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Research Paper

Compassionate use of convalescent plasma for treatment of moderate and severe pneumonia in COVID-19 patients and association with IgG antibody levels in donated plasma

Yasmin Maor[a,b], Daniel Cohen[c], Nir Paran[d], Tomer Israely[d], Vered Ezra[e], Ofra Axelrod[f], Eilat Shinar[g], Marina Izak[g], Galia Rahav[h,b], Naomi Rahimi-Levene[i,b], Baruch M Bazofin[j], Ram Gelman[k,l], Dror Dicker[b,m], Tal Brosh-Nissimov[n,o], Orli Megged[p], David Dahan[q], Avi Benov[l,r], Alona Paz[s], Kaykov Edward[t], Amit Moran[u], Ori Rogowski[v,b], Patrick Sorkine[w], Ami Mayo[x], Oren Zimhony[y,*], Jacob Chen[e,l,**]

[a] Infectious Disease Unit, Wolfson Medical Center, Holon, Israel
[b] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[c] School of Public Health, Tel Aviv University, Tel Aviv, Israel
[d] Department of Infectious Diseases, Israel Institute for Biological Research, Ness Ziona, Israel
[e] Medical directorate, Ministry of Health, Jerusalem, Israel
[f] Ministry of Health, Jerusalem, Israel
[g] Magen David Adom, National Blood Services, Ramat Gan, Israel
[h] Infectious Diseases Unit, Sheba Medical Center, Ramat Gan, Israel
[i] Shamir (Assaf Harofeh) Medical Center, Medical Director of the Blood Bank, Zerifin 703301, Israel
[j] Department of Anesthesiology and Critical Care Medicine, Hadassah Hebrew University Medical Center, Jerusalem, Israel
[k] Department of Medicine, Hebrew University-Hadassah Medical Center, Jerusalem, Israel
[l] Israel Defense Forces, Medical Corps, Tel Hasomer, Ramat Gan, Israel
[m] Internal Medicine D and Obesity Clinic Hasharon Hospital-Rabin Medical center, Petah Tiqva, Israel
[n] Infectious Diseases Unit, Assuta Ashdod University Hospital, Ashdod, Israel
[o] Faculty of Health Sciences, Ben-Gurion University of the Negev, Be'er-Sheva, Israel
[p] Pediatric Infectious diseases Unit, Shaare Zedek Medical Center, Jerusalem, Israel
[q] Respiratory ICU, Meir Medical Center, Kfar Saba, Israel
[r] The Azrieli Faculty of Medicine, Bar-Ilan University, Safed, Israel
[s] Infectious Unit, Bnei Zion Medical Center, Haifa, Israel
[t] Department of Geriatric Medicine, Galilee Medical Center, Naharia, Israel
[u] Department of head and neck surgery, the University of Texas, MD Anderson Cancer Center, Houston, Texas USA
[v] Department of Internal Medicine "C" Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
[w] General Intensive Care Unit, Mayanei Hayeshua Medical Center, Bnei Brak, Israel
[x] Department of Critical Care Medicine, Assuta Ashdod University Hospital, Ashdod, Israel
[y] Infectious Diseases Unit, Kaplan Medical Center, Rehovot, Israel, affiliated to the School of Medicine, Hebrew University and Hadassah, Jerusalem, Israel

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ABSTRACT

Background: We assessed outcome of patients with moderate and severe COVID-19 following treatment with convalescent plasma (CP) and the association with IgG levels in transfused CP.
Methods: A prospective cohort study. Primary outcome was improvement at day 14 defined as alive, not on mechanical ventilation, and moderate, mild, or recovered from COVID-19. Antibody levels in CP units were unknown at the time of treatment. IgG against the spike protein S1 was subsequently measured by ELISA. Neutralizing antibodies titers were determined in a subset. Outcome was assessed in relation to the mean antibody level transfused to the patients ($\leq$4.0 versus $>$4.0).
Findings: Of 49 patients, 11 (22.4%) had moderate, 38 (77.6%) had severe disease, 28 were ventilated. At day 14, 24 (49.0%) patients improved, 9 (18.4%) died, and 13 (26.5%) were ventilated. In 14/98 (14.3%) CP units

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* Corresponding author.
** Corresponding author at: Medical directorate, Ministry of Health, Jerusalem, Israel. 39 Yirmiyahu Street, Jerusalem 9446724, Israel.
E-mail addresses: oren_z@clalit.org.il (O.t.Z.s.e.c.t.t.a.t.w.D.C.T.s.b.a.". Zimhony), Jakob.chen@moh.gov.il (J. Chen).

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IgG was < 1.1 (cutoff calibration) and in 60 (61.2%) ≤4.0. IgG level and neutralizing antibody titer were correlated (0.85 $p < 0.001$). In patients receiving ≤4.0 antibody levels, 11/30 improved (36.7%) versus 13/19 (68.4%) in patients receiving >4.0 odds ratio (OR) 0.267 [95% confidence interval (CI) 0.079−0.905], $P = 0.030$. In patients diagnosed >10 days prior to treatment, 4/14 (22.4%) improved in the ≤4.0 antibody group, versus 6/7 (85.7%) in the >4.0 antibody group, OR 0.048 (95% CI, 0.004−0.520), $P = 0.007$. No serious adverse events were reported.

Interpretation: Treatment with CP with higher levels of IgG against S1 may benefit patients with moderate and severe COVID-19. IgG against S1 level in CP predicts neutralization antibodies titers.

Research in context

Evidence before this study

Previous studies suggested that convalescent plasma (CP) may be effective in improving survival rates in emerging viral infections, including Corona related SARS-CoV-1 infection (SARS) and Middle East respiratory syndrome (MERS). The benefit of CP for COVID-19 has not been established and was short of statistical significance in a small randomized study of severe and critical COVID-19 patients.

Added value of this study

This study demonstrated that in patients treated with CP the rate of improvement was significantly higher in patients who received higher IgG antibody titers against the spike protein, compared to patients who received CP with lower IgG titers. Treatment with higher IgG antibody titers was effective also in patients diagnosed more than 10 days prior to receiving treatment. Neutralizing antibodies titers were highly correlated to IgG against the S1 spike protein.

Implications of all the available evidence

Treatment with CP may benefit patients with moderate and severe COVID-19. The odds of success of CP therapy are in correlation with higher IgG levels against the spike protein which serves as a surrogate marker for neutralizing antibodies.

1. Introduction

Coronavirus disease 2019 (COVID-19) is a highly infectious pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of December 2019, it spread globally from Wuhan, China and on March 2020 was declared a pandemic outbreak [1].

Until May 21, 2020, SARS-CoV-2 infected more than 5 million people, in over 212 countries and territories, and resulted in more than 328,462 deaths [2]. By May 21, more than 16,670 people were infected in Israel, and 279 people have died [2].

In the absence of effective and recommended established therapy, treatment of COVID-19 to date has mainly been empirical and experimental in addition to meticulous supportive care. Recent observational and randomized studies involving patients with COVID-19 admitted to the hospital demonstrated mixed results regarding the efficacy of various antiviral and antimalarial drugs [3−6].

Remdesivir, a viral RNA polymerase inhibitor which was shown to shorten disease duration was recently approved by the Federal Drug Administration (FDA) [5,6]. However, it is likely that the global increasing demands and shortage of resources will preclude this promising anti-viral agent from many patients, globally.

Past studies demonstrated that convalescent plasma (CP) may be effective in improving survival rates in emerging viral infections, including Machupo virus (Bolivian hemorrhagic fever) [7], Juninvirus (Argentinian hemorrhagic fever) [8], Lassa fever [9], and Ebola virus [10]. CP therapy has been used to treat patients with SARS-CoV-1 infection (known also as SARS), Middle East respiratory syndrome (MERS), with promising results [11,12].

Currently, there are two reports from China regarding the use of CP for COVID-19 patients [13,14]. In a preliminary uncontrolled case series of five critically ill patients with COVID-19 and acute respiratory distress syndrome (ARDS), administration of CP containing neutralizing antibodies was followed by weaning off mechanical ventilation, reduction in viral loads, improved oxygenation and clinical stabilization [13]. A recent pilot study of 10 patients with severe COVID-19, described the usage of one 200 ml unit of CP with neutralizing antibody titers at or exceeding a 1:640 dilution. There were no serious adverse events in the recipients and all 10 patients had improvement in symptoms within 1−3 days of transfusion, with demonstrated radiological improvement in pulmonary lesions [14]. A small randomized study [15] that included patients with either severe or critical COVID-19 patients showed limited improvement with CP which was observed only for patients with severe rather than critical disease. The perceived benefit was short of statistical significance [15,16]. A systematic review of five small clinical studies summarizing the results of CP transfusion to critically ill COVID-19 patients concluded that CP appears to be safe and effective although the data base was small and the magnitude of effect was difficult to assess [17]. Despite some methodological limitations of these previous reports, and the small number of patients included, the data suggests the possibility of clinical benefit related to CP therapy.

The aim of this study was to describe the clinical outcome associated with CP transfusions for patients with moderate and severe COVID-19 in relation to antibody titer in the CP transfused. CP therapy was provided through a national compassionate program in Israel.

2. Methods

A prospective cohort study including the first 49 patients included in the cohort.

2.1. Patients

Physicians from all hospitals in Israel were eligible to apply for CP though a national compassionate use program. All patients who received CP during this program from its initiation on April 6th, 2020 to May 2nd, 2020 are included in this analysis.

The study was approved by the ethics committee of the Israeli Ministry of Health (0083-20-WOMC). To be included patients or their legal representatives signed informed consent forms.

Severity of COVID-19 was defined as follows:

- Patients with severe disease: patients with severe COVID-19 pneumonia, and/or in shock and/or requiring hemodynamic support, and/or requiring mechanical ventilation and or oxygen saturation at room air <90%.

- Patients with moderate disease: patients with respiratory rate> 30/minute and/or oxygen saturation at room air <93% and 90%.

Inclusion criteria for CP treatment were all the following:

- Diagnosis of COVID 19 confirmed by detection of SARS-CoV-2 RNA by real-time (RT) -PCR obtained from the nasopharynx or from deep suction.
- Lung infiltrates demonstrated by chest X-ray or computed tomography >50% of lung fields.
- Moderate or severe COVID-19.
- Informed consent.

Exclusion criteria were

- Age < 18 years.
- Time from symptom onset or diagnosis of COVID-19 by PCR >40 days.
- Neutropenia < 500.
- Active bacterial infection.
- Life expectancy due to other diseases less than 6 months.

A committee of three senior physicians reviewed all CP requests and approved them according to these criteria. Patients could receive antivirals but not remdesivir, and immune modulator drugs at the discretion of the treating physicians.

2.2. Donors of convalescent plasma

The National Blood services of Magen David Adom in Israel collected CP from volunteer donors who had recovered from COVID-19 according to the following criteria:

- Proven past illness with SARS-CoV-2 RNA by real time RT −PCR
- Recovery from SARS-CoV-2 defined as resolution of fever, respiratory complaints, and all other symptoms related to SARS-CoV-2 for at least 48 h, and two consecutive nasopharyngeal samples negative for SARS-CoV-2 RNA by real time RT −PCR tests that were taken at least 24 h apart
- Elapse of > 14 days since the last negative RT −PCR test.

All donors gave their informed consent to the donation through plasmapheresis procedure and had to meet the Israeli Ministry of Health standard criteria for plasma donation, which include seronegativity for Hepatitis B, C, HIV, HTLV and syphilis. Plasma was collected by apheresis procedure that was performed using a MCS+ 9000 (HAEMONETICS, USA). A 600 ml plasma volume was collected from each donor, which was further divided into three 200 ml plasma units and immediately stored at −30°C. At the time the study was conducted we did not have the ability to measure IgG level in the donated CP prior to transfusion to patients. Therefore, plasma aliquots from each donor were initially frozen at −30 °C for further evaluation for the presence of IgG against S1 and titer of neutralizing antibodies against SARS-CoV-2, following therapy with those CPs.

2.3. Determination of IGG level for S1 spike protein and neutralizing antibodies titer to SARS-CoV-2 in the donated plasma

The levels of serum IgG against the spike protein of SARS-Cov-2 were measured by an enzyme-linked immunosorbent assay (ELISA) using a validated commercial kit (Euroimmun AG, Luebeck, Germany, product number EI 2606-9601G), following the manufacturer's instructions. The assay detects SARS-CoV-2 IgG antibodies against the S1 domain of viral spike protein. The assay relies on an assay specific calibrator to report a ratio of specimen absorbance to calibrator absorbance. The final interpretation of positivity is determined by ratio above a threshold value. The assay is interpreted as positive when the ratio is ≥1.1. A larger ratio represents higher antibody levels [18,19]. To document the reliability of the IgG level readout, we determined the inter-assay variation of the calibrator, positive control, and negative control optical densities (ODs) in 15 different assays run in the same day or on different days. These measurements yielded the following mean, standard deviation and coefficient of variation values: mean=0.27, standard deviation (SD)=0.020, coefficient of variation (CV)=6.7% for calibrator; mean=0.8, SD=0.110, CV=13.8% for the positive control and mean=0.03, SD=0.002, CV=5.4% for the negative control. We also calculated the mean and standard deviation of the IgG final ratio in 25 serum samples tested on different days: mean=1.12, SD=0.230.

2.4. Serum neutralization assay of CP

Determination of neutralizing antibody titer in sera was conducted by plaque reduction neutralization test [20] with the following modifications: Vero E6 cells (ATCC® CRL-1586™) as target cells and SARS-CoV-2 virus were used. Vero E6 Cells were obtained from the American Type Culture Collection (Summit Pharmaceuticals International, Japan) and maintained according to the manufacturer guidelines. SARS-CoV-2 virus (GISAID accession EPI_ISL_406,862) was kindly provided by the Bundeswehr Institute of Microbiology, Munich, Germany. Virus titer was determined by plaque assay using Vero E6 cells and was conducted in a BSL3 facility in accordance with biosafety guidelines. Briefly Vero E6 cells in 12-well plates. Heat inactivated (56 °C, 20 min) serum samples were 2-fold diluted in MEM and incubated with equal volume of SARS-CoV-2 virus (150 pfu/ml) followed by infection of Vero E6 Monolayers in triplicate. Plaques were counted and $PRNT_{50}$ was determined as the dilution which reduced the number of plaques by at least 50% compared to control non-neutralized virus.

2.5. Treatment protocol

Frozen CP units were transported to the relevant hospital and were transfused to the patients after thawing, according to standard procedure. IgG antibody levels and neutralizing antibody titers were not known at the time of plasma administration and were measured later when these tests became available.

The first dose of 200 ml CP was transfused on the day of request (day 1), and if it was well tolerated it was followed 24 h later by a second unit of 200 ml. The second CP unit was sometimes from the same donor and at times from different donors according to availability.

2.6. Data collection

Data were collected prospectively from the various physicians using a predefined case report form.

2.7. Outcome measures

The primary outcome was the rate of improvement at day 14 after administration of the CP as compared between patients who received CP with mean antibody level in the two units ≤4.0 compared to the rate of improvement in patients who received CP units with a mean antibody level >4.0 as assessed by the Euroimmun ELISA kit. Improvement was defined as being alive, no need for mechanical ventilation, and change to moderate or mild COVID-19 clinical severity or recovered. Non-improvement was defined as death, or being on mechanical ventilation, or deteriorating clinical status.

Secondary outcomes were the rate of clinical improvement in the subgroups: patients with moderate disease severity at enrollment, patients with severe disease at enrollment, and patients requiring

mechanical ventilation at enrollment. We also assessed rates of improvement at various antibody levels.

Serious adverse events including anaphylaxis, other allergic reaction, and transfusion related acute lung injury were documented.

2.8. Statistical analysis

Analysis was performed using SPSS software version 25. We calculated the antibody ratio given to patients by calculating the mean ratio of IgG in the two CP units given to each patient. Descriptive statistics [mean, median, standard deviation (SD)] and interquartile range (IQR)] were used as appropriate. To assess correlations between antibody levels we used geometric means and the Pearson correlation. For continuous variables comparisons were done using the Mann-Whitney *U test*. For sample size 40 or less the exact significance was reported.

Categorical data was compared using Chi square or Fisher's exact test as appropriate. Significance was set at $p < 0.05$. Mantel-Haenszel was used to assess odds ratio (OR) and 95% confidence intervals (CI).

We assessed factors independently associated with improvement at day 14 using logistic regression. Independent variables found to be significantly associated with the dependent variable in a univariate analysis ($p < 0.1$) were entered into multivariate binary logistic regression analysis, (backwards conditional, P for entry <0.05, P for removal <0.10), with results presented as OR with a confidence interval (CI) of 95%. Statistical significance was set at $p < 0.05$.

Role of funding source: The study did not receive funding and relied on internal resources of the participating centers.

3. Results

3.1. Patients

Of 57 requests for CP, 54 (94.6%) applications were approved according to the inclusion and exclusion criteria (Fig. 1). Five patients were excluded from this analysis due to missing data that precluded the assessment of these patients. Thus, 49 patients comprise the cohort described here. Characteristics of patients are presented in Table 1. Thirty-five (71.4%) were males. The median age was 64.0 years (IQR 50.5–76.0). About a third had diabetes and hypertension. Clinical presentation was typical to COVID-19 infection and included fever in 69.4% of the patients, and cough in 83.7%. Eleven patients (22.4%) had moderate disease, and 38 (77.6%) had severe disease. In patients with severe disease, 28 (73.7%) were on mechanical ventilation at the time of recruitment to the study, (Fig. 1). The median time from PCR diagnosis to CP transfusion was 10.0 days (IQR 4.0–14.0). In 25 patients (51.0%), 10 days or more elapsed from PCR diagnosis to CP administration.

3.2. Antibody levels in CP units

The median level of antibody per donated CP unit was 3.15 (IQR 1.60–4.80, range 0.10–12.30). In 14 of 98 CP units (14.3%) antibody level was below 1.1 (the cutoff) and in 46 CP units (46.9%) antibody level was above 1.1 and below 4.0. Neutralizing antibodies activity was determined in 53 CP units (55.2%) from 29 donors. The median titer was 1:160 (IQR 1:160–1:640, range 1:20 to 1:2560). Neutralizing antibody titer was below the threshold of 1:160 in 8 CP units (15.1%) and equal or above 1:160 and below 1:640 in 31 CP units (58.5%). Moreover, taken as a continuous variable, the IgG ratio

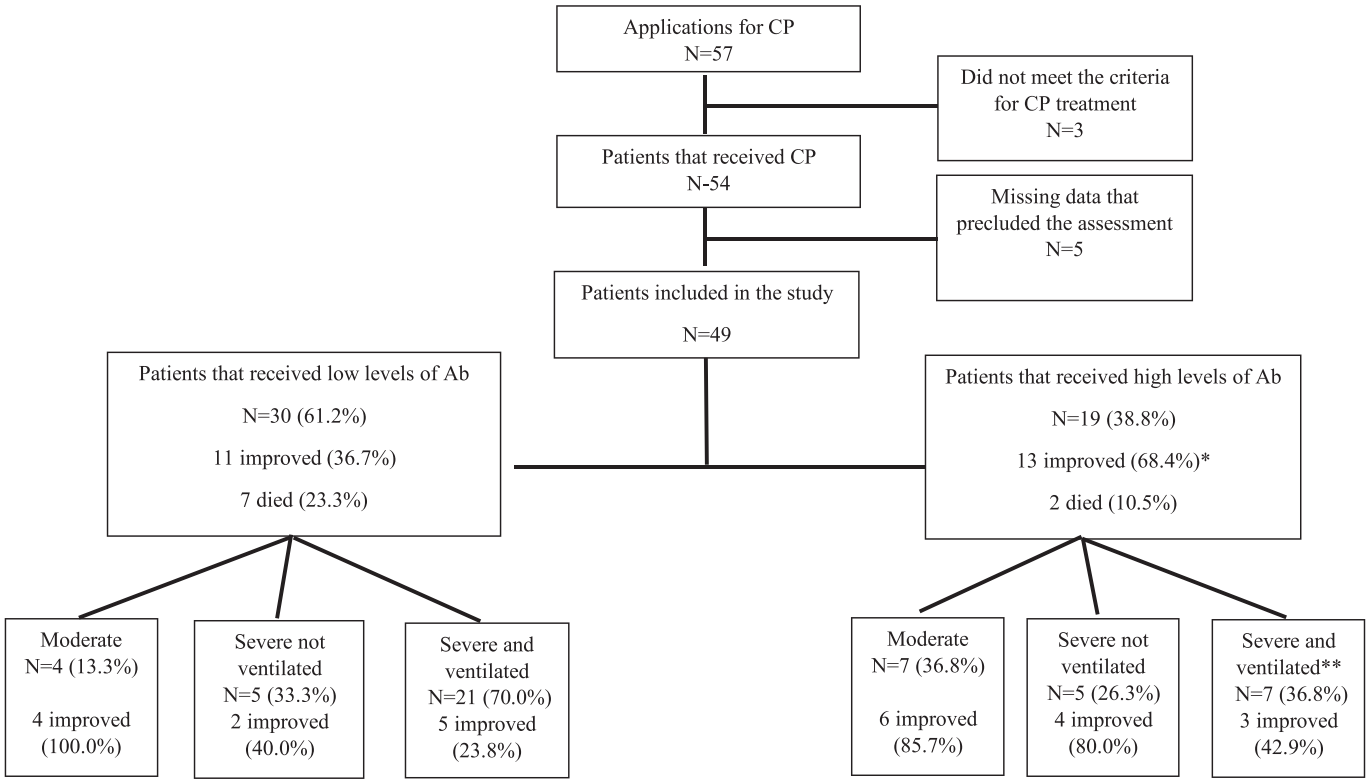

Fig. 1. Outcome in patients according to disease severity prior to CP transfusion and IgG against S1 level (≤4.0 or >4.0 of the calibration ratio) in CP

Patients were excluded from the CP program according to exclusion and inclusion criteria.

Patients were divided according to the mean antibody (Ab) level they received (≤4.0 or >4.0). Improvement was defined as being alive, not on mechanical ventilation and moderate or mild disease severity or recovered. Non improvement was defined as being dead or on mechanical ventilation or clinical deterioration.

*$P = 0.030$, **$P = 0.062$. CP – convalescent plasma.

Table 1
Patients' characteristics.

	All patients *N* = 49	Patients with severe disease N-38 (77.6%)	Patients with moderate disease *N* = 11 (22.4%)
Males – no. (%)	35 (71.4)	30 (78.9)	5 (45.5)
Age (years) – median (IQR)	64.0 (50.5–76.0)	64.0 (53.5–76.0)	59.0 (42.0–71.0)
Blood types – no. (%)			
O	21 (42.9)	17 (44.7)	4 (36.4)
A	24 (49.0)	18 (47.4)	6 (54.5)
B	1 (2.1)	1 (2.6)	0
AB	3 (6.1)	2 (5.3)	1 (9.1)
Times from positive SARS-CoV-2 PCR to receiving CP (days) – median (IQR)	10.0 (4.0–14.0)	10.0 (5.0–14.3)	6.0 (2.0–12.0)
Co-morbidity			
Obesity – no. (%)	16 (32.7)	10 (26.3)	6 (54.5)
Diabetes – no. (%)	15 (30.6)	13 (34.2)	2 (18.2)
Hypertension – no. (%)	18 (36.7)	13 (34.2)	5 (45.5)
Ischemic heart disease –no. (%)	7 (14.3)	6 (15.8)	1 (9.1)
Chronic lung disease –no. (%)	8 (16.3)	6 (15.8)	2 (18.2)
Chronic renal failure –no. (%)	7 (14.3)	6 (15.8)	1 (9.1)
Malignancy – no. (%)	6 (12.2)	4 (10.5)	2 (18.2)
Clinical symptoms at presentation			
Fever>38.0 °c – no. (%)	34 (69.4)	26 (68.4)	8 (72.7)
Cough – no. (%)	41 (83.7)	32 (84.2)	9 (81.8)
Muscle aches – no. (%)	12 (24.5)	9 (23.7)	3 (27.3)
Headache – no. (%)	9 (18.4)	5 (13.2)	4 (36.4)
Diarrhea – no. (%)	6 (12.2)	6 (15.8)	0
Vomiting – no. (%)	5 (10.2)	3 (7.9)	2 (18.2)
Clinical status prior to CP transfusion			
Ventilated – no. (%)	28 (57.1)	28 (73.7)	0
Fever – mean (SD)	37.7 (1.1)	37.6 (1.2)	39.4 (1.0)
MAP (mmHg) – mean(SD)	88.9 (19.5)	87.6 (21.6)	93.2 (10.6)
Laboratory results			
White blood cells (x10^3/liter) – median (IQR)	12.2 (5.4–17.2)	14.2 (8.8–17.6)	5.2 (3.3–6.4)
Absolute lymphocytes (x10^3/liter) – median (IQR)	0.780 (0.475–0.995)	0.715 (0.500–1.000)	0.800 (0.350–0.925)
Platelets (x10^3/liter) – median (IQR)	222.0 (146.3–350.5)	263.5 (149.5–358.0)	181.5 (132.3–254.3)
Serum creatinine (μmol/liter) mg/dL– median (IQR)	0.89 (0.70–1.20)	0.90 (0.67–1.31)	0.89 (0.70–1.03)
Aspartate aminotransferase (AST) (U/liter) – median (IQR)	44.5 (28.0–61.3)	48.0 (33.5–70.5)	22.7 (18.0–51.0)
Alanine aminotransferase (ALT) (U/liter) – median (IQR)	36.0 (20.0–65.8)	46.0 (24.0–80.5)	20.0 (14.0–43.0)
Lactate dehydrogenase (LDH) (U/liter) – median (IQR)	544.5 (402.0–737.5)	590.0 (424.0–739.0)	412.0 (321.0–507.0)
Creatinine kinase (CK) (mg/deciliter) – median (IQR)	109.0 (51.5–274.3)	181.0 (54.5–338.8)	87.0 (30.3–157.0)
C reactive protein (CRP) (mg/deciliter) – median (IQR)	13.6 (4.4–23.3)	13.6 (3.7–23.3)	12.0 (4.4–20.7)
D-dimers (μg/milliliter) – median (IQR)	1549.0 (728.8–5113.5)	1990.0 (792.0–8368.0)	819.0 (682.0–1660.0)
Ferritin ng/mL– median (IQR)	682.8 (275.0–1500.0)	922.9 (461.5–1527.0)	228.3 (128.3–924.3)

The values shown are based on available data. Temperature was available for 42 patients, MAP (mean arterial pressure) was available for 38 patients. Laboratory values for white-cell count, lymphocyte count, platelet count, AST and ALT were available for 46 patients, LDH for 46 patients, CK for 36 patients, CRP for 38 patients, D-dimers for 30 patients, and ferritin for 43 patients. SD denotes standard deviation. Patients with severe disease requiring mechanical ventilation and/or had ARDS and/or were in shock and/or required hemodynamic support. Patients with moderate disease did not qualify for severe disease definitions and had a respiratory rate> 30/minute, and/or room air oxygen saturation<93%.
IQR – interquartile range, SD – standard deviation.

determined by the commercial kit yielded a 0.85 Pearson r correlation ($p < 0.001$) with the plaque reduction neutralization test ($PRNT_{50}$) titers after logarithmic transformation of both variables.

3.3. Clinical outcome

The composite outcome of improvement (defined as being alive, not on mechanical ventilation and moderate or mild disease severity or recovered) was attained in 24/49 patients (49.0%) by day 14, (Fig. 1 and Table 2). At this time, nine patients (18.4%) died and 13 (26.5%) were on mechanical ventilation and three were in severe condition but not ventilated. Of 38 patients with severe disease, 14 (36.8%) improved, nine (23.7%) died, 13 (34.2%) were on mechanical ventilation and two (5.3%) were in severe condition without ventilation. Of 28 patients (57.1%) that were on mechanical ventilation at the time of CP transfusion eight (28.6%) improved, seven (25.0%) died, 12 (42.9%) remained on mechanical ventilation, and one (3.6%) remained in severe condition but was not ventilated. In the group of patients with moderate disease, 10 patients (90.9%) improved, one patient (9.1%) deteriorated to severe condition but did not require mechanical ventilation. There were no deaths in this group.

Table 2
Clinical improvement in correlation to the mean antibody level in the transfused CP.

	Mean IgG to S1 level ≤4.0	Mean IgG to S1 level >4.0	OR (95% CI)	P
All Patients *N* = 49	11/30 (36.7%)	13/19 (68.4%)	0.267 (0.079–0.905)	0.030
Severe Disease *n* = 38 (77.6%)	7/26 (26.9%)	7/12 (58.3%)	0.263 (0.062–1.109)	0.062
Severe disease, mechanical ventilation *n* = 28 (73.7%)	5/21 (23.8%)	3/7 (42.9%)		
Severe disease non ventilated *n* = 10 (26.3%)	2/5 (40.0%)	4/5 (80.0%)		
Moderate disease *n* = 11 (22.4%)	4/4 (100.0%)	6/7 (85.7%)		

Outcome was assessed 14 days after CP transfusion. Improvement was defined as being alive, not on mechanical ventilation and moderate or mild disease, or recovered. Patients that did not improve were defined as being dead, or on mechanical ventilation, or deteriorating.
CP – convalescent plasma. OR – odds ratio, CI – confidence interval.

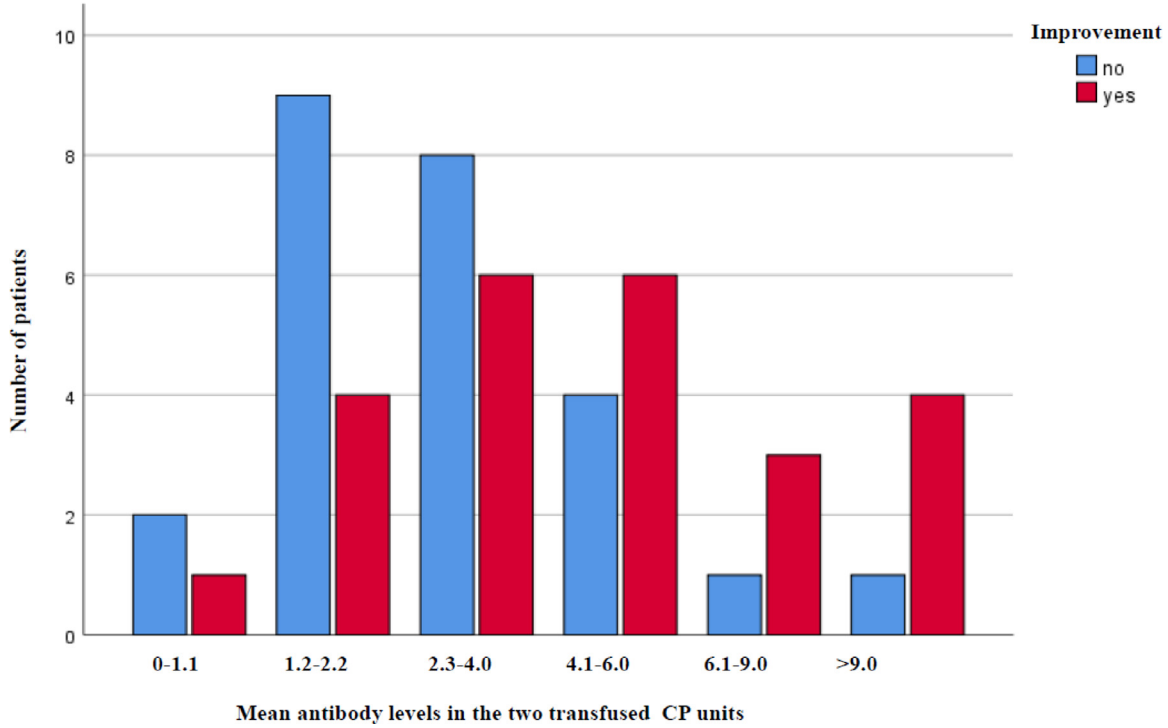

Fig. 2. Correlation between clinical improvement in patients and IgG against S1 levels in transfused CP.
CP – convalescent plasma.

3.4. Mean IGG against S1 in transfused CP and outcome

In the group of patients that received CP with a mean antibody level ≤4.0, 11 of 30 patients improved (36.7%) versus 13 of 19 patients (68.4%) in the group of patients that received CP with a mean antibody level > 4.0, OR 0.267 (95% CI 0.079–0.905), $P = 0.030$, Table 2. A similar pattern was observed in severe patients. Seven of 26 patients (26.9%) improved in the group that received CP with low antibody levels versus 7 of 12 (58.3%) in the group that received CP with high antibody levels, OR 0.263 (95% CI 0.062–1.109), $P = 0.062$. In severe patients that were ventilated at recruitment improvement was observed in 5 of 21 patients (23.8%) versus 3 of 7 (42.9%), respectively and in severe patients not requiring mechanical ventilation at the time of recruitment 2 of 5 improved (40.0%) versus 4 of 5 (80.0%), respectively. In patients with moderate severity of disease all improved in the group receiving CP with low antibody level versus 6 of 7 (85.7%) in the group receiving CP with high antibody level (Fig. 2).

The correlation of clinical outcome with neutralizing antibodies activity in transfused CP units was available only for 29 patients and was not as conclusive (Fig. 3).

There was an association between patients' improvement and antibody levels in correlation to the time that elapsed from diagnosis of COVID-19 (Figs. 4 and 5). In patients that received CP transfusion 10 days or less after the diagnosis, 7 of 12 improved (58.3%) in both groups (antibody titer ≤4.0 versus antibody titer >4.0). In patients that received the CP more than 10 days after diagnosis 4 of 18 (22.2%) improved in the group with low antibody titers versus 6 of 7 (85.7%) in the group that received high antibody titers, OR 0.048 (95% CI 0.004–0.520), $P = 0.007$.

3.5. Logistic regression for variables in association with clinical improvement

We identified four variables associated with improvement at day 14, (Table 3) using univariate analysis: female gender, severity of illness, D-Dimers, and antibody levels in CP.

In a multivariable logistic regression analysis of these variables only antibody level in administered CP and D-dimer were independently associated with improvement at day 14 (Table 3). Results were similar when antibody levels were entered into the model as a binary variable (≤4.0 versus >4.0), data not shown.

3.6. Adverse events

No serious adverse events were reported in this cohort. One patient developed a rash that responded to antihistamine therapy.

4. Discussion

This study suggests that higher IgG levels against S1 of SARS-CoV-2 in CP may confer improved outcome to patients with COVID-19. We demonstrated that the rate of clinical improvement at day 14 of patients who received CP with IgG above 4.0 was significantly higher compared to patients that received CP with lower IgG levels [68.4% versus 36.7%, OR 0.267 (95% CI 0.079–0.905), $p = 0.030$]. Moreover, there was a dose response correlation between improved patients' outcome and antibody levels in CP units measured both by ELISA, and by neutralizing antibody titers assessed in a subset of donors CPs. Benefit was also observed in patients with severe disease, including patients on mechanical ventilation and patients with prolonged illness. The magnitude of benefit was about 2-fold improvement in all subgroups except in the group of patients with moderate severity of illness where the vast majority would probably improve regardless of the amount of transfused antibodies. When controlling for other variables, including patient's severity of disease prior to CP treatment, only D-dimer levels, and IgG against S1 levels in the administered CP were significantly associated with improvement at day 14.

The concept of administrating CP to various infectious diseases is not new. Recently it was used to treat Ebola patients and eventually lead to identifying specific antibodies with high neutralizing activity against the virus and to production of monoclonal synthetic antibodies to treat Ebola [21]. Two small case series that administered CP plasma with high levels of neutralizing suggested improved outcome

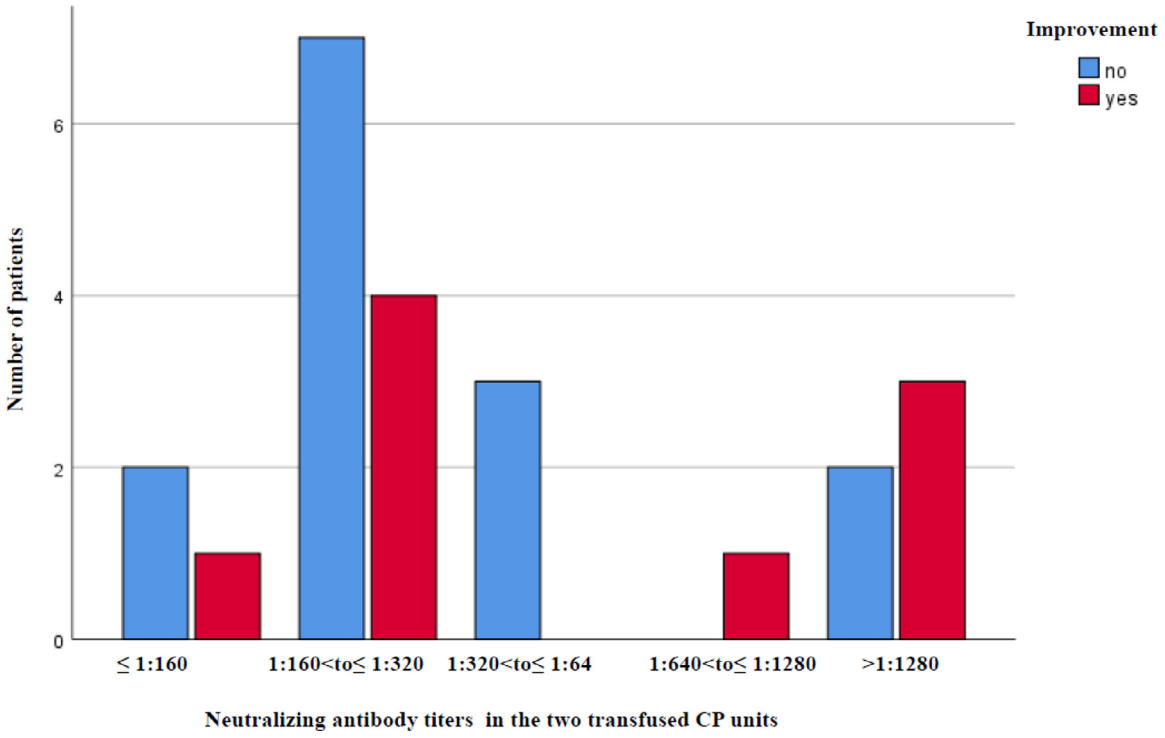

Fig. 3. Correlation between clinical improvement in patients and neutralizing antibody titers in transfused CP.
CP – convalescent plasma.

and reported no adverse events in patients with COVID-19 [13,14]. A randomized controlled study recently published showed some improvement that did not reach significance in 50 severe and critically ill patients [15]. This study reported a larger series of patients with a range of disease severity, and a large variation in length of time from COVID 19 diagnosis to CP transfusion. Beneficial effect was limited to patients with severe disease rather than critical disease [15,16]. Of note, patients included in this study [15] had a long disease duration (median 30 days from symptoms) prior to receiving CP whereas our patients received CP treatment at a median of 10 days from diagnosis. Prior studies demonstrated that CP is more effective when administered early during the disease course or as prophylaxis

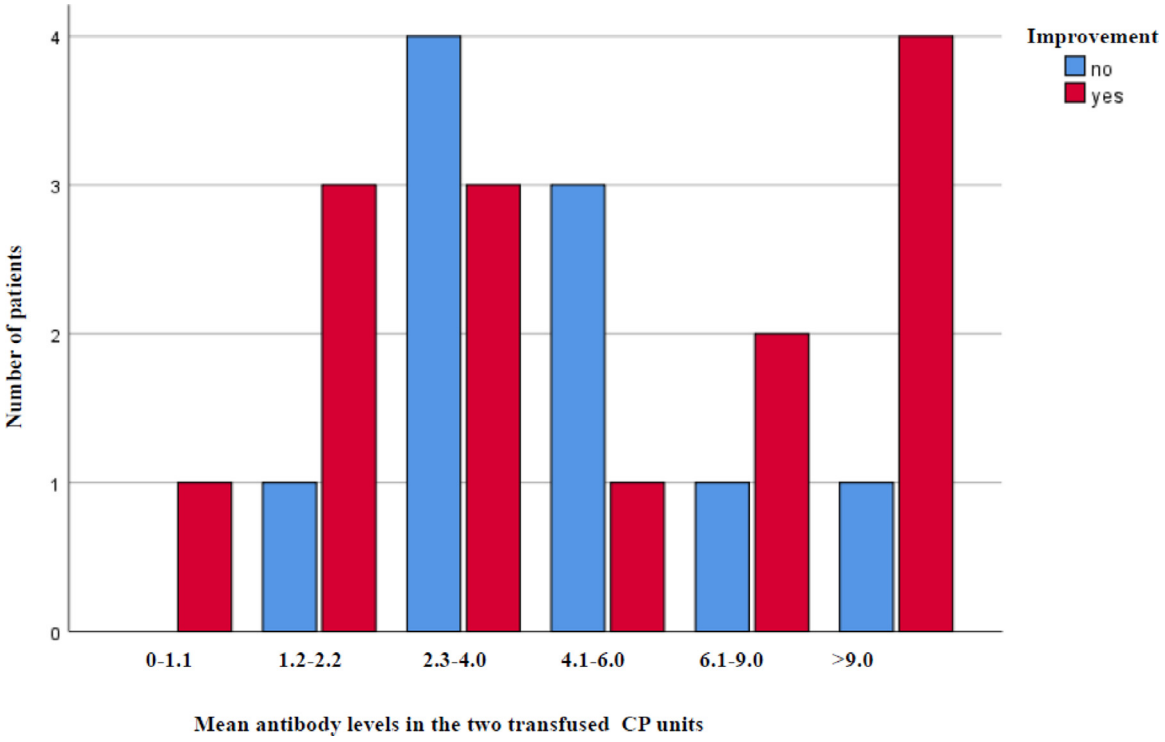

Fig. 4. Correlation between clinical improvement in patients who received the treatment less than 10 days after COVID-19 diagnosis, and IgG levels against S1 in transfused CP.
CP – convalescent plasma.

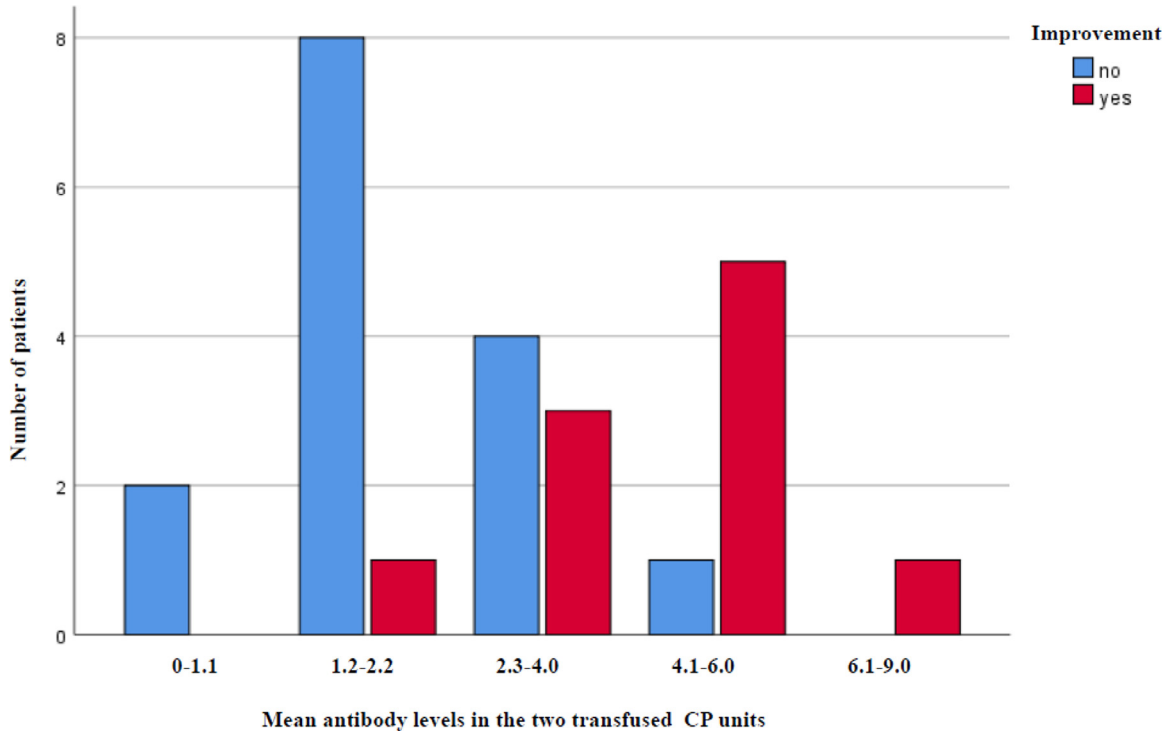

Fig. 5. Correlation between clinical improvement in patients who received the treatment 10 days or more after COVID-19 diagnosis, and IgG levels against S1 in transfused CP. CP – convalescent plasma.

closely after exposure to the infectious agent [22]. A previous large study of 80 patients in Hong Kong infected with SARS demonstrated that patients who were treated before day 14 had improved outcome as defined by discharge from hospital before day 22, supporting early administration of CP for optimal effect [23].

Our results, of improved outcome in patients with severe and prolonged COVID-19 following CP transfusion suggest that CP therapy, particularly when antibody levels are high, may be beneficial even beyond the earlier stages of disease. In patients with a shorter disease duration, the difference between patients receiving lower titers and higher antibody titers was not significant. We assume that this is due to the small number of patients included in our analysis. In addition, the efficacy of CP in moderate disease is difficult to assess from our data as well, as the vast majority of these patients are likely to recover without specific treatment, so a larger sample is needed to demonstrate efficacy of CP in moderate disease [22].

Table 3
Univariate and multivariate logistic regression analysis of variables associated with improvement 14days after CP transfusion.

	Univariate P	Multivariate OR (95% CI)	P value
Age	0.517		
Sex (male)	0.054*		
Obesity	0.480		
Diabetes	0.830		
Hypertension	0.629		
Ischemic heart disease	0.234		
Chronic lung disease	0.408		
Chronic renal failure	0.727		
Malignancy	0.105		
Disease severity (Severe disease)	0.010*		
Fever	0.307		
White blood cells ($x10^3$/liter)	0.107		
Absolute lymphocytes ($x10^3$/liter)	0.701		
Lactate dehydrogenase (LDH) (U/liter)	0.873		
C reactive protein (CRP) (mg/deciliter)	0.777		
D-dimers (μg/milliliter)	0.072*	1.000 (1.000–1.000)	0.089
Ferritin ng/mL	0.856		
IgG to S1 level in CP	0.039*	2.352 (1.036–5.338)	0.041

Univariate and multivariate logistic regression analysis of variables associated with improvement 14 days after treatment with CP. Nagelkerke $R^2 = 0.521$. $N = 30$.
CP – convalescent plasma. OR – odds ratio, CI – confidence interval.
* Variables that were entered into the multivariable logistic regression.

As already described by others [24,25] not all CP units contain measurable antibodies and 15% of donors did not have IgG against S1 IgG against S1 IgG against S1above the cutoff of the test employed. Therefore, it is important to measure antibody levels in plasma, optimally before donation, or before transfusion, to ensure efficacy of the treatment and to create a large enough CP inventory, allowing time for antibody testing before issuing the units for transfusion. This is now feasible as validated serological tests are now available [26,27].

We administrated two 200 ml CP units, 24 h apart. In one study that assessed CP treatment, five patients received 400 ml of CP with neutralizing antibodies with a titer ≥1:80 [13]. In a second study, 10 patients received a single CP unit containing neutralizing antibodies with a titer of >1:640 [14]. The median neutralizing antibody titer of S1-antibody in our study was 1:160 for a CP unit . Thus, antibody levels given in our study seem to be comparable or in a smaller dose than the one used by these studies. Our results support the adminstrating of neutralizing antibodies with a titer of 1:640 or higher, but as we only had information regrading neuralizing antibodies in 29 donors, so further study is warranted. It is expected that within months, hyper immune globulin with known titer of neutralizing antibodies against SARS2-CoV-2 will be available. This product is safer, and is presumed to have higher activity than CP. However, until this modality will be available a large inventory of CP with documented high antibody levels against SARS-CoV-2 can provide a promising therapeutic option.

Our data indicates that measuring IgG against the spike protein is sufficient to ensure response in most cases. We deduce this from the significant correlation between IgG levels against S1 and neutralizing

antibody titers in the subsets where both measurements were available. This is in accord with two other studies, [26,27] the latter described a 94.4% positive agreement between neutralizing IgG against S1 and 97.8% negative agreement with measurement of the S1 antibody level. Measuring neutralizing IgG against S1 in CP against SARS-CoV-2 requires a biosafety level 3 laboratory and is cumbersome, thus it is not feasible in most settings. In addition, the dose response association between IgG level and clinical outcome supports the idea that most CP donations with high antibody levels by semi quantitative ELISA tests will also contain a measurable titer of neutralizing antibodies. Yet, further study is needed to corroborate these results.

This study has several limitations. The number of patients described is small. The study lacks a control group, which limits CP efficacy assessment. As enrollment was through a compassionate program, there was heterogeneity regarding disease severity, length of disease and the standard of care given in different medical centers.

Of note, no patients in this cohort received remdesivir but some were treated with hydroxychloroquine. This could hamper our ability to separate the value of CP treatment from other interventions. Our assessment of outcome was conducted at day 14th, which might be too short for mortality rate determination

In addition, it is possible that physicians had a selection bias in referring patients to a compassionate use program, without strict criteria for eligibility to treatment. Therefore, we believe that the true value of CP treatment may be underestimated in this report.

In conclusion, our results support the beneficial effect of CP plasma for treatment of COVID-19 patients at various disease stages. The results emphasis the importance of measuring antibody levels in CP prior to transfusion to ensure adequate antibody level. We also demonstrated that it is possible to rely on semi quantitative ELISA for IgG against the S1 (by ELISA) without the need to perform complicated neutralizing antibody assays. Further randomized controlled studies are required to establish the role of CP in COVID −19. Nevertheless, in the situation of paucity of anti-viral therapies, the expected limited availability of remdesivir due to increased demands and limited resources in many settings, we propose to consider wider deployment of CP for patients with moderate and severe COVID-19 disease.

Contributors

Yasmin Maor: design, supervisory role, data collection analysis and interpretation, writing; Daniel Cohen: experimental work, data analysis and interpretation, writing; Nir Paran: experimental work, data analysis and interpretation, writing; Tomer Israely: experimental work, data analysis and interpretation; Vered Ezra: supervisory role, administrative and technical support; Ofra Axelrod: supervisory role, administrative and technical support; Eilat Shinar: data collection, supervisory role, writing; Marina Izak: data collection, supervisory role; Galia Rahav: data collection; Naomi Rahimi-Levene: data collection; Baruch M Bazofin: data collection; Ram Gelman: data collection; Dror Dicker: data collection; Tal Brosh-Nissimov: data collection; Orli Megged: data collection; David Dahan: data collection; Avi Benov: data collection; Alona Paz: data collection; Kaykov Edward: data collection; Moran Amit: data collection and writing; Ori Rogowski: data collection; Patrick Sorkine: data collection; Ami Mayo: data collection; Oren Zimhony: design, supervisory role, data analysis and interpretation, writing; Jacob Chen: design, supervisory role, data collection analysis and interpretation, writing.

Data sharing statement

The authors declare that all the data was collected through a national registry convalescent plasma therapy program and is available upon request.

Declaration of Competing Interest

The authors have declared that they have nothing to disclose.

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