTs have included patients at different stages of disease (Table 1). For example, 24.3% of patients in the study of Li et al. [13] were on mechanical ventilation (MCV) at the moment of the inclusion. Remaining subjects were on high-flow, low--flow or without requirement of oxygen. Since CP may help patients with non-severe disease [9], selection of patients by severity of disease is critical. Fusion of all these groups into one may not reflect the real effect of CP. In addition, sample size estimation may require an adjustment by this confounder. Thus, further RCTs should aim to estimate the efficacy of CP in every stage of disease with an appropriate sample size.

Little is known about the accurate plasma concentration of NAbs required to produce a significant clinical effect, as well as timing to transfusion. The US food and drug administration (FDA) recommended a minimal titer of NAbs of 1/160 to treat COVID-19 patients [19]. NAbs concentration varied across RCTs, and in some cases, NAbs were below the minimal cut-off proposed by the FDA (Table 1). The first completed RCT conducted in India by Agarwal et al. [17], CP with low NAbs concentration (i.e., 1/20) was transfused to some patients, whereas others received CP containing a higher concentration of antibodies. From an experimental perspective, variability in NAbs load may influence the results in a dose-response manner. It is unknown whether higher concentrations of NAbs may influence better clinical responses. RCTs including multiple dosage regimens are required to standardize CP therapy.

A recent case-control study showed that transfusion in the first 72 h of hospital admission may influence survival [20]. However, RCTs conducted so far have not included this variable in their analyses. We should recall that early hospital transfusion is different from transfusion at early stages of disease. Thus, this should call the attention on better methodological designs that include timing in transfusion and stratification by severity of disease.

Primary and secondary outcomes are different among RCTs. In addition, sample size estimation varies according to endpoints and some studies did meet the expected recruitment. Is it equivalent a study designed to estimate reduction in mortality to those intended to estimate the effect of CP on early hospital discharge? These estimates are completely different and pooling evidence from different designs may derive in biased conclusions about the real effect of CP in COVID-19. As shown in Table 1, most studies focus on mortality and early discharge. However, variability in inclusion criteria, and the clinical stage of disease, make studies not equivalent. Other outcomes such as intensive care unit (ICU) admission, and number of patients requiring orotracheal

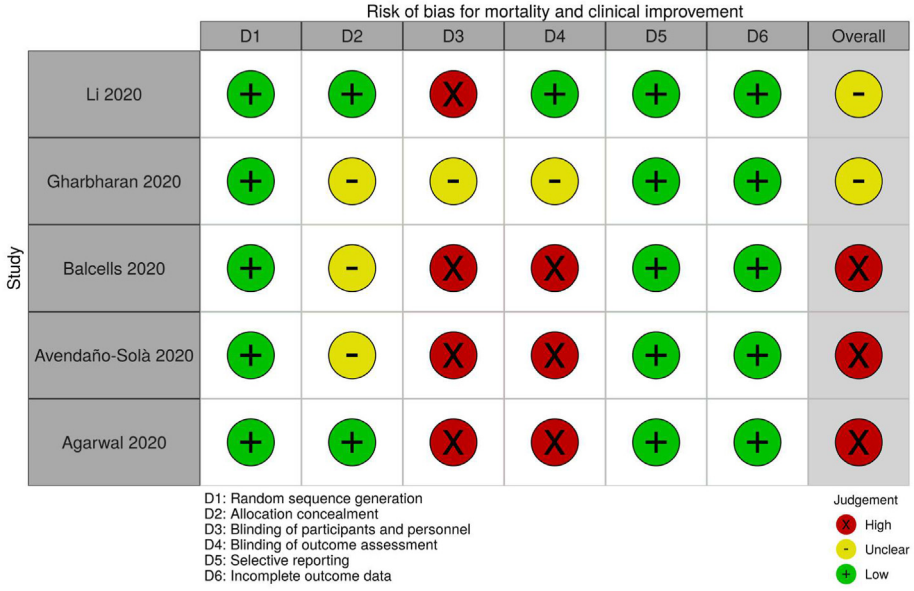

Fig. 1. Convalescent Plasma for COVID-19. Risk of bias in randomized controlled trials.

intubation haven been poorly studied. Thus, these outcomes should be considered given the current public health emergency and the high rate of ICU admission in this condition.

Risk of bias analysis revealed that none of the RCTs included in this review was on low risk of bias (Table 1). Lack of blinding of participants and personnel, and blinding of outcome assessment were the most common bias in these studies. In addition, there were not information about allocation concealment in 3 out of 5. These observations influence the decision to rate all the studies in either high or unclear risk of bias for mortality and clinical improvement outcomes (Fig. 1). This is of critical relevance since selection and measurement bias could have influenced the results of these trials. In addition, the current analysis allowed us to downgrade the quality of evidence in at least 2 points indicating that the evidence on CP is of low quality according to GRADE [12]. Double blinded RCTs are required to provide better evidence for the use of CP in COVID-19.

4. Additional potential cofounding factors

About 10% of critically ill patients with COVID-19 present antibodies against type I IFN [21]. In addition, other antibodies against other cytokines may also be found [21]. This is of critical relevance in production of CP, since some donors, especially those recovered from critical disease, may have this type of antibodies with unpredictable effects on CP receptors. In addition, it is unknown whether some CP contains pro-inflammatory cytokines that could exacerbate the disease. Thus, besides the measurement of NAbs, standards for cytokine concentration and cytokine autoantibodies are recommended.

Recent evidence has emerged on the evolutionary processes associated with adaptation of SARS-CoV-2 to humans. Two major strains of SARS-CoV-2 were described in Wuhan (i.e., strain "L" and "S") [22]. It has been suggested that S strain could be considered more aggressive. In the same line, two studies have shown that the non-synonymous mutation D614G in the spike gene is associated with an increased infectivity of SARS-CoV-2 [23,24]. Evolutionary models have suggested that the novel coronavirus could change infectivity and mortality over time influenced by lockdowns and other unpredictable evolutionary factors [25]. Altogether, these data indicate that efficacy of CP could be influenced by the evolutionary change of SARS-CoV-2 over time and argue for the inclusion of mutation analysis in epidemiological surveillance.

In the early 1950s, purification and concentration of immunoglobulins from healthy donors or recovered patients (i.e., intravenous immunoglobulins - IVIg), provided an option to treat serious infectious diseases as well as immune conditions including primary immunodeficiencies, allergies, and autoimmune diseases [9]. Recently an observational study on IVIg in COVID-19 showed that this treatment may improve hypoxia, hospital length and reduce progression to mechanical ventilation [26]. Since different concentrations of NAbs were found in this review (Table 1). Production of IVIg from recovered COVID-19 donors may provide an option to standardize doses and concentration of NAbs transfused.

5. Conclusions

Currently, RCTs on CP for COVID-19 are not comparable and exhibit a high risk of biased results. Inclusion criteria, severity of disease, NAbs concentration, blinding of personnel, participant and outcome assessment are some concerns that require attention in order to provide high quality evidence on this therapeutic option. In addition, other potentially confounding factors such as antibodies against IFN or deleterious inflammatory cytokines contained in CP require attention in the personalized selection of CP. Changes in SARS-CoV-2 lineages over time may also hinder a conclusion about the efficacy of CP in the current RCTs. There is no answer to the question about CP effectiveness in the current pandemic. The expected reduction in mortality secondary to viral fitness together with the "do less get more" approach may reduce the necessity of this therapy in the immediate future.

Availability of data and materials

Not applicable.

Funding

This work was supported by Universidad del Rosario (ABN-011), Bogota, Colombia.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Note added on proof

Manuscript by AlQahtani et al [27], published on Nov 4, reported an RCT in which 40 patients were studied. There was no difference in primary nor secondary outcomes at day 28 between CP and standard therapy groups. There was a high risk of bias due to lack of blinding, and low statistical power due to sample size.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgements

The authors thank all the members of the CREA for contributions and fruitful discussions.

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