Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Journal of Infectious Diseases 102 (2021) 244–246

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Perspective

The art of the possible in approaching efficacy trials for COVID19 convalescent plasma

Daniele Focosi[a,*], Albert Farrugia[b]

[a] North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy
[b] Faculty of Medicine and Medical Sciences, The University of Western Australia, Perth, Australia

ARTICLE INFO

Article history:
Received 2 October 2020
Received in revised form 20 October 2020
Accepted 23 October 2020

Keywords:
COVID19
Convalescent plasma
Randomized clinical trial

ABSTRACT

COVID-19 convalescent plasma (CCP) is widely used as a treatment. Although there are sufficient safety data, high-level evidence of efficacy is still lacking. We summarize here the results from randomized controlled trials (RCTs) published to date and analyze their flaws and biases. We then provide suggestions for the next round of CCP RCTs, discussing specification of CCP, therapeutic dose, timing, control arm, disease stage, and outcome measures.

Large observational studies, expanded access programs (EAP), and emergency use authorizations (FDA, 2020a, b) involving more than 100,000 recipients to date have conferred a high level of confidence in the safety of COVID-19 convalescent plasma (CCP) (Joyner et al., 2020a, b). The efficacy of CCP is currently based on three main lines of evidence:

1 A 1979 randomized control trial (RCT) demonstrating efficacy of CCP in 188 patients with Argentine hemorrhagic fever, where a case fatality rate of 16.5% was observed in patients treated with normal plasma versus 1.1% in those treated with immune plasma (Maiztegui et al., 1979);
2 Efficacy data from animal models (Imai et al., 2020; Sun et al., 2020); and
3 Results of case series, the largest of which is, without doubt, the US EAP: among patients younger than 80 who were treated within 72 h of diagnosis, there was reduced 7-day mortality correlated with higher neutralizing antibody (nAb) titers (Joyner et al., 2020a, b).

Nevertheless, to date there is no definitive proof of efficacy for any passive (polyclonal or monoclonal) antibody treatment against any respiratory pathogen (Mair-Jenkins et al., 2015; Subbarao et al., 2020). The yardstick for the assessment of therapeutic efficacy is the RCT, and in the setting of COVID-19 the five RCTs of CCP reported to date have produced inconclusive or negative results (Table 1), potentially due to lack of nAb titer assessment in donated units and/or late treatment. More encouraging findings have been reported from retrospective (Liu et al., 2020) or prospective (Salazar et al., 2020a, b) propensity-score-matched studies run in the US (again relying on CCP units not assessed for nAb titer), but the strength of evidence from this type of studies is lower than that from RCTs.

This body of data, however, has provided some useful pointers that are now used in the second round of RCTs, e.g., C3PO (NCT04355767), CONTAIN COVID-19 (NCT04364737), and PassI-tOnII (NCT04362176) in USA, TSUNAMI in Italy (NCT04393727), and RECOVERY in UK (NCT04381936).

We propose here a focus on the following parameters to further improve the design of the next round of RCTs, potentially leading to a more rigorous assessment of efficacy:

1 *Specification of the CCP.* Since a nAb dose–response relationship has been proven for CCP (Joyner et al., 2020a), the amount of the putative active entity (the nAb) requires quantification and should be available at a level that, as informed by data from the previous observations and RCTs, is thought sufficient for a therapeutic effect. In SARS, 5 mL/kg of plasma at a titer ≥1:160 was utilized (Cheng et al., 2005). Such titer needs to be specified through the viral neutralization assay (VNT), thus obviating any bias from the use of diverse surrogate tests having poor correlation with the VNT (Focosi et al., 2020a, b, c). Additionally, given the fast-declining kinetics of nAb titers, the VNT should be assessed on each donated unit in the case of repeated donations, rather than assuming the same value across multiple donations.
2 *Standardization of the therapeutic dose.* Currently published studies indicate a substantial variation in the dose administered, with many RCTs actually having fixed CCP volumes

* Corresponding author at: via Paradisa 2, 56124 Pisa, Italy.
E-mail address: daniele.focosi@gmail.com (D. Focosi).

https://doi.org/10.1016/j.ijid.2020.10.074

Table 1
Randomized controlled trials (RCTs) of COVID-19 convalescent plasma (CCP) reported to date. DOS, duration of symptoms; nAb, neutralizing antibodies.

RCT identifier	Country	Recruitment (out of expected)	Median DOS (days)	Median nAb in CCP units (in recipients)	Outcome	Ref
ChiCTR2000029757	China	103 (out of 200)	30	Not assessed	Benefit at day 28 only in noncritical patients	Li et al. (2020)
NCT04342182 (ConCOVID)	Netherlands	86 (out of 426)	30	1:160 (same as in recipients)	No benefit at day 15	Gharbharan et al. (2020)
CTRI/2020/04/024775 (PLACID)	India	464	6	1:40 (1:90 in recipients)	No benefit at day 28	Agarwal et al. (2020)
NCT04345523 (ConPlas-19)	Spain	81 (out of 278)	8	1:292	Reduction in mechanical ventilation and mortality at day 30	Avendano-Sola et al. (2020)
NCT04375098	Chile	58	<7	≥1:160	No benefit at day 30	Balcells et al. (2020)
IRCT20200325046860N1	Iran	189	<7	Not assessed	Shorter hospitalization, less need for mechanical ventilation	Abolghasemi et al. (2020)

independently of nAb titer or recipient body weight. Specifying the CCP with nAb titer should lead to the amount of nAb being adjusted for body viral load. Because the latter is difficult to estimate on the basis of viral loads in nasopharyngeal swabs, and viremia mostly occurs in severe patients only, body weight is the usual surrogate for body viral load. Based on largely arbitrary assumptions, Bloch et al. (2020) estimated that 250 ml of CCP with a titer ≥1:160 could be successful at neutralizing a viral infection for an average 80-kg adult. More precise mathematical models (as formerly developed for HCV (Neumann et al., 1998)) to estimate viral replication and nAb bioavailability in tissues where virus and host interact are necessary, eventually moving from stoichiometry between nAb titer and viral load in the *in vitro* setting of the VNT.

3 *Timing.* Whenever multiple doses are needed, most protocols suggest transfusion of 200–300-ml units 12–24 h apart. Whatever the actual therapeutic dose will be, the rationale for this timing is largely unknown given the small cumulative volume and hence the low risk for transfusion-associated circulatory overload (Joyner et al., 2020a, b). In addition to posing logistical hurdles for thawed plasma in terms of storage and regulatory approval, differences in timing are likely to affect the pharmacokinetics of nAb.

4 *Control arm.* This is currently largely based on no addition to standard of care or addition of normal saline (which cannot be double-blinded). We currently assume that the therapeutic benefit of CCP stems solely from the content of nAb to SARS-CoV-2. Although this may be a reasonable posture for purified hyperimmune immunoglobulin (IgG), unfractionated CCP contains many additional ingredients that together provide antiviral natural (e.g., isohemagglutinins (Focosi, 2020) or cross-reacting antibodies from previous respiratory betacoronavirus infections (Díez et al., 2020)) or anti-thrombotic activity (e.g., antithrombin-III (Gazzaruso et al., 2020)). Conversely, other components may provide pathologic effects (e.g., coagulation factors may exacerbate COVID-19-associated coagulopathy (Iba et al., 2020)). Hence the control should ideally consist of similarly processed plasma for transfusion from COVID-19-nonconvalescent donors, in similar volumes and with equivalent levels of possible biologically active proteins. This option would exclude using pharmaceutical-grade plasma as a control, which, although more standardized, differs from fresh frozen apheresis plasma in terms of clotting factor levels.

5 *Disease stage at the time of treatment.* This should be assessed using the WHO ordinal scale. The data available suggest that late stage disease (ICU admission/ventilator support) is unlikely to respond to CCP therapy (Agarwal et al., 2020), with several authors suggesting an optimal window as short as 44 h post-hospitalization for transfusing CCP (Salazar et al., 2020a, b). It is a well-known phenomenon that nAb titer correlates with the severity of symptoms in COVID-19 patients (Focosi et al., 2020a, b, c) and preliminary reports suggested that CCP was only effective in early stage disease (Focosi et al., 2020a, b, c), thus trials targeting late treatment in severe patients are unlikely to produce useful insights. Limiting RCTs to early stage disease, despite the limitations imposed on the modality, should allow a higher probability of success.

6 *Outcome.* The primary endpoint for any RCT should be the WHO ordinal scale for clinical improvement (WHO, 2020), supplemented by secondary end points such as virological clearance of nasopharyngeal or respiratory samples, blood, urine, or stool; admission to critical care unit; need for supplemental oxygen, mechanical ventilation/oxygenation, or ECLS; need for intravenous vasoactive medications; need for renal replacement therapy; death in critical care unit, death in hospital, and vital status (death) at 28 days; hospital-free days; ICU-free; and biological and immunological markers of illness. Sample power estimation should consider comorbidities as relevant stratifiers to be accounted for.

Other variables may be investigated, including antibody isotyping and IgG subclasses (Focosi et al., 2020a, b, c), but the abovementioned six points deserve major attention. Pathogen reduction technologies cause a decline of nAb titers which varies according to the platform used (Kostin et al., 2020) and can be missed if using surrogate tests (Tonn et al., 2020). In contrast, type of collection (apheresis vs recovered plasma) or storage temperature (Stadlbauer et al., 2020) do not affect nAb content.

Finalizing the intended recruitment of a given RCT is also a concern for a pandemic progressing with unpredictable trajectory. An alternative to localized recruitment is prospective, real-time pooling of worldwide data from individual RCTs of CCP in COVID-19 under a shared regulatory and statistical framework, which has been recently set up (http://nyulmc.org/compile) (Petkova et al., 2020). A similar initiative could stem from continental registries such as the European Union CCP Platform (https://www.euccp.dataplatform.tech.ec.europa.eu/).

Therapists may need to recognize the limitations posed by possible resistance from treaters and patients to the implications of randomization (Ledford, 2020) and accordingly tailor their expectations and designs. We must accept that the aim of treatment, particularly in the current phase of limited therapeutic options for COVID-19 disease, should be amelioration of patients' condition. If this can be achieved within the full framework of evidence-based medicine, this is preferable.

Conflict of interest

D.F. declares having no conflict of interest related to this manuscript. A.F. is currently providing services to companies developing therapies for COVID-19.

Funding

There was no funding source for this manuscript.

Ethical approval

This manuscript does not require ethical approval.

References

Abolghasemi H, Eshghi P, Cheraghali AM, Imani Fooladi AA, Bolouki Moghaddam F, Imanizadeh S, et al. Clinical efficacy of convalescent plasma for treatment of COVID-19 infections: results of a multicenter clinical study. Transfus Apher Sci 2020;102875, doi:http://dx.doi.org/10.1016/j.transci.2020.102875 [Epub ahead of print].

Agarwal A, Mukherjee A, Kumar G, Chatterjee P, Bhatnagar T, Malhotra P, PLACID Trial Collaborators. Convalescent plasma in the management of moderate COVID-19 in India: an open-label parallel-arm phase II multicentre randomized controlled trial (PLACID Trial). BMJ 2020;371:m3939.

Avendano-Sola C, Ramos-Martinez A, Munez-Rubio E, Ruiz-Antoran B, Malo de Molina R, Torres F, et al. Convalescent plasma for COVID-19: a multicenter, randomized clinical trial. medRxiv 2020; 2020.2008.2026.20182444.

Balcells ME, Rojas L, Le Corre N, Martínez-Valdebenito C, Ceballos ME, Ferrés M, et al. Early anti-SARS-CoV-2 convalescent plasma in patients admitted for COVID-19: a randomized phase II clinical trial. medRxiv 2020; 2020.2009.2017.20196212.

Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. J Clin Investig 2020;130(6):2757–65.

Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. Eur J Clin Microbiol Infect Dis 2005;24(1):44–6.

Díez J-M, Romero C, Gajardo R. Currently available intravenous immunoglobulin (Gamunex©-C and Flebogamma© DIF) contains antibodies reacting against SARS-CoV-2 antigens. bioRxiv 2020; 2020.2004.2007.029017.

FDA. Updated evidence to support the emergency use of COVID-19 convalescent plasma — as of 9/23/2020. Retrieved 26 September 2020, from. 2020. https://www.fda.gov/media/142386/download.

FDA. Decisional memo. Retrieved 6 September 2020, from. 2020. www.fda.gov/media/141480/download.

Focosi D. Anti-A Isohemagglutinin titers and SARS-CoV2 neutralization: implications for children and convalescent plasma selection. Br J Haematol 2020;190:e148–50.

Focosi D, Anderson AO, Tang JW, Tuccori M. Convalescent plasma therapy for COVID-19: state of the art. Clin Microbiol Rev 2020a;33(4), doi:http://dx.doi.org/10.1128/CMR.00072-20 [Epub ahead of print].

Focosi D, Mazzetti P, Pistello MM. Viral infection neutralization tests: a focus on SARS-CoV-2 with implications for convalescent plasma therapy. Rev Med Virol 2020b;, doi:http://dx.doi.org/10.1002/rmv.2170 [Epub ahead of print].

Focosi D, Tuccori M, Antonelli G, Maggi F. What is the optimal usage of Covid-19 convalescent plasma donations?. Clin Microb Infect 2020c;, doi:http://dx.doi.org/10.1016/j.cmi.2020.09.036 [Epub ahead of print].

Gazzaruso C, Valenti C, Coppola A, Gallotti P. Impact of convalescent and nonimmune plasma on mortality of patients with COVID-19. A potential role for antithrombin. Clin Microb Infect 2020;, doi:http://dx.doi.org/10.1016/j.cmi.2020.09.007 [Epub ahead of print].

Gharbharan A, Jordans CCE, GeurtsvanKessel C, den Hollander JG, Karim F, Mollema FPN, et al. Convalescent plasma for COVID-19. A randomized clinical trial. medRxiv 2020; 2020.2007.2001.20139857.

Iba T, Levy JH, Levi M, Connors JM, Thachil J. Coagulopathy of coronavirus disease 2019. Crit Care Med 2020;48(9):1358–64.

Imai M, Iwatsuki-Horimoto K, Hatta M, Loeber S, Halfmann PJ, Nakajima N, et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. Proc Natl Acad Sci U S A 2020;117(28):16587–95.

Joyner MJ, Bruno KA, Klassen SA, Kunze KL, Johnson PW, Lesser ER, et al. Safety update: COVID-19 convalescent plasma in 20,000 hospitalized patients. Mayo Clinic Proc 2020a;95:1888–97.

Joyner MJ, Senefeld JW, Klassen SA, Mills JR, Johnson PW, Theel ES, et al. Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: initial three-month experience. medRxiv 2020b; 2020.2008.2012.20169359.

Kostin AI, Lundgren MN, Bulanov AY, Ladygina EA, Chirkova K, Gintsburg A, et al. Impact of pathogen reduction technologies on immunological properties of the COVID-19 convalescent plasma. medRxiv 2020; 2020.2010.2002.20205567.

Ledford H. Evidence lags behind excitement over blood plasma as a coronavirus treatment. Nature 2020;584(7822):505.

Li L, Zhang W, Hu Y, Tong X, Zheng S, Yang J, 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;324:1–11.

Liu STH, Lin H-M, Baine I, Wajnberg A, Gumprecht JP, Rahman F, et al. Convalescent plasma treatment of severe COVID-19: a propensity score–matched control study. Nat Med 2020;, doi:http://dx.doi.org/10.1038/s41591-020-1088-9 [Epub ahead of print].

Mair-Jenkins J, Saavedra-Campos M, Baillie JK, Cleary P, Khaw FM, Lim WS, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. J Infect Dis 2015;211(1):80–90.

Maiztegui JI, Fernandez NJ, de Damilano AJ. Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome. Lancet 1979;2(8154):1216–7.

Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 1998;282(5386):103–7.

Petkova E, Antman EM, Troxel AB. Pooling data from individual clinical trials in the COVID-19 era. JAMA 2020;324(6):543–5.

Salazar E, Christensen PA, Graviss EA, Nguyen DT, Castillo B, Chen J, et al. Treatment of coronavirus disease 2019 patients with convalescent plasma reveals a signal of significantly decreased mortality. Am J Pathol 2020a;190:1680–90.

Salazar E, Christensen PA, Graviss EA, Ngyuen DT, Castillo B, Chen J, et al. Early transfusion of a large cohort of COVID-19 patients with high titer anti-SARS-CoV-2 spike protein IgG convalescent plasma confirms a signal of significantly decreased mortality. medRxiv 2020b; 2020.2010.2002.20206029.

Stadlbauer D, Baine I, Amanat F, Jiang K, Lally K, Krammer F, et al. Anti-SARS-CoV-2 spike antibodies are stable in convalescent plasma when stored at 4° Celsius for at least 6 weeks. Transfusion 2020;, doi:http://dx.doi.org/10.1111/trf.16047 [Epub ahead of print].

Subbarao K, Mordant F, Rudraraju R. Convalescent plasma treatment for COVID-19: tempering expectations with the influenza experience. Eur J Immunol 2020;50:1447–53.

Sun J, Zhuang Z, Zheng J, Li K, Wong RL-Y, Liu D, et al. Generation of a broadly useful model for COVID-19 pathogenesis, vaccination, and treatment. Cell 2020;182(3):734–43.

Tonn T, Corman VM, Johnsen M, Richter A, Rodionov RN, Drosten C, et al. Stability and neutralising capacity of SARS-CoV-2-specific antibodies in convalescent plasma. Lancet Microbe 2020;1(2):e63.

WHO. WHO R & D blueprint — novel coronavirus COVID19 therapeutic trial synopsis. Retrieved 1 October 2020, from. 2020. https://www.who.int/blueprint/priority-diseases/key-action/COVID-19_Treatment_Trial_Design_-Master_Protocol_synopsis_Final_18022020.pdf?ua=1.