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Treatment of B-cell depleted COVID-19 patients with convalescent plasma and plasma-based products

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 infected patients, receiving background anti-CD20 therapy, were treated with convalescent plasma or plasma-based products. Eight patients were included in the study, presenting with prolonged disease course and delayed viral clearance. CP/plasma-based products were offered as an add-on therapy to standard medical treatment. All patients showed remarkable clinical and laboratory improvement. In addition, polymerase chain reaction from nasopharyngeal swabs rapidly converted to negative following plasma administration. This study emphasizes the therapeutic efficacy of convalescent plasma and plasma-based products in a subgroup of immunocompromised patients with iatrogenic B-cell depletion.

1. Introduction

In the inevitable race to mitigate the coronavirus disease 2019 (COVID-19) burden, therapies for acute infection proved no significant efficacy in comparison to vaccination and infection control measures. While many treatments showed initial promise, only glucocorticoid therapy have held to the scrutiny of thorough research [1].

Passive immune therapy is a historically accepted therapeutic approach for the immediate management of viral infections. Relying on previous experience with several viral infections, including influenza and Ebola virus, therapy with convalescent plasma (CP) was utilized in the current pandemic [2,3]. As for now, only low-quality evidence supports its routine use [4,5]. Moreover, scarce evidence is available regarding the efficacy in B-cell depleted patients, secondary to anti-CD20 therapy.

Immunocompromised patients are prone to develop a prolonged disease course, persistent symptoms, severe manifestations, and decreased viral clearance [6,7]. Herein, we present eight patients with refractory COVID-19, secondary to iatrogenic B-cell depletion. Besides the similarity in disease characteristics, all patients showed a favorable response to the administration of CP, resulting in clinical and laboratory

improvement and successful viral clearance.

2. Materials and methods

Study subjects included were immunocompromised adult (age > 18 years) patients hospitalized at Hadassah Medical Center, between April 2020 to February 2021, with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, diagnosed based on clinical symptoms and nasal swabs, testing positive for real-time reverse transcriptase-polymerase chain reaction (PCR) assay for SARS-CoV-2.

Immunosuppression was secondary to the administration of anti-CD20 biological agents: Rituximab (RTX), Obinutuzumab or Oceralizumab, for the patients' background hematologic or autoimmune disorders.

CP/plasma-based products were offered as an add-on therapy to standard medical treatment: Antiviral therapy (Remdesivir), anticoagulation (LMWH), empiric antibiotics, and/or glucocorticoid therapy. All patients signed informed consent prior to plasma administration.

CP was collected from donors using standard apheresis methods, 14 days following the last negative nasopharyngeal PCR result. All Donors

Abbreviation: COVID-19, coronavirus disease 19; CP, convalescent plasma; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; PCR, polymerase chain reaction; RTX, Rituximab; LMWH, low molecular weight heparin; S protein, spike protein.

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were diagnosed with COVID-19 based on positive nasopharyngeal PCR results, and had to fulfill the Israel Ministry of Health criteria for disease recovery. Plasma units were tested for IgG antibodies against SARS-CoV-2 spike (S) protein, with an approximate titer of greater than 1:100. Plasma was tested for transmissible viruses (HIV, HBV, HCV, etc.) and preserved at -80°C . The protocol comprised the administration of two units of ABO compatible plasma in a 24 h interval. Intravenous transfusion rate was standard, 100 ml/h, and the patients were monitored for adverse reactions. One patient received Kamada Hyper-Immunoglobulin (IgG).

3. Results

Since the beginning of the COVID-19 pandemic, a cohort of eight patients receiving anti-B-cell therapies were treated in our institution with convalescent plasma or plasma-based products (Table 1).

Patients' mean age was 58.6 years, with a female predominance of 62.5%. The majority of patients received anti-B-cell therapies due to underlying haematological malignancies (5/8 patients). Six patients developed COVID-19-related symptoms within one month of the administration of the last dose of the anti-B-cell agent. Initially, patients presented with prolonged fever and PCR positivity (patients 1–5) and received CP or plasma-based products late in the disease course (35–60 days). Following initial therapeutic improvement, plasma was administered earlier in therapy to patients 6–8, (between 4 and 14 days), resulting in a shorter disease course. Prior to CP/plasma-based product

administration, the plasma titer of SARS-CoV-2 antibodies were either low or undetectable. 50% of patients had undergone serologic testing post-administration, presenting a noticeable increase in their titers. All patients improved clinically after receiving CP or plasma-based products. It is noteworthy that where tested, nasopharyngeal swabs converted to negative at an accelerated rate after plasma administration, signifying effective viral clearance.

Herein, we present two cases in detail, one with a prolonged disease course and late CP administration (Table 1: case 2), and the other with a shorter course and early treatment with CP (Table 1: case 8).

3.1. Case 2

A 47-year-old female was diagnosed with COVID-19 after developing abdominal pain and fever. While initially subsiding, fever recurred after three weeks, appearing daily, along with an unremitting cough. Five months earlier, the patient was diagnosed with Burkitt's lymphoma, for which she received R-CODOX-M/R-IVAC protocols, with a favorable response. The last RTX dose was given one month prior to contracting COVID-19. Due to the persistence of fever and cough, the patient was admitted for further evaluation. Initially, she was empirically treated with ertapenem and azithromycin for the coverage of a suspected bacterial superinfection. However, a thorough assessment, including physical examination, blood, urine, and sputum cultures, as well as a whole-body CT scan, was significant only for pulmonary ground glass opacities (GGO), compatible with COVID-19. Serologic testing for SARS-

Table 1
Patients characteristics and outcomes.

| Patient No., age and sex | Indication for B-cell depletion therapy | Anti B-cell agent (approx. time of last dose before symptoms) | Prominent disease features | Approx. days from symptoms to plasma administration (days) ^a | SARS-CoV-2 antibodies prior to treatment ^b (AU/ml) | SARS-CoV-2 antibodies post-treatment ^b (AU/ml) (approx. days post) | Clinical outcome (Clearance time ^c) |
|------------------------------|---|---|---|---|---|---|---|
| 1, 62 y/o F | MZL | RTX (10 days) | Fever, respiratory symptoms, prolonged PCR positivity | 40 | IgG 7.19 IgM 0.395 | IgG 23.7 (3 days) | Improvement ^d (unknown) |
| 2, 47 y/o F | Burkitt lymphoma | RTX (1 month) | Abdominal pain, prolonged fever, prolonged PCR positivity | 45 | IgG undetectable | IgG 6.89 (1 day) | Improvement (2 days) |
| 3, 58 y/o F | FL | Obinutuzumab (1 month) | Desaturation, recurrent fever, recurrent PCR positivity | 60 | IgG undetectable | Not measured | Improvement (same day) |
| 4 ^e , 63 y/o M | FL, relapse | Obinutuzumab, (1 month) | Prolonged fever and PCR positivity, mild desaturation | 35 | IgG undetectable | IgG 3.83 (40 days) | Improvement after second course (Not achieved at 45 days) |
| 5, 43 y/o M | GPA | RTX (1 week) | Prolonged fever, respiratory symptoms, recurrent PCR positivity | 50 | Not measured | IgG 6.89 (2 months) | Improvement (same day) |
| 6 ^f , 67 y/o F | CLL | RTX (1 month) | Fever, malaise, respiratory symptoms | 7 | Treated with anti-SARS-CoV-2 hyperimmune globulin IgG undetectable | Not measured | Improvement (unknown) |
| 7, 65 y/o M | MS | Ocrelizumab (7 months) | Fever, malaise, respiratory symptoms - severe COVID-19 | 14 | IgG undetectable | Not measured | Improvement (2 days) |
| 8, 64 y/o F | RA | RTX (3 months) | Fever, respiratory symptoms - severe COVID-19 | 4 | IgG undetectable | Not measured | Improvement (10 days) |

^aAll patients received two convalescent plasma doses unless otherwise indicated. ^bAntibodies to S-protein, LIASION© assay: IgM < 1.1 AU/ml negative, IgM > 1.1 AU/ml positive. IgG < 12 AU/ml negative, IgG > 15 AU/ml positive. ^cApproximal days from plasma to 1st of 2 consecutive negative nasal swab PCR spaced at least 1 day apart). ^dClinical improvement relates to defervescence, abating respiratory symptoms, weaning oxygen support and shortened hospitalization ^eReceived one dose of convalescent plasma and a few weeks later a second course of two convalescent plasma doses. ^fTreated with Kamada hyperimmune immunoglobulins (IgG). **Abbreviations:** SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; MZL, marginal zone lymphoma; RTX, polymerase chain reaction; FL, follicular lymphoma; GPA, granulomatosis with polyangiitis; CLL, chronic lymphocytic leukemia; MS, multiple sclerosis; COVID-19, coronavirus disease 19; RA, rheumatoid arthritis.

CoV-2 was negative, suggesting an unresolved SARS-CoV-2 infection. Therefore, two doses of CP were added to background therapy with Remdesivir and LMWH. Soon thereafter, remarkable clinical recovery ensued, with complete defervescence C-reactive protein decreased from 18.8 mg/dl on the day of plasma administration to 1.1 mg/dl Table 1.; 72 h later. PCR-based nasopharyngeal swabs, being positive for 45 days, converted to negative within 2 days.

3.2. Case 8

A 64-year-old female was diagnosed with an asymptomatic COVID-19 after a positive nasopharyngeal swab for SARS-CoV-2. Her medical history was significant for rheumatoid arthritis, treated with RTX and leflunomide. The last rituximab dose was administered 3 months earlier.

After an asymptomatic course during the first week, she started to suffer from high-grade fever, fatigue, and dyspnea. She was admitted to the hospital for respiratory distress and severe desaturation in room air, with the need for supplemental oxygen through high-flow AIRVO. Her lab tests were notable for lymphopenia, high inflammatory markers, and elevated d-dimer. Serology for SARS-CoV-2 was negative, and chest X-ray (CXR) displayed diffuse alveolar infiltrates.

The patient was initially treated with intravenous methylprednisolone, prophylactic LMWH, and empiric antibiotics (for a presumed bacterial superinfection). At the 4th day of her hospitalization, and in light of her B-cell depleted status, she received two sequential doses of CP. Within 5 days, pronounced clinical improvement ensued, with resolution of fever and respiratory distress, and a marked decline in the need for supplemental oxygen. Her nasopharyngeal swabs, which were positive for 25 days, turned negative within 10 days of CV administration.

4. Discussion

Passive immunotherapy was introduced in the late 19th century as a potential therapy for a variety of viral infections. Nowadays, it represents a notable and relevant treatment option in the COVID-19 pandemic. While the administration of CP or plasma-based products is indicated regardless of the patient's immunological status, efficacy was proved to be highest upon use in the early stages of COVID-19. The administration to elderly patients (>65 years old) within 72 h of symptom onset has achieved a 48% reduction in progression to severe respiratory disease [8].

In our study, CP/plasma-based products were administered more than 72 h of symptom onset, due to the prolonged disease course presented by our immunocompromised hosts. Despite the delay between symptom-onset and administration, remarkable efficacy in clinical and laboratory outcomes was achieved.

Host immune response against viral pathogens is mainly mediated by T-cell lymphocytes and natural killer cells. In addition to their role in humoral immunity and production of neutralizing antibodies, B-cells also serve as professional antigen presenting cells, inducing T-cell activation and differentiation [9]. Hence, humoral and cellular immunity is affected by anti-CD20 treatment. B-cell depleted patients present with decreased viral clearance and a prolonged disease course.

Plasma therapy has a number of benefits, including the prevention of new infection, antibody-mediated viral suppression, viral clearance, as well as accelerated infected-cell clearance via complement activation, antibody-dependent cellular cytotoxicity (ADCC), and phagocytosis [10]. All of the latter emphasize the potential immunological yield of CP administration as a therapeutical option for B-cell depleted patients.

Notably, B-cell depletion could positively influence disease severity. It results in a reduction of non-neutralizing antibodies, thus posing a decreased risk for developing antibody-dependent enhancement

syndrome, cytokine storm, and acute respiratory distress syndrome [7,11]. Accordingly, depletion in peripheral B-cells results direct reduction of cytokine production, including IL-6. In concordance, most patients in the current study were diagnosed with mild COVID-19, mainly presenting with refractory fever, prolonged disease course, persistent PCR positivity.

We have herein presented our institutional experience with CP/ plasma-based product therapy in B-cell depleted immunocompromised patients. Following administration, all patients achieved clinical and laboratory improvement. Accelerated viral clearance also occurred, as reflected by the accelerated rate of negative nasopharyngeal PCR swabs. No transfusion reaction was documented, indicating evident safety. Study results are aligned with up-to-date reports on safety and efficacy of CP therapy in B cell depleted patients [12].

Our study holds some limitations. Firstly, the sample size is small. Secondly, patients received additional COVID-19 targeted therapy, such as Remdesivir and corticosteroids, thus potentially adding to the therapeutic benefit of plasma administration. In addition due to the retrospective aspect of this study, data is lacking regarding anti-SARS-CoV-2 antibodies following CP administration in 50% of patients. Furthermore, anti-SARS-CoV-2 antibodies measurements were performed in different timelines post treatment with CP.

To conclude, anti-CD20 targeted therapies continue to be applied worldwide for a variety of haematological and autoimmune disorders. In the era of the COVID-19 pandemic, patients receiving these therapies are prone to persistent SARS-CoV-2 infection. CP/ plasma-based products offer a safe and effective therapeutic option for such patients. Further evidence is still needed to establish the clinical benefit of such therapy.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

References

- [1] National Institution of Health: Coronavirus Disease 2019 (COVID-19) Treatment Guidelines [Available from, <https://www.covid19treatmentguidelines.nih.gov/therapeutic-management/>, 2021.
- [2] I.F. Hung, K.K. To, C.K. Lee, K.L. Lee, K. Chan, W.W. Yan, et al., Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection, *Clin. Infect. Dis.* 52 (4) (2011) 447–456.
- [3] Y. Cheng, R. Wong, Y.O. Soo, W.S. Wong, C.K. Lee, M.H. Ng, et al., Use of convalescent plasma therapy in SARS patients in Hong Kong, *Eur. J. Clin. Microbiol. Infect. Dis.* 24 (1) (2005) 44–46.
- [4] S. Sarkar, K.D. Soni, P. Khanna, Convalescent plasma is a clutch at straws in COVID-19 management! A systematic review and meta-analysis, *J. Med. Virol.* 93 (2) (2021) 1111–1118.
- [5] N. Devasanapathy, Z. Ye, M. Loeb, F. Fang, B.T. Najafabadi, Y. Xiao, et al., Efficacy and safety of convalescent plasma for severe COVID-19 based on evidence in other severe respiratory viral infections: a systematic review and meta-analysis, *Cmaj.* 192 (27) (2020) E745–e55.
- [6] Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, et al. Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. *N. Engl. J. Med.* 383(2020). p. 2291–3.
- [7] I. Kos, B. Balensiefer, S. Roth, M. Ahlgrim, M. Sester, T. Schmidt, et al., Prolonged course of COVID-19-associated pneumonia in a B-cell depleted patient after rituximab, *Front. Oncol.* 10 (2020) 1578.
- [8] R. Libster, G. Pérez Marc, D. Wappner, S. Coviello, A. Bianchi, V. Braem, et al., Early high-titer plasma therapy to prevent severe Covid-19 in older adults, *N. Engl. J. Med.* 384 (7) (2021) 610–618.
- [9] X. Chen, P.E. Jensen, The role of B lymphocytes as antigen-presenting cells, *Arch. Immunol. Ther. Exp.* 56 (2) (2008) 77–83.
- [10] E.M. Bloch, S. Shoham, A. Casadevall, B.S. Sachais, B. Shaz, J.L. Winters, et al., Deployment of convalescent plasma for the prevention and treatment of COVID-19, *J. Clin. Invest.* 130 (6) (2020) 2757–2765.
- [11] G. Novi, M. Mikulska, F. Briano, F. Toscanini, F. Tazza, A. Uccelli, et al., COVID-19 in a MS patient treated with ocrelizumab: does immunosuppression have a protective role? *Mult. Scler. Relat. Disord.* 42 (2020) 102120.
- [12] T. Hueso, C. Poudroux, H. Péré, A.L. Beaumont, L.A. Raillon, F. Ader, et al., Convalescent plasma therapy for B-cell-depleted patients with protracted COVID-19, *Blood.* 136 (20) (2020) 2290–2295.