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Analysis of the IgG subclass profile and IgG sum-total discrepancy in COVID-19 convalescent plasma donors: A single-centre prospective cohort study

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ABSTRACT

Introduction: Although IgG1 and IgG3 have been shown to be the dominant subclasses in the acute phase of SARS-CoV-2 infection, little is known about the distribution of IgG subclasses during the recovery phase of COVID-19. The aim of the study was to analyze the profile of IgG subclasses in COVID-19 convalescent plasma donors.
Methods: A total of 36 convalescent plasma donors were included in the analysis. IgG and IgG subclass levels were measured using a nephelometric assay in plasma samples obtained directly from the plasma container.
Results: Although there was no significant difference in the concentration of IgG subclasses between the study and control groups, the contribution of IgG1 to the total IgG pool between the study and control groups was statistically significant ($p = 0.0478$). In addition, there was a discrepancy between the total IgG and IgG sum values in the study group, exceeding 15 % in 19,4 % of samples ($n = 7$), while in the control group no samples with a sum/ total IgG difference > 15 % were observed.
Conclusions: The selective affinity of the IgG1 subclass for the polyclonal anti-IgG reagent may interfere with the determination of total IgG and should be considered when interpreting the results of enzyme immunoassays
Data Availability: The data that support the findings of this study are available on request from the corresponding author.

1. Introduction

The functioning of the immune system during SARS-CoV-2 infection is the result of the dynamics of viral replication, kinetics of specific antibodies and cytokines, and the patient's individual characteristics, such as age and comorbidities [1]. Although most attention is paid to the functioning of the immune system in the phase of active SARS-CoV-2 infection, the regulation of the immune response in the recovery phase seems to be no less important [2]. The level and profile of IgG subclasses seem to be important in this context, especially considering the proven role of IgG deficits on the severity of COVID-19 [3,4].

Immunoglobulin G (IgG), which is a molecule composed of polypeptide chains (two identical copies of light and heavy chains), exists in the form of four subclasses: IgG1, IgG2, IgG3 and IgG4. The distribution of particular subclasses is variable depending on whether the organism is in a state of homeostasis or its disturbance (e.g. viral or bacterial infection) [5]. Viral infections primarily stimulate IgG1 and IgG3 response [6]. Accordingly, during SARS-CoV-2 infection, it is the IgG1 and IgG3 molecules themselves that constitute the dominant subclass of antibodies specific for the spike (S) protein of the virus as well as its receptor-binding domain (RBD) [7]. IgG1 triggers complement dependent cytotoxicity (CDC) by binding to the C1q subunit, and also has the ability to bind to specific Fc receptors causing antibody dependent cellular cytotoxicity (ADCC) [8]. IgG3 also triggers CDC, showing a higher affinity for C1q compared to other IgG subclasses [9]. In addition, IgG3 has a high affinity for immune response effector cells (neutrophils,

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monocytes, macrophages and natural killer (NK) cells) to which it binds via FcgRIIa, FcgRIIIa, and FcgRIIIb receptors, thus regulating their activity [10].

IgG subclass profiles have already been analysed in COVID-19 patients with particular emphasis on the severity of the disease [11–15]. IgG deficiency was associated with faster disease progression and the necessity of hospitalization in the ICU department, and in addition, patients with IgG deficiency had a higher incidence of acute kidney injury, mortality, ICU stay time and total hospitalization time [11]. When analysing patients with COVID-19 pneumonia, a correlation was observed between IgG2 deficiency and the increased need for mechanical ventilation (with a relative risk of 3.38 % and 95 % confidence interval range of 1.61–7.09) [12]. Moreover, as expected, the severe course of the disease was associated with an increased percentage of the IgG1 and IgG3 subpopulations and a different distribution of IgG1 glycoforms [13,14]. Interestingly, the analysis of the avidity of antibodies seems to indicate that the severe course of SARS-CoV-2 infection is associated with low IgG avidity against the virus RBD protein [15]. It can therefore be assumed that also in the recovery phase of COVID-19, some trends in the profile of the IgG subclasses may be present. However, the distribution of IgG subclasses during COVID-19 recovery has not been analysed so far.

Interestingly, in addition to the analysis of IgG subclasses, the relationship of total IgG concentration (determined directly by analytical methods) and IgG sum (calculated indirectly as a result of summing up the concentration of individual IgG1–4 subclasses), seems to be important in the context of immunity disorders. Although few studies have been conducted to assess the IgG total / sum relationship, it seems that the discrepancies between these variables may be as high as > 20 % [16]. To our knowledge, the relationship between IgG total and IgG sum has not been assessed in the COVID-19 convalescent population so far.

In this cohort study, we attempted to assess the profile of IgG subclass during the recovery period of COVID-19 and analyse the relationship between the sum of the IgG subclasses (IgG sum) and the total IgG.

2. Materials and methods

2.1. Study population

A total of 36 donors, who donated convalescent plasma by apheresis in Regional Centre for Transfusion Medicine (Bialystok, Poland) were subjected to prospective analysis. The following inclusion criteria have been applied: i) history of SARS-CoV-2 infection confirmed by both positive nucleic acid testing of nasopharyngeal swab specimens using the reverse transcription polymerase chain reaction (RT-PCR) and positive ELISA testing for anti-SARS-CoV-2 IgG S1 antibodies (only donors meeting both criteria were included in order to minimize the risk of disrupting the analysis by donors misdiagnosed with COVID-19), ii) no history of COVID-19-specific treatment, including remdesivir or convalescent plasma transfusion, iii) fulfillment of the donor eligibility criteria for plasma donation by apheresis. Healthy plasma donors (n = 9) with no history of SARS-CoV-2 infection, negative for SARS-CoV-2 S1 IgG antibodies constituted the control group. Plasma was collected with a DigiPla 80 plasmapheresis device (Sichuan Nigale Biomedical Co. Ltd., China). All donors were tested routinely for HBV/HCV/HIV with serology and NAT, and for T. pallidum with serology. Measurements of IgG total and IgG1–4 subclasses were performed on plasma samples obtained directly from plasma collection bag.

2.2. IgG assay

Measurement of total IgG and IgG1–4 concentration was performed by a nephelometric assay, on a BN II System nephelometric analyser (Siemens Healthcare GmbH, Germany), using the Siemens N Antisera to Human IgG, Siemens N AS IgG1/IgG2 and Siemens N Latex IgG3/IgG4 kits (according to the manufacturer's instructions).

2.3. Ethics committee approval

This prospective study was approved by the Ethical Committee of Medical University of Bialystok (protocol number APK.002.219.2020). Written informed consent was obtained from participants in accordance with the Declaration of Helsinki for Human Research.

2.4. Statistical methods

Statistical analysis was performed using GraphPad Prism (GraphPad Software). The results were summarized as medians and ranges. The Kruskal-Wallis test was used to compare the concentration and proportion of total IgG and IgG subclasses. The IgG total – sum differences were analysed using the Bland and Altman method. Correlation between variables was analyzed using the Spearman's correlation coefficient. P values < 0,05 were considered statistically significant.

3. Results and discussion

3.1. Clinical characteristics

A total of 36 COVID-19 convalescent plasma (CCP) donors and 9 healthy controls were included in the analysis. The median age of CCP donors was 42.5 years with 29 males (76,7 %) and 7 females (23,3 %). In terms of the severity of COVID-19, 16 donors with a history of mild disease and 20 asymptomatic donors were analyzed (no donors with a history of moderate / severe COVID-19 were identified in the study group). The severity of COVID-19 was determined based on National Institute of Health (NIH) guidelines [17], defining asymptomatic disease as positive nucleic acid testing despite no symptoms consistent with COVID-19 (due to possible exposure to an infected or potentially infected person) and mild disease as a variable combination of the following symptoms: fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell without accompanying shortness of breath, dyspnea, or abnormal chest imaging.

3.2. IgG subclass distribution

The conducted analyzes did not reveal a significant difference in the concentration of IgG total and IgG subclasses when comparing study and control groups (Table 1), with IgG1 being a dominant IgG subclass (Fig. 1B), which is consistent with previous studies on IgG subclasses in the convalescent phase of infection [18]. However, there was a statistically significant difference in the contribution of IgG1 to the total IgG pool between the test and control groups, and also between the subgroup with a total IgG difference> 15 % (p = 0.0478) (Table 1). Additionally, the analysis of concentration frequency distribution of the individual IgG subclasses showed a greater dispersion of values in the study group compared to the control group (Fig. 1A), which may be related to the different days post disease onset (DPO) time in individual study participants [19]. At the same time, the IgG results need to be interpreted with caution as the higher skewness to the left in study group shown in Fig. 1A suggests that the distribution of results may not be comparable to the control group, as confirmed by the Blend-Altmann plot presented in Fig. 1D.

3.3. IgG sum – total analysis

There was a significant correlation between the total IgG and IgG sum in both study (rho = 0.908) and control groups (rho = 0.781) (Fig. 1C), as well as between total IgG and IgG 1 subclass (rho = 0.921 and rho = 0.721, respectively) (Fig. 1D). This observation is consistent with previous studies carried out in the adult and pediatric population, however, it should be noted that in case of adults it was a heterogeneous

Table 1
Concentration and proportion of total IgG and IgG subclasses in study group and healthy control.

IgG subclass	Control (n = 9)		Study group (n = 36)		Study subgroup (> 15 % IgG total/sum difference) (n = 7)	
	Plasma level [g/l]	**Proportion of total IgG [%]**	**Plasma level [g/l]**	**Proportion of total IgG [%]**	**Plasma level [g/l]**	**Proportion of total IgG [%]**
IgG1	5.25 ^{ns} (3.70–6.17)	68.44 * (54.25–82.72)	5.78 ^{ns} (2.35–8.06)	63.07 * (47.53–87.32)	5.34 ^{ns} (4.09–6.26)	72.23 * (48.07–78.32)
IgG2	2.19 ^{ns} (1.73–2.51)	30.45 ^{ns} (21.84–38.65)	2.54 ^{ns} (0.90–3.50)	27.43 ^{ns} (15.02–40.33)	1.38 ^{ns} (0.96–3.46)	20.60 ^{ns} (17.82–33.94)
IgG3	0.31 ^{ns} (0.09–0.43)	4.29 ^{ns} (1.26–5.35)	0.32 ^{ns} (0.08–0.94)	3.39 ^{ns} (1.23–8.83)	0.35 ^{ns} (0.08–0.48)	3.99 ^{ns} (1.46–6.31)
IgG4	0.51 ^{ns} (0.06–1.12)	6.58 ^{ns} (0.77–15.73)	0.36 ^{ns} (0.04–2.23)	4.21 ^{ns} (0.40–19.69)	0.13 ^{ns} (0.04–1.42)	2.29 ^{ns} (0.47–13.93)
IgG total	7.56 ^{ns} (6.21–8.36)	NA	9.09 ^{ns} (4.24–12.90)	NA	8.53 ^{ns} (4.97–10.20)	NA
IgG sum	8.06 ^{ns} (6.41–9.54)	NA	8.84 ^{ns} (3.67–14.09)	NA	7.52 ^{ns} (5.25–10.19)	NA

*p-value: < 0.05; ^{ns}non-significant (p-value: >0.05)
Results are reported as median (range); n = number of samples; NA - not applicable; the Kruskal-Wallis test for nonparametric analysis was used to compare the results

population in terms of the underlying disease, and in the case of children, only samples from patients with suspected immune disorders were analyzed [20,21]. In accordance with the published studies, the D value, defined as the difference between the sum of the IgG1–4 subclasses and the IgG total value, was also analyzed. In study group 19,4 % of samples (n = 7) had > 15 % difference between IgGsum and IgG total, of which in 4 samples IgG sum exceeded IgG total value while in 3 samples IgG sum was lower than the IgG total (Fig. 1E). Importantly, no samples with an IgG sum/ total difference > 15 % were observed in the control group (Fig. 1E). In addition, all samples in control group had IgG sum values higher than IgG total, while in study group 15 samples (42,8 %) had IgG sum above the IgG total and 20 samples (57,2 %) had IgG sum below IgG total (Fig. 1E). The extended correlation analysis showed a moderate positive correlation between the concentration of IgG2, IgG3, IgG4 and D-value (Fig. 1F). This is in line with previous studies, which also found a dependence of the D value on the concentration of the individual IgG subclass [21]. It is indicated that the reason for this relationship may lie in the selective affinity of the antiglobulin reagent used in the tests to determine the IgG total value [21]. Although the assays are developed with the use of polyclonal anti-IgG molecules, which by definition capture all IgG subclasses, it cannot be ruled out that the IgG1 subclass, dominant during SARS-CoV-2 infection, has a stronger affinity for the anti-IgG used in the test [7]. At the same time, a negative moderate correlation was found between the concentration of IgG1, IgG3 and the number of days post disease onset (DPO) (Fig. 1F). The decrease in antibody concentration with time after SARS-CoV-2 infection is already a relatively well-known phenomenon, characterized in various patient populations depending on the course of COVID-19 [22]. Particularly with regard to asymptomatic infections, both the initial antibody concentration and the rate of antibody reduction appear to be related to either the initial viremia or the impaired ability of the virus to replicate, thus inducing an enhanced serological response [23].

3.4. Study limitations

Undoubtedly, there were limitations to our study. First of all, presented study group was limited to donors with a mild or asymptomatic disease course. While this allowed for the homogeneity of the study group to be maintained, we need to emphasize that the distribution of subclasses and dependencies of IgG total - sum in the subpopulation with a history of severe COVID-19 may differ, taking into account the proven increase in the share of the IgG1 and IgG3 subclasses in the total IgG pool, along with the severity of the disease [7,19]. This seems to be confirmed by observations made during studies of the IgG profile in other infectious diseases, where an increase in acute phase proteins correlated with an increase in IgG concentration [24]. Secondly, it should be remembered that despite the significant influence of viral infections on the concentration of IgG and its subclass, their level may depend, for example, on gender, daily habits (smoking, alcohol consumption), metabolic factors (lipid profile) and comorbidities [25–27]. In addition, the methodology was limited to one type of nephelometric assay, which makes it difficult to relate the obtained results to other commercially available assays for quantitative determination of IgG and its subclasses. Finally, the follow-up time did not allow us to draw firm conclusions on the fluctuation in IgG subclasses throughout whole COVID-19 convalescent period.

4. Conclusion

In summary, the conducted study allowed us to assess the distribution of IgG subclasses during the COVID-19 convalescence period and to assess the phenomenon of discrepancy in the results of IgG total and the sum of IgG subclasses. In our opinion, this may be of importance when interpreting the results of enzyme immunoassays in the course of infectious diseases, which may be modified due to the increased concentration of IgG subclasses specific for a given infectious agent.

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CRedit authorship contributions statement

Tomasz Wasiluk: Investigation, Writing – original draft, Writing – review & editing. **Magdalena Sredzinska:** Methodology Writing – review and editing. **Anna Rogowska:** Writing – review & editing. **Agnieszka Zebrowska:** Writing – review & editing. **Barbara Boczkowska-Radziwon:** Writing – review & editing. **Anna Stasiak-Barmuta:** Methodology, Resources. **Piotr Radziwon:** Conceptualization, Writing – review & editing.

Conflict of interest

All authors have seen and approved the study submitted. No part of the submitted work has been published or is under consideration for publication elsewhere. The authors have no competing interests.

Ethics approval statement

Study was approved by the Bioethical Committee of the Medical University of Bialystok (protocol number: APK.002.219.2020).

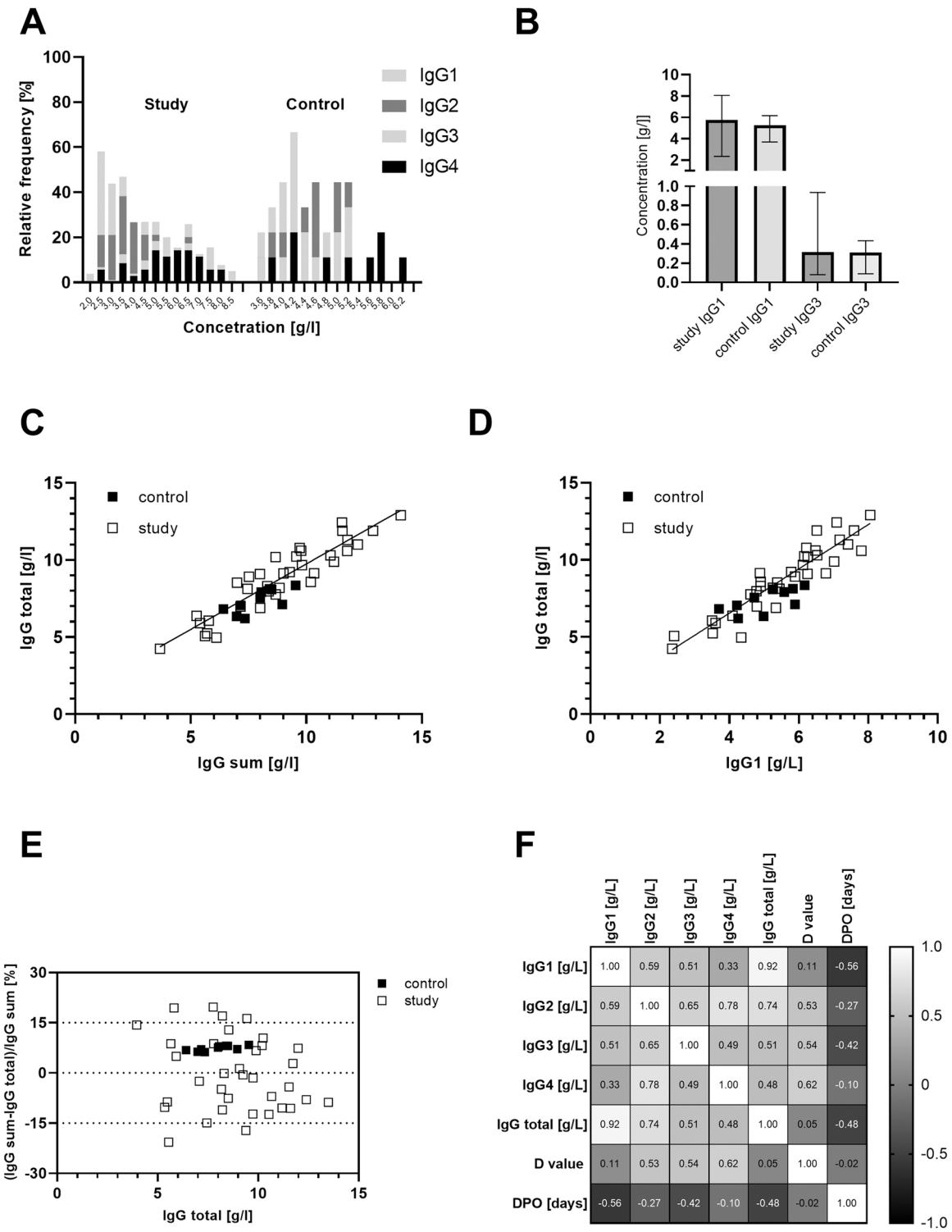

Fig. 1. IgG subclass analysis. (A) IgG subclass frequency distribution in study and control groups. (B) Absolute levels of IgG1 and IgG3 in study and control groups. (B, C, D) IgG sum – IgG total relationship. (B) Difference analysis of IgG measurement presented as Bland–Altman plot - expressed as a percentage of total IgG for each sample. The mean difference of 15 % are shown by dashed lines. (C) Regression analysis presented as a scatter plot of total IgG against sum of IgG subclasses (IgG sum). (D) Regression analysis presented as a scatter plot of IgG1 against sum of IgG subclasses (IgG sum). (E) Correlation analysis of IgG1–4 concentration, D value and days post disease onset (DPO).

Patient consent statement

All study subjects provided written informed consent prior to study enrollment.

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