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Novel protocol for selection of SARS-CoV-2 convalescent plasma donors

Novi protokol za izbor davaoca plazme nakon SARS-CoV-2 infekcije

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Abstract

Background/Aim. Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) 2019 infection represents a global problem. At this moment, in October 2020, there is no vaccine or efficient treatment for infected patients. Treatment with blood plasma rich with anti-SARS-CoV-2 specific antibodies might be a safe, and effective therapy for COVID-19 patients. **Methods.** A total of 768 patients were analyzed in this study, whose samples were collected in a time interval from May 1, 2020, till August 15, 2020. Patients were enrolled in the study from COVID-19 hospitals and out-clinics. In-house ELISA tests were developed to measure the concentration of anti-S1S2 spike and anti-nucleoprotein (np) (IgG, IgA, IgM) SARS-CoV-2 antibodies. Blood convalescent plasma was selectively collected from recovered patients according to specific antibodies concentration. **Results**. The highest concentrations of anti-S1S2 spike or anti-np specific IgG antibodies were detected in patients with the moderate/heavy clinical form of the infection. An extremely high concentration of anti-S1S2 spike IgG and anti-np IgG was demonstrated in 3% and 6% of patients who recovered from severe COVID-19, respectively. Of tested hospitalized patients, 63% and 51% had modest levels of anti-S1S2 spike and anti-np, respectively. After 60 days, in our selected donors, concentrations of anti-S1S2 spike IgG and anti-np IgG antibodies increased in 67% and 58% of donors, respectively. **Conclusion.** In-house developed ELISA tests enable a novel protocol for selecting convalescent blood plasma donors recovered from SARS-CoV-2 infection.

Key words:
antibody specificity; clinical protocols; covid-19 serotherapy; enzyme-linked immunosorbent assay; SARS-CoV-2; plasma; tissue donors.

Apstrakt

Uvod/Cilj. Infekcija *Severe Acute Respiratory Syndrome Corona Virus 2* (SARS-CoV-2) u 2019. godini predstavlja globalni problem. U ovom trenutku (oktobar 2020. godine), ne postoji vakcina, niti efikasan tretman zaraženih bolesnika. Primena krvne plazme bogate antitelima specifičnim za SARS-CoV-2 može da bude sigurna i efikasna terapija za bolesnike sa COVID-19. **Metode.** Ispitivanjem je obuhvaćeno ukupno 768 bolesnika čiji su uzorci krvne plazme bili prikupljeni u vremenskom intervalu od 1. maja 2020. do 15. avgusta 2020. godine. Bolesnici, uključeni u studiju, su bili iz COVID-19 bolnica i ambulanti. *In-house* ELISA testovi su razvijeni za merenje koncentracije antitela na S1S2 *spike* i antitela na nukleoprotein (anti-np) (IgG, IgA, IgM) SARS-CoV-2. Krvna plazma rekonvalescenata je selektivno sakupljana prema koncentraciji specifičnih antitela. **Rezultati.** Najviše koncentracije anti S1S2 *spike* ili anti-np IgG specifičnih antitela detektovane su kod bolesnika sa srednje teškom/teškom kliničkom formom infekcije. Ekstremno visoke koncetracije anti S1S2 *spike* IgG i anti-np IgG nađene su kod 3%, odnosno 6% bolesnika oporavljenih od teškog oblika COVID-19. Od ispitanih hospitalizovanih bolesnika, 63% i 51% su imali minimalne vrednosti anti S1S2 *spike* i anti np antitela, redom. Nakon 60 dana, u plazmi izabranih donora koncentracija anti S1S2 *spike* IgG i anti-np antitela porasla je kod 67%, odnosno 58% donora, redom. **Zaključak.** *In-house* razvijeni ELISA testovi omogućavaju novi protokol za odabir davaoca krvne plazme oporavljenih od SARS-CoV-2 infekcije.

Ključne reči:
antitela, specifičnost; protokoli, klinički; covid-19 seroterapija; elisa; SARS-CoV-2; plazma; tkivo, davaoci.

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Introduction

To date (October 2020), there are no drugs approved by the US Food and Drug Administration (FDA) for treating patients with coronavirus disease 2019 (COVID-19). Current clinical research includes measures for infection prevention and control and, also, supportive care, including oxygen and mechanical ventilation support when necessary. A myriad of drugs that have been approved for other indications are used, as well as a variety of new drugs whose effects are being studied in several hundreds of clinical trials that are underway worldwide.

Scientists around the world are working tirelessly, and information about the mechanisms of transmission, the clinical spectrum of the disease, new diagnostics, and strategies for prevention and therapy is spreading rapidly. In general, there are many unknowns regarding the virus-host interaction, the development of the epidemic, the possibilities and success of treatment, and any research in this field is extremely important.

Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) is spread primarily by respiratory droplets - close face-to-face contact. The infection can be spread by asymptomatic, presymptomatic, and symptomatic carriers. The average time from exposure to the virus to the onset of symptoms is 5 days, and 97.5% of people develop symptoms within 11.5 days. The most common symptoms are high fever, dry cough, and difficulty breathing. Radiographic and laboratory abnormalities, such as lymphopenia and elevated lactate dehydrogenase, are common but nonspecific. The diagnosis is made by detecting SARS-CoV-2 by polymerase chain reaction (PCR), even though false-negative test results can occur in 20% to 67% of patients, depending on the quality and timing of the test. The disease may have an asymptomatic or fulminant course, characterized by sepsis and acute respiratory failure. Approximately 5% of patients with COVID-19, i.e., 20% of those hospitalized, have serious symptoms that require intensive care. More than 75% of patients hospitalized with COVID-19 require additional oxygen, which includes the best therapeutic measures for the treatment of acute hypoxic respiratory failure [1].

In current trials, antiviral therapy, immune modulators, and anticoagulants are being tested. The death rate from COVID-19 varies significantly depending on age, ranging from 0.3 deaths per 1,000 patients (age 5 to 17 years) to 304.9 deaths per 1,000 cases in patients aged 85 years or older in the United States of America (USA). Among patients hospitalized in the intensive care unit, the mortality rate is up to 40%. At least 120 SARS-CoV-2 vaccines are being prepared. Until an effective vaccine is available, the primary methods for reducing the spread are face masks, social distancing, as well as the application of all therapeutic modalities [1].

Since some 10% of patients fail to cope with this disease despite the applied measures, doctors initiated collecting and therapeutic application of convalescent plasma from recovered COVID-19 patients after 14 days from the end of the disease. The idea is old, and it is based on the use of plasma rich in IgG antibodies, which would facilitate the fight, support immunity, and alleviate the clinical picture. Four to six or eight weeks after infection, there should be enough antibodies in the patient's blood to neutralize the virus and theoretically limit the infection [1].

Plasma donors can be convalescents who have had a confirmed positive PCR test for the virus, no symptoms for 14 days, negative PCR test at the time of donation, and have a high titer of specific neutralizing IgG antibodies. In most cases, it is a period of one month [2].

Treatment with plasma from patients recovered from viral infections was first reported during the 1918 influenza pandemic. The first report of 5 critically ill patients with COVID-19 treated with convalescent plasma containing neutralizing antibodies showed an improvement in clinical status in all participants. This was reflected as a combination of changes in body temperature, assessment of organ involvement, oxygen partial pressure, viral load, serum antibody titers, routine blood biochemical index, acute respiratory distress syndrome (ARDS), ventilation, and extracorporeal membrane oxygenation monitored before and after administration of convalescent plasma [3].

However, a subsequent multicenter, randomized clinical trial in China of 103 patients with severe COVID-19 found no statistical significance in the time to clinical improvement within 28 days in patients receiving convalescent plasma compared to standard treatment (51.9% compared to 43.1%, respectively) [4]. Since the study was discontinued, this limited the possibility of detecting a clinically important difference. Alternative approaches include the use of convalescent hyperimmune globulin produced from plasma and monoclonal antibodies directed to SARS-CoV-2 [5, 6].

The virulence of a particular virus is often considered related to the immune response it encounters in the human body. For COVID-19, the immune response is divided into two phases. The initial phase is thought to involve the development of a specific immune response needed to eliminate the virus and stop the disease from progressing. It is, therefore, important to provide treatments that have previously stimulated an immune response, such as antibodies and immunomodulators [2, 6]; there may be a loss of 20% of its own antibodies that will resume a few days later. The immune response, however, weakens with age, making the elderly particularly vulnerable. If the immune response is weak or damaged due to other complications such as cardiovascular disease and diabetes, the virus multiplies and can lead to tissue damage. The second phase of the immune response leads to the damage of the cells causing pneumonia. Such infection is very dangerous since pneumonia causes respiratory disorders, making it difficult for individuals to breathe on their own. Different therapies are tested in different phases of the disease, making it important to identify the exact phase of the patient’s disease before starting the treatment [1].

The World Health Organization warns that there is no evidence that the presence of antibodies means that you are protected from reinfection with COVID-19. The level of immunity and how long the immunity lasts are still unknown. Ongoing studies will eventually reveal more data on this.

Due to all of the above, any research related to COVID-19 therapy is crucial, including the collection and administration of convalescents' plasma to patients.

Patients with a resolved viral infection will develop an immune response with polyclonal antibodies to various viral antigens, and some of these polyclonal antibodies, if the patient has them in high titers, will probably neutralize the virus and prevent more severe forms of the infection. Doctors are trying to use this fact in a therapeutic sense, and that is why the idea of collecting plasma (rich in antibodies) from the convalescents after COVID-19 came about [1–13].

The potential danger of using such plasma in terms of side effects in recipients, including but not limited to allergic reactions, acute lung injury, and circulatory overload in patients with cardiac disorders, must also be mentioned here [14–16].

Our institution also worked on testing the antibody titers in patients who overcame COVID-19 and developed its own protocol for collecting plasma by a manual technique using a system of multiple bags. Since plasma transfusion is a routine medical procedure, no new medical approvals are required to perform it. In fact, the same basic concept was used to treat several Ebola patients with convalescent serum during the 2014–2015 epidemic [17].

Methods

Specific antibody detection

Specific anti-S1S2 SARS-CoV-2 antibodies and anti-nucleoprotein (np) SARS-CoV-2 antibodies were quantified with a home-based ELISA test. Specific SARS-CoV-2 S1S2 and SARS-CoV-2 np (Sino Biological, EU) antigens were coated on polystyrene microwells (0.5 ug/mL, coat buff, 100 uL/well, overnight, 4 ºC). After 3 washing cycles (phosphate buffer saline – PBS, 0.01% Tween) microwells were blocked (PBS, 1% bovine serum albumin – BSA, 1 h, room temperature – RT), and after further washing cycles, patient samples were incubated in duplicates (1/100 diluted, 100 uL/well, 3 h RT, shaking, 60 revolutions – rot/min). Secondary antibodies were incubated after washing cycles (goat anti-human IgG, IgA, or IgM Southern Biotech, USA, 100 uL/well, 1.5 h RT, shaking, 60 rot/min). Final washing cycles were followed with substrate incubation (TMB solution, Siemens, EU, 100 uL/well, 15 min RT, dark), and after stopping (Stop solution, Siemens, EU, 50 μL/well) optical density of each sample was determined at 450 nm (Synergy HT, EU spectrophotometer). The concentration of every sample was expressed in equivalent units (EU)/mL and determined from the standard curves obtained with monoclonal antibodies specific for S1S2 or np (Sino Biological, EU). Equivalent unit is the concentration of tested antibodies from a patient's plasma sample that had the same, i.e. equivalent value of optical units in the ELISA test as the monoclonal antibody specific for the S1S2 domain and the monoclonal antibody specific for the SARS-CoV-2 nucleoprotein.

Patients

A total number of 768 patients, whose samples were collected in the time interval from May 1, 2020, till August 15, 2020, were analyzed in this study. The first group were recovered patients from COVID-19 hospitals (n = 457) and the second group were patients from COVID-19 out-clinics (n = 311). Serum samples frozen from volunteer healthy donors were used as a negative control (n = 160). All control negative samples were collected in the period April – August 2019, a time period long before any signs of the SARS-CoV-2 pandemic.

Clinical data collection

Data were collected after fulfilling the electronic questionnaire (www.covidmirage.com).

Convalescent plasma collection

Plasma collection was performed in patients who had undergone COVID-19, had specific anti-S1S2 SARS-CoV-2 antibodies and np SARS-CoV-2 antibodies present in the circulation, and wanted to donate their plasma to treat other people. Four to six or eight weeks after infection, there should be enough antibodies in the patient's blood to neutralize the virus and theoretically limit the infection [1]. Before collecting plasma, each donor was tested for markers of transfusion-transmitted infections (hepatitis B, hepatitis C, HIV 1/2, and syphilis) by ELISA tests and PCR technique, checked for the titer of specific anti-S1S2 SARS-CoV-2 antibodies and np SARS-CoV-2 antibodies and their blood group was determined (ABO and Rhesus factor). The procedure consists of placing a venous catheter (Cell Connect CC1, Fresenius Medical Care, Germany) into the cubital vein (most often), attaching a multiple bag system (TH, Jiaxing Tianhe Pharmaceutical Co., Ltd, China) containing 63 mL of anticoagulant-preservative CPD/SAG-M solution – composition: citric acid – 0.299 g; sodium citrate – 2.63 g; monobasic sodium phosphate – 0.222 g and dextrose – 2.55 g in a primary bag while accompanying (satellite) bag contained 100 mL of optimal SAGM additive solution (containing: NaCl – 877 mg; adenine – 16.9 mg; dextrose – 900 mg and mannitol – 525 mg), intended for resuspension of concentrated erythrocytes. After that, 450 mL of blood is taken from a voluntary donor, centrifugated (Jouan, Thermo Scientific France) at a speed of 3,500 rpm for 10 min at a temperature of 4 ± 2 °C, and separated into components, i.e., cell suspension and plasma, manually. During the centrifugation time, the donor is given via *iv* catheter 0.9% NaCl solution to maintain cannula patency and volume recovery. In the further procedure, the cellular elements of the blood are returned to the donor after centrifugation, and after that, the procedure of taking another unit of whole blood is repeated. An average of 611.47 (310–680) mL of convalescent plasma was thus collected from each donor, and the procedure took an average of 90 min. Plasma was frozen at -60 °C within 6 h maximum and stored in freezers at -40 ± 5 °C (Fiocchetti, Frigoriferi Scientifici, Italy) with a shelf life of three years. Whole blood (450 ± 45 mL) for the preparation of plasma units was taken from donors (aged 25 to 55 years), unreactive to markers of transfusion-transmitted diseases (hepatitis

B and C, AIDS and lues), performed by ELISA tests and PCR technique, with orderly clinical and laboratory findings.

Statistical analysis

A comparison of antibody concentration between investigated groups was performed with the Mann Whitney test (Figure 1). Data analysis was performed with the software package StatGraph Prism 6.

Results

Average anti-SARS-CoV-2 antibody concentration in investigated groups

As previously explained, we have quantified specific anti-S1S2 spike and anti-np antibodies in COVID-19 patients with heavy or mild clinical picture treated during 3–5 months. All our hospitalized patients demonstrated heavy (but not critical) clinical presentation, with an average of 11 days of hospital treatment. As expected, sera from hospitalized patients contained significantly more anti-S1S2 spike-specific IgG (Figure 1A) and anti-np IgG antibodies (Figure 1B) compared to the group treated out of hospital facilities. Similarly, the group with severe clinical form presented significantly more anti-S1S2 spike specific IgA (Figure 1C) and anti-np IgA antibodies (Figure 1D) compared to the less severe COVID-19 group. Interestingly, the group with severe COVID-19 symptoms demonstrated a significantly higher average concentration of anti-S1S2 spike specific IgM (Figure 1C) and anti-np IgM antibodies (Figure 1D) compared to another group.

Selection of convalescent plasma donors according to the concentration of SARS-CoV-2 specific antibodies

Widely accepted criteria for selection of convalescent plasma donors are levels of specific anti-SARS-CoV-2 IgG antibodies, most frequently antibodies to envelope antigens. In our group of hospitalized patients, 225 had detectable anti-

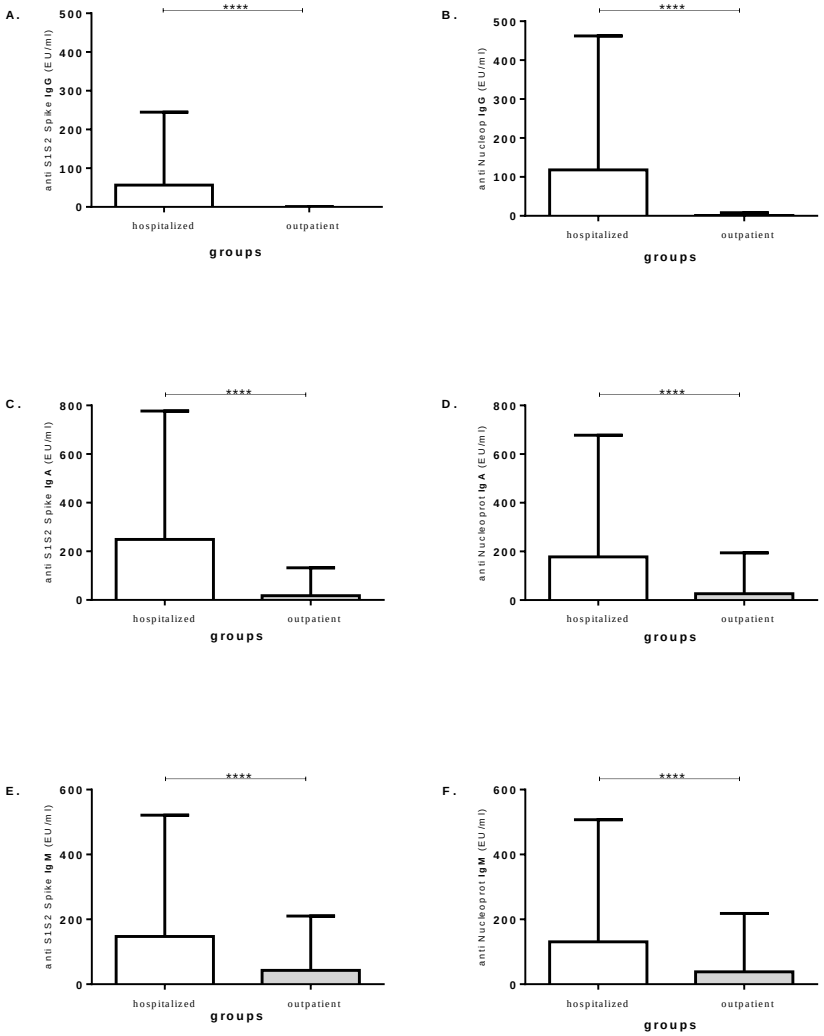

Fig. 1 – Average concentration of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) specific antibodies in investigated patients. All values are expressed as mean ± standard deviation (**$p < 0.0001$, Mann Whitney test). EU – equivalent units.**

S1S2 spike specific IgG antibodies, while 202 had detectable anti-np IgG antibodies. The stratification according to the concentration of specific potential anti-SARS-CoV-2 antibodies in plasma donors are shown is Table 1. Patients that demonstrated a level of specific anti-SARS-CoV-2 antibodies of IgG class in hundreds or thousands EU/mL were selected as convalescent plasma donors. Several patients with a high concentration of specific anti-SARS-CoV-2 antibodies had to be excluded as potential donors according to medical indications (cardiovascular disease, hemophilia).

Time-related concentration change of SARS-CoV-2 specific antibodies in samples of convalescent plasma donors

Until the end of August, we have selected and collected plasma rich in specific anti-SARS-CoV-2 antibodies from 12 people (12/225, 5.3% of patients cured of severe COVID-19). All of these patients had at least two points of specific antibody measurements, with a time interval of no less than 60 days. Interestingly, the concentration of anti-S1S2 spike IgG antibodies increased in 67% of our plasma donors, parallel with the increase of anti-np IgG antibodies in 58% of donors (Table 2). The concentration of anti-S1S2 spike and anti-np of IgA class increased in 50% of all donors. Interestingly, while anti-S1S2 spike IgM concentration decreased in donors, the concentration of anti-np IgM antibodies again was increased in our plasma donors.

Discussion

Convalescent plasma has a strong historical advantage and good biological value. Although this therapeutic approach is promising, it has not yet been shown to be safe in the treatment of COVID-19. Data after transfusion of ABO-compatible human convalescent plasma COVID-19 to 5,000 hospitalized adults with severe or life-threatening COVID-19, of which 66% were in the intensive care unit, were analyzed. The incidence of all serious adverse events, including mortality (0.3%), in the first 4 hours after transfusion was < 1%. Of the 36 reported adverse reactions, 25 were convalescent plasma related, including mortality (n = 4), circulatory overload associated with transfusion (n = 7), acute lung injury associated with transfusion (n = 11), and severe allergic reaction after transfusion (n = 3). However, physicians estimate that only 2 of 36 reactions are definitely associated with convalescent plasma transfusion. The mortality rate after the 7th day was 14.9%. Given the lethal nature of COVID-19 and the large population of critically ill patients included in these analyzes, the mortality rate does not appear to be too high. These early indicators suggest that convalescent plasma transfusion is safe in hospitalized patients with COVID-19 [13–16].

Table 1

Identification of potential convalescent plasma donors according to concentration of specific anti severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) antibodies

Ab conc. (EU/mL)	Patients, n (%)	
	anti-S1S2 spike IgG	anti-nucleoprotein IgG
> 1,000	5 (3)	10 (6)
> 100	19 (8)	31 (15)
> 10	59 (26)	57 (28)
5–10	142 (63)	104 (51)

Ab conc. − antibody concentrations; EU – equivalent units.

Table 2

Time related concentration change of severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) specific antibodies in samples of convalescent plasma donors

Patient's ID, 1st sample	Patient's ID, 2nd sample (after 60 days)	Anti-S1S2 spike Ab			Anti-nucleoprotein Ab		
		IgG	IgA	IgM	IgG	IgA	IgM
617	1,009	▲	▲	▼	▼	▼	▼
666	1,010	▲	▲	▼	▲	▲	▲
778	1,011	▲	▲	▲	▲	▲	▲
664	1,012	▲	▲	▼	▲	▲	▲
12	723	▲	▼	▲	▼	▼	▼
956	1,182	▼	▼	▼	▲	▼	▼
256	1,090	▼	▼	▼	▼	▼	▼
950	1,249	▼	▼	▲	▼	▼	▲
1,030	1,275	▲	▲	▲	▲	▲	▲
367	1,042	▲	▼	▼	▼	▼	▼
119	1,288	▼	▼	▼	▲	▲	▲
1,065	1,287	▲	▲	▲	▲	▲	▲
Increase		67%	50%	42%	58%	50%	58%

Ab – antibody; EU – equivalent units; ID – identity of a patient (donor) indicated by the original protocol number (e.g., donor number one gave the first sample under number 617, and under number 1009, donated plasma for the second time).

In addition to the antiviral mechanisms of neutralizing antibodies, the immunomodulatory effects of plasma components may be beneficial. Several small and large studies have shown the effects of convalescent plasma on the treatment of severe viral illness [diphtheria, scarlet fever, pertussis, poliomyelitis, measles, mumps, flu, Ebola, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS)] as fever, nausea, allergic reactions, blood-borne pathogens transmission, and some serious adverse events such as transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), and antibody-dependent enhancement (ADE) [13–16, 18–20].

In June 2020, the USA Department of Defence began an action to collect plasma units from patients who had fully recovered from COVID-19 in order to support the development of effective treatment. The goal is to collect 10,000 units of this plasma until September 10, 2020 [19].

Until October 2020, there were no licensed vaccines or targeted therapies against the virus itself. Plasma with anti-SARS-CoV-2 antibodies, obtained from recovered individuals with confirmed COVID-19 infection, has been primarily collected using apheresis devices and stored in blood banks in some countries to be administered to patients with COVID-19 in order to reduce the need for intensive care and a lower mortality rate. Therefore, it is necessary to point out some important issues related to convalescent plasma and its use in the treatment of patients as a form of anti-viral therapy. The protective effect can last for weeks and months. After the donor's assessment, 200–600 mL of plasma can be collected with apheresis devices (used in the world and in our country). The donation interval may vary among countries. Hence, there is the necessity of testing antibody titer values. Although limited published studies are not prospective or random until vaccination or targeted antiviral therapy is approved, plasma therapy appears to be a safe and likely effective treatment for critically ill patients with COVID-19. It can also be used for prophylactic purposes, but the safety and efficacy of this approach should be tested in randomized clinical trials, and a conclusion reached [20].

Eligibility criteria for plasma donors may vary from country to country but certainly include, above all, a safe procedure, health and antibody titers checks, and consent to the procedure [20–22]. The antibody titer will vary according to the duration between the time of collection and the onset of infection. According to literature data, the titer ranges from 1: 1,000 [20] to 1: 160 and 1: 640 [22]. In previous studies, it has been observed that seroconversion occurs between 8 and 21 days after the onset of symptoms. In clinical trials, one plasma unit was given initially (200 mL) and repeated after 12 h. The duration of antibody efficacy is not known, but it is estimated that it will last for weeks to several months [20–24]. On August 23, 2020, the FDA issued an approval for the emergency use of convalescent plasma COVID-19 for the treatment of hospitalized patients with COVID-19. There are insufficient data to recommend for or against the use of convalescent plasma for the treatment of COVID-19. Available data suggest that serious adverse reactions after administration of COVID-19 convalescent plasma are rare and consistent with the risks associated with plasma infusions in other indications. The long-term risks of treatment with convalescent plasma COVID-19 and whether its use reduces the immune response to SARS-CoV-2, making patients more susceptible to reinfection, have not been assessed. Convalescent plasma should not be considered the standard of care for the treatment of patients with COVID-19 [25].

Prospective, well-controlled, adequately initiated randomized trials are needed to determine whether convalescent plasma is effective and safe for the treatment of COVID-19.

All this confirms the fact that it is necessary to collect and store certain quantities of convalescent plasma in order to provide reserves, which encouraged us to provide plasma reserves for the treatment of COVID-19 patients with modest funds and without additional costs.

Conclusion

In-house developed ELISA tests enable a novel protocol for the selection of convalescent blood plasma donors. According to our data, it is necessary to recruit and test a large number of patients who have recovered from severe COVID-19 in order to have a sufficient number of appropriate convalescent plasma donors.

REFERENCES

1. *Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC.* Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA 2020; 324(8): 782–93.
2. *Park A.* Blood Plasma Treatment for Coronavirus Set to Get Its First Trial Run in New York. Available from: https://time.com/5809955/blood-plasma-coronavirus/[cited 2020 March 26]
3. *Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J,* et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. JAMA 2020; 323(16): 1582–9.
4. *Li L, Zhang W, Hu Y, Tong X, Zheng S, Yang J,* et al. Effect of Convalescent Plasma Therapy on Time to Clinical Improvement in Patients With Severe and Life-threatening COVID-19: A Randomized Clinical Trial. JAMA 2020; 324(5): 460–70.
5. *Wang C, Li W, Drabek D, Okba NMA, van Haperen R, Osterhaus ADME,* et al. A human monoclonal antibody blocking SARS-CoV-2 infection. Nat Commun 2020; 11(1): 2251.
6. B*rouwer PJM, Caniels TG, van der Straten K, Snitselaar JL, Aldon Y, Bangaru S,* et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science 2020; 369(6504): 643–50.
7. Food and Drug Administration. Coronavirus disease 2019 (COVID-19) EUA nformation. 2020. Available at: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/ emergency-use-authorization#covid19euas. [accessed 2020 August 31].
8. *Grady D.* Blood Plasma From Survivors Will Be Given to Coronavirus Patients. Available from: www.nytimes.com › health › plasma [updated 2020 August 4].

9. The COVID-19 Treatment Guidelines Panel's Statement on the Emergency Use Authorization of Convalescent Plasma for the Treatment of COVID-19. Available from: https://www.covid19treatmentguidelines.nih.gov/ [updated 2020 September 1]
10. *Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR*, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med 2020; 172(9): 577–82.
11. *Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F,* et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clin Infect Dis 2020; 71(15): 778–85.
12. *Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y,* et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clin Infect Dis 2020; 71(16): 2027–34.
13. National Institutes of Health website. Coronavirus disease 2019 (COVID-19) treatment guidelines. Available from: https://www.covid19treatmentguidelines.nih.gov/ [accessed 2020 July 1].
14. *Joyner MJ, Wright RS, Fairweather D, Senefeld JW, Bruno KA, Klassen SA*, et al. Early safety indicators of COVID-19 convalescent plasma in 5000 patients. J Clin Invest 2020; 130(9): 4791–7.
15. U.S. Food and Drug Administration. Convalescent plasma letter of authorization. Available from: https://www.fda.gov /media/141477/download. [accessed 2020 August 31].
16. U.S. Food and Drug Administration. EUA 26382: Emergency Use Authorization (EUA) Request. Available from: www.fda.gov/media/141481/download [accessed 2020 September 6].
17. *Edwards T , Semple MG , De Weggheleire A , Claeys Y , De Crop M, Menten J* et al. Design and analysis considerations in the Ebola_Tx trial evaluating convalescent plasma in the treatment of Ebola virus disease in Guinea during the 2014–2015 outbreak. ClinTrials 2016; 13(1):13–21.
18. *Choi JY*. Convalescent Plasma Therapy for Coronavirus Disease 2019. Infect Chemother 2020; 52(3): 307–16.
19. Convalescent Plasma Collection Program. Available from: health.mil › Coronavirus › COVID. [accessed 2020 September 29]
20. *Yiğenoğlu* TN, *Hacıbekiroğlu* T, *Berber* I, *Dal* MS, *Baştürk* A, *Namdaroğlu* S. Convalescent plasma therapy in patients with COVID-19. J Clin Apher 2020; 35(4): 367–73.
21. *Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S,* et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol 2020; 92(9): 1518–24.
22. *Duan K, Liu B, Li C, Zhang H, Yu T, Qu Y*, et al. The Feasibility of Convalescent Plasma Therapy in Severe COVID-19 Patients:a Pilot Study. medRxiv 2020; doi: 10.1101/2020.03.16.20036145.
23. *Casadevall A, Scharff MD*. Return to the past: the case for antibody-based therapies in infectious diseases. Clin Infect Dis 1995; 21(1): 150–61.
24. *Casadevall A*. Passive antibody administration (immediate immunity) as a specific defense against biological weapons. Emerg Infect Dis 2002; 8(8): 833–41.
25. *Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC*. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA 2020; 324(8):782−93.

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