A Prospective Phase II Study on The Use Of Convalescent Plasma Monotherapy For The Treatment of Severe Covid-19 Disease: A Preliminary Report.

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Abstract

Currently, there are no effective treatments for novel corona virus disease 2019 (COVID-19). In this study, we report the preliminary results on the efficacy and safety of convalescent plasma (CP) infusion, as monotherapy in 9 patients with severe COVID-19 disease. The median time from symptom onset to CP transfusion was 6 days. All symptoms improved significantly after a median of 8 days. In 6/9 patients, symptomatic improvement was observed already after the 1st dose of CP transfusion. Laboratory parameters associated with disease severity tended to significantly decrease over time and lymphocyte counts significantly increased on day 14. All patients exhibited significant increases in SARS-CoV-2 IgA and IgG antibodies starting on day 7 through day 21 after CP infusion with concurrent reduction of SARS-CoV-2 RNA on days 7 and 14 with 44.4% % of the patients having undetectable SARS-CoV-2 RNA on day 14. After a median follow-up of 66 days, all patients remain alive. Eight patients recovered completely and were discharged from hospital after a median duration of hospitalization of 21 days. No severe adverse events were observed. In conclusion, this preliminary report suggests that CP infusion monotherapy administered early in the disease course may be a safe and effective strategy for patients with severe COVID-19 disease. Trial registration: NCT04408209. Registered 05 May 2020- Retrospectively registered, (https://clinicaltrials.gov/ct2/show/***NCT04408209***?term=NCT04408209&draw=2&rank=1).

1. Background

The SARS-Cov-2 coronavirus outbreak, which first occurred in Wuhan, China, on 12 December 2019, is now a global threat. On 11 March 2020, the World Health Organization (WHO) declared it a pandemic disease and according to the epidemiological data on June 24 the virus has affected, 9,24 millions of people and resulted in 477.000 deaths worldwide in over 100 countries [1].The virus causes a severe form of pneumonia called corona virus disease 2019 (COVID-19).

In 80% of the cases the disease is mild but in patients with comorbidities an increased likelihood of a severe form of chest infection as well as increased mortality are expected. The most common symptoms in well documented cases include fever (88%), dry cough (68%), fatigue (38%), productive cough (33%), shortness of breath (19%), sore throat (14%), headache (14%), myalgia and arthralgia (15%). Less common symptoms include diarrhea (4%) and vomit (5%) [2]. Regarding the mortality of the disease there are no conclusive data but according to the observations from China, Italy and South Korea the total mortality was 2.3%, 2.8%, 0.5% respectively and increased with increasing age with a peak value after 80 years of age (14.8%, 8.2%, 3.7% respectively).

Until now there is no effective treatment for this disease or vaccine strategy and treatment options mainly include disease prevention, prompt diagnosis, follow up and supportive measures despite the fact that a variety of new drugs are being currently tested in various clinical trials, the results of which are eagerly awaited.

Taking into consideration the high contagiousness of the virus SARS-CoV-2 and the increased mortality of patients with comorbidities there is a demanding need for immediately available therapies. Towards this direction the passive immunization of patients using convalescent plasma (CP) from patients fully recovered from COVID-19 is a therapeutic option immediately available since a considerable number of patients has fully recovered from the disease and could be used as a source of CP containing high titers of antibodies specific for the virus [3].

The administration of CP or hyperimmune globulins (hyper-IG) from patients recovered from viral infections has already been successfully used in the past during the epidemics of SARS, MERS, Influenza A H1N1 resulting in reduction of the duration of hospitalization and reduction of mortality [4–6]. Additionally, during the epidemic of Ebola virus in 2014 the WHO has adopted the administration of CP as an empirical treatment during the period of exacerbation of the disease [7].

Published data on the use of CP for the treatment of COVID-19 infection are gradually increasing and include mostly case series with small number of cases and conflicting results [7–13]. The largest experience comes from a recently published open label randomized trial from 7 centers in Wuhan China, including 103 patients with COVID-19 with severe or life threatening disease. The trial was terminated early after 103 of a planned 200 patients were enrolled, due to its negative results. The authors state that the interpretation of the findings may be limited by the early termination of the study [14].

The aim of our prospective phase II study is to investigate the safety and efficacy of CP administration obtained by plasmapheresis from patients recovering from SARS-CoV-2, for the treatment of severe COVID-19.

2. Methods

Study design

This study is a multicenter phase II trial (identifier number NCT04408209), conducted at 5 hospitals in Athens. The study conforms to the principles outlined in the Declaration of Helsinki, it was approved by all Institutional Review Boards of participating hospitals and adheres to the CONSORT guidelines. All patients provided written informed consents.

The primary endpoint was survival on day 28. The secondary endpoints were: time to clinical improvement (i.e. patients not fulfilling the criteria for severe disease), safety, duration of hospitalization, duration of stay in the intensive care unit (ICU), duration of ventilation support /ECMO if applicable,

time until negative SARS-CoV-2 PCR (nasal/pharyngeal swab), predictive value of comorbidities and inflammation markers on mortality, length of stay in the ICU and length of hospital stay, feasibility of collection of plasma units from donors recovered from SARS-CoV-2, titer of anti-SARS-CoV-2 antibodies in the infused plasma units, investigation of the titer of anti-SARS-CoV-2 antibodies in the patients before the infusion of CP on days 1–7 and subsequently weekly until day 35.

Patients

From May 6, 2020, to July 24, 2020, 11 patients were enrolled in this study. The results on the first 9 patients for whom the endpoints could be evaluated are reported. All patients were diagnosed as having grade 4 COVID-19 disease according to the WHO criteria as formulated in February 2020 [15]. The diagnosis was confirmed by real-time RT-PCR assay of the nasopharyngeal swab. Inclusion and exclusion criteria are described in Supplementary Table 1.

Clinical and laboratory parameters were registered for the first 7 days and on a weekly basis there after until day 28. Anti-SARS-CoV2 antibody titles were determined on days 1–7, and on days 14 and 28. RT-PCR for SARS-CoV2 from the nasopharyngeal swab was performed on days 1, 4, 7, 10, 14, 21 and 28.

All patients received treatment with single donor CP ABO identical that included the infusion of 200–233 ml of CP in 30–60 minutes on days 1, 3 and 5. The CP was infused within 1 hour after thawing.

Donors of CP

Nine donors who had recovered from SARS-CoV-2 infection were invited to donate their plasma after written informed consent was obtained. All donors had been previously diagnosed with RT-PCR assay confirmed COVID-19 and subsequently proven to have cleared SARS-CoV-2 from nasopharyngeal mucosa by 2 negative RT-PCR results from nasal or pharyngeal swabs. The two RT-PCR assays should be at least 1 week apart. All donors tested negative for hepatitis B virus, hepatitis C virus, HIV, and syphilis at the time of blood donation. The donors had been well (asymptomatic) for at least 2 weeks, with detectable serum IgG and IgA anti-SARS-CoV-2 antibodies. Plasmapheresis was performed according to institutional SOPs. Using the platelet and plasma collection kits of Trima Accel 15% of the donor's blood volume was collected safely, without administering replacement fluid. Each donor’s plasma of total volume 600–700 ml was divided in equal doses of 200–233 ml, transferred to the National Center for Blood Donation where they were preserved as Fresh Frozen plasma and administered upon request to the participating hospitals. Each plasma unit of total volume 600–700 ml was administered to one patient in 3 divided doses.

Detection of anti-SARS-CoV2 antibodies

The IgG and IgA antibodies in the sera of the patients were determined semiquantitavely by the Anti-SARS-CoV-2 IgG ELISA and Anti-SARS-CoV-2 IgA ELISA (Euroimmun Medizinische Labordiagnostika AG) respectively. The method detects antibodies against the recombinant Spike protein of the virus (S1 domain). Both assays were performed on the automated EUROIMMUN Analyzer I (Euroimmun Medizinische Labordiagnostika AG), according to the manufacturer’s protocol. The Anti-SARS-CoV-2 IgG ELISA has 90% sensitivity (95% CI 74.4–96.5) and 100% specificity (95% CI 95.4–100). (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance). Patients’ index values were calculated by dividing patients’ sera optical density (OD) values by the mean of the duplicate calibrator OD values. The results are interpreted as positive if the index value is > 1.1, negative if < 0.9 and borderline between 0.8 and 1.1. Quality control material supplied by the manufacturer was analyzed in each run. Serial analysis of the samples of each patient was performed on the same run in order to have a more accurate comparison between samples.

RNA Extraction and Real Time RT-PCR for SARS-CoV-2

Nasopharyngeal and/or oropharyngeal swabs were collected and transferred to the Microbiology laboratory, immersed in an appropriate virus transport medium (e.g. UTM Viral Transport, Copan Diagnostics Inc., Brescia, Italy). Flocked swabs made from synthetic material are preferred for sample collection in order to maximize viral recovery. Lower respiratory tract samples (e.g. bronchoalveolar lavage or aspirates, sputum, etc.) were also accepted.

Automated purification of viral RNA from either viral transport medium, or lower respiratory tract samples is performed using the QIAsymphony DSP virus/pathogen mini kit on the QIAsymphony SP platform (QIAGEN, Hilden, Germany). A Real Time, one – step Reverse Transcription – PCR, specific for ORF1ab gene of SARS-CoV-2 and for N gene of all, or other coronaviruses is finally performed using the VIASURE SARS-CoV-2 Real Time PCR Detection Kit (CerTest Biotec SL, Zaragoza, Spain).

Statistics

Descriptive statistics, comparisons using the Wilcoxon signed-rank test and correlations assessed using Spearman’s rank correlation coefficients were performed with IBM SPSS v.25 software. Non-parametric tests were used, due to the small number of studied cases.

3. Results

CP donors

CP was collected by plasmapheresis from 9 patients (7 males and 2 female). Median age was 55 years (IQR, 45 to 63 years). Median time from diagnosis to plasmapheresis was 50 days (IQR, 38 to 56 days). Median levels of IgA and IgG antibodies were 7.52 (range: 1.68–12.35) and 10.96 (range: 2.48–12.68) respectively.

Patients' characteristics

From May 7, 2020 to July 24, 2020, 9 patients (4 males and 5 females) with WHO grade 4 COVID-19 disease were enrolled and received CP transfusion. Patient characteristics at diagnosis are shown in Table 1. Median age was 61 years (interquartile range [IQR], 60–62 years). Median time from symptom onset to hospital admission and CP transfusion was 1 day (IQR, 0–3 days) and 6 days (IQR, 4–7 days), respectively. Most common symptoms at disease onset were fever (8 cases), cough (7 cases), shortness of breath (3 cases) and headache (3 cases), while less common symptoms included diarrhea (2 cases), loss of taste (2 cases), loss of smell (2 cases), arthralgias (1 case), myalgias (1 case), and sore throat (1 case). Four patients were overweight, 4 had normal weight and 1 was obese. Four patients had comorbidities: 1 pulmonary fibrosis, 1 essential hypertension and diabetes mellitus, 1 chronic obstractive disease (COD) and lung cancer and 1 essential hypertension only. Antibacterial treatment was used in 5 patients with coexistent bacterial infection. One patient received treatment with tocilizumab (single dose of 8 mg/kg) after clinical deterioration and intubation, 6 days after the first CP infusion.

Table 1
Clinical characteristics of patients

Patient no	Sex	Age, y	BMI category	Time to admission from symptom onset *(days)*	Time to CP treatment from symptom onset *(days)*	Principal symptoms	Oxygen support	SOFA score	Comorbidities
1	M	62	Overweight	0	4	Fever, cough, shortness of breath, headache	Low-flow nasal cannula	4	None
2	F	60	Overweight	1	3	Fever, cough, malaise	Low-flow nasal cannula	4	None
3	M	60	Overweight	0	6	Fever, cough, lack of taste, lack of smell, diarrhea	High-flow nasal cannula	7	Pulmonary fibrosis
4	F	62	Overweight	5	6	Fever, cough	Low-flow nasal cannula	7	None
5	M	52	Normal weight	11	18	Cough, headache	High-flow MV	7	Hypertension, diabetes mellitus
6	F	29	Normal weight	0	11	Fever, myalgias, arthralgias, headache, lack of taste, lack of smell	None	2	None
7	F	73	Obese	3	5	Fever, cough, sore throat	Low-flow nasal cannula	3	Hypertension
8	F	61	Normal weight	3	7	Fever, cough, shortness of breath	Low-flow nasal cannula	5	None
9	M	70	Normal weight	1	1	Fever, shortness of breath	High-flow MV	5	COD, lung cancer

On computer-assisted tomography (CT), all patients showed bilateral ground-glass opacities and/or pulmonary parenchymal consolidation with predominantly subpleural and bronchovascular bundle distribution. Seven patients had multiple lobe involvement, and five patients had interlobular septal thickening. At the time of enrolment no patient had life threatening disease.

Effects of CP transfusion

Improvement of clinical symptoms. All symptoms, including fever and cough, improved significantly after a median of 8 days (IQR, 6.5–15.5 days). In 6 out of 9 patients (66.6%), symptomatic improvement was observed already after the 1st CP dose. Prior to CP transfusion, 8/9 patients needed oxygen treatment: 3 patients were on Venturi Mask and 5 received low-flow nasal cannula oxygenation as shown in Table 2. Following CP treatment, 4 patients experienced deterioration of their respiratory function associated with progression of infection and received mechanical ventilation after 2, 3, 5 and 7 days after the 1st dose of CP transfusion, respectively. All patients were extubated after 2, 5, 7 and 43 days, respectively. Median length of stay in ICU was 9.5 days.

Table 2
Comparison of SOFA score and oxygen support before and after CP transfusion

	Day 1: before CP transfusion		After CP transfusion day 7		After CP transfusion day 14		After CP transfusion day 21		After CP transfusion day 28		After CP transfusion day 35	
Patient no	SOFA score	Oxygen support	SOFA score	Oxygen support	SOFA score	Oxygen support	SOFA score	Oxygen support	SOFA score	Oxygen support	SOFA score	Oxygen support
1	4	nasal cannula	2	nasal cannula	0	None	0	None	0	None	0	None
2	4	nasal cannula	0	None	0	None	0	None	0	None	0	None
3	7	MV 50%	8	Mechanical ventilation	7	Mechanical ventilation	5	Mechanical ventilation	6	Mechanical ventilation	2	Mechanical ventilation
4	7	nasal cannula	5	Mechanical ventilation	2	MV 40%	2	nasal cannula	2	nasal cannula	2	nasal cannula
5	7	MV 60%	5	nasal cannula	0	None	0	None	0	None	0	None
6	2	None	2	None	0	None	0	None	0	None	0	None
7	3	nasal cannula	4	Mechanical ventilation	2	nasal cannula	0	None	0	None	0	None
8	5	MV 35%	2	None	2	nasal cannula	2	nasal cannula	0	None	0	None
9	5	nasal cannula	6	Mechanical ventilation	4	MV 35%	3	nasal cannula	0	None	0	None

MV = Venturi Mask

Of the 4 remaining patients needing oxygen supply, all became free of need for oxygen administration within a median time of 9.5 days following CP infusion (5–28 days). Oxygen saturation improved consistently from pre-treatment levels through day 14 (median pre-treatment: 96%, day 7: 97%, day 14: 98%). (Fig. 1)

SOFA score after CP therapy decreased gradually to zero in 6/9 patients within a median time of 17.5 days (14–21 days), while in the remaining 3 patients it improved consistently within a median of 35 days. SOFA score significantly decreased on day 7 (2 vs 5, p = 0.047), on day 14 (2 vs 5 p = 0.011), on day 21 (0 vs 5, p = 0.017), and on day 28 compared to day 1 (0 vs 5, p = 0.027) as shown (Table 2, Fig. 1).

Changes in laboratory parameters after CP transfusion. Median lymphocyte count increased significantly after CP transfusion compared to baseline levels, starting on day 14 [median 2.10×10^9/lt vs 1.39×10^9/lt (p = 0.036), and remained significantly higher throughout days 21 and 28 [1.95×10^9/lt, p:0.035; and 2.165×10^9/l, p = 0.028, respectively). As shown in Fig. 1 and Table 3, several inflammatory markers including CRP, fibrinogen, LDH, and IL-6 decreased significantly on days 7, 14, 21, and 28 compared to baseline.

Table 3
Comparison of laboratory parameters before (day 1) and after (day 7 and day 14) CP transfusion

Parameters	Day 1: before CP transfusion	Day 7 after CP transfusion	Day 14 after CP transfusion	Day 21 after CP transfusion	P value
Lymphocytes, *(10^9/L, NR: 1.1- 4.0)*	1.39 (0.45–1.69)	1.53 (0.33–2.1)	2.10 (0.4–3.6)#	1.95 (0.98–2.14)**	0.036# 0.035**
Platelets, *(10^9/L, NR: 130–400)*	204 (133–522)	395 (343–572)*	433.5 (314–966)#	273 (217–298)**	0.008* 0.025# 0.025**
CRP, *(mg/L, NR: 0.00–6.00)*	23.1 (0.3–146)	4.135 (0.1–284)	2.025 (0.1–161)	NA	-
Fibrinogen, *(mg/dL, NR: 200–400)*	538 (334–1026)	495 (367–811)	330 (320–420)#	367 (282- 472.2)**	0.018# 0.036**
LDH, *(U/L, NR: 135–225)*	294 (162–354)	212 (173–467)	235 (138–303)	146 (140–235)**	0.012**
Ferritin, *(ng/mL, NR: 13–150)*	411 (264–1406)	600 (215–1848)	513 (86- 2413)	479 (313–847)	-
Intereukin-6, *(pg/mL, NR: < 7)*	34.6(18.8–70.6)	10.2 (1.5- 259.5)	5.0 (1.5–14)	NA	-
SaO_2, *(%, NR: ≥ 95)*	96 (93–98)	97 ((94–98)	98 (97–98)#	NA	0.011#
NA: not applicable					
*: p value for the comparison between Day 1 and Day 7.					
# : p value for the comparison between Day 1 and Day 14.					
**: p value for the comparison between Day 1 and Day 21.					

Increase of IgA and IgG antibodies and decrease of SARS-CoV-2 RNA

IgA and IgG antibody levels were determined at baseline and days 7, 14, 21, and 28 in 8/9 patients. All evaluable patients exhibited significant increases in SARS-CoV-2 IgA and IgG antibodies at days 7, 14, and 21 compared to pre-treatment levels (Fig. 2). Median IgA levels were: pre-treatment, 1.44 (0.48–7.75); day 7, 12.9 (5.34-45), p = 0.012); day 14, 28.75 (5.45–72.2), p = 0.008; day 21, 33.64 (5.56–69.2), p = 0.018, (all p values compared to baseline). Median IgG levels were: pre-treatment, 0.39 (0.2-8.0); day 7, 6.02 (0.81–39.32), p = 0.008; day 14, 9.585 (4.78–38.84), p = 0.012; day 21, 9.52(6.46–13.59), p = 0.018. A nonsignificant trend to increased IgA levels was observed on day 28 compared to day 1 [45.74 (4.11–56.4), p = 0.08], whereas IgG levels increased significantly [10.42 (7.79–13.4), p = 0.045]. Compared to day 7, IgG and IgA levels were significantly increased at day 14 (p = 0.038 and p = 0.015, respectively), while IgG was significantly increased also at day 21 (p = 0.018). Median time of detection of antibodies following CP infusion was 11 days from symptom onset (8–19 days). No significant correlation was found between levels of antibodies in the infused CP and recipient antibody levels on days 7, 14 and 21.

Concurrently, 9/9 patients showed reduction of SARS-CoV-2 RNA, as shown by increased Ct values at day 14 and day 21 compared to day 1 [day 14: 33.71 (26.99-45) p = 0.028, day 21: 45 (36.1–45) vs day 1 : 26.66 (19.22–37.53]. However, there was no significant correlation between the increase of SARS-CoV-2 antibodies and the reduction of viral RNA.

As shown in Table 4, 2/9 (22.2%) patients had undetectable SARS-CoV-2 RNA on day 7, while 4/9 (44.4%) had undetectable RNA on day 14 following CP infusion. Median time to PCR SARS-CoV-2 negativity was 14 days after CP infusion.

Table 4
Comparison of serum IgA and IgG antibodies and SARS-Cov-2 RNA status before and after (day 7 and day 14) CP transfusion.

	Day 1: before CP transfusion			Day 7 after CP transfusion			Day 14 after CP transfusion		
Patient no	Serum IgA antibodies	Serum IgG antibodies	Serum SARS-CoV-2 RNA load (Ct value)	Serum IgA antibodies	Serum IgG antibodies	Serum SARS-CoV-2 RNA load (Ct value)	Serum IgA antibodies	Serum IgG antibodies	Serum SARS-CoV-2 RNA load (Ct value)
1	0.49	0.2	19.22	6.74	6.02	29.26	> 14	12.29	26.99
2	7.75	8.0	29.23	> 14	12.29	22.98	> 14	11.60	Negative
3	1.66	0.42	24.32	> 14	3.62	28.99	> 14	7.09	27.14
4	0.48	0.28	24.26	NA	NA	29.13	NA	NA	31.2
5	NA	NA	NA	> 12	39.32	Negative	> 12	38.84	Negative
6	1.47	0.91	37.53	5.72	4.27	Negative	5.45	5.97	Negative
7	1.24	0.2	23.24	5.02	0.81	33.1	> 12	4.78	35.42
8	2.91	0.4	34.0	43.5	11.63	31.0	57.8	12.55	32.0
9	0.6	0.26	29.0	5.34	4.19	NA	5.59	6.6	Negative
NA: not available									

Outcomes

After a median follow-up of 66 days (IQR 59–70 days), 9/9 patients remain alive. Eight patients recovered completely and were discharged from hospital after a median hospital length of stay of 21 days (IQR, 15.75 days to 27.5 days). One patient with a history of pulmonary fibrosis was extubated and remains hospitalized in regular ward on low oxygen for 72 days, as of today.

No correlation was found between comorbidities and length of hospital or ICU stay or between the pre-treatment levels of antiSARS-CoV2 antibodies and disease severity.

Adverse events

No adverse events related to CP transfusion have been recorded in any patient throughout the follow-up period with the exception of a mild erythema in one patient following the infusion of the first CP unit resolved upon administration of antihistaminic drugs.

4. Discussion

In this preliminary report of a prospective multicenter study, we present initial safety and potential efficacy results of CP monotherapy administered within less than 10 days from symptom onset in 9 patients with at least grade 4 COVID-19 disease. Regarding clinical outcomes, all patients remain alive after a median follow-up of 66 days and have shown clinical improvement, while 8/9 patients have been discharged from the hospital and 4/4 requiring mechanical ventilation have been extubated. Moreover, length of hospital stay of 21 days in our study was similar to previous comparable studies (13) excluding ones that enrolled critically ill patients (such as mechanically ventilated patients) in which length of stay was substantially longer [8, 14].

Our data are in accordance with several previous small case series, where CP infusion resulted in clinical and laboratory improvement, as well as clearance of viremia in a substantial proportion of enrolled patients. However, in all previous case series, patients received combinations of antiviral and anti-inflammatory therapies including steroids in addition to CP infusion, thereby further limiting any conclusions as to the contribution of this treatment to the observed favorable outcomes [8, 9, 13]. On the other hand, the only randomized trial of CP as add-on therapy to standard treatment versus standard treatment alone, in patients with severe or life threatening COVID-19, has recently reported no significant effect of added CP therapy compared to standard treatment alone regarding time to clinical improvement during a follow up of 28 days [14]. However, several points have to be carefully considered, including the premature closure after approximately 50% of planned patients' enrollment, possibly rendering the study underpowered to detect any significant differences between the CP and control arms with respect to primary efficacy analysis. Importantly, however, in a subgroup analysis of the study that included only patients with severe but not critical disease, CP infusion resulted in a significantly higher percentage of clinically improved patients within 28 days and a significant rate of PCR negativity for the virus within 72 hours. This finding is strongly suggestive of the utility of CP therapy only if used early in the disease process.

The importance of early administration of CP treatment is further supported by another study of critically ill COVID-19 patients, which also showed no significant reduction of mortality with CP therapy [12]. Moreover, in the trial by Li *et al.*, the median interval between the onset of symptoms and

randomization was 30 days, which appears rather long overall [14]. However, there are no robust data in the literature about the optimal time of administration of CP for COVID-19, while significant variation exists between different studies. In the study by Salazar *et al.*, median time from symptom onset to transfusion was 10 days and from hospitalization to transfusion 2 days, similar to our study [13]. In the study by Duan *et al.* [9], median time from symptom onset to CP infusion was longer (16.5 days), while in the study of Shen et al. in critically ill patients, plasma was infused between 10 and 22 days after admission [8]. In our study, we have enrolled patients within no more than 10 days from symptom onset as per the protocol, taking into the consideration that CP treatment would reasonably be clinically useful only if used early.

Regarding the dose of CP and titer of antiviral antibodies in the CP, we did not use any cutoff value since there is no evidence from the literature about the optimal dose and titer of antibodies to maximize efficacy of CP infusion in COVID-19. In most clinical trials one to two units have been proposed for treatment. In the previously discussed randomized study, only the plasma units with a SARS-Cov-2-RBD–specific IgG titer of at least 1:640 were used at the dose of 4–14 ml/kg, while 95% of patients received a single dose of 200 ml [14]. In our study, larger volumes of 600–700 ml single donor CP were used for each patient, divided into 3 doses, aiming at maximizing the antibody activity of CP, as we did not use any cutoff values of the titer of antiviral antibodies and the titer of neutralizing antibodies was not determined.

Another important issue is the optimal time for CP collection from the donors. The durability of antibody response to SARS-CoV2 is not known but the data from SARS indicate that neutralizing antibodies are short-lived [16]. Therefore, the selection of donors for CP donation should include recently recovered patients that are expected to have high titers of antiviral neutralizing antibodies. In our trial the median time of plasma donation from the diagnosis of the disease was 43 days a time point where almost all patients are expected to have developed a durable antibody response [17].

In our study, we observed a significant decrease of laboratory parameters associated with disease severity early after CP infusion including CRP, IL-6, LDH and fibrinogen. Moreover, lymphocyte count significantly increased. Lymphopenia is a predictive biomarker of disease severity [18–19] and therefore, the observed increase of lymphocyte count may be suggestive of disease improvement following CP infusion.

Regarding IgG and IgA antiSARS-CoV2 antibodies, 5/9 patients had detectable antibodies before CP infusion. There was no correlation between the severity of the disease and the titer of antibodies at baseline, although there are some reports that support this association [20].

The median time of seroconversion in our trial was 11 days from symptom onset, in accordance with data from the literature for patients not treated with CP, in whom seroconversion time was reported as 13 days [20], 9–12 days [21], and 11 days following the onset of symptoms [17]. However the anti-SARS-Vo2 antibodies detected could be either donor or recipient derived and no clear conclusion can be drawn about true seroconversion time. A gradual significant increase of IgG and IgA antibodies titer was consistently observed in all patients following CP infusion, on days 7,14,21 compared to day 1 whereas on day 28 the anti-SARS-CoV2 IgA levels were not significantly different compared to day 1 and only IgG levels were still increased with a borderline significance. The early robust increase of anti-SARS-CoV2 levels followed by a moderate increase on day 28 suggests that the CP strongly contributes to the antibody response in addition to patients' intrinsic response. However no correlation was found between the level of antibodies in the transfused units and the level of anti-SARS antibodies on days 7,14, and 21.

Regarding the dynamics and type of antibody response in a report of 217 samples obtained from 32 patients the IgM antibody response occurred and peaked earlier than IgG, the IgM began to decline at week 3 while the IgG persisted and was maintained [22]. In addition in another report, of 285 patients three types of seroconversion were observed: synchronous detection of IgG and IgM, IgM detection earlier than IgG, and IgM detection later than IgG [20]. In our study, we observed a synchronous type of seroconversion however the contribution of either donors' or recipients' derived antibodies to this response cannot be determined .

The increase of anti-SARS-CoV-2 antibodies following the CP infusion was associated in our study with a reduction of the viral load as detected by the increased Ct values of the PCR analysis for viral RNA, although no linear correlation could be shown between anti-SARS-CoV-2 antibodies titer increase and viral load reduction. Within a median follow up of 66 days, PCR negativity was obtained at 14 days in 4/9 (44,4%) patients and the median time to PCR negativity was 14 days. This time is rather longer compared to the data from the literature. In the study by Shen *et al.*, 3/5 critically ill patients were PCR negative on day 3 and all patients were negative on day 12 following CP treatment [8]. In addition, in the study by Duan *et al.*, SARS-CoV-2 RNA was decreased to an undetectable level in all 7 patients by day 6 after its administration [9]. In the randomized trial, CP infusion was significantly associated with a negative conversion rate of viral PCR at 72 hours in 87.2% of the CP group vs 37.5% in the control group [14]. The longer time to PCR negativity in our trial may be attributed to the earlier administration of CP, which could possibly represent a higher viral load before infusion.

Regarding CP safety, no adverse events were observed following the infusion with the exception of a mild rash that resolved with antihistaminic drugs in one patient. There were no cases of transfusion related acute lung injury (TRALI) neither of transfusion associated circulatory overload possibly due to dividing the total dose of 600 ml of CP to 3 doses. No cases of antibody dependent enhancement (ADE) were reported in accordance with previous reports [8–9, 11, 13–14]. ADE represents a well-recognized effect in many viral illnesses [23–24], has been described in human clinical dengue [25] and is characterized by the facilitation of viral entry into the cells by antibodies or the enhancement of viral toxicity by antibodies.

Our study has limitations. First, it is a nonrandomized uncontrolled study, which precludes any definite conclusions regarding efficacy of examined therapy. However, due to the severity of cases, patient randomization to CP infusion- a therapeutic strategy known to offer benefit in previous epidemics- versus no treatment would not be considered ethical, especially when dealing with a life threatening infection. In view of the encouraging

preliminary findings reported herein, the study is planned to continue in order to include a planned number of 100 patients, who will be compared to an historical control group of age-, comorbidities- and COVID-19- severity matched patients.

In conclusion, this preliminary report suggests that CP infusion monotherapy administered early in the disease course could be a safe and possibly an effective strategy for patients with severe COVID-19 disease, resulting in symptomatic and disease severity-related laboratory improvement, prompt increase of antiviral antibody levels and reduction of viral load.

Abbreviations

SARS; Severe acute respiratory syndrome

SARS-Cov-2; Severe acute respiratory syndrome coronavirus 2

IgA; ImmunoGlobulins A

IgG; ImmunoGloboulins G

IgM; ImmunoGlobulins M

RNA; ribonucleic acid

MERS; Middle East respiratory syndrome

H1N1; Hemagglutinin Type 1 and Neuraminidase Type 1

ECMO; Extracorporeal membrane oxygenation

PCR; Polymerase Chain Reaction

RT-PCR; Reverse transcription polymerase chain reaction

ABO; Blood group system consisting of groups A, AB, B and O

SOPs; Standard Operating Procedures

ELISA; Enzyme-linked immunosorbent assay

IBM SPSS; IBM Software for Statistical analysis

mg/Kg; Milligram Per Kilogram

SOFA; Sequential Organ Failure Assessment

CRP; C-reactive Protein

LDH; Lactate Dehydrogenase

IL-6; Interleukin-6

RBD; Receptor-binding domain

Declarations

Ethics approval and consent to participate

This study is a multicenter phase II trial (identifier number NCT04408209), conducted at 5 hospitals in Athens. The study conforms to the principles outlined in the Declaration of Helsinki and it was approved by all Institutional Review Boards of participating hospitals.

- Institutional Review Board of “Attikon” University General Hospital; ref. number: 185/ 14-4-20
- Institutional Review Board of “Evangelismos” General Hospital; ref. number: 162/ 23-4-20
- Institutional Review Board of “St Savvas” Oncology Hospital; ref. number: 12843/ 22-4-20
- Institutional Review Board of “Sotiria” General and Chest Diseases Hospital of Athens; ref. number: 10647/ 21-4-20
- Institutional Review Board of “Alexandra” General Hospital; ref. number: 245/ 14-4-2020.

All patients provided written informed consents to participate in the study.

Consent for publication

Written Consent for study publication was obtained from all participants.

Availability of data and material

Row data are available upon request

Competing interests

All authors have nothing to disclose.

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Author contributions

V.P.: contributed to the literature review, study design, data collection, analysis and interpretation, and writing the manuscript.

M.P.: contributed to the literature review, study design and interpretation

S.G.P.: contributed to the literature review, study design, data collection, analysis and interpretation, and writing the manuscript.

A.A., A.K., A.B., M.P., E.K., and G.P. contributed to data collection, analysis and interpretation.

E.G., A. M., K. S., C.M., G.P., A.M., S.T. and E.T. contributed to study design, data analysis and interpretation.

M-A. D.: contributed to study design, data analysis and interpretation and writing the manuscript

All authors have read and approved the manuscript.

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Not Applicable

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Figures

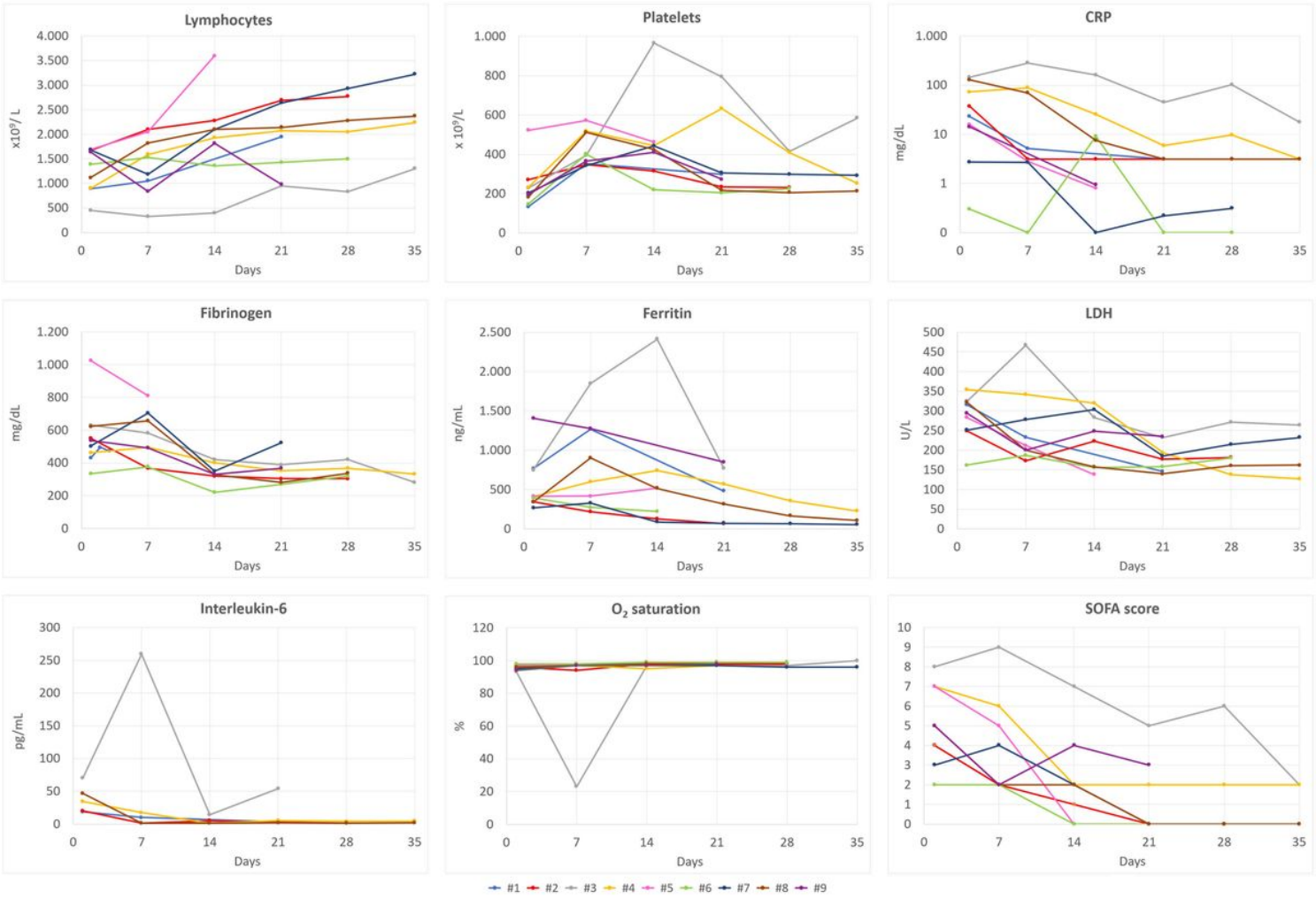

Figure 1

Laboratory parameters, Oxygen Saturation and SOFA score before and after the infusion of CP

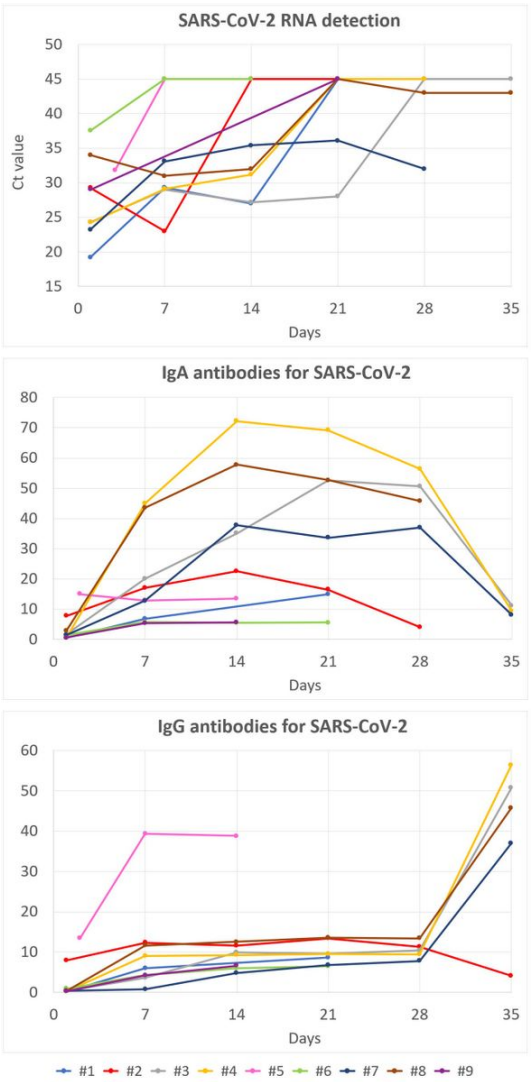

Figure 2

Ct values of real time PCR for SARS-CoV-2 RNA of the nasopharyngeal swab and titers of IgA and IgG anti-SARS-CoV-2 antibodies before and after the infusion of CP

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable1.doc
- CONSORTextensionforPilotandFeasibilityTrialsChecklist.doc