sciendo

Original Study • DOI: 10.2478/ahem-2025-0006 • AHEM • Vol. 79 Issue 1/2025 • 35–49

AHEM

Advances in Hygiene and Experimental Medicine
Postępy Higieny i Medycyny Doświadczalnej

The assessment of parameters of convalescent plasma and their impact on COVID-19 symptoms

Original Study

Agnieszka Kuś[1], Szymon Wieczorek[2], Jarosław Dybko[3], Małgorzata Szymczyk-Nużka[4], Aleksandra Butrym[5], Krzysztof Dudek[6], Grzegorz Mazur[1], Siddarth Agrawal[1]*

[1] Department and Clinic of Internal Medicine, Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Wroclaw, Poland
[2] Faculty of Medicine, Wroclaw Medical University, Wroclaw, Poland
[3] Department of Hematology and Cellular Transplantation, Lower Silesian Oncology Center, Wroclaw, Poland
[4] Regional Centre of Transfusion Medicine and Blood Bank (RCTMBB-W), Wroclaw, Poland
[5] Department of Cancer Prevention and Therapy, Wroclaw Medical University, Wroclaw, Poland
[6] Faculty of Mechanical Engineering, Wroclaw University of Science and Technology, Wroclaw, Poland

Received 29 July, 2024, Accepted 19 February, 2025

Abstract

Background. The rapid spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, known as COVID-19, has been a dreadful public health problem with no specific treatment strategy. Using convalescent plasma (CP) with neutralizing antibodies has been analyzed as a potential strategy to reduce mortality and the severity of illness.
Materials and Methods. Our open-label, multi-center, single-arm trial aimed to evaluate the effects of CP transfusion and the risk factors for mortality following CP among 108 COVID-19-infected patients. Each patient was transfused with 200 mL of CP with a confirmed neutralization activity from 44 recovered donors. The efficacy of COVID-19 treatment was defined as the desired change in clinical parameters after CP administration.
Results. Our results showed that leukocyte counts increased significantly after CP transfusion ($p < 0.001$), and hemoglobin levels improved over time ($p = 0.006$). In contrast, inflammation indicators, including C-reactive protein (CRP) or procalcitonin, decreased after CP transfusion ($p < 0.001$). Post hoc analysis revealed significant improvements in several serological parameters, including hematocrit, lymphocytes, and platelet counts, between baseline (V0) and 28 days post-CP transfusion (V3). High-titer IgA, IgM, and IgG anti-SARS-CoV-2 antibodies persisted during 28 days of CP transfusion ($p < 0.001$).
Conclusions. Overall, these findings contribute to optimizing COVID-19 treatment approaches, providing valuable information on the effectiveness of convalescent plasma therapy and its associated factors.

Keywords

plasma transfusion • COVID-19 • convalescent plasma • anti-SARS-CoV-2 neutralization antibodies • serologic parameters

1. Introduction

Since late 2020, when the COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started, no one expected such an incredibly negative impact on healthcare systems, the effects of which are evident until today. To date, the virus has infected approximately 660 million people worldwide and led to over 6 million deaths [1]. Due to other underlying comorbidities, including cardiovascular or respiratory disease, diabetes, and hypertension, older people are at the highest risk of infection [2].

To date, the United States Food and Drug Administration (FDA) has authorized certain antiviral medications, monoclonal antibodies, and molecular drugs to treat COVID-19, including paxlovid, remdesivir, bebtelovimab, molnupiravir, etc. [3]. Furthermore, more than 300 drugs are being investigated under clinical trials worldwide [4–6]. Due to the historical success of similar to SARS-CoV-2 infectious diseases[7] and its overall safety, convalescent plasma (CP) has been employed as a promising strategy for COVID-19 treatment. The active ingredient of CP is neutralizing antibodies (nAb) against SARS-CoV-2, which consists of serum collected from previously infected individuals. Neutralizing antibodies neutralize the virus, avoiding further infection and improving clinical outcomes. Furthermore, it provides passive immunomodulatory properties that optimize the exaggerated inflammatory cascade [4]. This strategy is significantly critical for life-threatening patients who require a fast and robust response to lesser symptoms of COVID-19 infection [5].

Several analytical parameters must be determined to establish the basic efficacy of CP therapy. Since comprehensive assessment of clinical and laboratory parameters is not feasible in large-scale Atrials, smaller investigational studies are needed to define suitable outcomes and parameter measures[8]. Our study aimed to evaluate the efficacy of CP among COVID-19-infected patients and determine possible factors associated with its efficacy. These effects were analyzed by assessing 1) the

* *E-mail: siddarth@agrawal.pl*

probability of overall survival, 2) the duration of hospitalization, and 3) the changes in the distribution of laboratory test results in the subsequent visits after CP administration. This study is beneficial for better understanding the potential of CP as a therapeutic tool against severe COVID-19 infection.

2. Materials and methods

2.1. Characteristics of convalescent plasma donors

Three hundred patients who recovered from COVID-19 infection were offered to participate in this open-label, multi-center, phase IV, single-arm trial (ClinicalTri-als.gov number NCT04642014). The recovery criteria included body temperature normalization for more than three days, resolution of respiratory tract symptoms, and two consecutively negative results of the SARS-CoV-2 RT-PCR assay test. Forty-four donor patients (5 non-pregnant women and 39 men) aged between 21 and 59 years old (Me=42 years old) met the inclusion criteria; their blood was collected after four weeks post-onset of illness. The donor patients reported typical symptoms of COVID-19 infection, excluding two who had an asymptomatic disease. The duration of symptoms was 2 to 33 days (Me = 14 days). Eight donors smoked cigarettes, and the mean body mass index (BMI) was 29.4 kg/m^2. Most donors had blood group A Rh + (13/44, 29.5%), followed by 0 Rh + (11/44, 25.0%), B Rh + (8/44; 18.2%) and AB Rh + (7/44; 15.9%). The donor's blood was collected four weeks post-onset of illness. 600 ml ABO-compatible plasma sample was harvested during apheresis from each donor, and each sample was divided and stored at 200 mL aliquots without any detergent or heat treatment. The plasma-neutralizing titers ranged from 700 to 2000 (Me = 2000). Further details can be found in the clinical trial database (NCT04642014).

2.2. Characteristics of convalescent plasma recipients

We analyzed the results of 108 patients (34 women and 74 men) with COVID-19 who received convalescent plasma (CP) between December 15, 2020, and February 28, 2022. The median age of patients treated with CP was 54,9 years old (range 23-88); 25% of patients were older than 66. According to the WHO Interim Guidance, the plasma recipients were diagnosed with COVID-19 with real-time RT-PCR assay confirmation. The inclusion criteria to receive CP involved at least one of the following: respiratory distress with tachypnea ≥ 30 breaths per minute, oxygen level less than 94% in resting state, partial pressure of oxygen (pO_2) ≤ 80 mm Hg. Patients who met the criteria received one dose (200ml) of ABO-compatible inactivated CP with a confirmed neutralization activity.

Each CP dose delivered to one recipient came from an individual donor. The efficacy of COVID-19 treatment, defined as the desired change in clinical parameters after CP administration, was measured by (1) the time from CP transfusion to death for any reason, (2) duration of the hospitalization, and (3) the results of laboratory tests performed in four subsequent visits (V0 – results directly prior the CP administration; V1 – after three days; V2 – after seven days; V3 – after 28 days of CP administration). Clinical parameters included in our study were as follows: the level of hematocrit, monocytes, leukocytes, erythrocytes, hemoglobin, platelets, neutrophils, eosinophils, C-reactive protein (CRP), procalcitonin, ferritin, mean corpuscular hemoglobin (MCH), D-dimer, lactate dehydrogenase (LDH), pH, pO_2, pCO_2, HCO_3, Arterial Blood Gas (BE), O_2, sodium, and potassium, as well as mean platelet volume (MPV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW). Furthermore, CP recipients were tested with regard to the level of IgA anti-SARS-CoV-2, IgM anti-SARS-CoV-2, and IgG anti-SARS-CoV-2 antibodies after CP transplantation.

2.3. Inclusion criteria/ variables

The studied variables were (1) the time from the onset of COVID-19 symptoms to CP administration, (2) plasma donor antibody titers, (3) age, and (4) body mass index (BMI). The cut-off values of these risk factors of death were determined based on the ROC curve analysis and summarized in Table 1.

2.4. Statistics

The impact of the risk factors studied was analyzed using correlation and regression coefficients to evaluate the relationships between the number of days since CP transfusion and laboratory test results. Kaplan-Meier survival analysis was conducted to identify factors influencing overall survival probability following CP administration. The normality of data distribution was tested using the Shapiro-Wilk test. Continuous variables were described using medians and interquartile ranges, and comparisons were performed using the Mann-Whitney U test or Friedman test for non-parametric data. Pearson's chi-squared test was used for categorical variables. Post-hoc analyses were performed to further investigate significant differences identified by the Friedman test. The Wilcoxon signed-rank test with Bonferroni correction was used. Statistical analyses were performed to evaluate relationships between variables, including Spearman's and Pearson's correlation coefficients. ROC curve analysis was used to assess predictive accuracy for key parameters. All analyses were performed using the statistical software package Statistica (v13.0). Results are presented as point estimates and 95% confidence intervals (±95% CI), and a p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Overall survival probability analysis

Including all studied variables, age was the only factor influencing the overall survival probability of 28 days with COVID-19 infection

Table 1. The results of the ROC curves analysis of variables for the assessment of the likelihood of death caused by COVID-19 infection

Variable (parameter)	Cut-off value	Sensitivity	Specificity	AUC (95% CI)	p value
Duration from the first COVID-19 symptom to CP administration (days)	<9	0.510	0.500	0.598 (0.388-0.807)	p = 0.797
Plasma titers	<2000	0.625	0.400	0.536 (0.342-0.730)	p = 0.670
Age (years old)	≥68	0.750	0.840	0.842 (0.706-0.978)	p < 0.001
BMI (kg/m^2)	≥30	0.500	0.760	0.577 (0.372-0.781)	p = 0.136

Table 2. Post-hoc pairwise comparisons of overall survival probability based on hematocrit, monocyte, and pCO_2 levels after CP transfusion

Subgroups	Hematocrit	Monocytes	pCO2
Normal vs Below standard	p = 0.257	p = 0.006	p = 0.653
Normal vs Above standard	p < 0.001	p = 0.283	p = 0.039
Below vs Above standard	p = 0.019	p = 0.512	p = 0.021

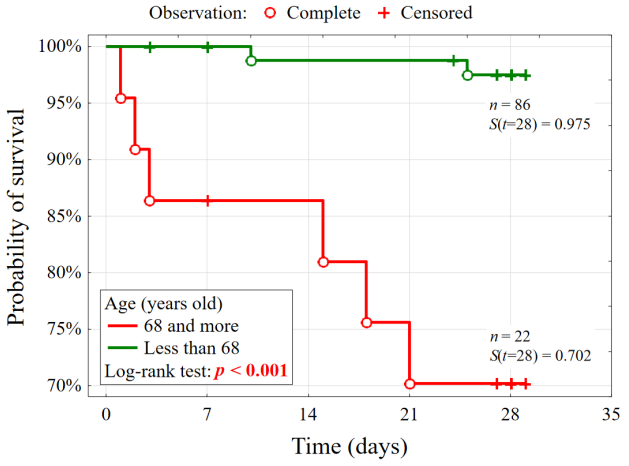


Figure 1. Kaplan-Meier survival curve demonstrating the probability of survival at 28 days post-CP transfusion stratified by age groups (≥ 68 years vs. < 68 years). The log-rank test revealed a significant difference in survival rates (p < 0.001), with younger patients displaying a higher survival probability (97.5% vs. 70.2%)

(S(t)=28) after CP transfusion. COVID-19-infected recipients younger than 68 were more likely to survive 28 days after CP transfusion (p < 0.001; Fig. 1).

Furthermore, we compared the overall survival probability rates of COVID-19-infected patients based on different laboratory parameters. Kaplan-Meier survival analysis showed significant differences in survival probability depending on hematocrit (p = 0.002, Fig. 2A), monocyte (p = 0.017, Fig. 2B), and pCO_2 (p = 0.042, Fig. 2C) levels. Post-hoc analysis (Table 2) revealed that patients with hematocrit levels above the standard had significantly lower survival probability compared to those with normal levels (p < 0.001), while those below standard had a better survival rate than those above standard (p = 0.019). For monocytes, survival probability was significantly higher in the normal group compared to the below-standard group (p = 0.006). Regarding pCO_2, survival probability was lower in the above-standard group than normal (p = 0.039) and below-standard groups (p = 0.021).

3.2. Duration of the hospitalization

The duration of hospitalization from CP transfusion to potential recovery or death among 108 COVID-19-infected patients ranged from 2 to 67 days (Me = 10, SD = 8 days). Table S1 presents the results of the median probability of overall survival of COVID-19 infection in the groups with different risk factors of death. Patients admitted to the hospital due to COVID-19-associated dyspnea were likelier to stay there for extended periods (p = 0.047) (Figure S1).

3.3. Results of laboratory tests performed in subsequent visits.

Taking into account four subsequent visits after CP administration, a significant change in the distribution of leukocytes (p<0.001, Fig. 3A) and hemoglobin (p = 0.006, Fig. 3B) levels were observed (Table S2). This ratio is shifting towards an increase in the level of the analyzed clinical parameters. For instance, during CP transfusion, 13 patients had leukocyte levels above standards; after 28 days, this parameter was observed in 36 patients (Fig. 3A). Considering hemoglobin, the number of patients with standard results increased over time. Subsequently, the number of patients with lower standard results decreased (Fig. 3B). These results were also confirmed while assessing the mean daily leukocytes and hemoglobin counts after CP transfusion (from 0 to 12 days) (p<0.001, Fig.3C and p=0.024, Fig. 3D, respectively). There was a more rapid increase in leukocytes than hemoglobin levels.

Furthermore, our study revealed a statistically significant relationship between the distribution of the number of patients differing in assessing the level of serological parameters on consecutive days of visits. Analyzed biochemical tests included the hematocrit (p=0.009, Fig. S2A), lymphocytes (p<0.001, Fig. S2B), neutrophils (p=0.032, Fig. S2C), and eosinocytes (p<0.001, Fig. S2D) levels (Table S2). The distribution of patients in these laboratory tests is related to their normalization in each visit;

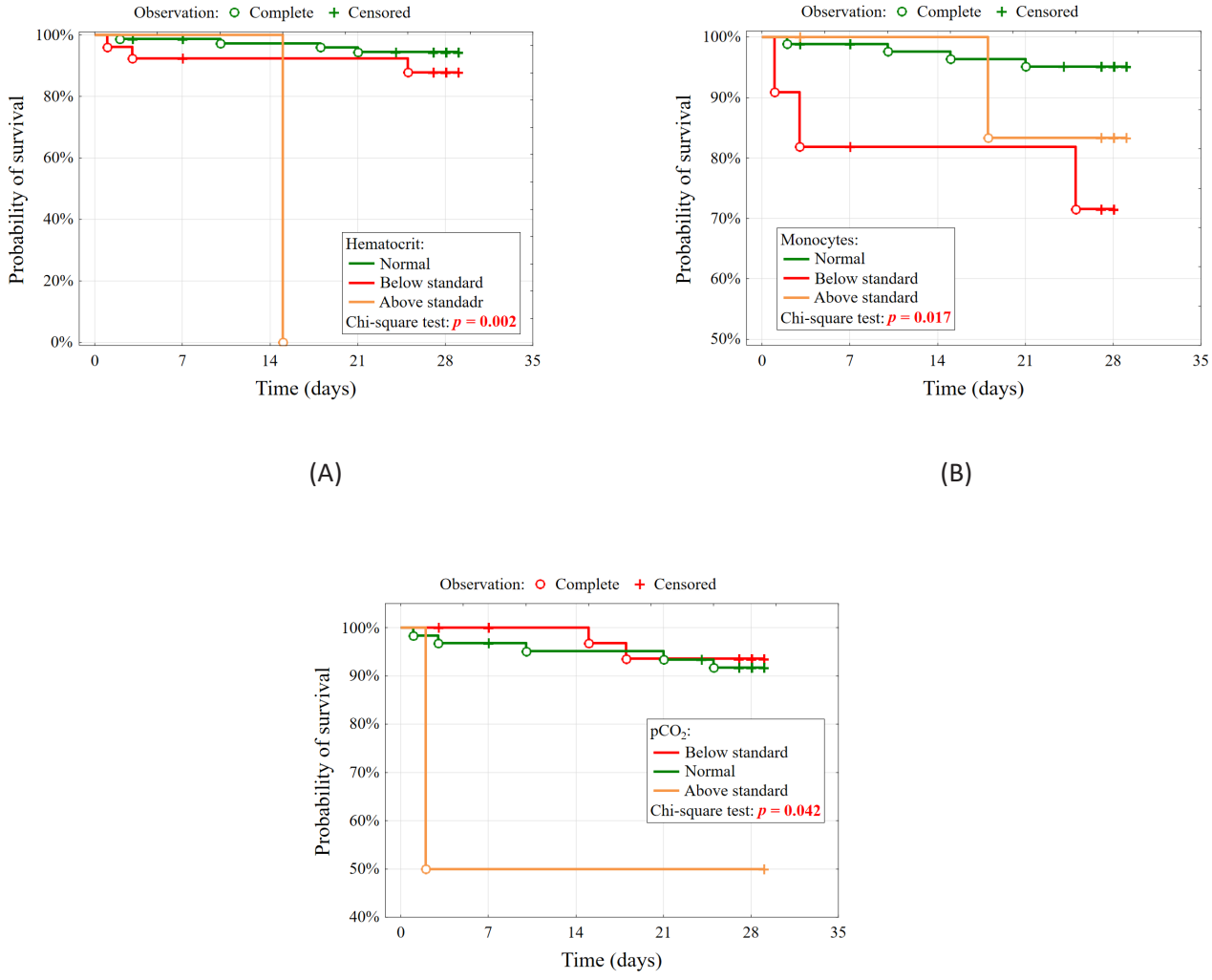
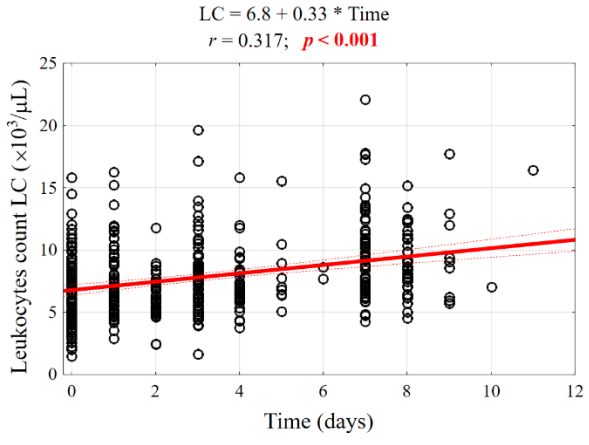


*Figure 2. Kaplan-Meier curves among COVID-19 patients differ by the assessment of the laboratory test results: A) hematocrit level (p = 0.002), B) monocytes level (**p** = 0.017), and C) pCO2 (**p** = 0.042) and their associations with survival probability, supported by log-rank tests for statistical significance. V0: baseline (pre-CP transfusion), V1: 3 days post-transfusion, V2: 7 days post-transfusion, V3: 28 days post-transfusion*

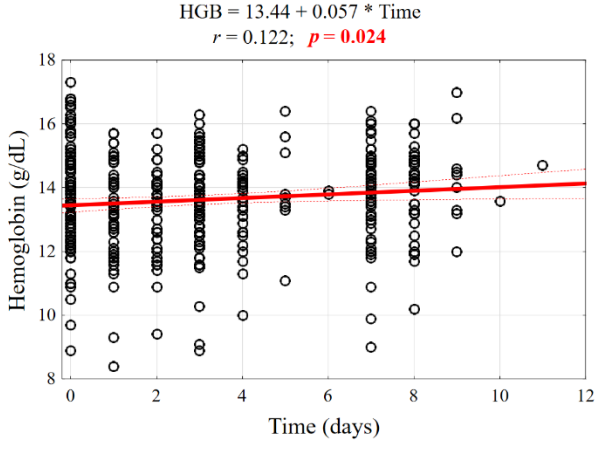
due to the decrease in the number of patients with results below standards over time, more of them had normal biochemical parameters. For instance, during CP administration, 27 patients (27/108; 25.0%) and 79 patients (79/108; 73.1%) had hematocrit levels below standards and normal, respectively. After 28 days, the number of patients with lower standards was decreased to 14 patients (14/108; 13.9%), and the number of patients having normal hematocrit levels was enhanced to 85 patients (84.2%) (Fig. S2A). Furthermore, as shown in Table S2, a statistical correlation between the distribution of the number of patients was also found while analyzing mean corpuscular hemoglobin (MCH) (p=0.005, Fig. S2E), platelets (p<0.001, Fig. S2F), and monocytes (p=0.004, Fig. S2G) levels over time. Although there was no increase in the number of patients with normal results, the increase in the level of each biochemical parameter may be determined by evaluating the ratio between the number of patients having results below and above standards. For instance, over time, after 28 post-CP administrations, the number of patients with blood platelet levels above standards increased from 4 (4/108; 3.7%) to 38 (38/101; 37.6%), while the number of patients with blood platelet levels below standards decreased from 16 (16/108; 14.8%) to 1 (1/101; 1.0%, Table S2). These data show the increased level of different biochemical parameters after CP administration. It is worth considering the sizes in the

(A)

(B)



(C)



(D)

Figure 3. The distribution of patients differed in leukocyte levels (A) across four visits (pre-CP and three post-CP follow-ups). A significant leukocyte increase is observed (Friedman ANOVA, χ^2 = 145.1, $p < 0.001$). Panel B focuses on hemoglobin levels over the same period, showing progressive normalization (Friedman ANOVA, $\chi 2 = 18.3$, $p = 0.006$). Panels C and D present correlation analyses between leukocyte counts and time post-transfusion, with p-values of < 0.001 and 0.024, respectively. V0 – results directly before the CP administration; V1 – after three days; V2 – after seven days; V3 – after 28 days of CP administration

following groups: normal, below standards, and above standards of each biochemical parameter. Although statistical significance is observed, several groups are too limited to report any correlations, which may result in false positive results.

Considering the inflammation indicators, since the CP plasma administration, there was a decrease in the CRP (p <0.001, Fig. 4A, and Fig. 4B, Table S3) and procalcitonin (p <0.001, Fig. 4C and Fig. 4D, Table S3) levels in each visit. For instance, 105 patients had CRP levels above standards prior CP administration (105/109; 96.3%); after 28 days, this number was decreased to 57 patients (57/100; 57.0%, Fig. 4A). Consistently, the number of patients with procalcitonin levels above standards has decreased from 55 (55/103; 52.9%) to 14 (14/97; 14.4%, Fig. 4C). Post hoc analysis using the Wilcoxon signed-rank test with Bonferroni correction confirmed significant decreases in CRP and procalcitonin levels between baseline (V0) and all subsequent visits (V1, V2, and V3). Therefore, CP plasma decreased the inflammation indicators levels, as shown by CRP and procalcitonin results.

In addition, as shown in Fig. 5 and Table S3, the significant role of CP administration on COVID-19 treatment was also observed by the increase of the IgA anti-SARS-CoV-2 (p < 0.001, Fig. 5A), IgM anti-SARS-CoV-2 (p < 0.001, Fig. 5B), and IgG anti-SARS-CoV-2 levels (p < 0.001, Fig. 5C). Prior CP administration, 73 patients had positive results of IgA anti-SARS-CoV-2 and IgG anti-SARS-

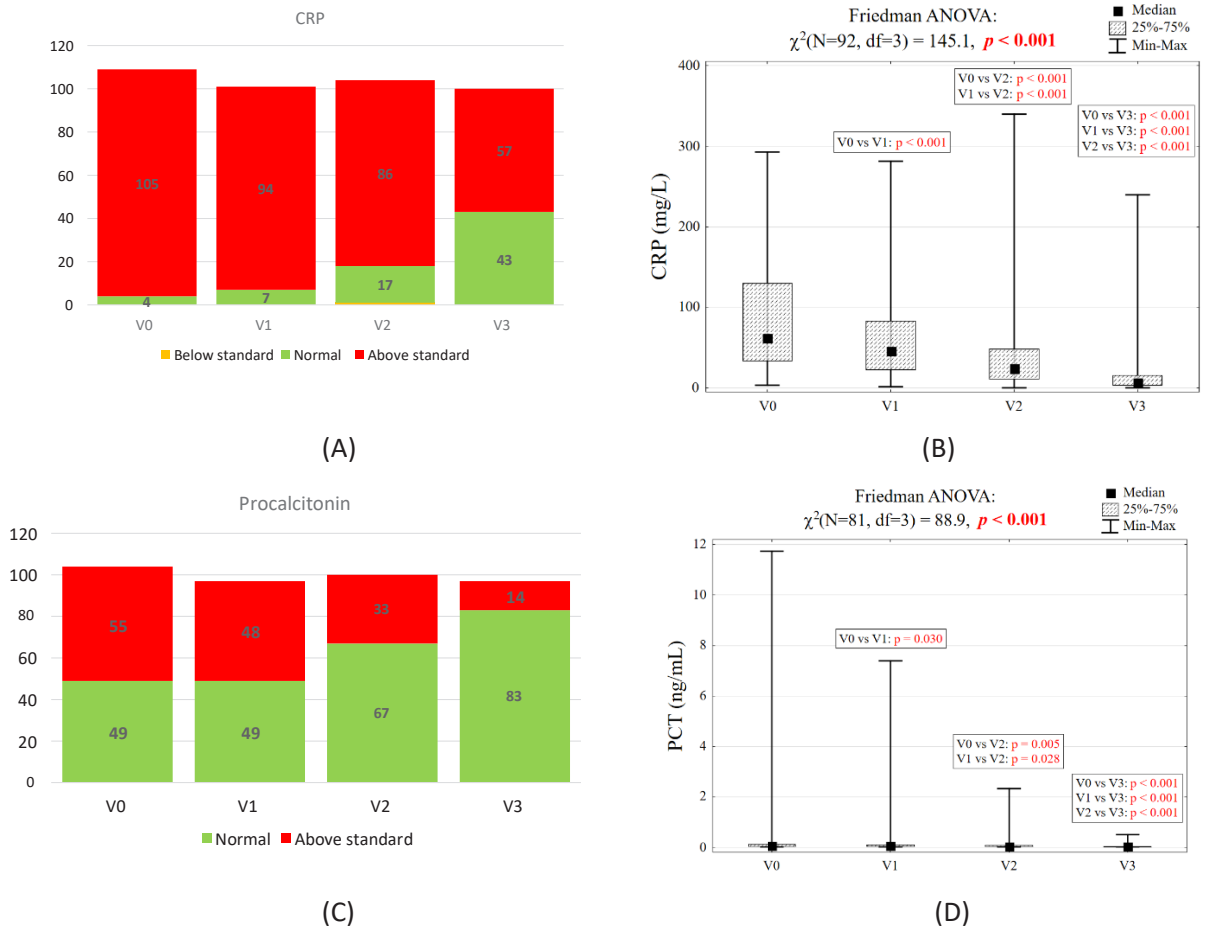


Figure 4. Patients' structure differs in assessing the level of inflammation indicators on consecutive days. Panels A and B illustrate CRP levels, with significant declines from baseline to post-CP follow-ups (Friedman ANOVA, $\chi 2 = 145.1$, $p < 0.001$). Panels C and D depict procalcitonin trends, similarly showing reductions (Friedman ANOVA, $\chi 2 = 88.9$, $p < 0.001$). Subpanels include bar graphs with standard deviations and group comparisons via post-hoc tests. Post hoc tests confirmed significant differences between V0 and all subsequent time points for both indicators. V0 – results directly prior the CP administration; V1 – after three days; V2 – after seven days; V3 – after 28 days of CP administration

CoV-2 (73/108; 66.4%) and 58 patients had positive results of IgM anti-SARS-CoV-2 (58/108; 52.7%). After 28 days of CP plasma administration, the number of patients with positive results of IgA, IgM, and IgG anti-SARS-CoV-2 was increased significantly (95/96; 99.0% for IgA anti-SARS-CoV-2 and IgG anti-SARS-CoV-2, p<0.001 and 82/96; 85.4% for IgM an-ti-SARS-CoV-2, p<0.001, respectively). Post hoc analysis confirmed significant increases in all antibody levels between baseline (V0) and 28 days post-CP administration (V3), highlighting the sustained production of high-titer antibodies.

4. Discussion

For over a century, convalescent plasma (CP) therapy has been applied to prevent and treat many infectious diseases. This strategy was successfully used in the treatment of SARS, Middle East respiratory syndrome (MERS), and 2009 influenza A (H1N1), leading to increased efficacy, safety, and disease progression [9–12]. In a meta-analysis of 32 studies of SARS coronavirus infection and severe influenzas, Mair-Jenkins et al. determined a significant reduction of mortality of recipients after CP administration, especially in those receiving CP early after the first symptom onset [7]. However, no association between CP therapy and increased survival was observed in 84 Ebola virus-infected patients. It is possible due to the absence of data on neutralizing antibody titration for stratified analysis [13]. Therefore, due to the virological and clinical similarity among SARS, MERS, and SARS-CoV-2, it is reasonable to work on determining the role of CP therapy in COVID-19 treatment.

CP obtained from recovered COVID-19 patients consists of many neutralizing antibodies leading to eradicating the SARS-CoV-2 virus from blood circulation and pulmonary tissues [14]. In the early stages of the COVID-19 pandemic, reports from open-label trials and case series provided the safety and effectiveness of CP transfusion to patients with COVID-19 infection[15]. Therefore,

the World Health Organization (WHO) has provided guidelines for the usage of CP plasma and standardized donor selection, which was further supported by an Emergency Use Authorization (EUA) from the United States Food and Drug Administration (FDA) [16]. However, this initial enthusiasm has been extinguished after publishing many randomized clinical trials, large platform trials, and systematic reviews showing no or only modest efficacy of CP plasma in COVID-19-infected patients. Therefore, on the day of publishing this article, WHO and FDA are consistent in their statement; they do not recommend using CP in patients with severe and non-severe COVID-19 [7]. Nevertheless, due to conflicting results, CP therapy still needs further consideration. This investigational medicinal product is an object of more than 140 registered clinical trials worldwide [17]. Our study adds to the growing body of evidence supporting the efficacy of CP among COVID-19-infected patients.

Due to the large-scale study group (108 patients were considered), the results obtained are reliable and convincing. For instance, leukocyte counts tended to increase and remain high until 28 days after CP transfusion ($p < 0.001$, Fig. 3A). In contrast, inflammation indicators such as C-reactive protein (CRP) or procalcitonin tended to decrease after CP transfusion (CRP - $p < 0.001$, Fig. 4A, and Fig. 4B, and procalcitonin - $p < 0.001$, Fig. 4C and Fig. 4D). This phenomenon is thought to result from the immunomodulatory effect of CP such as providing passive immunity by blocking inflammatory cytokines, autoantibodies, and complement pathways [18].

Results from our study are in accord with another study conducted in Pakistan by Khan et al., who observed decreased CRP levels over 72 hours after CP administration among patients with moderately severe ($p < 0.030$) and severe ($p < 0.014$) COVID-19 infection[19]. In a separate study of ten patients with severe COVID-19, seven of them showed increased lymphocyte counts (0.65×109/L vs. 0.76×109/L) and decreased CRP levels (55.98 mg/L vs. 18.13 mg/L) over 12 days after CP transfusion [20]. Furthermore, from 136 patients, Alsharidah et al. reported an improvement in lymphocyte counts from seven days after CP transfusion in patients with moderate COVID-19 infection and eleven days in patients with severe disease. Simultaneously, CRP levels declined throughout the first 14 days after CP transfusion [21]. The clinical improvement observed by the rapid decline of CRP levels was also assessed among 30 COVID-19-infected patients nine days after CP transfusion. Studied patients also had increased fibrinogen and D-dimer levels after four days of CP therapy, suggesting the emergence of coagulopathy [8]. This disease has been frequently observed in patients with COVID-19 and is associated with subsequent thromboembolic events and severe outcomes. This result is inconsistent with our findings; there is no statistical significance in the D-dimer and fibrinogen levels after CP administration. Therefore, according to our study, there is no increased risk of coagulopathy while receiving CP. Furthermore, in Italy, Franchini et al., showed improvement in clinical, functional, radiologic, and laboratory parameters among nineteen elderly patients over 65 years old with COVID-19 infection 14 days after CP transfusion. There was a significant decrease in CRP values at all the time points analyzed (from a median baseline level of 7.40 mg/L to a median level of 0.73 mg/L at day 14 after CP infusion) [22]. These results, as seen by CRP levels, suggest the anti-inflammatory properties of CP. Thus, CP therapy could improve clinical outcomes by enhancing laboratory parameters among patients with COVID-19 infection, which is mostly seen by decreased CRP levels and increased leukocyte counts.

Notably, it is beneficial to monitor laboratory tests, especially for patients at high risk of developing chronic diseases, such as hypertension or diabetes, and for elderly adults over 67 years old, who, according to our results, are less likely to survive 28 days after CP transfusion than their younger counterparts ($p < 0.001$; Fig. 1). Therefore, older than 67 years old adults are predicted to live shorter than 28 days after CP transfusion compared to younger counterparts, potentially despite the improvement of clinical parameters. In contrast, in a study composed of extremely old (median age, 87 years) patients with COVID-19 infection, Franchini et al. determined that CP reduced the mortality risk by 65% compared with a control population after 14 days of CP transfusion [22]. Nevertheless, due to the gap in the knowledge of SARS-CoV-2 pathogenesis and strategies for its treatment, it is worth considering other factors potentially affecting the efficacy of CP transfusion, including administered antiviral agents. For instance, the combination therapy with CP transfusion and hydroxychloride was already determined by Xu et al., but their results did not show optimal improvement, and specific treatment needs further analysis [23].

Antibodies increase the affinity and neutralization capacity of CP plasma over time. Even if low-titered, they may be highly effective [8]. Our study revealed a significant increase of IgA, IgM, and IgG anti-SARS-CoV-2 antibodies that persisted after 28 days of CP transfusion ($p<0.001$). Therefore, the question appears whether this increase contributes to reducing viral load. Although the answer will be described in further perspective of the study, according to the literature, we assume these correlations may be observed. In a group of 35 322 CP transfused COVID-19-infected patients, Joyner et al. found that for patients receiving high IgG plasma (>18.45 S/Co), the mortality rate after seven days of CP transfusion was 8.9%. For recipients with medium IgG plasma (4.62 to 18.45 S/Co), the mortality rate was 11.6%. However, for recipients with low IgG plasma (<4.62 S/Co), the mortality was 13.7% ($p=0.048$). Thus, the higher the anti-SARS-CoV-2 IgG concentration level, the lower the mortality rate due to COVID-19 infection [24]. This study did not provide the role of time from CP transfusion to the mortality rate, which could affect the final anti-SARS-CoV-2 IgG concentration. Consistent with our findings,

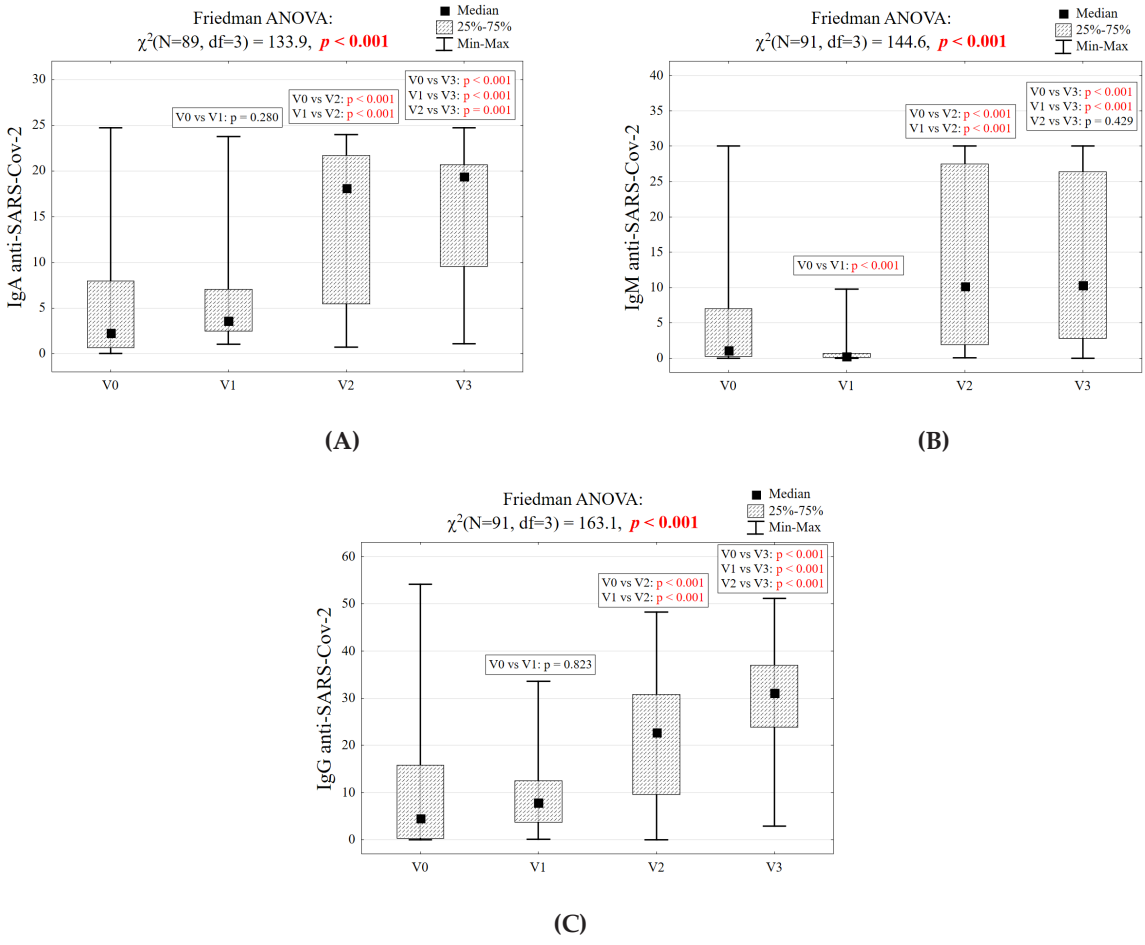


*Figure 5. The results of Friedman tests representing the level of antibodies against: A) IgA – anti - SARS-CoV-2, B) IgM – anti-SARS-CoV-2, and C) IgG – anti-SARS-CoV-2 in the following days of visits (Friedman ANOVA, **p** < 0.001 for all). Post hoc analysis revealed significant differences between baseline (V0) and 28 days post-CP transfusion (V3) for all antibody types. V0 – results directly prior the CP administration; V1 – after three days; V2 – after seven days; V3 – after 28 days of CP administration (Friedman ANOVA, p < 0.001 for all)*

Arreita et al. determined that following CP therapy, anti-SARS-CoV-2 IgG increased significantly at 24 hours, and high levels were sustained at 7- and 21-day [25]. Furthermore, Franchini et al. showed an increase in anti-SARS-CoV-2 IgG levels three days after CP transfusion, reaching a plateau on day 7 and then increasing again by day 14 [22]. These studies lead to the conclusion that the persistence of anti-SARS-CoV-2 IgG antibodies over time (even 28 days – as shown in our study – Fig. 5C) after CP transfusion may reduce viral load and hence reduce mortality rate (as indicated by Joyner et al. [24]) due to COVID-19 infection.

Furthermore, according to our study, there is an increased level of an-ti-SARS-CoV-2 IgA antibodies that persisted even for 28 days after CP transfusion (Fig. 5A). Consistently, our recently published data revealed that patients who received plasma with IgA > 1.15 AU / ml displayed an eight-fold increase in survival compared to patients who received CP with IgA <1.15 AU / ml (OR = 7.92 95% CI 1.20-52.3). Moreover, we found IgA affects the level of IgG antibodies in donor plasma; a decrease in the levels of IgG accompanied by increases in IgA levels [26]. Nevertheless, the question still requiring further consideration is whether increased levels of IgA during CP transplantation impact the overall survival of COVID-19-infected patients or if it is due to the direct correlation with other antibodies. However, considering that studies evaluating the efficacy of CP therapy concentrate predominantly on examining total Ig or IgG-specific anti-SARS-CoV-2 antibody levels [27]. Our findings, taken together, may shed new light on the potential mechanisms of action of CP through the activity of IgA.

While analyzing the therapeutic role of CP among COVID-19-infected patients, we wondered if donor sex is the factor affecting the efficacy of CP. In our study, CP plasma came from 5 non-pregnant women and 39 men. To the best of our knowledge, most studies show no statistical correlation between the sex of donors and the efficacy of CP plasma [28]. However, articles

dominating males [29,30] or female [31] donors are also observed. In a study by Schmidt et al., female CP donors had higher mean initial anti-SARS-CoV-2 antibody levels than males. However, the rate of decline was more rapid in females, perhaps suggesting that lower initial titers in males might be offset by a slower rate of decline [31]. In contrast, Jain et al. demonstrated that higher levels of anti-nucleocapsid IgG S/C ratios were associated with males, older age, Hispanic ethnicity, and fewer days between symptom onset and first donation [32]. Mehew J. consistently found that higher neutralizing antibody levels were observed in males, older donors, and previously hospitalized donors [33]. The discrepancies in the association of CP donor sex with antibody response could result from several factors. One of them is a methodological workflow of the study; some studies evaluated neutralizing antibody levels, others anti-NC IgG levels using the Abbott assay [31]. Furthermore, females tend to have more robust immune responses to infection, which is thought to be caused by genetic and hormonal influences on the immune system [34]. Additionally, testosterone has been found to suppress immune function [35]. Therefore, differences in antibody levels can result from biological factors. Nevertheless, further studies are required to determine sex discrepancies in the neutralizing SARS-CoV-2 antibody.

Due to the emergence of new COVID-19 variants and the decreasing efficacy of existing vaccines, finding alternative strategies for SARS-CoV-2 treatment is a critical point in disease progression [36]. While plasma products (e.g., hyperimmune globulin, monoclonal antibodies) and/or vaccination are successful therapeutic options, human CP is the only medicinal product that is immediately available for use to prevent and treat COVID-19, especially in low- and middle-income countries, where it is challenging to get more expensive drugs [37]. The other significant advantage of CP is versatility, with the potential to respond to new emerging variants [38]. Therefore, our results provide the basis for investigating the effects of CP plasma on the elimination of novel SARS-CoV-2 variants from COVID-19-infected patients.

The presented study has some limitations. First, the median time from the onset of COVID-19-related symptoms to CP transfusion was 16 days. To answer the question of whether changes in the values of clinical outcomes come from the therapeutic effects of CP or are a result of the natural kinetics of viremia, further studies are needed. Secondly, the randomization represented by the control group was not included in this study; this study group is needed to determine the benefits or disadvantages of CP transfusion in COVID-19 patients. Third, at the time of analysis, some patients were still hospitalized (the duration of hospitalization ranged from 2 to 67 days (median *Me* = 10, SD = 8 days)). This variation in hospitalization duration introduces potential bias, as the outcomes for patients still hospitalized at the time of data collection could not be fully assessed. Last but not least, specific SARS-CoV-2- directed drugs were not considered when analyzing clinical parameters after CP administration.

5. Conclusions

In conclusion, this open-label, multi-center, single-arm study shows a potential therapeutic effect of CP transfusion among COVID-19-infected patients. One dose of CP with antibodies with neutralizing activity can rapidly improve clinical outcomes by increasing leukocyte counts and decreasing inflammation biomarkers, including C-reactive protein and procalcitonin. Prompt CP administration within the initial 9 days post-symptom onset correlated with accelerated improvements in blood leukocyte and platelet levels. Decreases in inflammation indicators (CRP and procalcitonin) and robust increases in anti-SARS-CoV-2 antibodies post-CP administration underscore the therapeutic potential of this intervention. These findings contribute valuable insights into factors influencing CP efficacy. Consideration of specific laboratory markers and timely administration is crucial for optimizing the benefits of CP therapy in severe COVID-19 cases. However, the optimal dose, treatment time point, and detailed changes of each diagnostic value need to be further investigated.

Authors' contribution

Conceptualization: Grzegorz Mazur; Writing original draft: Agnieszka Kuś; Methodology: Szymon Wieczorek, Jarosław Dybko, Małgorzata Szymczyk-Nużka; Writing, review and editing: Aleksandra Butrym, Siddarth Agrawal; Formal analysis: Krzysztof Dudek; Supervision: Grzegorz Mazur, Siddarth Agrawal;

ORCID

Agnieszka Kuś https://orcid.org/0000-0002-6258-6823
Szymon Wieczorek https://orcid.org/0000-0002-7849-1300
Jarosław Dybko https://orcid.org/0000-0003-3555-2887
Małgorzata Szymczyk-Nużka https://orcid.org/0000-0002-5729-6717
Aleksandra Butrym https://orcid.org/0000-0002-8199-2018
Krzysztof Dudek https://orcid.org/0000-0001-8206-6238
Grzegorz Mazur https://orcid.org/0000-0002-7611-8115
Siddarth Agrawal https://orcid.org/0000-0003-1118-5090

Funding

This research was funded by the Medical Research Agency in Poland, grant nr 2020.ABM.COVID19.0005.

Conflicts of interest

The authors have no conflicts of interest to declare.

Ethics approval

This study was approved by the Bioethics Committee of Wroclaw Medical University, approval nr UR/DBL/D/347/2020.

References

[1] COVID-19 Map - Johns Hopkins Coronavirus Resource Center 2021. Available at: https://coronavirus.jhu.edu/map.html (Accessed November 06, 2022).

[2] Fischer JC, Zänker K, Van Griensven M, Schneider M, Kindgen-Milles D, Knoefel WT, Lichtenberg A, Tamaskovics B, Djiepmo-Njanang FJ, Budach W, et al. The role of passive immunization in the age of SARS-CoV-2: An update. European Journal of Medical Research. 2020;25(1).

[3] Centers for Disease Control and Prevention (CDC). COVID-19 Treatments and Medications 2022. Available at: https://archive.cdc.gov/www_cdc_gov/coronavirus/2019-ncov/your-health/treatments-for-severe-illness.html (Accessed November 06, 2022).

[4] Franchini M, Glingani C, De Donno G, Lucchini G, Beccaria M, Amato M, Castelli GP, Bianciardi L, Pagani M, Ghirardini M, et al. Convalescent plasma for hospitalized COVID-19 patients: A single-center experience. Life. 2022;12(3).

[5] Tobaiqy M, Qashqary M, Al-Dahery S, Mujallad A, Hershan AA, Kamal MA, Helmi N. Therapeutic management of patients with COVID-19: a systematic review. Infection Prevention in Practice. 2020;2(3).

[6] Heustess AM, Allard MA, Thompson DK, Fasinu PS. Clinical management of covid-19: A review of pharmacological treatment options. Pharmaceuticals. 2021;14(6).

[7] Mair-Jenkins J, Saavedra-Campos M, Baillie JK, Cleary P, Khaw FM, Lim WS, Makki S, Rooney KD, Nguyen-Van-Tam JS, Beck CR, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: A systematic review and exploratory meta-analysis. Journal of Infectious Diseases. 2015;211(1):80–90.

[8] Marconato M, Abela IA, Hauser A, Schwarzmüller M, Katzensteiner R, Braun DL, Epp S, Audigé A, Weber J, Rusert P, et al. Antibodies from convalescent plasma promote SARS-CoV-2 clearance in individuals with and without endogenous antibody response. Journal of Clinical Investigation. 2022;132(12).

[9] Zhou B, Zhong N, Guan Y. Treatment with convalescent plasma for influenza A (H5N1) infection. New England Journal of Medicine. 2007;357(14):1450–1.

[10] Ko JH, Seok H, Cho SY, Ha YE, Baek JY, Kim SH, Kim YJ, Park JK, Chung CR, Kang ES, et al. Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: A single centre experience. Antiviral Therapy. 2018;23(7):617–22.

[11] Cheng Y, Wong R, Soo YOY, Wong WS, Lee CK, Ng MHL, Chan P, Wong KC, Leung CB, Cheng G. Use of convalescent plasma therapy in SARS patients in Hong Kong. European Journal of Clinical Microbiology and Infectious Diseases. 2005;24(1):44–6.

[12] Hung IFN, To KKW, Lee CK, Lee KL, Chan K, Yan WW, Liu R, Watt CL, Chan WM, Lai KY, et al. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clinical Infectious Diseases. 2011;52(4):447–56.

[13] van Griensven J, Edwards T, de Lamballerie X, Semple MG, Gallian P, Baize S, Horby PW, Raoul H, Magassouba N, Antierens A, et al. Evaluation of convalescent plasma for ebola virus disease in Guinea. New England Journal of Medicine. 2016;374(1):33–42.

[14] Marano G, Vaglio S, Pupella S, Facco G, Catalano L, Liumbruno GM, Grazzini G. Convalescent plasma: New evidence for an old therapeutic tool? Blood Transfusion. 2016;14(2):152–7.

[15] Joyner MJ, Wright RS, Fairweather D, Senefeld JW, Bruno KA, Klassen SA, Carter RE, Klompas AM, Wiggins CC, Shepherd JRA, et al. Early safety indicators of COVID-19 convalescent plasma in 5000 patients. Journal of Clinical Investigation. 2020;130(9):4791–7.

[16] World Health Organization (WHO). IMAI District Clinician Manual: Hospital care for adolescents and adults. World Health Organization. 2011;2(January):780.

[17] Müller-Olling M, Vahlensieck U, Hilger A. Heterogeneity in COVID-19 convalescent plasma clinical trials. Clinical Pharmacology and Therapeutics. 2022;111(5):995–1000.

[18] Lünemann JD, Nimmerjahn F, Dalakas MC. Intravenous immunoglobulin in neurology-mode of action and clinical efficacy. Nature Reviews Neurology. 2015;11(2):80–9.

[19] Khan TNS, Mukry SN, Masood S, Meraj L, Devrajani BR, Akram J, Fatima N, Maqsood S, Mahesar A, Siddiqui R, et al. Usefulness of convalescent plasma transfusion for the treatment of severely ill COVID-19 patients in Pakistan. BMC Infectious Diseases. 2021;21(1).

[20] Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, Zhou M, Chen L, Meng S, Hu Y, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proceedings of the National Academy of Sciences of the United States of America. 2020;117(17):9490–6.

[21] Alsharidah S, Ayed M, Ameen RM, Alhuraish F, Rouheldeen NA, Alshammari FR, Embaireeg A, Almelahi M, Adel M, Dawoud ME, et al. COVID-19 convalescent plasma treatment of moderate and severe cases of SARS-CoV-2 infection: A multicenter interventional study. International Journal of Infectious Diseases. 2021;103:439–46.

[22] Franchini M, Glingani C, Morandi M, Corghi G, Cerzosimo S, Beduzzi G, Storti A, Di Stasi V, Rastrelli G, Vignozzi L, et al. Safety and efficacy of convalescent plasma in elderly COVID-19 patients: The RESCUE trial. Mayo Clinic Proceedings: Innovations, Quality & Outcomes. 2021;5(2):403–12.

[23] Xu TM, Lin B, Chen C, Liu LG, Xue Y. Non-optimal effectiveness of convalescent plasma transfusion and hydroxychloroquine in treating COVID-19: A case report. Virology Journal. 2020;17(1).

[24] Joyner MJ, Senefeld JW, Klassen SA, Mills JR, Johnson PW, Theel ES, Wiggins CC, Bruno KA, Klompas AM, Lesser ER, et al. Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: Initial three-month experience. medRxiv: the preprint server for health sciences. 2020.

[25] Arrieta A, Galvis AE, Morphew T, Ehwerhemuepha L, Osborne S, Enriquez C, Imfeld K, Hoang J, Nieves D, Ashouri N, et al. Safety

and antibody kinetics of COVID-19 convalescent plasma for the treatment of moderate to severe cases of SARS-COV-2 infection in pediatric patients. Pediatric Infectious Disease Journal. 2021;40(7):606–11.

[26] Kuś A, Wieczorek S, Dybko J, Szymczyk-Nużka M, Butrym A, Makuch S, Mazur G, Agrawal S. Potential of high-titre IgA convalescent plasma to improve survival and symptoms in COVID-19 patients. European Journal of Clinical Investigation. 2023;53(2):13928.

[27] Verkerke H, Saeedi BJ, Boyer D, Allen JW, Owens J, Shin S, Horwath M, Patel K, Paul A, Wu SC, et al. Are we forgetting about IgA? A re-examination of coronavirus disease 2019 convalescent plasma. Transfusion. 2021;61(6):1740–8.

[28] Ghadami L, Hasibi M, Asadollahi-Amin A, asanjarani B, Farahmand M, Abdollahi H. Convalescent plasma therapy in patients with severe COVID-19, A single-arm, retrospective study. Microbial Pathogenesis. 2022;165.

[29] Wang X, Guo X, Xin Q, Pan Y, Hu Y, Li J, Chu Y, Feng Y, Wang Q. Neutralizing antibody responses to severe acute respiratory syndrome coronavirus 2 in coronavirus disease 2019 inpatients and convalescent patients. Clinical Infectious Diseases. 2020;71(10):2688–94.

[30] Jin JM, Bai P, He W, Wu F, Liu XF, Han DM, Liu S, Yang JK. Gender differences in patients with COVID-19: Focus on severity and mortality. Frontiers in Public Health. 2020;8.

[31] Schmidt AE, Vogel P, Chastain CA, Barnes T, Roth NJ, Simon TL. Analysis of 52 240 source plasma donors of convalescent COVID-19 plasma: Sex, ethnicity, and age association with initial antibody levels and rate of dissipation. Journal of Clinical Apheresis. 2022;37(5):449–59.

[32] Jain S, Garg K, Tran SM, Rask IL, Tarczon M, Nandi V, Kessler DA, Strauss D, Sachais BS, Yazdanbakhsh K, et al. Characteristics of coronavirus disease 19 convalescent plasma donors and donations in the New York metropolitan area. Transfusion. 2021;61(8):2374–83.

[33] Mehew J, Johnson R, Roberts D, Harvala H. Convalescent plasma for COVID-19: Male gender, older age and hospitalisation associated with high neutralising antibody levels, England, 22 April to 12 May 2020. Eurosurveillance. 2020;25(45).

[34] Fairweather DL, Rose NR. Women and autoimmune diseases. Emerging Infectious Diseases. 2004;10(11):2005–11.

[35] Foo YZ, Nakagawa S, Rhodes G, Simmons LW. The effects of sex hormones on immune function: a meta-analysis. Biological Reviews. 2017;92(1):551–71.

[36] Rahimi-Levene N, Shapira J, Tzur I, Shiloah E, Peer V, Levin E, Izak M, Shinar E, Ziv-Baran T, Weinberger M, et al. Predictors of mortality in COVID-19 patients treated with convalescent plasma therapy. PLoS ONE. 2022;17(7 July).

[37] Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, Van Buskirk C, Grossman BJ, Joyner M, Henderson JP, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. Journal of Clinical Investigation. 2020;130(6):2757–65.

[38] Hartmann J, Bloch EM, Burnouf T. Experience with COVID-19 convalescent plasma provides vital guidance to future pandemics. Transfusion. 2022;62(3):681–4.

Supplementary Materials

Table S1. Analysis of hospitalization duration across various patient subgroups stratified by key clinical and demographic parameters, including time between symptom onset and CP administration, donor plasma titer levels, age, BMI, and presence of dyspnea at hospital admission

Patients subgroups	Me (Q1 – Q3)	Min - Max	p
Time between symptoms and CP administration <9 days	11 (9 - 14)	2 – 67	0.057
Time between symptoms and CP administration □9 days	10 (8 – 12)	3 – 31	
Donor plasma titer = 2000	10 (8 - 14)	2 – 67	0.907
Donor plasma titer < 2000	10 (8 - 13)	3 – 52	
Age < 68 years old	10 (8 - 13)	2 – 52	0.510
Age □ 68 years old	12 (8 - 16)	2 – 67	
BMI □ 30 kg/m^2	10 (8 - 14)	2 – 67	0.684
BMI > 30 kg/m^2	10 (9 - 13)	2 – 27	
Hospitalization time (days): Dyspnea during the admission to the hospital	10 (9 - 14)	2 – 67	0.047
Lack of dyspnea	9 (6 - 10)	4 – 18	

***Me** – median (50%); Q1 – lower quartile (25%); Q3 – upper quartile (75%); p – significance level of the Mann-Whitney U test*

Table S2. The number (percentage) of plasma recipients differed in categories depending on the results of biochemical, serological parameters in the subsequent visit days (V0 - before CP administration, V1 - after 3 days, V2 – 7 days, V3 – after 28 days of CP administration)

	V0 Before	V1 After 3 days	V2 After 7 days	V3 After 28 days	Chi-squared test
Leukocytes count	N = 108	N = 102	N= 101	N = 101	
Below standard	14 (13.0%)	4 (3.9%)	3 (3.0%)	1 (1.0%)	χ^2 = 38.2
Standard	81 (75.0%)	83 (81.4%)	82 (81.2%)	64 (63.4%)	df = 6
Above standard	13 (12.0%)	15 (14.7%)	16 (15.8%)	36 (35.6%)	p < 0.001
Hemoglobin	N = 108	N = 111	N = 101	N = 101	
Below standard	39 (36.1%)	50 (49.0%)	36 (35.6%)	23 (22.8%)	χ^2 = 18.3
Standard	69 (63.9%)	52 (51.0%)	64 (63.4%)	78 (77.2%)	df = 6
Above standard	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	p = 0.006
Hematocrit	N = 108	N = 102	N = 101	N = 101	
Below standard	27 (25.0%)	37 (36.3%)	25 (24.8%)	14 (13.9%)	χ^2 = 17.0
Standard	79 (73.1%)	65 (63.7%)	76 (75.2%)	85 (84.2%)	df = 6
Above standard	2 (1.9%)	0 (0.0%)	0 (0.0%)	2 (2.0%)	p = 0.009
MCV	N = 108	N = 102	N = 101	N = 101	
Below standard	3 (2.8%)	2 (2.0%)	2 (2.0%)	1 (1.0%)	χ^2 = 1.61
Standard	98 (90.7%)	91 (89.2%)	93 (92.1%)	93 (92.1%)	df = 6
Above standard	7 (6.5%)	9 (8.8%)	6 (5.9%)	7 (6.9%)	p = 0.952
MCH	N = 108	N = 102	N = 101	N = 101	
Below standard	3 (2.8%)	2 (2.0%)	2 (2.0%)	1 (1.0%)	χ^2 = 18.6
Standard	104 (96.3%)	100 (98.0%)	99 (98.0%)	93 (92.1%)	df = 6
Above standard	1 (0.9%)	0 (0.0%)	0 (0.0%)	7 (6.9%)	p = 0.005
Platelets	N = 108	N = 102	N = 101	N = 101	
Below standard	16 (14.8%)	6 (5.9%)	1 (1.0%)	1 (1.0%)	χ^2 = 69.8
Standard	88 (81.5%)	88 (86.3%)	80 (79.2%)	62 (61.4%)	df = 6
Above standard	4 (3.7%)	8 (7.8%)	20 (19.8%)	38 (37.6%)	p < 0.001
Neutrophiles	N = 108	N = 101	N = 100	N = 101	
Below standard	12 (11.1%)	7 (6.9%)	5 (5.0%)	2 (2.0%)	χ^2 = 13.8
Standard	49 (45.4%)	57 (56.4%)	64 (64.0%)	54 (53.5%)	df = 6
Above standard	47 (43.5%)	37 (36.6%)	31 (31.0%)	45 (44.6%)	p = 0.032
Limphocytes	N = 108	N = 101	N = 100	N = 101	

Table S2. The number (percentage) of plasma recipients differed in categories depending on the results of biochemical, serological parameters in the subsequent visit days (V0 - before CP administration, V1 - after 3 days, V2 – 7 days, V3 – after 28 days of CP administration)

	V0 Before	V1 After 3 days	V2 After 7 days	V3 After 28 days	Chi-squared test
Below standard	85 (78.7%)	71 (70.3%)	59 (59.0%)	31 (30.7%)	$\chi^2 = 57.4$
Standard	20 (18.5%)	28 (27.7%)	39 (39.0%)	65 (64.4%)	df = 6
Above standard	3 (2.8%)	2 (2.0%)	2 (2.0%)	5 (5.0%)	$p < 0.001$
Monocytes	N = 108	N = 101	N = 100	N = 101	
Below standard	11 (10.2%)	9 (8.9%)	4 (4.0%)	3 (3.0%)	$\chi^2 = 18.9$
Standard	90 (83.3%)	84 (83.2%)	84 (84.0%)	76 (75.2%)	df = 6
Above standard	7 (6.5%)	8 (7.9%)	12 (12.0%)	22 (21.8%)	p = 0.004
Eosynocytes	N = 108	N = 101	N = 100	N = 101	$\chi^2 = 26.2$
Below standard	42 (38.9%)	28 (27.7%)	22 (22.0%)	9 (8.9%)	df = 3
Standard	66 (61.1%)	73 (72.3%)	78 (78.0%)	92 (91.1%)	$p < 0.001$
Basocytes	N = 108	N = 101	N = 100	N = 101	$\chi^2 = 8.07$
Below standard	4 (3.7%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	df = 3
Standard	104 (96.3%)	101 (100.0%)	99 (99.0%)	101 (100.0%)	p = 0.045
CRP	N = 109	N = 101	N = 104	N = 100	
Below standard	0 (0,0%)	0 (0,0%)	1 (1,0%)	0 (0,0%)	$\chi^2 = 71,4$
Standard	4 (3,7%)	7 (6,3%)	17 (16,3%)	43 (43,0%)	df = 6
Above standard	105 (96,3&)	94 (84,7%)	86 (82,7%)	57 (57,0%)	$p < 0,001$
Prokalcytoniny	N = 104	N = 97	N = 100	N = 97	$\chi^2 = 39,2$
Standard	49 (47,1%)	49 (50,5%)	67 (67,0%)	83 (85,6%)	df = 3
Above standard	55 (52,9%)	48 (49,5%)	33 (33,0%)	14 (14,4%)	$p < 0,001$
Ferrytyna	N = 102	N = 96	N = 97	N = 96	
Below standard	0 (0,0%)	0 (0,0%)	1 (1,0%)	0 (0,0%)	$\chi^2 = 9,78$
Standard	12 (11,8%)	5 (5,2%)	6 (6,2%)	14 (14,6%)	df = 6
Above standard	90 (88,2%)	91 (94,8%)	90 (92,8%)	82 (85,4%)	p = 0,134
D-dimer	N = 106	N = 101	N = 97	N = 95	$\chi^2 = 6,56$
Standard	38 (35,8%)	23 (22,8%)	21 (21,6%)	27 (28,4%)	df = 3
Above standard	68 (54,2%)	78 (77,2%)	76 (78,4%)	68 (71,6%)	p = 0,087
LDH	N = 106	N = 98	N = 99	N = 97	
Below standard	1 (0,9%)	0 (0,0%)	1 (1,0%)	1 (1,0%)	$\chi^2 = 23,2$
Standard	16 (15,1%)	13 (13,3%)	23 (23,2%)	37 (38,2%)	df = 6
Above standard	89 (84,0%)	85 (86,7%)	75 (75,8%)	59 (60,8%)	$p < 0,001$
pH	N = 104	N = 84	N = 89	N = 86	
Below standard	3 (2,9%)	1 (1,2%)	0 (0,0%)	1 (1,2%)	$\chi^2 = 7,24$
Standard	32 (30,8%)	37 (44,0%)	29 (32,6%)	28 (32,5%)	df = 6
Above standard	69 (66,3%)	46 (54,8%)	60 (67,4%)	57 (66,3%)	p = 0,299
PO_2	N = 104	N = 84	N = 89	N = 85	
Below standard	64 (61,5%)	56 (66,7%)	63 (70,8%)	55 (64,7%)	$\chi^2 = 7,25$
Standard	34 (32,7%)	17 (20,2%)	19 (21,3%)	22 (25,9%)	df = 6
Above standard	6 (5,8%)	11 (13,1%)	7 (7,9%)	8 (9,4%)	p = 0,298
PCO_2	N = 104	N = 84	N = 89	N = 86	
Below standard	38 (36,5%)	17 (20,2%)	20 (22,5%)	20 (23,2%)	$\chi^2 = 9,69$
Standard	63 (60,6%)	66 (78,6%)	67 (75,3%)	63 (73,3%)	df = 6
Above standard	3 (2,9%)	1 (1,2%)	2 (2,2%)	3 (3,5%)	p = 0,138
HCO_3	N = 104	N = 84	N = 89	N = 86	
Below standard	7 (6,7%)	1 (1,2%)	0 (0,0%)	1 (1,2%)	$\chi^2 = 18,8$
Standard	91 (87,5%)	75 (89,3%)	79 (88,8%)	69 (80,2%)	df = 6
Above standard	6 (5,8%)	8 (9,5%)	10 (11,2%)	16 (18,6%)	p = 0,005
BE	N = 102	N = 84	N = 89	N = 86	
Below standard	6 (5,9%)	1 (1,2%)	0 (0,0%)	0 (0,0%)	$\chi^2 = 17,8$
Standard	70 (68,6%)	49 (58,3%)	52 (58,4%)	58 (67,4%)	df = 6

Table S2. The number (percentage) of plasma recipients differed in categories depending on the results of biochemical, serological parameters in the subsequent visit days (V0 - before CP administration, V1 - after 3 days, V2 – 7 days, V3 – after 28 days of CP administration)

	V0 Before	V1 After 3 days	V2 After 7 days	V3 After 28 days	Chi-squared test
Above standard	26 (25,5%)	34 (40,5%)	37 (41,6%)	28 (32,6%)	p = 0,007
O_2	N = 104	N = 84	N = 88	N = 85	
Below standard	76 (73,1%)	70 (83,3%)	73 (83,0%)	62 (72,9%)	χ^2 = 6,47
Standard	27 (26,0%)	14 (16,7%)	15 (17,0%)	22 (25,9%)	df = 6
Above standard	1 (1,0%)	0 (0,0%)	0 (0,0%)	1 (1,2%)	p = 0,372

Table S3. Number (percentage) of plasma recipients differing by the categories depending on the SARS-CoV-2 antibodies levels during a subsequent visits (V0 - test before CP administration, V2 - after 7 days, V3 - after 28 days of CP administration)

	V0 Before	V2 After 7 days	V3 After 28 days	Chi-square test
IgA anty-SARS-CoV-2	N = 108	N = 97	N = 96	
Negative	34 (30.9%)	5 (5.2%)	0 (0.0%)	χ^2 = 52.8
Inconsistent	3 (2.7%)	2 (2.0%)	1 (1.0%)	df = 4
Positive	73 (66.4%)	90 (92.8%)	95 (99.0%)	p < 0.001[a]
IgA anty-SARS-CoV-2	N = 108	N = 97	N = 96	χ^2 = 73.3
Me [Q1; Q3]	2.3 [1; 8]	18.1 [5; 22]	19.4 [10-21]	df = 2
Min - Max	0 - 25	1 - 24	1 - 25	p < 0.001[b]
IgM anty-SARS-CoV-2	N = 108	N = 98	N = 96	χ^2 = 32.5
Negative	52 (47.3%)	19 (19.4%)	14 (14.6%)	df = 2
Positive	58 (52.7%)	79 (80.6%)	82 (85.4%)	p < 0.001[a]
IgM anty-SARS-CoV-2	N = 108	N = 97	N = 96	χ^2 = 83.3
Me [Q1; Q3]	1.1 [0; 7]	10.2 [2; 27]	10.3 [3; 26]	df = 2
Min - Max	0 - 30	0 - 30	0 - 30	p < 0.001[b]
IgG anty-SARS-CoV-2	N = 108	N = 98	N = 96	χ^2 = 53.5
Negative	38 (34.2%)	6 (6.1%)	1 (1.0%)	df = 2
Positive	73 (65.8%)	92 (93.9%)	95 (99.0%)	p < 0.001[a]
IgG anty-SARS-CoV-2	N = 108	N = 97	N = 96	χ^2 = 125.8
Me [Q1; Q3]	4.5 [0.3; 16]	22.7 [10; 31]	31.2 [24; 37]	df = 2
Min - Max	0 – 54.1	0 – 48.3	2.9 – 51.2	p < 0.001[b]

Me – median (50%); Q1 – lower quartile (25%); Q3 – upper quartile (75%); Min – minimum; Max – maximum; a – Chi-square independence test; b – Friedman test

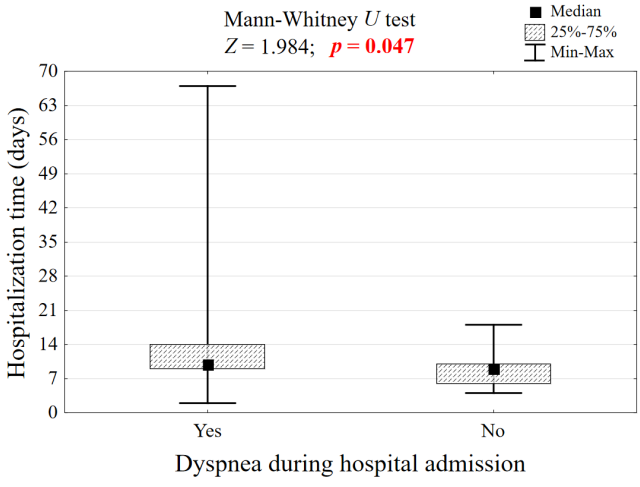
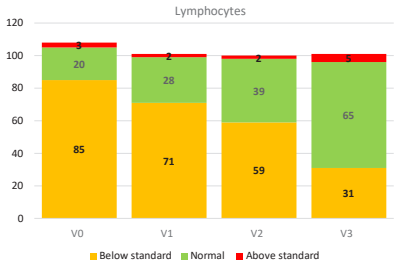
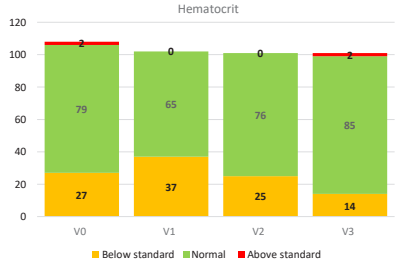


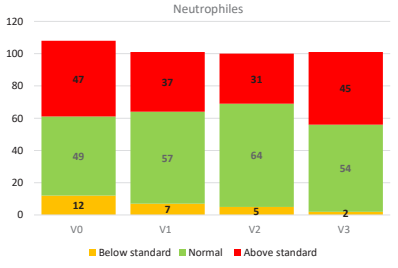
*Figure S1. Duration of hospitalization among patients admitted due to COVID-19-associated dyspnea compared to those admitted for other reasons. The boxplot demonstrates a significantly longer duration of hospitalization for patients with dyspnea (Mann-Whitney U test, **p** = 0.047)*



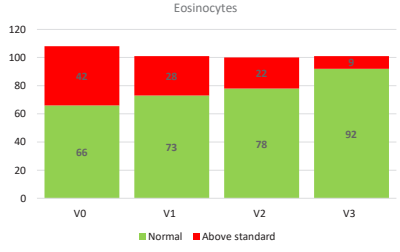
(A)



(B)

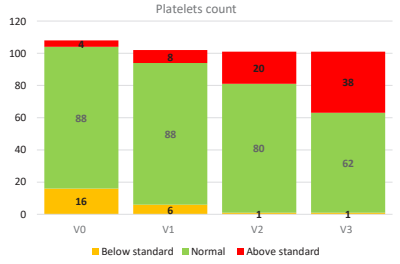


(C)

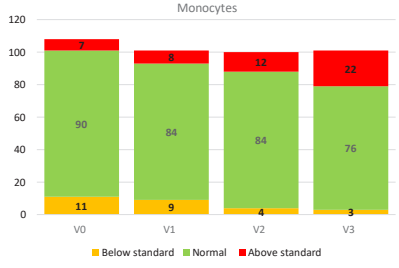


(D)

(E)



(F)



(G)

Figure S2. The structure of number of patients differing in the assessment of the level of serological response in the consecutive days of the visits including A) hematocrit, B) lymphocytes, C) neutrophiles, D) eosinocytes, E) MCH (Mean corpuscular hemoglobin), F) platelets, G) monocytes levels. V0- first visit prior CP administration, V1 – subsequent visit after 3 days, V2 – subsequent visit after 7 days, V3 – subsequent visit after 28 days of CP administration