Convalescent plasma treatment, which is passive polyclonal antibody administration to treat patients with active coronavirus disease-2019 (COVID-19), is suggested and investigated in many research protocols as a promising therapy approach for coronavirus disease. This approach may decrease COVID-19 associated mortality by providing immediate immunity to deteriorating patients and by reducing viral spread early on [1-3]. Early indicators suggest that transfusion of convalescent plasma is safe in hospitalized patients with COVID-19 [4], therefore it is of utmost importance that blood establishments satisfy both safety and efficacy demands by selection qualified blood donors and by taking biosafety measures for protection from severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) during whole blood or plasmapheresis donation as well as during manufacturing procedures [5,6].

The possibility of SARS-CoV-2 blood viremia in convalescent donors and whether detection of it by reverse-transcriptase polymerase chain reaction (RT-PCR) testing is important to evaluate, although there is no established knowledge that COVID-19 is transmitted through blood or blood products, and the risk of transmission of SARS-CoV-2 by transfusion remains theoretical [7]. However, this testing is important to assess as a safety measure for the blood screening process in every convalescent donor as was recently recommended [8]. As an alternative, pathogen reduction technology (PRT) has been suggested to be effective in reducing infectious pathogen load in blood products and pooled plasma [9,10] and may also serve as another safety procedure.

In addition to safety measures, it is important that SARS-CoV-2 convalescent plasma contain neutralizing antibodies against the virus to be efficient in eliminating it. One of the best overall quantitative correlations to the neutralizing titer was obtained with the Euroimmun IgG (Lubeck, Germany) ELISA assay [11] when compared with other seven commercially available immunoassays. Therefore, we used the Euroimmun IgG ELISA assay to screen all of our blood units and to choose efficient plasma with high antibody level.

In the current study we reported our experience in preparing COVID-19 convalescent plasma. We recruited patients recovered from proven COVID-19 infection, collected whole blood from those who met our inclusion criteria for convalescent plasma donation, and prepared safe SARS-CoV-2 pathogen free and positive SARS-CoV-2 IgG hyper immune plasma as well as other safe blood components.

**ABSTRACT**

**Background:** During the coronavirus disease-2019 (COVID-19) pandemic outbreak our blood bank developed protocols to guarantee accurate blood components to COVID-19 patients.

**Objectives:** To provide convalescent whole blood donor screening strategies for patients recovering from COVID-19.

**Methods:** We recruited COVID-19 recovering patients who met our defined inclusion criteria for whole blood donation. All blood units were screened for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) RNA by real time reverse transcription polymerase chain reaction (RT-PCR) and SARS-CoV-2 immunoglobulin G (IgG) antibodies against the S1 domain.

**Results:** We screened 180 blood units from patients recovering from COVID-19. All results were negative for SARS-CoV-2 RNA and 87.2% were positive for SARS-CoV-2 IgG antibodies in the plasma.

**Conclusions:** Blood component units from recovering COVID-19 patients are safe. Plasma units with positive IgG antibodies could serve as an efficient passive immunization for COVID-19 patients. Moreover, in the face of increased transfusion demand for treatment of anemia and coagulation dysfunction in critical ill COVID-19 patients, red blood cells units and random platelets units from convalescent donors can be safely transfused.

**KEY WORDS:** blood bank, blood donors, coronavirus disease-2019 (COVID-19), infectious disease
Potential convalescent donors were recruited through local networking and local media. Subjects were urged to donate blood for convalescent plasma after recovering from COVID-19 symptoms. Donors were requested to answer a health-related questionnaire to ensure their eligibility and were provided with information related to the blood donation. A blood management computerized system upgraded the blood supply chain.

Inclusion criteria for convalescent whole blood donors included:

• Meet all criteria for volunteer blood donation
• Age between 18 and 65 years
• Prior diagnosis of COVID-19 documented by a positive nasopharyngeal swab
• Complete resolution of all COVID-19-related symptoms for at least 28 days or two negative repeated SARS-CoV-2 RNA nasopharyngeal swabs for at least 14 days before the time of blood donation
• Male donors and never-pregnant female donors
• Signed blood donor written consent

Exclusion criteria for convalescent whole blood donors included:

• Failure to pass standard volunteer blood donor screening criteria required by the Israel Ministry of Health
• Inadequate venous access for phlebotomy

Biosafety protective measures against exposure to SARS-CoV-2 were taken during whole blood donation. Our protocol allowed the collection, manufacturing, and storage of convalescent plasma for 1 to 5 years and storage of leukocyte depleted RBC units and random platelets for 42 and 5 days, respectively. Final products were specifically labeled as COVID-19 convalescent plasma/blood and stored in a dedicated location.

Nucleic acids obtained from whole blood of SARS-CoV-2 survivors were extracted using MagnaPure 96 instrument (Roche, Germany) according to the manufacturer instructions. RT-PCR reactions, using primers corresponding to the SARS-CoV-2 envelope (E) gene, were performed as previously described [12]. The qRT-PCR reactions were performed in 25 μl Ambion Ag-Path Master Mix (Life Technologies, USA) using TaqMan Chemistry on the ABI 7500 instrument.

Immunoassays for detection of anti-SARS-CoV-2 IgG in convalescent blood

Immunoassays for detection of anti-SARS-CoV-2 IgG in convalescent blood was conducted by Euroimmun (Lubeck, Germany). The Euroimmun (EI) anti-SARS-CoV-2 IgG ELISA is based on a recombinant S1 protein from the SARS-CoV-2 spike protein. Testing was performed according to manufacturer instructions. Index values (signal to cut-off [S/Co] ratios) of < 0.8, ≥ 0.8 to < 1.1, and ≥ 1.1 were interpreted as negative, indeterminate, and positive, respectively. All testing was performed on AGILITY® automated ELISA analyzers (DYNEX Technologies Inc., Chantilly, VA, USA) [13]. The control group was comprised of 20 healthy laboratory employees.

RESULTS

Whole blood units were collected from 180 recovering COVID-19 patients, 153 males and 27 females, mean ± SD age 34.2 ± 13.65 years, within up to 45 days from the active disease.

All whole blood units were found negative for SARS-CoV-2 RNA. Of them, 157 donors were positive for SARS-CoV-2 IgG with high serum IgG antibodies, median 5.7 (range 1.1–15). SARS-CoV-2 IgG antibodies were not present in any of the 20 healthy control subjects (median 0.2, range 0.17–0.38), leading to sensitivity of 87.2 % and specificity of 100%.

It is worth noting that since March 2020 COVID-19 wards were opened in our hospital and within the 3 following months the Sheba blood bank administered 64 convalescent plasma units to 38 hospitalized COVID-19 patients. As blood PCR for COVID-19 was negative, other blood components such as red blood cell units and random platelets were also administered as needed.

DISCUSSION

Blood banks need to address the demand for COVID-19 convalescent plasma units. Many blood centers like ours have implemented special infrastructures for blood collections for constructing inventories of convalescent plasma to meet the growing demand. To satisfy requests for hyper immune plasma we defined the requirements for the recruitment and the selection of COVID-19 convalescent blood donors and established strict standards to distribute efficient and safe products under challenging regulatory and logistical requests. We preferred whole blood donation and manufacturing components and not plasmapheresis, because our plasmapheresis unit is fully occupied with single donor platelet pheresis donors for bone marrow transplantation and hematology patients and because our donors are more at ease with whole blood donation than being connected to a plasmapheresis machine for 2 hours. Our findings show that no concern exists from COVID-19 convalescent donors to the laboratory staff, as all our donors were recruited under strict protocol and the processing of the blood was done in a biosafety environment.
All units were screened and found negative for the nucleic acid-based detection of SARS-CoV-2 and thus no risk of occult COVID-19 infection and no viral transmission exists from blood products. Therefore, we suggest that viral inactivation procedures are not needed during plasma preparation procedures from COVID-19 convalescent donors.

Although neither the U.S. Food and Drug Administration (FDA) [14] nor the European Center for Disease Control recommend pathogen reduction technologies for convalescent plasma, some blood banks use solvent/detergent for large pools of plasma [15], or photo-inactivation in the presence of a photosensitizer for single-unit inactivation. Ragan and colleagues [16] recently reported good reductions of SARS-CoV-2 viral titers in whole blood and plasma when SARS-CoV-2 was used to inoculate these units that then underwent treatment with riboflavin and UV light. They suggested that this reduction may help protect the blood supply during the COVID-19 pandemic. As we had to deliver rapidly convalescent plasma units during the pandemic situation, we preferred the quicker way to test donor's blood for SARS-CoV-2 RT-PCR within a day.

The results of a meta-analysis showed that the vast majority of infected individuals elicited anti-spike antibodies within 2 weeks after onset and high levels of antibodies lasted for 1–3 months after disease onset [17]. Therefore, our blood collection and infrastructure protocol yielded high levels of anti-COVID-19 antibodies, as all our donors were early COVID-19 recovered within up to 45 days from the active disease. Our findings showed 100% specificity and 87.2% sensitivity for the antibody testing when measured at least 14 days after symptom onset. Another study reported serum IgG to SARS-CoV-2 spike protein high sensitivity and specificity using the Euroimmun manufacturer's cutoff of 1.1 units [18].

Validated serologic assays of donor units are crucial for patients infected with COVID-19 convalescent plasma. The FDA has recommended [19] that convalescent plasma with a virus neutralizing antibody titer greater than 1:160 to be used for therapeutic transfusion. However, assay to determine viral neutralizing antibody titers was not available in our institution and instead the Euroimmun assay proved to have high sensitivity and specificity in our analyses. Moreover, it was shown to have the highest correlations with neutralizing antibody titers and high discriminative capacity for detecting these neutralizing antibodies when compared to the performance of commercial EIAs to detect IgG or total antibodies to SARS-CoV-2 [20].

Our findings support the suggestion to give convalescent plasma to hospitalized patients with positive nasopharyngeal RT-PCR and negative or low anti S1-SARS-COV-2 antibodies. Specifically, we recommend treating early in the course of the disease, especially patients with additional chronic diseases or immune-compromised situations that are at high risk of mortality. It is of note that antibody-dependent enhancement (ADE) is only a theoretical concern related to passive immunization in COVID-19 patients, and ADE hyper inflammatory immune-mediated deterioration has not been reported in adult and pediatric patients receiving convalescent plasma for SARS-CoV-2 [21-23]. Nevertheless, we recommended the SARS-COV-2 antibodies status of patients before administrating convalescent plasma.

CONCLUSIONS

Increased transfusion demand for treatment of anemia and coagulation dysfunction in patients with severe COVID-19 disease, especially those placed on extracorporeal membrane oxygenation [24,25], shows the importance of giving critically ill patients other blood components from convalescent donors with negative test results. We recommend implementing this strategy.

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References
Humans are exposed to influenza virus throughout their lifetime through a combination of infections and vaccinations. It remains unclear whether different exposure routes induce distinct influenza-specific immunological memory. Dugan and colleagues found that infection-induced antibodies reacted to non-neutralizing epitopes of influenza virus, whereas vaccination-induced antibodies reacted to neutralizing epitopes. Infection-induced antibodies also preferentially responded to influenza strains present during an individual’s childhood. Passive transfer of vaccination-induced antibodies, but not infection-induced antibodies, protected mice in a model of influenza infection. These findings demonstrate that existing influenza-specific memory and route of exposure influence influenza immunity.

Tumors evade antitumor T cells by various mechanisms. Young and associates used a CRISPR screen to show that tumor necrosis factor (TNFa) and autophagy play a role in the T cell-mediated killing of tumor cells. Pharmacologic or genetic inhibition of autophagy in tumor cells increased TNFa-mediated T cell killing of tumor cells. Deletion of the gene Rb1cc1 in tumor cells improved the efficacy of immune checkpoint blockade in a mouse tumor model. However, deleting the TNFa receptor in tumor cells partially abrogated the improved efficacy of immune checkpoint blockade in the absence of Rb1cc1. Thus, autophagy inhibition may improve T cell–mediated immunotherapies in patients who have cancer.