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Review Article

Presentation of Neutralizing Antibodies in Single- or Pooled-Convalescent Immune Plasma from Donors to Prevent the Current SARS-CoV-2 Pandemic

Fatih Ozcelik [1*], Muhterem Ercan [2], Bulent Barıs Guven [3], Mehmet Zahit Ciraci [4]

[1] Department of Medical Biochemistry, Sultan 2. Abdulhamid Han Training and Research Hospital, University of Health Sciences Turkey, Istanbul, Turkey

[2] Department of Physiology, Hamidiye Faculty of Medicine, University of Health Sciences Turkey, Istanbul, Turkey

[3] Department of Anesthesia and Reanimation, Sultan 2. Abdulhamid Han Training and Research Hospital, University of Health Sciences Turkey, Istanbul, Turkey

[4] Department of Medical Biochemistry, Hamidiye Faculty of Medicine, University of Health Sciences Turkey, Istanbul, Turkey

*** Correspondence:** Fatih Ozcelik, University of Health Sciences Turkey, Sultan 2. Abdulhamid Han Training Hospital, Department of Medical Biochemistry, 34668, Istanbul, Turkey Tel: +90 216 5422020 Fax: +90 216 5422761

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Abstract

As there is no specific and efficient treatment yet, the fight against the COVID-19 pandemic is being carried out with a great difficulty today. The use of immune plasma is considered as a promising option since there is an expectation that it will reduce mortality, as in influenza pandemics experienced in 1918 and 2009. However, the safety and effectiveness of this treatment option against SARS-CoV-2 viruses are not certainly known. In addition, the optimal obtaining methods and protection time of neutralizing antibodies in passive immunization are not fully known. Therefore, it would be especially useful to investigate the most effective neutralizing antibody collection methods without disrupting the overall structure and effectiveness of the antibodies subject to in the use of the convalescent immune plasma. For this purpose, we found it appropriate to prepare a broad review on the structure and properties of antibodies, as well as the principles and storage conditions of antibodies to be used in passive immunization.

Keywords:
Single or Pooled Convalescent Immune Plasma; COVID-19; Apheresis; Neutralizing Antibodies

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Introduction

The antibodies produced by the plasma cells of the immune system and called immunoglobulin are molecules made of glycoprotein. Immunoglobulin isotypes include two light chains (LCs), each called kappa (κ) and lambda (λ), weighing 25 KDa, and two heavy chains (HCs), each weighing 50 KDa. Both light and heavy chains of the immunoglobulin molecule are divided into variable (in the heavy chain: VH and in the light chain: VL) and constant (CH and CL) domains. These domains in the fragment antigen-binding (Fab) in the antibody are called complementary-determining regions (CDRs). CDRs form the basis of the immunological specificity of the antibody [1,2]. The three hypervariable regions (L1, L2 and L3; H1, H2 and H3) within each variable domain are responsible for the antigen-specific recognition function (Figure 1). Antibody-antigen interaction works with structural interaction motifs and amino acid residues. Antibody binding spaces are segmented at the motif level, not at the amino acid level. The vast majority of these are localized to CDRs [3]. The part of the antibody that communicates with the immune system through complement and cell receptors is the Fc region. The Fc portion is located at the base of the Y-shaped antibody. The Fab parts, which interact with the antigen, are located at both upper ends of the Y. The main function of the humoral system is that plasma cells produce antibodies with these two regions. Humoral specificity represents the ability of antibodies to selectively interact with the antigen via epitope-paratope. The paratopic regions of the antibody, which recognize the epitope region of the antigen, are rich in important amino acid residues such as tyrosine and serine, and the antibody-antigen interaction in general is modular. Evolutionarily, it is advantageous to have short motifs instead of long motifs in these regions because short motifs reduce the potential escape area for antigens. However, these short motifs make it difficult for the immune system to distinguish the alien from the self. Of the CDRs regions, only the VH-CDR3 region is mandatory for antibody-antigen interaction. Other regions are thought to be necessary for fine tuning in antibody recognition [4–8]. Specifically, the amino acid trend detected in CDRs in antibodies differs between antibody-antigen complexes. Antibodies recognize antigens by Fab regions. In this recognition, the paratope and the epitope regions are connected very precisely. Thanks to this binding, the marked antigen (bacteria, virus or other pathogens) is recognized and destroyed by the immune system including macrophages, or some of them are neutralized directly [9–11]. This binding of the antibody breaks the potential of the pathogens that cause disease.

Configuration of Antibodies and Disruptive Effects

Antibodies with globular protein structure are organized in a significant proportion with structures such as α-helix and β-sheet. In the rest, β-bends or coils can be observed. These structures are not random but relatively more regular structures. The side chains of the secondary structures which close to each other combine to form super secondary structures (motifs). When antibodies or proteins are denatured,

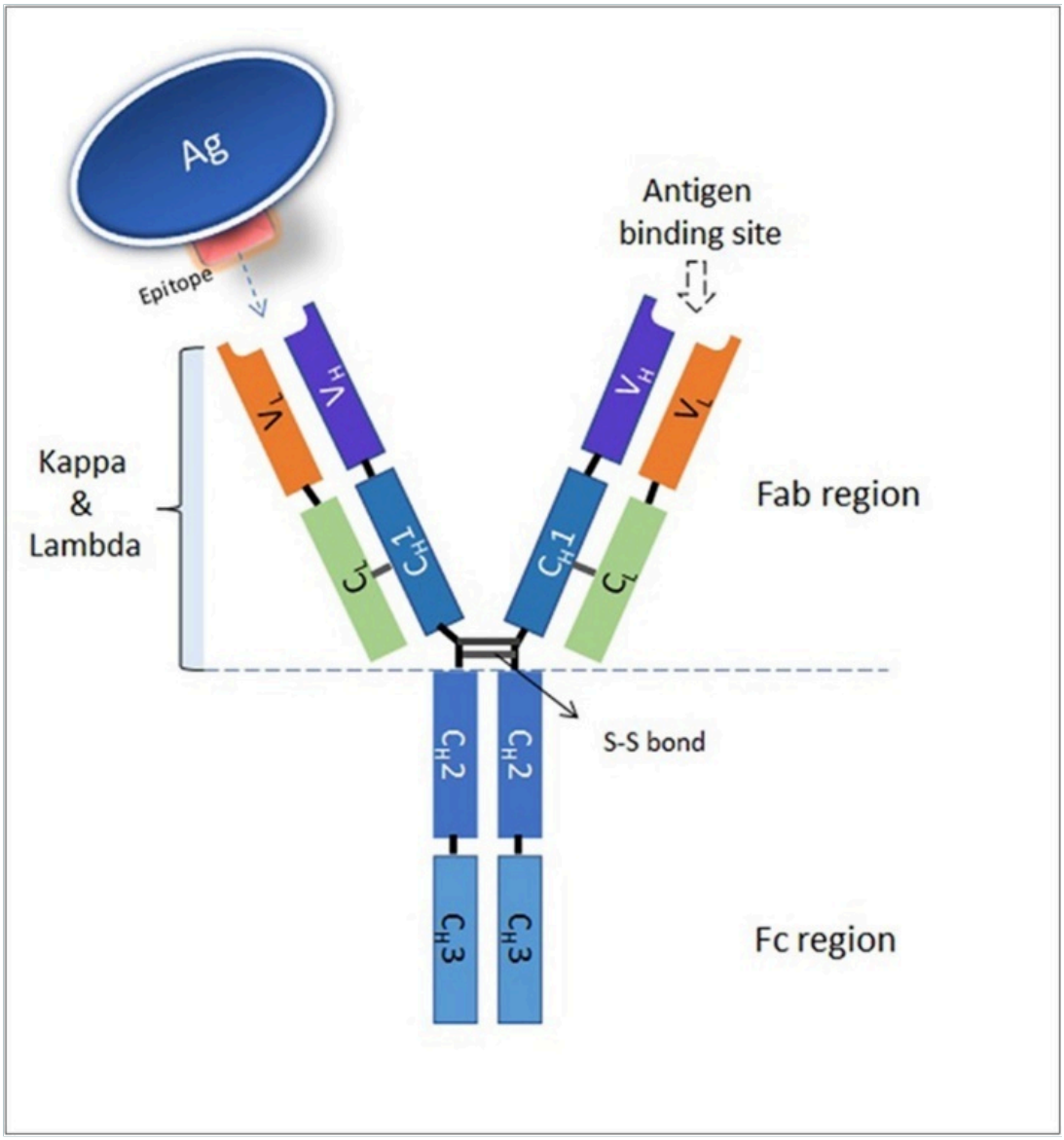

Figure 1. Antibody structure and functional Fc and Fab regions. C: Constant domain, V: Variable domain, H: Heavy chain, L: Light chain

random coils dominate the structure. The hydrophobic side chains of amino acids which make up these globular proteins, are embedded inside and hydrophilic groups are located on the surface of the protein. This structural organization creates the maximum number of hydrogen bonds, disulfide bonds and ionic interactions and ensures proper folding of proteins to perform their functions and increases their durability. Furthermore the chaperones play a major role in the formation of the three-dimensional structure of proteins during their synthesis [12–17]. Although the primary structure of proteins is formed with highly resistant peptide bonds, secondary, tertiary, and quaternary structures are composed of noncovalent weak bonds such as hydrogen, ionic and van der Waals forces, except disulfide. All these complex modular structures are sensitive to chemical, radioactive, heat, pressure, friction, oxidant and heavy metals (such as mercury, lead, zinc, manganese and cadmium). It has been reported that the stability and function of methionine and tryptophan residues of antibodies are sensitive to UV light, high temperature and reactive oxygen radicals [18–21]. It has also been found that freezing during storage of antibodies or other proteins can result in protein aggregation by increasing the acidification of the solution, affecting protein functions and disrupting the secondary and tertiary structure [22–24]. However, it is believed that this effect will not be a problem for many polyclonal IgGs with a pI value (isoelectric point) of 4.7-7.0, except those with a pI value of 7.0. In general, antibodies should be stored in a sterile environment at ≤ 4 ° C [25]. Moreover, storage at room temperature can often lead to disruption and immobility of

antibodies due to microbial proliferation. Storage of lyophilized monoclonal antibodies at ≤ -20 ° C and the addition of sucrose (or trehalose) and / or polyols (glycerol and sorbitol) to the formulation retains their activity for a long time (3-5 years) [25,26]. For any reason, proteins and antibodies that are damaged structurally or functionally are rapidly destroyed in the body. These endogenous proteins are marked by ubiquitin and shipped for destruction.

Depending on the characteristics and storage conditions of the antibody, the shelf life of the antibody can range from 3 days to one year at + 4 ^{0}C. However, some types of antibodies can be stored for much longer. To protect the activity, prevent the aggregation and extend the storage life, concentrated substances (~ 1 M) such as sucrose are added in a suitable temperature and pH environment [26–28]. The stability of proteins is also related to the amount of aromatic amino acid residues that can absorb UV light in the environment. It was determined that the tryptophan residue of the IgG exposed to UV turned into glycine or glycine hydroperoxide [29,30]. Therefore, the use of UV light exposed antibodies is not recommended. Antibodies can be affected by physical or mechanical trauma due to high frequency vibration, vortexing, shaking, defrosting, pump operation and filtration under pressure. It is also reported that such effects can disrupt the structure and function of antibodies and cause their aggregation [31–34]. The effect of exposure to various nonpolar surfaces and frictional interaction may impair the functions of proteins although the exact mechanisms are not fully understood [32,35,36]. Therefore, it should be noted that antibody obtaining procedures should contain less procedures as much as possible without containing irradiation and mechanical stresses.

Neutralizing Antibody Types and Mechanism of Action

Immunoglobulins can be secreted by B cells (plasma cells) or can be found as transmembrane proteins on the non-polar surface of the B cells. Immunoglobulins have the ability to combine with the specific antigen (immunogen) that triggers their production [13–17]. Antibodies can neutralize viruses by combining with neuraminidase (N) and hemagglutinin (H) (resembling sialic acid residues in red blood cells), which allows viruses to attach to respiratory epithelial cells. Then they can neutralize them [9,37]. The recognition of antigens by B cells occurs with B cell receptors or immunoglobulin, while recognition by the T cell occurs only with the surface receptor. In the early period of exposure to the pathogen, IgM antibody is synthesized, while in the late period, the lower molecular weight IgG antibody is synthesized. The largest of the antibodies, IgM is a pentamer with ten antigen binding sites. Due to its' larger size, it can show its effectiveness only in intravascular space. However, IgG antibody can easily circulate effectively between intravascular spaces and interstitial tissues. Secretarial IgA is located on the mucous surfaces where it is produced. This antibody forms the first line of defense against mucosal entering pathogens such as influenza, rhinovirus and human immunodeficiency virus (HIV). It is thought that IgA antibodies may also be effective against the novel corona virus (SARS-CoV-2), which enter through mucosal surfaces [38–42]. IgM class macromolecules are potent agglutinator agents. They can quickly activate the complement system in the classical way. They are of great importance in the early phase of the primary immune response, as they are rapidly produced in a few days to provide protection when a foreign antigen enters the body. IgG, which has a longer life than IgM, is the dominant serum antibody. IgG also directly contributes to an immune response, including neutralization of toxins and viruses [43–45]. Thanks to its specific antigen recognition function, this antibody provides protective immunity in blood and tissue against many types of microorganisms (bacteria, viruses and some fungi). In a study conducted by Castilla et al., cloning of a monoclonal antibody capable of neutralizing the corona virus, an infectious gastroenteritis agent, was achieved. These chimeric immunoglobulins were expressed in murine myeloma or COS cells, which are fibroblast-like cell lines derived from monkey kidney tissue. It was found that these secreted recombinant antibodies reduced the infectious virus more than 10^4 times. The researchers found that IgA can neutralize 50 times more TGEV than recombinant monomeric IgG containing the same antigenic binding site [40]. These findings are in line with the knowledge that IgA in mucosal secretions provides the initial immunological barrier against many pathogens, especially viruses that invade the mucosal surfaces of the body [39,46,47]. All these data suggest that neutralizing antibodies developed after corona virus infection may be effective in limiting the virus.

Collection of Convalescent Immune Plasma and Adverse Reactions

While animal viruses infect cells by binding to the cell type specific cell surface receptor, many antibodies that neutralize viruses directly inhibit viral binding to surface receptors. As with neutralization of toxins, high affinity IgA and IgG antibodies from these antibodies were found to be very important [9]. In this context, antibody response developed by symptomatic or non-symptomatic people to combat the COVID-19 pandemic caused by the SARS-CoV-2 virus is considered as an option in treatment worldwide because no specific treatment and vaccine has been yet found in the fight against COVID-19 [48,49]. For this reason, it is recommended to use convalescent immune plasma, which is thought to contain high number of neutralizing antibodies. This approach is conceptually based on passive immunotherapy. However, the safety and efficacy of the convalescent immune plasma for COVID-19 therapy has not yet been confirmed. In addition, the duration of protection of antibodies with protective properties is uncertain. There are several methods of collecting the convalescent immune plasma from donors recovered from COVID-19 disease. The first is the provision of blood components by the conventional (classical) method. In fact, the principles used in the preparation of the fresh frozen plasma component today are the same as in this method. Similar methods were used in 1918 to obtain blood products in the Spanish flu pandemic. The other method is the convalescent immune plasma supplied using digital technology apheresis device. These two methods have either advantages and disadvantages [50–53].

Studies on H1N1 influenza pandemic, which started in Spain in 1918 and 2009, showed that blood transfusions applied at that time reduced mortality in influenza patients complicated by pneumonia [50,51,54]. In addition, early transfusions have been reported to provide significant improvement in clinical signs and symptoms. However, significant side effects can be observed due to the use of convalescent immune plasma. Among these, allergic reactions, anaphylaxis, transfusion-related acute lung injury (TRALI) and hemolysis due to transfusion antibodies (especially against A and B blood group antigens) are the most feared side effects [55,56]. However, in many studies, no serious side effects related to the convalescent immune plasma used for patients with H5N1 influenza and Severe acute respiratory syndrome (SARS) have been reported. It has been reported that mostly mild fever and chills are observed [57–59].

Whole blood, which is a mixture of different types of cells, colloids and crystalloids, is used by separating it into components according to need. Whole blood can be conventionally separated into blood components as erythrocyte concentrate, platelet concentrate, fresh frozen plasma and cryoprecipitate by the classical method (Figure 2). The same blood components can be obtained directly from the donor blood circulation using an apheresis device. In the apheresis method, besides these components, granulocyte and peripheral blood hematopoietic stem cell collection and therapeutic plasma exchange can be performed [55,60]. Apheresis method is based on the difference of size / specific weights of blood cells. In this method, the centrifugation that distinguishes according to the specific weight and the filtration principle that distinguishes according to their sizes are used (Figure 3). Chromatography principle can be added to these principles depending on situation. In the principle of centrifugation, the device provides continuous blood flow from the patient to the centrifuge. An anticoagulant (usually citrate) is added before centrifugation. The centrifuge rotates at 2000–2500 rpm to separate the various components of anticoagulated blood by their density or specific weight. The separation takes place in a non-selective plasma layer with respect to the axis of rotation, the buffy layer of platelets, lymphocytes, monocytes, granulocytes and the most distant erythrocytes. The effectiveness of separation of various blood components depends on the dimensions of the centrifuge and the speed (revolutions per minute) and duration of the centrifuge, which creates the force of gravity. The filtration principle is also used in hemodialysis. Microprocessors in apheresis devices control the blood flow rate of the device, the amount of citrate anticoagulant mixed with the whole blood, the rotational speed of the centrifuge, the separation of the blood component and the rest of the components returned to the donor [52,61–64]. Whole blood from is directed to the membrane with different sized pores (0.2-0.65 micron) at a given pressure and flow rate. Smaller structures from these pores can pass through the filter. It is an extremely comfortable and fast process.

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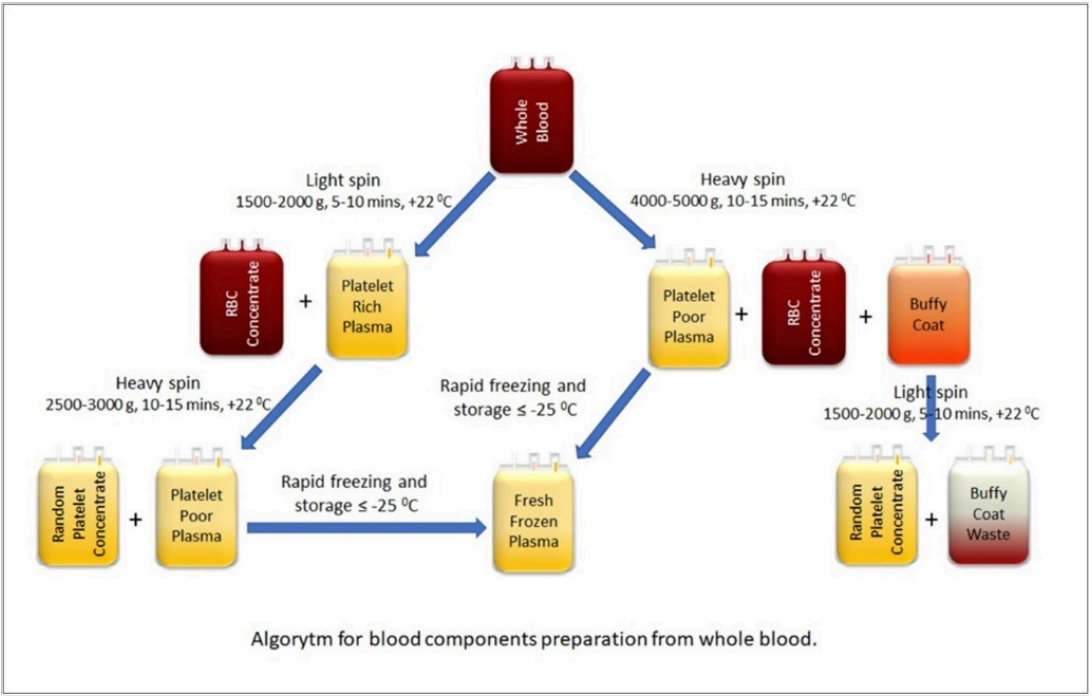

Figure 2. Separation of blood components from the whole blood by classical-conventional method. Whole blood can be separated by two different methods depending on whether the first centrifugation speed is heavy and light spin. In the Buffy coat method, centrifugation starts with heavy spin.

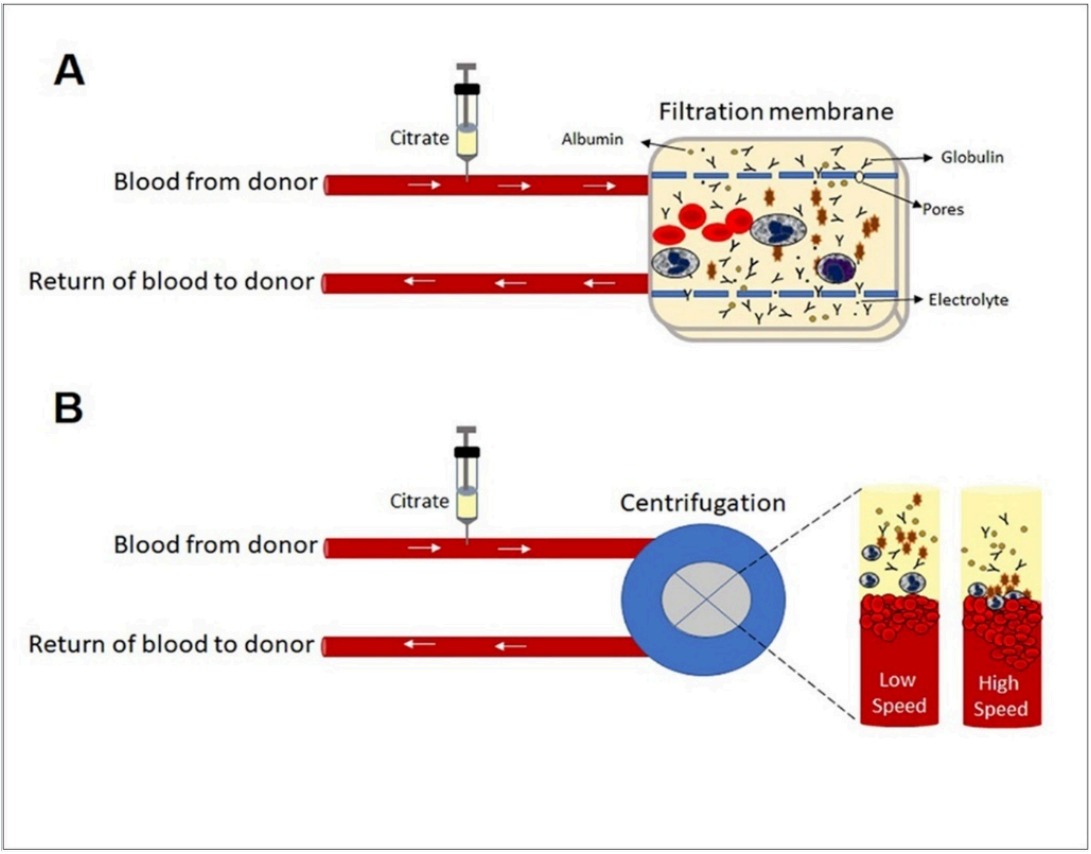

Figure 3. Centrifugation and filtration principle in collecting immune plasma component with apheresis device.

Apheresis gives the advantage of being able to collect a higher amount of plasma (ranging up to 200-600 ml) from a single person, while preventing unnecessary red cell loss from the donor. However, a maximum of 150-200 ml of convalescent immune plasma can be obtained from 1 unit of whole blood (approximately 450 ml) collected randomly by classical conventional method. However, a maximum of 150-200 ml of convalescent immune plasma can be obtained from 1 unit of whole blood (approximately 450 ml) collected randomly by classical conventional method. However, as mentioned above, we think that the application of IV citrate during filtration in apheresis method, the small porous structure of the filter membrane, the risk of contact with the active surface, physical and mechanical effects due to filtration under the pump and pressure may partially impair the structure and functions of the antibodies and cause aggregation. In addition, applied irradiation (gamma rays or UVC sterilization) or chemical pathogen inactivation procedures to kill viruses and bacteria in plasmas obtained by classical or apheresis method and freezing-thawing procedures may result in a higher amount of neutralizing antibody loss [67,68]. By the way, it is reported that the Coronaviruses can remain stable at -25 ^{0}C.

Current researchers show that people with SARS-CoV-2 infection develop neutralizing antibodies against to the virus. This neutralizing formation varies depending on viruses and individual differences in the immune system. While some people respond strongly to the same pathogen, some people may have very low levels of neutralizing antibodies in their blood [48,69–74]. Also, it is not clear whether the antibodies against SARS-CoV-2 provide immunity against the subsequent infection. To clarify this issue, Grzelak et al conducted a study in which people who had COVID-19 infection investigated the presence of seroconversion and neutralizing antibodies. In this study, they investigated antibodies against nucleoprotein (N) or trimeric Spike (S) virus proteins by ELISA method. Antibodies against the virus were detected 4-15 days after the onset of the disease. They found seropositivity in 29% of people with several symptoms (within 15 days after symptoms) and in 3% of healthy donors selected from the region with the COVID-19 outbreak [48]. These findings were evidence that neutralizing antibodies were produced at a level that could be measured against the virus. Generally, it is believed that the neutralization of viruses by antibodies occurs when antibodies bind to the proteins of the virus that attach to cell receptors. Thus, antibodies prevent the virus from adhering to epithelial cells. Again, mucosal secretion antibodies of type IgA have been found to prevent virus attachment to the cell by a similar mechanism [46,75–78]. In a study conducted to take advantage of the neutralization properties of antibodies, transient expression genes of flavivirus were transplanted into vertebrate cells to obtain antibodies that protect against virus infection [76]. These and similar studies show that neutralizing antibodies are important option in resistance to the virus.

It is known that in patients with immune system depression or weakness, COVID-19 disease progresses seriously, and the neutralizing antibodies against SARS-CoV-2 virus cannot sufficiently be created. It is believed that the use of neutralizing antibodies originated from those who have previously had this disease would be beneficial in patients with COVID-19. Plasma donors can be considered as candidates at least 14 days after all symptoms have improved and the COVID-19 PCR test has become negative. Donor candidates may become donors after compulsory tests and standard procedures established by regional committee. It is especially emphasized that male donors should be primarily selected to prevent the risk of Transfusion Related Acute Lung Injury (TRALI) [48,52]. However, we think that the effectiveness of neutralizing antibodies in immune plasmas to be obtained from donors will vary depending on the methods of collection, storage conditions and whether they are exposed to radiation or chemical agents that disrupt the antibody structure. Apheresis devices allows the collection more often and amount of plasma by preventing the collection of unnecessary components; however, during this procedure plasma undergoes a heavy filtering and certain filtering process. Furthermore, a freezing procedure is required in order to stock the immune plasma for longer time periods. In addition, pathogen inactivation processes (radioactive irradiation: gamma irradiation and biochemical treatment) to prevent transmission of infections can disrupt the conformational structure of antibodies in the immune plasma. In this way, the neutralizing antibodies that are denatured or whose load is altered should not be expected to play an effective role in virus inactivation. According to our experience and feedback, the expected benefit could not obtain if plasma collected by apheresis method. However, the results have not been compared with the classical conventional methods yet. Our research on this topic will be presented later. This situation contrasts with the results of blood transfusions used during the Spanish virus pandemic in

1918. The main reason might be the utilization of different collection methods and the area of expertise. Because the immune plasmas used in Spanish virus outbreak were randomly collected convalescent immune plasma by classical methods and were not subjected to pathogen inactivation or freezing. In the other word, neutralizing antibodies were used where in vivo conditions maintained significantly.

Due to a significant number of COVID-19 donors failed to produce sufficient neutralizing antibodies may be the answer to why immune plasma use has failed [48,52]. We propose that such a problem can be solved by pooling of convalescent immune plasma from at least 3 or 4 donors. In this way, the possibility of the success of neutralizing antibodies in the immune plasma will be increased 3 to 4 times. Since such an approach may cause volume loading in a patient with heart failure and volume overload, the condition of the patient must be evaluated before the administration and the amount should be adjusted case by case. Another suggestion is to give pooled convalescent immune plasmas without freezing and exposure to pathogen inactivation (irradiation or biochemical treatment) in order to protect neutralizing antibodies. However, we also know that such an application will be a subject to a lot of criticism by clinicians. The method we recommend is like the use of pooled platelet suspension applied today. In addition, the risk of transfusion reaction due to transfusion of pooled convalescent immune plasma formed from 3 or 4 donors is not higher than the pooled platelet suspension. Fever or mild allergic reactions that are mostly related to protein can be eliminated by using acetaminophen or antihistamine agents depending on the patients' condition [79,80]. Again, the extent to which the pathogen inactivation procedure is required in patients with high viral load and the danger of not performing pathogen inactivation is controversial. The benefit-harm situation should be considered here. Another situation to be investigated is the effectiveness of pooled convalescent immune plasma use. Unfortunately, studies comparing the classical conventional and apheresis method of convalescent immune plasma are quite limited.

Conclusion:

In the fight against the COVID-19 pandemic, obtaining the collected neutralizing antibodies with the method that will suffer the least is important for the success of the treatment. In addition, studies in which apheresis and classical methods are compared to single donor and pooled options are needed to determine the most appropriate collection method.

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