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Effect of Convalescent Plasma Therapy on Mortality and Viral Load in Severely Ill Patients with COVID-19

Jan Moravec[1], Martin Müller[1], Petr Turek[2], Michal Moravec[1], Tomáš Nejtek[1], Roman Zazula[1]

[1]Department of Anesthesiology and Intensive Care, First Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic; [2]Department of Blood Transfusion, Thomayer University Hospital, Prague, Czech Republic

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Abstract: The use of convalescent plasma (CP) appeared to be a promising, easily available and safe way of treatment of severe COVID-19 at the onset of the pandemic in early 2020. Conducted in 2020 and 2021, our study of 52 severely to critically ill COVID-19 patients who received CP plasma as a treatment and of 97 controls found no difference in 30-day or 90-day mortality rates. A significant viral load drop in most patients (4.7 log10 [$p<0.001$] copies/ml) was observed following CP administration. Retrospective analysis of selected inflammatory markers and immunoglobulins showed higher C-reactive protein levels among the study group, and their decrease on Day 7.

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Mailing Address: Jan Moravec, MD., Department of Anesthesiology and Intensive Care, First Faculty of Medicine, Charles University and Thomayer University Hospital, Vídeňská 800, 140 59 Prague 4, Czech Republic; e-mail: jan.moravec@ftn.cz

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Introduction

The history of convalescent plasma (CP)/serum therapy goes back to administration of horse serum for diphtheria in the 1890s (Behring, 1890; Bracha and Tan, 2011). The first widespread use of CP as a therapy came with the 1918 Spanish influenza pandemic caused by the A/H1N1 virus. A later meta-analysis found a lower mortality risk in patients infected with the Spanish influenza who later developed pneumonia and received CP treatment (Luke et al., 2006). Prior to introduction of antibiotics, convalescent serum obtained from immunized animals was widely used to battle serious bacterial infections. The antibiotic boom after the Second World War substantially reduced clinical use of CP therapy.

The novel COVID-19 disease brought CP into the spotlight of therapeutic options in the early pandemic. The use of plasma collected from recovered patients seemed to be a feasible, readily available and safe treatment.

Those who have recovered from COVID-19 produce and increase the levels of specific antibodies within three weeks following the onset of the first symptoms. Initially, IgM and IgA antibodies are created, followed by IgG antibodies about a week later. The IgM and IgA levels begin to fall after three weeks since the first symptoms occurred. The protective effect of IgG antibodies lasts longer: their levels decrease after about six months. Nevertheless, there is a high inter-individual variability in both the serum levels and production length for each antibody type (Mallano et al., 2022).

These antibodies can help neutralize the virus and modify the inflammatory response. Therefore, the use of COVID-19 convalescent plasma containing anti-SARS-CoV-2 antibodies was considered a suitable experimental therapy for this disease (Wang et al., 2020).

Material and Methods

Approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine and the Thomayer University Hospital and conducted at the DAIC (Department of Anesthesiology and Intensive Care, First Faculty of Medicine, Charles University and Thomayer University Hospital), our study researched and evaluated experimental treatment of severely ill COVID-19 inpatients with CP collected from recovered donors. The patients received two transfusion units of CP (approx. 200–250 ml each) from different donors. The study protocol reflected the expert guidelines of CSARIM (Czech Society for Anesthesiology, Resuscitation and Intensive Medicine) (Balík et al., 2020). The patients signed a consent about the nature and the extent of proposed plasma therapy, laboratory parameters to be monitored and expected benefits and risks of the study. Where the informed consent could not be obtained from the patients, it was signed by a closed relative.

The anti-SARS-CoV-2 convalescent plasma was collected in three large hospitals, all of them located in the Czech capital of Prague (Thomayer University Hospital, Institute of Haematology and Blood Transfusion, and General University Hospital in

Prague). The plasma donation was mandated by complete recovery from laboratory-confirmed COVID-19, at least 14 days since recovery or end of isolation/quarantine, good general health, eligibility and standard requirements for blood donation (Turek, 2020).

Given the urgency of the situation, the presence of anti-SARS-CoV-2 antibodies in the CP was only confirmed by a rapid test (by Innovita) before its administration to the first 4 patients. The plaque reduction neutralization test (virus neutralisation test) and IgG/IgA ELISA test to determine the levels of antibodies were taken subsequently. The plaque reduction neutralization tests were performed at the Central Military Health Institute, Těchonín, Czech Republic.

Plaque reduction neutralization test: to determine antibody titer, the plasma sample is incubated in different diluted concentrations with a standard concentration of virus suspension, and subsequently added to the cell culture. The pathogens that have not been neutralized by the antibodies then infect the cells. The result is read under microscope as the cytopathic effect of the virus. A negative result means a lower antibody titer in the sample than the dilution.

Specific antibody levels: simultaneously with the plaque reduction neutralization test, the levels of IgG anti-SARS-CoV-2 antibodies were measured at the Thomayer University Hospital and the Institute of Haematology and Blood Transfusion using the ELISA test (by Euroimmun, Lübeck, Germany), which contains the recombinant S1 spike protein domain and intern calibrator allowing for a semi-quantitative reading. A later comparison showed a correlation between the neutralization test and the ELISA test results (Figure 1) (Turek et al., 2020). From November 2020, only CP with a COVID-IgG index value > 3.7, and from January 2021, only CP with a COVID-IgG levels > 80 BAU (binding antibody units)/ml (equivalent to an index value of approx. 4.5) were administered to patients.

Viral load: a sample of nasopharyngeal swab or tracheal aspirate culture was taken before the first CP transfusion to establish the SARS-CoV-2 viral load using the real-

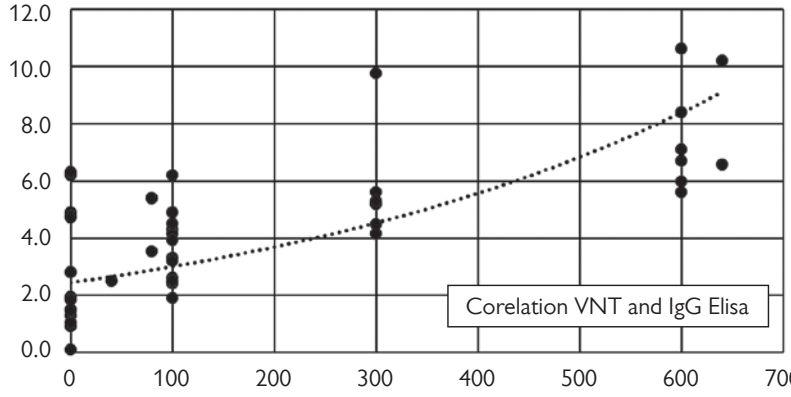

Figure 1 – ELISA virus neutralisation test.

Table 1 – COVID-19 severity by NIH

Severity	Definition by NIH
Asymptomatic or presymptomatic infection	PCR SARS-CoV-2 positivity without any symptoms.
Mild illness	Patients with mild illness may exhibit a variety of signs and symptoms (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell). They do not have shortness of breath, dyspnea on exertion, or abnormal imaging.
Moderate illness	Moderate illness is defined as evidence of lower respiratory disease during clinical assessment or imaging, with $SpO_2 \geq 94\%$ on room air at sea level.
Severe illness	Patients with COVID-19 are considered to have severe illness if they have $SpO_2 < 94\%$ on room air at sea level, $PaO_2/FiO_2 < 300$ mm Hg, a respiratory rate > 30 breaths/min, or lung infiltrates > 50%.
Critical illness	Acute respiratory distress syndrome, virus-induced distributive (septic) shock, cardiac shock, an exaggerated inflammatory response, thrombotic disease, and exacerbation of underlying comorbidities caused by SARS-CoV-2 infection.

Coronavirus Disease 2019 (COVID-19) Treatment Guidelines
NIH – National Institutes of Health

time PCR technique (RT-PCR). The second RT-PCR test was performed on Day 7 following the CP application. Viral load was not measured in the control group due to technical and logistic reasons.

Study population: the study group consisted of DAIC patients with severe or critical illness as defined by the National Institutes of Health (NIH) Guidelines (Table 1) (Coronavirus Disease 2019 [COVID-19] Treatment Guidelines). The control group comprised severely and critically ill patients who were admitted in our department in the same time period and received the same treatment as the study group except CP therapy.

The primary outcome of our study was 30-day and 90-day mortality rates in both groups. As the secondary outcome, we evaluated the non-specific immunoglobulin levels (IgA, IgM, IgG), inflammatory parameters (C-reactive protein – CRP, procalcitonin – PCT, and interleukin 6 – IL-6), leucocytes and their populations (neutrophils, lymphocytes and neutrophil-to-lymphocyte ratio – NLR). The blood samples were taken before CP administration and on Day 7. In the control group the tests were taken on the admission day, and on Day 7 intensive care unit (ICU) stay.

The statistic comparison of the discrete data was performed by Pearson's chi-squared test with Yates' correction for continuity. The effect of CP on the development of the individual parameters under observation was tested by a linear mixed effect model. R software, version 4.1.3 (Vienna, Austria), with the RStudio

interface was used for the statistical analysis. Statistically significant was considered $p<0.05$.

Results

From April 2020 to March 2021, CP was administered to 52 patients hospitalised at DAIC with PCR confirmed COVID-19, bilateral viral pneumonia and respiratory failure requiring treatment with either high flow oxygen therapy, non-invasive ventilation or mechanical ventilation. One patient was treated solely with conventional oxygen therapy. 50 patients obtained 2 transfusion units of CP from different donors, with a minimum interval of 2 hours between each unit. 2 patients only received one transfusion unit (due to a transfusion-associated circulatory reaction in 1 patient, and a critical condition following cardiopulmonary resuscitation with a very poor prognosis in another patient). Along with CP, the patients were treated with low molecular weight heparin and systemic corticosteroids. Further medication, which reflected the current knowledge and drug availability at the time, is included in Table 2. The average age of patients treated with CP was 65.2 ± 13.3 years (average age ± standard deviation). There were 37 men (71%) and 15 women (29%) in the study group.

Out of the 97 patients in the control group, 95 required either high flow oxygen therapy or non-invasive ventilation or mechanical ventilation, 2 patients received only conventional oxygen therapy. The average age of controls was 67.5 ± 10.7 years. There were 64 men (66%) and 33 women (34%) in the control group.

The data on 30-day and 90-day mortality rates were obtained from all patients both in the study and control group. 30-day mortality among CP-treated patients was 40.4%, and 48.5% in control group (Figure 2). 90-day mortality was 55.8% in the study group and 56.7% among controls (Figure 3). No statistically significant difference was found in both 30-day mortality ($p=0.44$) and 90-day mortality ($p=1$).

Table 2 – Overview of medication

Treatment	Number of patients receiving in study group	Number of patients receiving in control group
Low molecular weight heparin	52 (100%)	97 (100%)
Systemic corticotherapy	52 (100%)	94 (97%)
Remdesivir	29 (56%)	32 (33%)
Favipiravir	5 (10%)	19 (20%)
Hydroxychlorochine + isoprinosine	3 (6%)	0
Remdesivir + ivermectin	2 (4%)	2 (2%)
Favipiravir + ivermectin	2 (4%)	1 (1%)
Remdesivir + isoprinosine	1 (2%)	0
Ivermectin + isoprinosine	1 (2%)	0
Baricitinib	0	2 (2%)

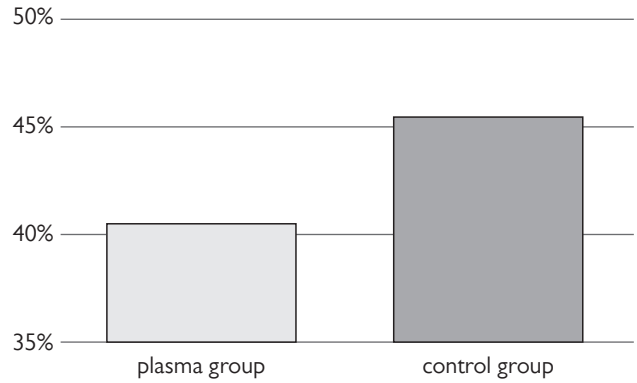

Figure 2 – 30-day mortality.

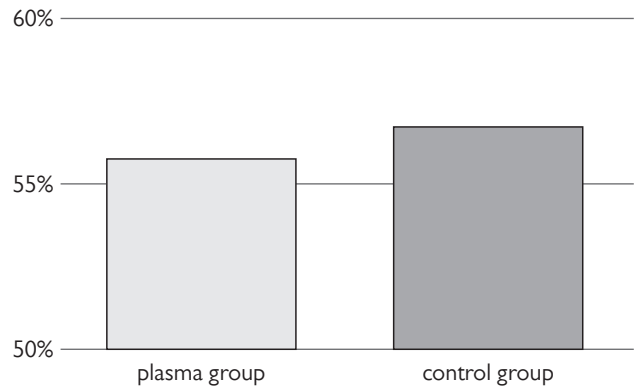

Figure 3 – 90-day mortality.

The median value (first quartile; third quartile) of length of hospital stay was 16.0 (10.8; 24.0) days in the study group. In the control group, the length of hospital stay was only 13.0 (8.0; 19.0) days. The difference in the length of hospital stay between the study and control groups is borderline statistically significant (p=0.04).

The median length of mechanical ventilation was 284 (155; 485) hours in the study group, and 262 (151; 347) hours in the control group. The difference in the length of mechanical ventilation was not statistically significant (p=0.053).

Complete data on viral load were obtained from 28 patients: their levels dropped in 23 patients, rose in 4 of them, and remained unchanged in 1 patient. 11 patients became PCR SARS-CoV-2 negative on Day 7. On average, the viral load decreased by 4.7 log10 ($p<0.001$) copies/ml over the 7 days following CP administration (Figure 4).

A comparison was made between the levels of inflammatory markers measured in the study and control groups on T 0 (patient's admission to DAIC, that is, before CP administration), and on Day 7 (eighth day of ICU stay). The following data are presented as median values (first quartile; third quartile).

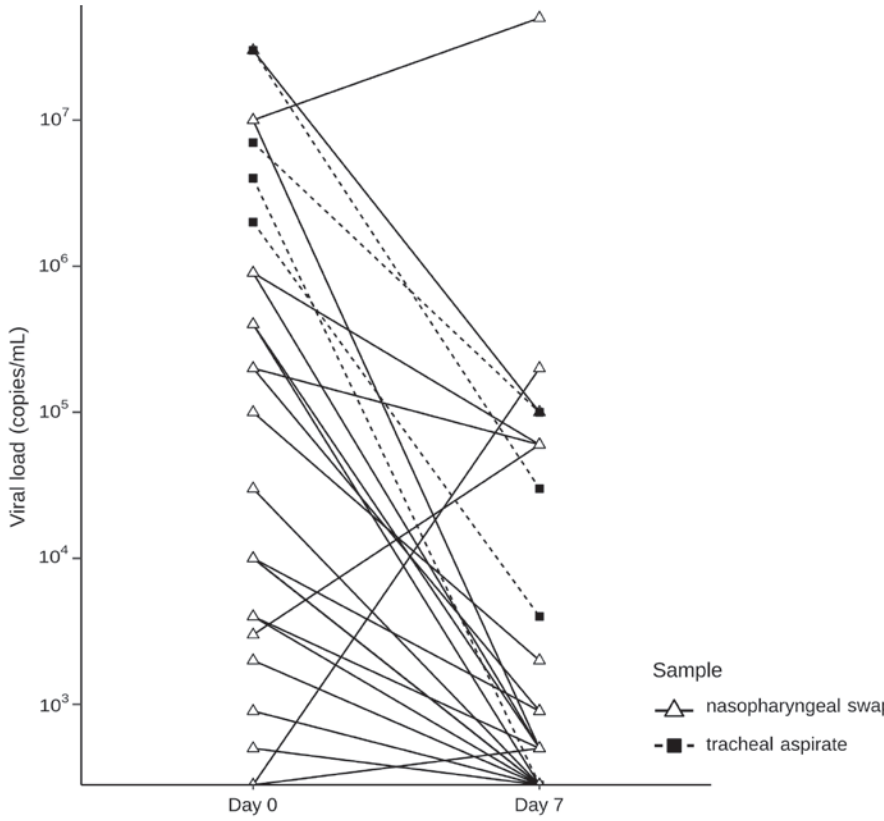

Figure 4 – Viral load over time.

Complete data on C-reactive protein values were obtained on T 0 from all patients, on Day 7 the data is missing from 11 patients in the study group and from 22 patients in the control group. On the admission day, the CRP levels were significantly higher in the study group – 171.9 (113.5; 234.9) mg/l, than in the control group – 113.2 (41.0; 157.0) mg/l, p<0.001. On Day 7, the CRP levels were lower in the study group – 83.6 (21.4; 134.0) mg/l, compared to the control group – 98.1 (21.8; 134.4) mg/l, with an average decrease of 74.1 mg/l (p<0.001) in the study group. Among the controls, the decrease in the CRP levels was insignificant (p=0.26) (Figure 5).

We also compared the procalcitonin levels, which, however, did not show any significant difference between the groups on T 0, nor was there any significant change in PCT levels on Day 7.

A significantly lower level of leucocytes was found in the study group on T 0 (p=0.009). On Day 7, however, the change in the leucocyte levels was insignificant both in the study and control group (Figure 6).

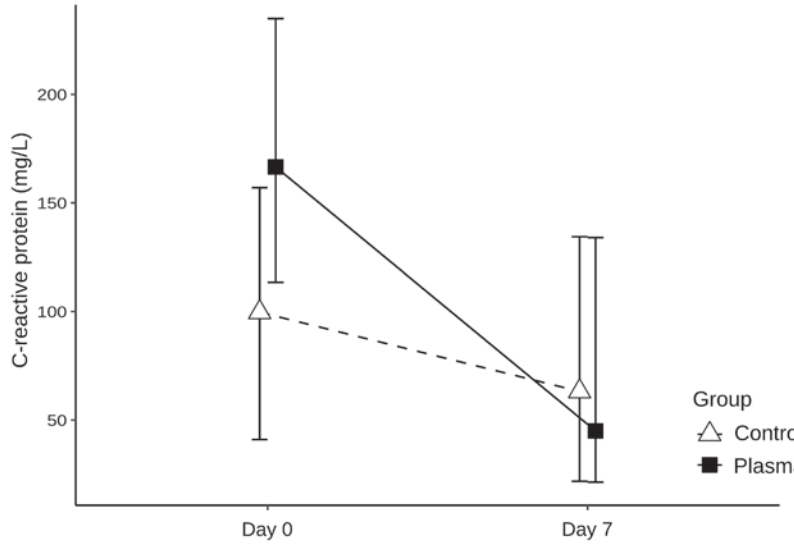

Figure 5 – C-reactive protein.

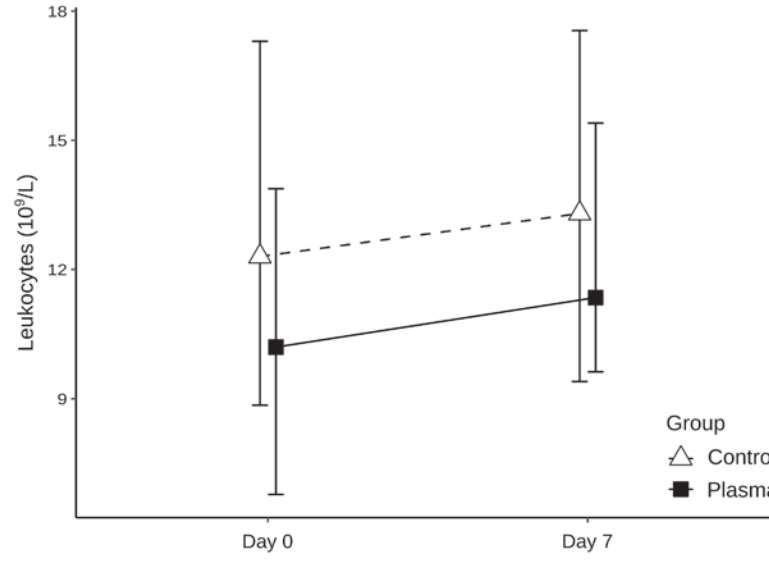

Figure 6 – Leucocytes.

A comparison was made of leucocyte populations, namely lymphocytes. No significant difference was found in the absolute number of lymphocytes between the groups, whether on T 0 or on Day 7.

Another potentially interesting inflammatory marker that we evaluated was neutrophil-to-lymphocyte ratio (NLR), the high levels of which are associated with

higher mortality (Vafadar Moradi et al., 2021). We did not find any statistically significant difference in the NLR levels between the study and control groups either on T 0 or on Day 7.

We also chose to examine the levels of individual immunoglobulins (IgA, IgM, and IgG) as potentially interesting markers of antibody immunity response. Despite a rise in the IgM levels among the controls on Day 7, we found no statistically significant difference between the groups (p=0.07). The IgA and IgG levels showed no significant difference between the groups both on Day 0 and Day 7.

Discussion

At the turn of spring 2020, the world, was hit by the COVID-19 pandemic. With the speed of the pandemic onset, it was necessary to prepare the inpatient wards for a surge of patients requiring an isolation regime, and also actively search the available resources for methods to treat the novel disease. Convalescent plasma appeared to be a relatively quickly available, proven and inexpensive option for treatment. Our hospital was among the first ones in the Czech Republic to use convalescent plasma as a treatment of COVID-19 patients. Before the first administration of CP, a study protocol was designed to allow for a retrospective analysis of the CP efficacy.

Despite the well-known complications of blood plasma transfusion as such, a serious adverse reaction (hypotension) only occurred in one out of 102 CP administrations. None of the patients developed a circulatory overload that would lead to heart failure, allergic or anaphylactic reaction, transfusion-related acute lung injury, haemolytic reaction or transfusion-related infection. Although our study did not prove the CP efficacy, it also did not prove to be harmful for the patients and increase morbidity and/or mortality.

Owing to the limited availability of CP and logistic obstacles of the plaque reduction test in the early pandemic, some patients received CP without establishing its titer value from neutralisation effect. Subsequent tests revealed a low antibody titer in several CP units that had been administered, which could have been the other potential confounding factor for non-superiority of CP over standard treatment (see Methods above).

The crucial limitation of our study is the absence of data on the dynamics of viral load in the control group. Its comparison with the data on the study group could have revealed the effect of CP on the clearance of the virus. On a sample of 231 patients, Fajnzylber et al. (2020) showed a viral load median value of 4.4 log10 in sputum, and its decrease over time in most patients both in sputum and nasopharyngeal swab. However, a systematic review on viral load and disease severity by Dadras et al. (2022) found that even relationship between COVID-19 severity and viral load is inconclusive.

The other limitation of the study is the absence of randomisation. At the time of the study, the indication criteria for administration were based on the expert

guidelines of CSARIM, therefore only patients with a severe course were included in the study in accordance with the guidelines (Balík et al., 2020).

There are probably several reasons for the unsatisfactory outcome of CP treatment. One of them might be timing: CP was applied to patients who already required high flow oxygen therapy or ventilatory support and their illness has reached an advanced stage where the inflammation was difficult to control. Some studies describe the cytokine-storm rather than the direct cytopathogenic effect of SARS-CoV-2 to be the primary factor of the tissue damage and respiratory failure (Yang et al., 2021). According to some studies, application of a different immunomodulation treatment, namely IL-6 inhibitor (e.g., tocilizumab), leads to a better prognosis and shorter hospitalisation (RECOVERY Collaborative Group, 2021), while other studies have not proven its effect on mortality rate (Rosas et al., 2021).

Despite that the fact, that there is currently no evidence for benefit of CP therapy for COVID-19, which was also the conclusion of our study, there are still weak recommendation for use a CP (Coronavirus Disease 2019 [COVID-19] Treatment Guidelines) and some studies suggested a potential benefit for immunocompromised patients when administrated early (Writing Committee for the REMAP-CAP Investigators et al., 2021; Denkinger et al., 2023) and CP holds the potential to evolve in real-time with virus and retain activity against new variants unlike monoclonal antibodies.

Conclusion

Despite the statistically insignificant difference in 30-day mortality, the 90-day mortality rate was practically the same in both groups.

This finding corresponds with the outcomes of other large multicentric studies (Bégin et al., 2021; Writing Committee for the REMAP-CAP Investigators et al., 2021).

The viral load decrease following CP treatment was evident in most patients, but it could have been caused by many factors, and its evaluation would require comparison with the control group.

At the onset of the COVID-19 pandemic, convalescent plasma appeared to be a promising treatment with the unavailability of targeted therapy and rapid growth in the patients' numbers. Today, the therapeutic potential of convalescent plasma seems to be minimal. Currently, there is an array of accessible antiviral drugs, monoclonal antibodies and immunomodulatory agents. Although CP administration did not result in better clinical outcome, it was not harmful for the patients (did not increase mortality). According to the NIH, CP therapy is not recommended for immunocompetent patients anymore (Coronavirus Disease 2019 [COVID-19] Treatment Guidelines). According to the multidisciplinary expert position of CSARIM, CP therapy is not recommended for patients receiving any ventilation support or on high flow oxygen therapy (Bohoněk et al., 2021).

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Writing Committee for the REMAP-CAP Investigators; Estcourt, L. J., Turgeon, A. F., McQuilten, Z. K., McVerry, B. J., Al-Beidh, F., Annane, D., Arabi, Y. M., Arnold, D. M., Beane, A., Bégin, P., van Bentum-Puijk, W., Berry, L. R., Bhimani, Z., Birchall, J. E., Bonten, M. J. M., Bradbury, C. A., Brunkhorst, F. M., Buxton, M., Callum, J. L., Chassé, M., Cheng, A. C., Cove, M. E., Daly, J., Derde, L., Detry, M. A., De Jong, M., Evans, A., Fergusson, D. A., Fish, M., Fitzgerald, M., Foley, C., Goossens, H., Gordon, A. C., Gosbell, I. B., Green, C., Haniffa, R., Harvala, H., Higgins, A. M., Hills, T. E., Hoad, V. C., Horvat, C., Huang, D. T., Hudson, C. L., Ichihara, N., Laing, E., Lamikanra, A. A., Lamontagne, F., Lawler, P. R., Linstrum, K., Litton, E., Lorenzi, E., MacLennan, S., Marshall, J., McAuley, D. F., McDyer, J. F., McGlothlin, A., McGuinness, S., Miflin, G., Montgomery, S., Mouncey, P. R., Murthy, S., Nichol, A., Parke, R., Parker, J. C., Priddee, N., Purcell, D. F. J., Reyes, L. F., Richardson, P., Robitaille, N., Rowan, K. M., Rynne, J., Saito, H., Santos, M., Saunders, C. T., Serpa Neto, A., Seymour, C. W., Silversides, J. A., Tinmouth, A. A., Triulzi, D. J., Turner, A. M., van de Veerdonk, F., Walsh, T. S., Wood, E. M., Berry, S., Lewis, R. J., Menon, D. K., McArthur, C., Zarychanski, R., Angus, D. C., Webb, S. A., Roberts, D. J., Shankar-Hari, M. (2021) Effect of convalescent plasma on organ support-free days in critically ill patients with COVID-19: A randomized clinical trial. *JAMA* **326**, 1690–1702.

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