Memory B cells and serum immunoglobulins are associated with disease severity and mortality in patients with COVID-19

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INTRODUCTION

The unprecedented COVID-19 pandemic, which has affected the entire world for more than 1 year, has caused over 160 million confirmed cases and over 3 million deaths.1 The causative agent of the disease is a novel single-stranded RNA coronavirus (SARS-CoV-2) that humans have not previously encountered. The clinical expression of COVID-19 may vary from an asymptomatic form to a severe disease with acute respiratory distress syndrome, multiorgan failure and death.2 This variable clinical spectrum of COVID-19 severity indicates that host immune responses to SARS-CoV-2 play an essential role in determining the clinical course after initial infection.3 Similar to T and Natural Killer (NK) cells, B cells are critical in the clearance of cytopathic viruses and development of the memory response that prevents reinfections.4 Memory B cells respond rapidly to reinfections by inducing the growth of plasma cells. Long-term protection against infections is provided by plasma cells and memory B cells. After elimination of infectious agents, plasma cells continue antibody secretion and cause serological memory.5 Antibodies have an essential role in the immune response to viruses and there is a predisposition to viral infections in antibody-deficient diseases such as common variable immunodeficiency (CVID).6 Although not all viral agents cause serious infections, viruses such as enteroviruses and cytomegalovirus (CMV) reportedly increase morbidity and mortality rates in patients with CVID and X-linked agammaglobulinaemia (Bruton’s disease).7 The changes in host immune cells can vary based on different viral infection types and viral pathogenic mechanisms.8 However, the roles of B cells and antibodies in response to SARS-CoV-2 infection have not been clarified.

In the present study, the relationship between COVID-19 disease severity and B cell subsets and serum immunoglobulin levels was examined. In addition, humoral immunity status and its association with severity of COVID-19 were characterised.

METHODS

Study design

This retrospective cohort study was conducted at the Necmettin Erbakan University Meram Faculty of Medicine Hospital. Among patients hospitalised for COVID-19 confirmed by the laboratory between March and November 2020, those who met the following inclusion criteria were included in the study: over 18 years of age and laboratory analyses of immunoglobulin levels and number of cells in B lymphocyte subsets performed within 72 hours after admission. The exclusion criteria were the following: use of drugs (corticosteroids, anticonvulsants, ciclosporin, anti-B cell therapies, eg, rituximab, ofatumumab, alemtuzumab) and other immunosuppressive treatments or presence of a disease (an immune deficiency, coeliac disease, inflammatory bowel disease, a malignancy or an autoimmune disease) at the time of COVID-19 diagnosis that might affect serum immunoglobulin levels.

ABSTRACT

Purpose of the study The aim of this study was to investigate the relationship of B cell-mediated immunity with disease severity and mortality in patients with COVID-19.

Study design In this retrospective cohort and single-centre study, 208 patients with laboratory-confirmed COVID-19 were recruited. A COVID-19 severity score, ranging from 0 to 10, was used to evaluate associations between various factors. Serum immunoglobulin levels and the number of cells in B lymphocyte subsets were measured and their association with disease severity and mortality in patients with COVID-19 examined.

Results The median age of the patients was 50 (35–63) years and 88 (42%) were female. The number of deceased patients was 17. The median COVID-19 severity score was 8 (6–8) in deceased patients and 1 (0–2) in survivors. Deceased patients had significantly lower levels of total B lymphocytes, naive B cells, switched memory B cells, and serum IgA, IgG, IgG1, and IgG2 than recovered patients (all p<0.05). In addition, a significant negative correlation was found between the number of these parameters and COVID-19 severity scores. Decrease in the number of total B cells and switched memory B cells as well as lower serum IgA, IgG and IgG2 levels were independent risk factors for mortality in patients with COVID-19.

Conclusion In the present study, the prognosis of patients with COVID-19 was shown to be associated with the B cell subset and serum immunoglobulin levels.
and B lymphocyte subset levels; use of drugs or a history of disease that may affect international normalised ratio (INR) levels; presence of chronic kidney disease; and use of drugs or a history of disease that may affect mean arterial pressure levels. A total of 297 patients were enrolled based on the inclusion criteria and 89 were excluded for reasons shown in figure 1. Consequently, 208 patients with confirmed COVID-19 were finally enrolled in the study.

Data collection
Patient age, gender, medical history and laboratory values from electronic medical records were collected. A COVID-19 severity score ranging from 0 to 10, consisting of age, oxygen saturation, mean arterial pressure, blood urea nitrogen, C reactive protein and INR for disease severity of patients, was used for analysis. Mean arterial pressure, blood urea nitrogen, C reactive protein and international normalised ratio (INR) were measured via multiple-colour flow cytometry. Staining was performed using the following antibodies: human monoclonal anti-CD27-phycocerythrin (BD Biosciences, Erembodegem, Belgium), anti-CD19-allophycocyanin (BD Biosciences) and anti-IgD-fluorescein isothiocyanate (BD Biosciences) according to the manufacturer’s instructions. Cells were analysed on a BD FACs Canto II Flow Cytometry System (BD Biosciences). The B cell subsets were defined as follows: naive B cells (CD19+ IgD− CD27−), non-switched memory B cells (CD19+ CD27+ IgD−) or switched memory B cells (CD19+ CD27+ IgD+). The absolute number of cells was calculated by multiplying the relative proportion of a particular B cell population with the absolute number of lymphocytes obtained based on an automatically analysed differential white cell count (Mindray BC-6200 Auto Hematology Analyzer; Nanshan, Shenzhen, China) performed on the same day.

Statistical analysis
Continuous variables are presented as median with IQR and categorical variables as numbers and percentages in each category. Mann-Whitney U test was used to evaluate continuous data, and χ² test was used to evaluate categorical data when comparing the death and recovery of patients with COVID-19. Spearman’s correlation test was used to assess the association between B lymphocyte subset counts, serum immunoglobulin levels and COVID-19 severity scores. Univariate and multivariate logistic regression analyses were used to identify risk factors for mortality in patients with COVID-19. All variables with p values <0.1 on univariate analysis were entered into forward, stepwise multivariate logistic regression analysis. The SPSS statistical package (V22.0) and GraphPad Prism statistical software (V8.0; GraphPad Software, San Diego, California, USA) were used for all analyses. P<0.05 was considered statistically significant.
IgG (p=0.004), IgG1 (p=0.003) and IgG2 (p=0.016) were negatively correlated with COVID-19 severity scores. The number of CD19+ total B lymphocytes (p=0.003), CD27+ IgD+ naive B cells (p=0.033) and CD27+ IgD− switched memory B cells (p=0.030) and serum levels of IgA (p=0.041), IgG (p=0.004), IgG3 (p=0.003) and IgG4 (p=0.016) were negatively correlated with COVID-19 severity scores. The number of switched memory B cells and IgM, IgG3 and IgG4 differences were not observed (p<0.001). The protective function of the T cell subsets is determined by chemokines and cytokines secreted from antigen-presenting cells. Concomitantly, the protective function of the

### RESULTS

#### Clinical and laboratory features of patients

A total of 208 patients with COVID-19 were included in the present study. The median age was 50 years (IQR, 35–63 years) and 88 (42%) were female. Between-gender difference in COVID-19-related mortality was not observed (p=0.541). The number of deceased patients was 17 and the number of survivors was 191. The median COVID-19 severity score was 8 (6–8) in deceased patients and 1 (0–2) in survivors. Among the deceased patients, 23.5% (n=4) had moderate (4–6) and 76.5% (n=13) high (7–10) COVID-19 severity scores. The clinical and laboratory findings of deceased patients and survivors are summarised in table 2.

#### Comparison of B cell indicators between deceased and recovered patients with COVID-19

Deceased patients had significantly lower levels of CD19+ total B lymphocytes (p=0.002), CD27+ IgD+ naive B cells (p<0.001) and CD27+ IgD− switched memory B cells (p<0.001) than recovered patients. Significant differences in the number of CD27+ IgD− non-switched memory B cells were not observed between groups (p=0.130). Serum IgA, IgG, IgG3 and IgG4 levels were significantly lower in deceased patients than in recovered patients (p=0.020, p=0.018, p=0.021 and p=0.006, respectively). Between-group IgM, IgG3, or IgG4 differences were not observed (p=0.078, p=0.360 and p=0.220, respectively; figure 2).

#### Correlation analysis between COVID-19 severity scores and B cell indicators

The number of CD19+ total B lymphocytes (p=0.003), CD27+ IgD+ naive B cells (p=0.033) and CD27+ IgD− switched memory B cells (p=0.030) and serum levels of IgA (p=0.041), IgG (p=0.004), IgG3 (p=0.003) and IgG4 (p=0.016) were negatively correlated with COVID-19 severity scores.

#### DISCUSSION

In the present study, both decreased numbers of total B cells and switched memory B cells were independent risk factors for mortality in patients with COVID-19. Furthermore, lower serum IgA, IgG and IgG3 levels were independent risk factors for mortality in patients with COVID-19. In addition, 76.5% of deceased patients had a high COVID-19 severity score (≥7). A significant negative correlation was observed between number of total B cells, naive B cells and switched memory B cells, serum IgA, IgG, IgG3 and IgG4, and COVID-19 severity scores.

Both T cells and B cells have essential roles in the clearance of viral infections. The effector function of T cell subsets is determined by chemokines and cytokines secreted from antigen-presenting cells. Concomitantly, the protective function of the
humoral immune response with regard to controlling infection is mainly focused on causing plasma cells to produce neutralising antibodies. IgM is the first antibody to appear in the humoral immune response and can be detected 3 days after acute infection. Subsequently, high-affinity IgG responses develop and mediate long-term memory against the virus. After infection
recovery, plasma cells continue to secrete antibodies, which have serological memory. Memory B cells formed during the initial infection can rapidly respond to recurrent infections by forming high-affinity plasma cells. Long-term protection is achieved via stimulation of long-lived plasma cells and memory B cells. Virus-specific memory B and T cells are the main components of protective immune memory. The antiviral activities of memory B cells have been demonstrated in experimental animal studies. Mice that failed to develop an adaptive immune response were exposed to CMV. Memory B cells taken from immunised animals were transferred to T and B cell-exposed to CMV. Memory B cells taken from immunised animals were shown to emerge within a month of infection. In previous studies, SARS-CoV-2-specific memory B cells were shown to emerge within a month of infection. In addition, baseline levels of switched memory B cells have been associated with shorter symptom duration in patients with COVID-19. These data indicate that a protective memory response occurs prior to the formation of memory B cells in at least some patients with COVID-19. Possibly, memory B cells identified in some individuals were produced in response to previous coronavirus infections. As a group, coronaviruses are likely to generate cross-reactive B and T cell responses. In addition, during the primary infection, T cells assist the differentiation of B cells in the germinal centre and mediate a larger pool of memory B cells. However, patients whose B cells received insufficient T cell help may have poor germinal centre reactions, fewer antigen-specific antibodies and a longer symptomatic disease period. The effects of T cells on the germinal centre may affect the B cell response to SARS-CoV-2. In severe COVID-19 cases, serious impairments in germinal centre formation have been observed due to defects in T cell functions.

In severe SARS-CoV-2 infection, T cell and B cell lymphopaenia may be observed due to viral-induced immunosuppression or direct cytopathic effects of the virus. In addition, immunoglobulin deficiency may be common in patients with COVID-19 with a severe clinical course. Furthermore, patients with COVID-19 with IgG deficiency reportedly had a worse clinical course than patients with normal IgG levels. In a previous study in which the kinetics of antibody response and the association with prognosis in patients with COVID-19 were investigated, the level of IgG against SARS-CoV-2 spike surface glycoprotein (S-IgG) was lower in intensive care patients than in other patients. Antibodies may show antiviral activity by direct antimicrobial effects such as viral neutralisation and antibody-dependent cellular cytotoxicity or by modulation of the inflammatory response. CVID, where impaired B cell differentiation and defects in immunoglobulin production are the main presentation, is the most common form of symptomatic primary immunodeficiency. Patients with CVID are susceptible to rhinovirus, norovirus and herpesvirus infections. Similar results were obtained in a previous study describing the clinical findings of patients with primary antibody deficiency and COVID-19. High fever and dyspnoea were detected in all five patients with CVID included in the study. Three of the patients with CVID were admitted to intensive care and one died. In a multicentre study on this subject, 9 of 94 patients with primary immunodeficiency and COVID-19 died. Among the deceased patients, six had a diagnosis of primary antibody deficiency (four CVID, one isolated IgG deficiency, one IgA and IgG2 deficiency). Among these patients, most of those admitted to the intensive care unit were subjects with primary antibody deficiency. In the present study, parallel results were obtained with previous studies in which the importance of B lymphocytes and antibodies in immune response against SARS-CoV-2 was demonstrated.

Intravenous immunoglobulin (IVIG) treatment administered to patients with COVID-19 may have positive effects on fever and other symptoms as well as lymphopaenia. In addition, the immunomodulatory effect of IVIG treatment on most inflammatory cells such as monocytes has been reported, and pooling plasma from thousands of donors could potentially contain antibodies against other coronavirus strains that are cross-reactive with SARS-CoV-2. Immunoglobulins are the most important elements of the humoral immune response and IgG is the most abundant in serum. IgG is divided into four subclasses based on molecular structure: IgG1, IgG2, IgG3, and IgG4. IgG1 deficiency often accompanies general antibody deficiencies. IgG2 deficiency is associated with recurrent viral and bacterial infections. In IgG3 deficiency, the susceptibility to viral infections of the urinary system may increase. In IgG4 deficiency, the tendency to chronic lung diseases is increased. Although IgG4 only functions in neutralisation, IgG1, IgG2 and IgG3 have neutralisation and antibody-dependent enhancement effects. Similar to total IgG against SARS-CoV-2, IgG subclass responses can be detected until late in the infection. Among the IgG subclasses against spike protein, IgG3 is the most dominant.
have essential roles in the innate immune response against viral agents, and interferon-γ (IFN-γ) activates IgG production. IFN-γ levels decrease in SARS-CoV-2 infection and may be associated with low IgG2 levels. IFN-γ-targeted and IgG2-targeted therapies have been suggested as a potential method to prevent harmful aberrant inflammatory reactions in patients with COVID-19. The increased IgG2 levels in these patients may be associated with the decreased activation of immune cells such as neutrophils, eosinophils, monocytes and dendritic cells and reduced cytokine release from these cells. IgG2 also blocks the migration of neutrophils to the lungs via the complement pathway. Consequently, the lungs may be protected from the harmful effects of the aberrant immune response by correlating reduced neutrophilia in the lungs with a reduced systemic inflammatory response.28 29

IgA is principally responsible for the mucosal immunity of the respiratory and gastrointestinal systems, which are the main routes of entry of SARS-CoV-2 and other pathogens. IgA is highly protective against SARS-CoV-2 infection. Anti-SARS-CoV-2 monoclonal IgA binds to the viral spike protein and blocks receptor binding via competitive inhibition; the antibody can neutralise the virus on mucosal surfaces before it enters the cells. However, the virus may cross the epithelial barrier if there is a high density of viral particles or a defect in mucosal immunity, which is reflected in reduced IgA levels.30 31 A strong positive correlation between selective IgA deficiency (SIgAD) and COVID-19 infection has been reported worldwide. In Japan, where SIgAD is rare, SARS-CoV-2 infection and mortality rates are low. The frequency of SIgAD in the USA is 40-fold that in Japan. In the USA and other Western countries with much higher rates of SIgAD than Japan, SARS-CoV-2 infection and mortality rates are also higher.32

The present study had several limitations. First, B cell parameters of patients with COVID-19 were examined only in the acute phase of the disease. Investigating peripheral B lymphocyte subclasses and immunoglobulins in the acute phase and after recovery from disease would have been ideal. Second, SARS-CoV-2-specific memory B cells were not examined. Measuring the levels of memory cells specific to the disease is important when trying to understand the immunopathogenesis of COVID-19. Finally, conducting the study with larger patient groups (both for recovered and deceased patients) and healthy control groups may increase its power.

Despite these limitations, to the best of our knowledge, the current study is the first where the association between B cell subsets and immunoglobulin levels (also IgG subgroups) with severity score and mortality risk in patients with COVID-19 was investigated. Therefore, the results of this study may be useful for future studies where the above-mentioned limitations are taken into account to understand more fully the significance of B cell-mediated immunity against SARS-CoV-2.

CONCLUSION

In the present study, B cell-mediated immunity was shown to be vital to the host immune response against SARS-CoV-2. Total B cells, memory B cells, and total serum IgG, IgG1, IgG2, and IgA levels were shown to be associated with severity scores and mortality in patients with COVID-19. Examining the baseline levels of B cell subsets and serum immunoglobulin levels of patients during acute SARS-CoV-2 infection may be helpful in the early detection of severe patients and in determining aggressive treatment strategies, such as high-dose IVIG, in a timely manner.

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REFERENCES

1. World Health Organization Web Page. Coronavirus disease Dashboard. Available: https://covid19.who.int/ [Accessed 23 May 2021].

2. Chen X, Zhao B, Qu Y, et al. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 level in critically ill patients with coronavirus disease 2019. *Clin Infect Dis* 2020;71:1937–42.

3. Quinti I, Mezzaomi O, Milito C. Clinical management of patients with primary immunodeficiencies during the COVID-19 pandemic. *Expert Rev Clin Immunol* 2021;17;163–8.

4. Huang AT, García-Carreras B, Hitchings MDT, et al. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun* 2020;11:1–16.

5. Vabret N, Britton GJ, Gruber C, et al. Immunology of COVID-19: current state of the science. *Immunity* 2020;52:910–41.

6. Quinti I, Lougaris V, Milito C, et al. A possible role for B cells in COVID-19: Lesson from patients with agammaglobulinemia. *J Allergy Clin Immunol* 2020;146:211–3.

7. Jones TPW, Buckland M, Breuer J, et al. Viral infection in primary antibody deficiency syndromes. *Rev Med Virol* 2019;29:e2049.

8. Wang E, Nie J, Wang H, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis* 2020;221:1762–9.

9. Altschuld DJ, Unda SR, Benton J, et al. A novel severity score to predict inpatient mortality in COVID-19 patients. *Sci Rep* 2020;10:1–8.

10. Prompetchara E, Kettley C, Palaga T. Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. *Asian Pac J Allergy Immunol* 2020;38:1–9.

11. Hou H, Wang T, Zhang B, et al. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. *Clin Transl Immunology* 2020;9:e136.

12. Thevaram I, Nguyen THD, Koutsakos M, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. *Nat Med* 2020;26:453–5.

13. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol* 2010;17:1055–65.

14. Klenovsek K, Weisel F, Schneider A, et al. Protection from CMV infection in immunodeficient hosts by adoptive transfer of memory B cells. *Blood* 2007;110:3472–9.

15. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020;181:1489–501.

16. Wilson R, Stapper C, Dugan H, et al. Distinct B cell subsets give rise to antigen-specific antibody responses against SARS-CoV-2. *Res Sq* 2020;rs.3:rs.80476.

17. Newell KL, Clemmer DC, Cox JB, et al. Switched and unswitched memory B cells detected during SARS-CoV-2 convalescence correlate with limited symptom duration. *PloS One* 2021;16:e0244855.

18. Mateus J, Grifoni A, Tarke A, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* 2020;370:89–94.

19. Kaneko N, Kuo H-H, Boucau J, et al. Loss of Bcl-6-expressing T follicular helper cells and germinal centers in COVID-19. *Cell* 2020;183:143–57.

20. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020;71:762–8.

21. Husain-Syed E, Vardas I, Wilhelm J, et al. Immunoglobulin deficiency as an indicator of disease severity in patients with COVID-19. *Am J Physiol Lung Cell Mol Physiol* 2021;320;1:LS90–9.

22. Sun B, Feng Y, Mo X, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in patients with COVID-19 patients. *Emerg Microbes Infect* 2020;9:490–4.

23. Casadevall A, Joynier MJ, Pirofski L-A. SARS-CoV-2 viral load and antibody responses: the case for convalescent plasma therapy. *J Clin Invest* 2020;130:5112–4.

24. Janssen LMA, van der Flier M, de Vries E. Lessons learned from the clinical presentation of common variable immunodeficiency disorders: a systematic review and meta-analysis. *Front Immunol* 2021;12:620709.

25. Wang Q, Zhang L, Kuwahara K, et al. Immunodominant SARS coronavirus epitopes in humans elicited both enhancing and neutralizing effects on infection in non-human primates. *ACS Infect Dis* 2016;2:361–76.

26. Pasternak G, Lewandowicz-Uzysztyksa A, Pents K. Analysis of differences between total IgG and sum of the IgG subclasses in children with suspected immunodeficiency – indication of determinants. *BMC Immunol* 2018;19:1–8.

27. Goh YS, Chavatte J-M, Lim Jieling A, et al. SARS-CoV-2 viral load and antibody responses: the case for convalescent plasma therapy. *J Clin Invest* 2020;130:5112–4.

28. Janssen LMA, van der Flier M, de Vries E. Lessons learned from the clinical presentation of common variable immunodeficiency disorders: a systematic review and meta-analysis. *Front Immunol* 2021;12:620709.

29. Wang Q, Zhang L, Kuwahara K, et al. Immunodominant SARS coronavirus epitopes in humans elicited both enhancing and neutralizing effects on infection in non-human primates. *ACS Infect Dis* 2016;2:361–76.

30. Pasternak G, Lewandowicz-Uzysztyksa A, Pents K. Analysis of differences between total IgG and sum of the IgG subclasses in children with suspected immunodeficiency – indication of determinants. *BMC Immunol* 2018;19:1–8.

31. Pasternak G, Lewandowicz-Uzysztyksa A, Pents K. Analysis of differences between total IgG and sum of the IgG subclasses in children with suspected immunodeficiency – indication of determinants. *BMC Immunol* 2018;19:1–8.

32. Goh YS, Chavatte J-M, Lim Jieling A, et al. SARS-CoV-2 viral load and antibody responses: the case for convalescent plasma therapy. *J Clin Invest* 2020;130:5112–4.

33. Janssen LMA, van der Flier M, de Vries E. Lessons learned from the clinical presentation of common variable immunodeficiency disorders: a systematic review and meta-analysis. *Front Immunol* 2021;12:620709.

34. Wang Q, Zhang L, Kuwahara K, et al. Immunodominant SARS coronavirus epitopes in humans elicited both enhancing and neutralizing effects on infection in non-human primates. *ACS Infect Dis* 2016;2:361–76.

35. Pasternak G, Lewandowicz-Uzysztyksa A, Pents K. Analysis of differences between total IgG and sum of the IgG subclasses in children with suspected immunodeficiency – indication of determinants. *BMC Immunol* 2018;19:1–8.

36. Goh YS, Chavatte J-M, Lim Jieling A, et al. SARS-CoV-2 viral load and antibody responses: the case for convalescent plasma therapy. *J Clin Invest* 2020;130:5112–4.

37. Janssen LMA, van der Flier M, de Vries E. Lessons learned from the clinical presentation of common variable immunodeficiency disorders: a systematic review and meta-analysis. *Front Immunol* 2021;12:620709.

38. Wang Q, Zhang L, Kuwahara K, et al. Immunodominant SARS coronavirus epitopes in humans elicited both enhancing and neutralizing effects on infection in non-human primates. *ACS Infect Dis* 2016;2:361–76.

39. Pasternak G, Lewandowicz-Uzysztyksa A, Pents K. Analysis of differences between total IgG and sum of the IgG subclasses in children with suspected immunodeficiency – indication of determinants. *BMC Immunol* 2018;19:1–8.

40. Goh YS, Chavatte J-M, Lim Jieling A, et al. SARS-CoV-2 viral load and antibody responses: the case for convalescent plasma therapy. *J Clin Invest* 2020;130:5112–4.

41. Janssen LMA, van der Flier M, de Vries E. Lessons learned from the clinical presentation of common variable immunodeficiency disorders: a systematic review and meta-analysis. *Front Immunol* 2021;12:620709.