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RESEARCH ARTICLE

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Assessment of anti-SARS-CoV-2 antibodies level in convalescents plasma

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Abstract

Despite extensive vaccination, the quantity of patients infected with the SARS-CoV-2 virus and its variants continues to grow worldwide. Treating patients with a severe course of COVID-19 is a difficult challenge. One of the generally accepted and specific therapy methods is the use of plasma rich in anti-SARS-CoV-2 antibodies. On the other hand, assessing the antibodies level depending on the time after infection allows for vaccine-decision. The study marked the level of anti-SARS-CoV-2 IgG antibodies in 351 COVID-19 convalescent residents of one geographical region in Poland. The study group included blood donors. The studies were cross-sectional and extended to a questionnaire to determine infection severity. These data were compiled statistically. The study considered epidemiological factors, the time from the end of the infection, and infection severity. The fastest increase of the antibodies level was observed up to 59 days after COVID-19, and it was statistically significantly higher among men. Higher levels of antibodies were found among people above the average age in both men and women. There was an increase in the level of antibodies since the onset of the disease in men, while in women, it decreased. The antibodies level was also found to depend on the severity of the course of COVID-19 infection. The optimal group of plasma donors in the studied geographical region is men and women above 39 years old. after a more severe infection. The titer of antibodies increases with time from the disease.

KEYWORDS

convalescent plasma, COVID-19, SARS-CoV-2, treatment

1 | INTRODUCTION

The plasma of COVID19 convalescents is rich in anti-SARS-CoV-2 antibodies, and its use in the treatment of a severe course of this infection is widely accepted. Passive increasing of the body's immune defense is based on multicenter observations of reduced mortality risk among transfused plasma patients with a high concentration of antibodies than those who received plasma with a low concentration of antibodies. Increased awareness of the health, society, and economy-connected harm caused by COVID-19 and an increasing sense of solidarity led to the growing number of donating blood COVID-19 convalescent patients.[1,2] Determining the optimal group of donors and the optimal period for donation have considerable significance for preparing the plasma specimen.

This study aims to determine the IgG anti-SARS-CoV-2 antibody titers in COVID-19 convalescents in the Pomeranian region of Poland, depending on the epidemiological factors, time since recovery (isolation), and the severity of the disease.

2 | MATERIALS AND METHODS

We recruited COVID-19 convalescents (infection was confirmed by Polymerase chain reaction [PCR] analysis of nasal swabs) who reported donating blood at the Regional Center of Blood Donation and Treatment in Gdańsk (Poland). The inclusion criteria were: confirmed SARS-CoV-2 infection, 18–56 years of age, normal complete blood count (hemoglobin, hematocrit, erythrocyte, and leukocyte formula, platelets), normal blood pressure, pulse, and body temperature. In addition, the IgG anti-SARS-CoV-2 antibody titers were measured, and a detailed survey was conducted regarding symptoms such as chills, dry cough, musculoskeletal pain, conjunctivitis, fever (defined as ≥38°C), fatigue, dyspnea, diarrhea, and smell/taste disturbances. The exclusion criteria were: autoimmune diseases, anti-HLA antibodies in the blood (postpregnancy or posttransfusion), active infection or oncological illness, history of viral disease (particularly HIV, Hepatitis B, and C), or infection with *Treponema pallidum*, being under the influence of psychoactive substances.

We divided the entire sample of participants into two subgroups depending on the severity of their COVID-19 illness. The severe course of COVID-19 was defined as ≥5 symptoms, whereas mild illness was defined as ≤4 symptoms.

Participation in our study was voluntary. It was conducted after the scheduled blood donation, whose purpose was to obtain plasma rich in anti-SARS-CoV-2 antibodies used to treat patients severely ill with COVID-19. Blood was collected 10–393 days after the 14-day isolation period. None of the participants had prior anti-SARS-CoV-2 vaccination, and none were hospitalized due to a severe course of COVID-19. Blood tests were performed using the MAGLUMI 800 device: test SARS-CoV-2 S-RBD IgG (Snibe Diagnostic; test result <1 AU/ml was nonreactive, whereas ≥1 AU/ml was reactive). Serological tests were performed using the in vitro chemiluminescent kit for the quantification of S-RBD IgG neutralizing antibodies (nAb) against SARS-CoV-2, intended for serum and plasma testing on automatic analyzers of the MAGLUMI series in accordance with the recommendations of the manufacturer of the SNIBE DIAGNOSTIC test. After collecting blood from the examined person, it was placed in test tubes with a separating gel or clot activator. After centrifugation (>10 000 RCF for 10 min), a sample (10 µl volume) containing no fibrin or other solids was collected. Then the sample, along with the buffer and magnetic particles coated with the recombinant S-RBD antigen, were mixed and incubated, which resulted in the formation of immune complexes. After magnetic field precipitation, the supernatant was removed and washed. After the addition of ABEI-labeled anti-human IgG antibodies, the sample was subjected to another incubation and precipitation followed by washing to remove unbound proteins from the sample. Finally, the chemiluminescence reaction was initiated and the light signals were measured with a photomultiplier for 3 s as a relative light unit (RLU) that is proportional to the SARS-CoV-2 S-RBD IgG concentration. All tests were performed after the manufacturer recommended calibration with quality control as well as precautions and safety measures for in vitro diagnostics. The sensitivity of the test (according to the manufacturer) in the case of a test performed 15 days after the onset of symptoms is 100.0% and its specificity is 99.6% (CE REF 30219017 M).[3] Our study protocol was approved by the local independent Bioethics Committee (NKBBN 199/2021). The obtained results were analyzed using the χ^2 test (Statistica 13.3 StatSoft Pl.). Statistical significance was accepted at $p < 0.05$. Excel software was used to illustrate the obtained results and determine the trends (Microsoft Corporation).

3 | RESULTS

We included a total of 351 COVID-19 convalescents in our study (305 males and 46 females), whose ages ranged from 18 to 63 (mean age 39). The obtained results were divided into four groups depending on the number of days since the isolation of the antibody titers (Table 1).

We noted an increasing trend in anti-SARS-CoV-2 antibody titers depending on the time since infection (Table 1, Figure 1). The highest increase in the average antibody titer was observed in convalescents from Group I versus II (increase by 82,4%). In addition, we noted an increase of 27.1% between Group II and III and between III and IV 6.4%. The total increase in antibody titer between Group I versus IV was 146.6%.

Based on the mean or median age, the group of convalescents was divided into two groups: <39 and ≥39 years of age. The results were illustrated in Figure 2. Participants who were at or above the mean age had higher antibody titers than those who were younger. Among the older (≥39 lat years of age) participants, the line of the trend was increasing, whereas it was horizontal among the younger (<39) ones. We noted a statistically significant difference between the minimum and maximum values of antibody titers depending on age.

A similar correlation was noted in terms of sex. Average antibody titers were lower among females than among males (statistically significant difference $p < 0.05$). In addition, among the female participants, we noted a decrease in the antibody titers depending on the time since infection. In contrast, among the males, this correlation was positive (increasing titers, Figure 3).

We noted higher antibody titers among the male and female participants above the mean age (statistically significant difference, $p < 0.05$). In addition, among the male participants, the difference between anti-SARS-CoV-2 antibody titers increased with time since

TABLE 1 Levels of IgG anti-SARS-CoV-2 antibody in the studied group of patients depending on the time after COVID-19

	<29 days	30–59 days	60–89 days	>90 days
Group size	27	204	95	25
Average antibody titer*	1:349 41	1:637 27	1:809 64	1:861 52

*Statistical significance $p < 0.05$.

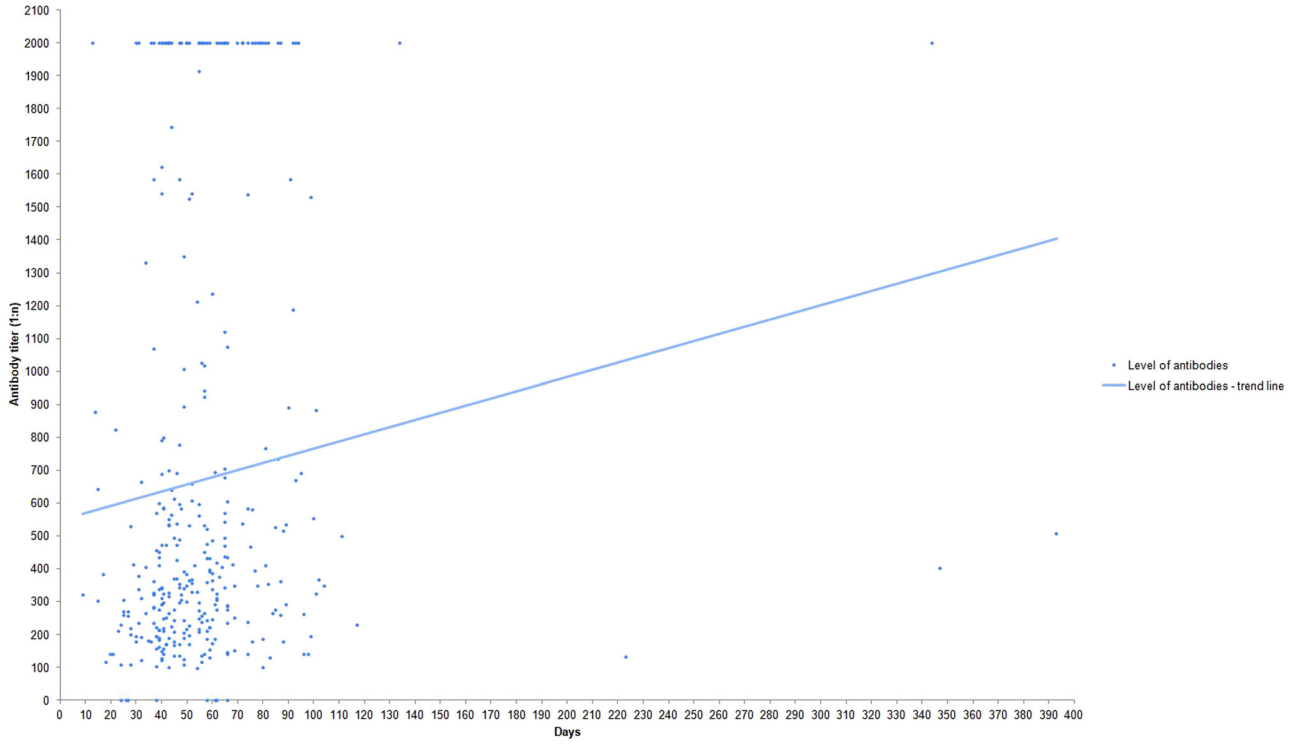

FIGURE 1 Anti-SARS-CoV-2 IgG antibodies among COVID-19 convalescents depending on the time since infection

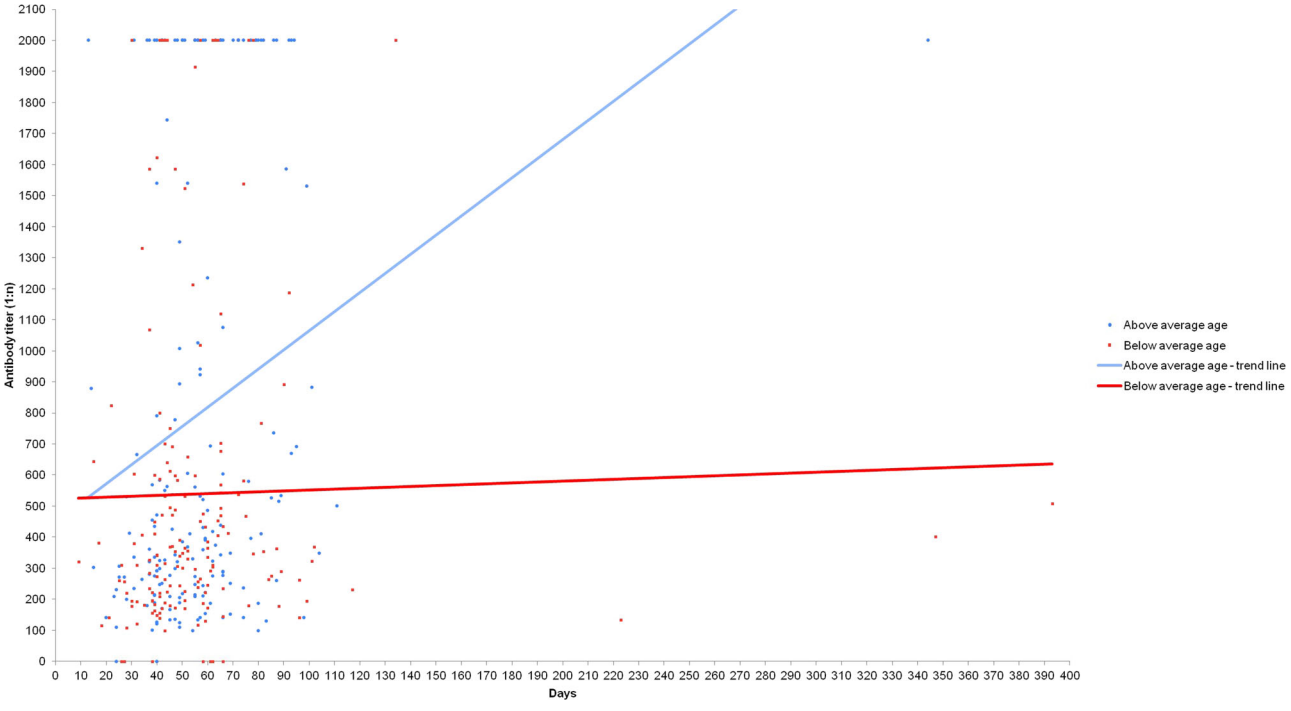

FIGURE 2 Anti-SARS-CoV-2 antibody titers depending on age

infection, whereas among the female participants, we observed an inverse correlation (Figures 4 and 5).

We divided the entire sample of participants into two subgroups depending on the severity of their COVID-19 illness. The severe course of COVID-19 was defined as ≥5 symptoms, whereas mild illness was defined as ≤4 symptoms. We noted a difference in antibody titers' minimum and maximum values depending on the severity of illness (Figure 6). These titers were higher among participants who

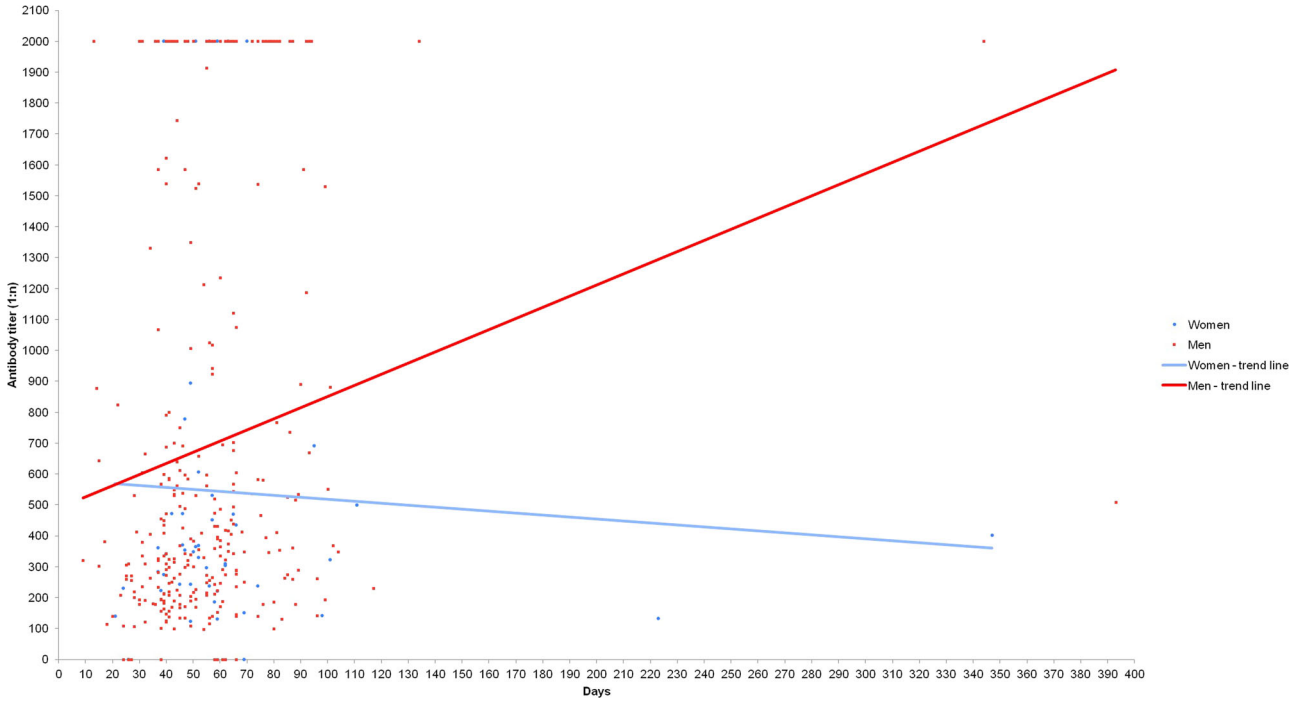

FIGURE 3 Anti-SARS-CoV-2 antibody titers depending on sex

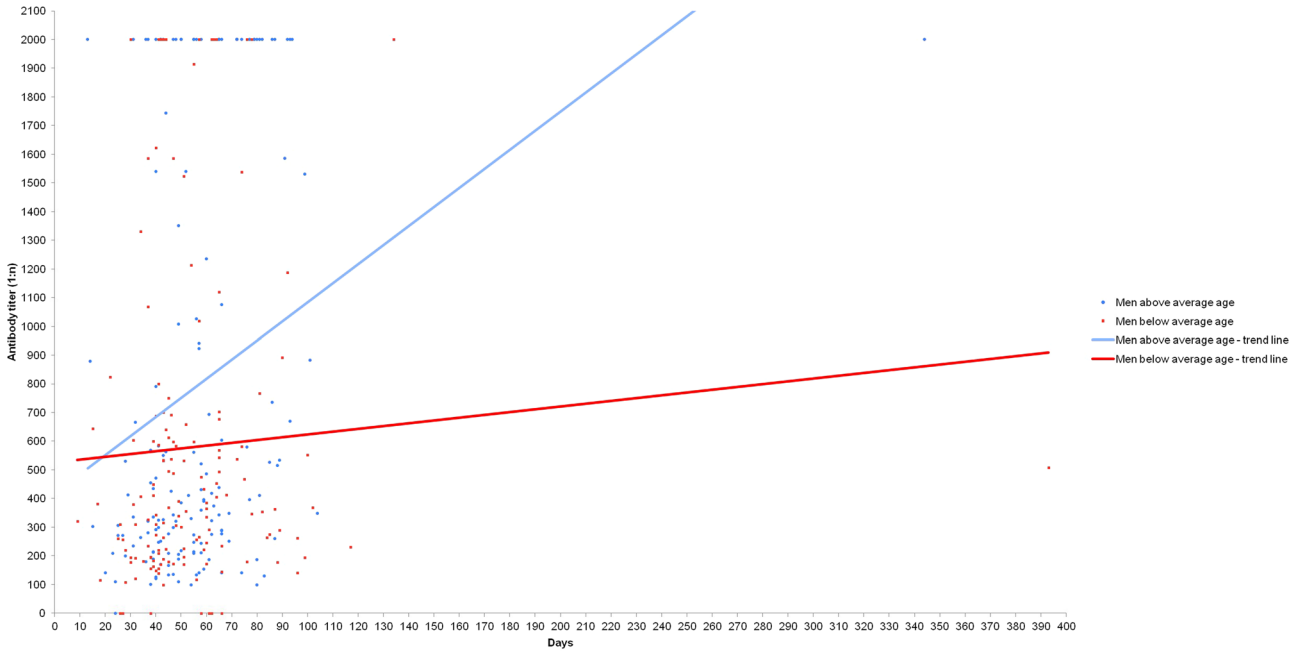

FIGURE 4 Anti-SARS-CoV-2 antibodies among males, depending on mean age

recovered from a more severe course of COVID-19 when measured early (<60 days since the end of isolation; statistically significant difference $p < 0.05$). However later, after 140 days, we noted an inverse correlation (higher antibody titers among participants after mild illness), which was not statistically significant and based on a small sample.

The anti-SARS-CoV-2 antibody titers were the highest in males and females above the mean or median age (≥39 years of age) and had a more severe course of COVID-19 (Figure 7). It is noteworthy that in female participants, we noted the higher antibody titers only in the early period (<2–3 months since the end of isolation; Figure 5).

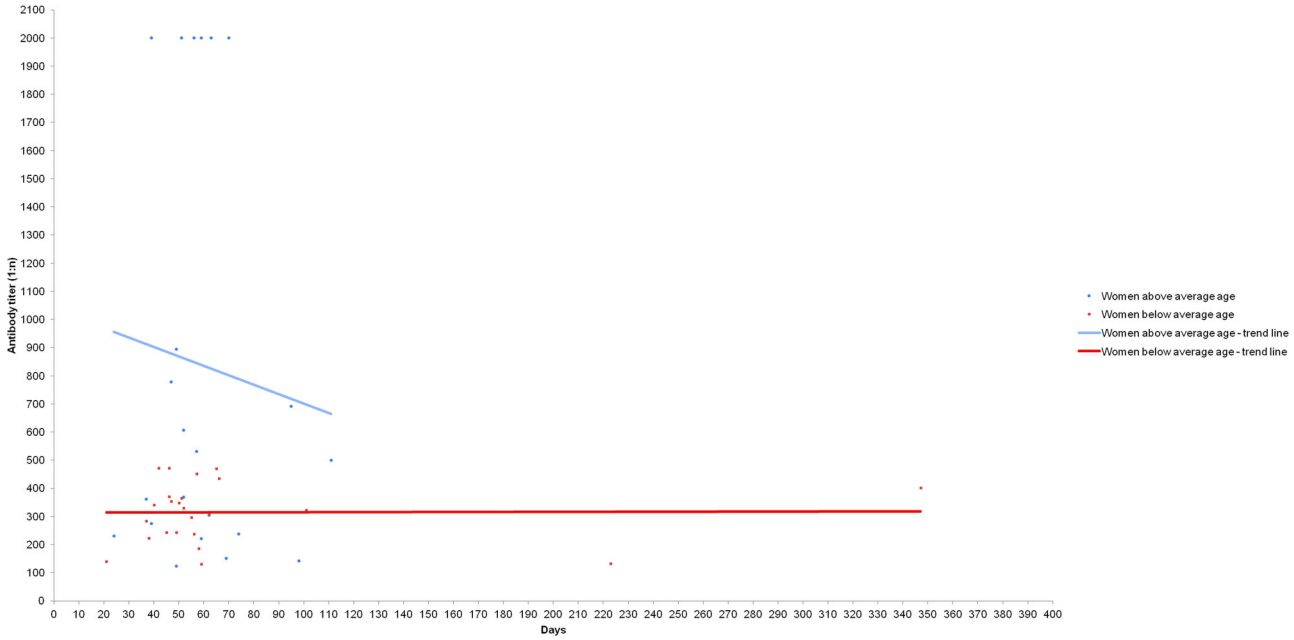

FIGURE 5 Anti-SARS-CoV-2 antibodies among females, depending on mean age

4 | DISCUSSION

Passive immunotherapy based on transfusing antibody-rich plasma obtained from convalescents is one of the strategies in treating infectious diseases. The effectiveness of plasma from convalescents has been demonstrated in treating hepatitis A and B, rabies, cytomegalovirus, and respiratory syncytial virus pneumonia.[4–7] In addition, this method helps treat patients with severe course of illness and postexposure prophylaxis.[4,5,8] Many authors confirm the effectiveness of convalescent plasma in preventing and treatment of a severe course of COVID-19. However, others did not find a statistically significant influence of it on mortality.[8–11]

In Poland, the supply, production, and storage of plasma are conducted by Regional Centers of Blood Donation and Treatment. Plasma donors are recruited from COVID-19 convalescents in whom high titers of specific anti-SARS-CoV-2 antibodies are expected. Flisiak et al. accepted antibody titer >1:500 as "high."[3] However, the salient questions about who the optimal donors are and when is the optimal time to collect their plasma remains unanswered. In their analysis of the *Spanish influenza* epidemic, Luck et al. noted that the plasma richest in antibodies was collected from convalescents 7–60 days after the end of infection symptoms.[4] Chen et al. reported a decrease in anti-SARS-CoV-2 IgG antibodies in the third month since recovery from COVID-19.[12] Klein et al. had similar results.[8] In our study, the convalescents had the recommended antibody titer (>1:500) after 30 days since the end of isolation. Our study participants donated plasma in various periods since recovery. Therefore, we had the opportunity to measure antibody titers for a long time, except for one male participant who donated blood 11 times within 6 months (due to continually high anti-SARS-CoV-2 titer, we could not obtain repeated antibody titer measurements in the same convalescent and asses individual trends. However, in this particular convalescent, it is not possible to exclude the possibility of reinfection. In available literature is no widely available and generally agreed-upon best test for measuring neutralizing antibodies, and the antibody titers in convalescent plasma from patients who have recovered from COVID-19 are highly variable.[13] The level of 27.4 AU/ml (the result is the same as the level 1:500) was defined on the basis of research conducted by the Polish Nationality Center for Blood and Blood Treatment - Maglumi and DiaSorin SARS-CoV-2 S-RBD IgG tests.

In our study, we noted higher antibody titers in convalescents who donated plasma later after infection, and these were mainly higher in those who were older and in males. These results are congruent with those published by Klein et al.[8] We first excluded anti-HLA antibodies (postpregnancy or post-transfusion) in all the collected blood samples, thus explaining the small number of female participants in our study. Furthermore, the weaker response to immunization, lack of anti-HLA antibodies despite a history of pregnancies might go hand-in-hand with low anti-SARS-CoV-2 antibody titers. Thus, as many as 60%–70% of female convalescents were excluded from our study due to the presence of anti-HLA antibodies.

Weisber et al. and Ko et al. noted that the antibody titers were higher in patients who had a more severe course of illness.[14,15] Our results were similar, with initial antibody titers 60% higher in participants who recovered from severe COVID-19 (≥5 symptoms) than those after mild illness (1:500 vs. 1:800). After 5–6 months since infection, the antibody titers in both groups became similar. Klein et al. noticed a similar trend: antibody titers were significantly higher in hospitalized patients (implying a more severe course of illness).[8] Robbiani et al. concluded that the observed difference in

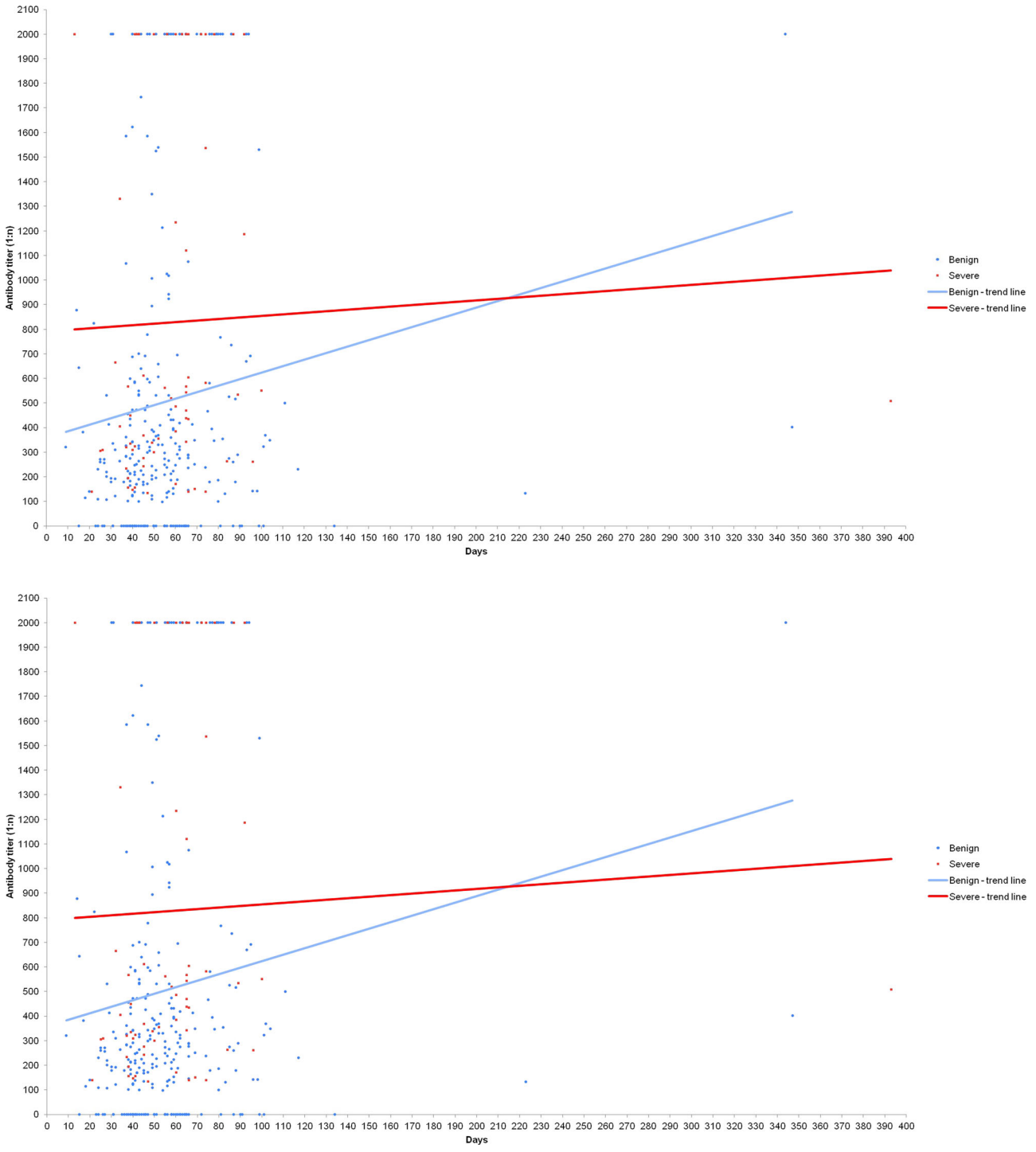

FIGURE 6 Anti-SARS-CoV-2 antibody titers depending on the severity of the disease

antibody titers is due to a more severe course of COVID-19 in older males and with higher mortality in that group.[16] Scully et al. sought to explain this difference via the influence of estrogen, testosterone, and progesterone on the immune response and the course of illness.[17] This correlation should also be considered as a possible explanation for the decreased antibody titers among older women in our study.

The presence of specific antibodies confirms past infection. However, it remains unclear how effective they will be in other patients. In their study about *Lassa fever*, Jahrling et al. assessed the quality of plasma via specific IgG antibody titers and the neutralizing test. The authors noted that the most effective plasma was obtained from convalescents after 8 months since recovery and had high antibody and neutralizing test titers.[18] Our study similarly observed the

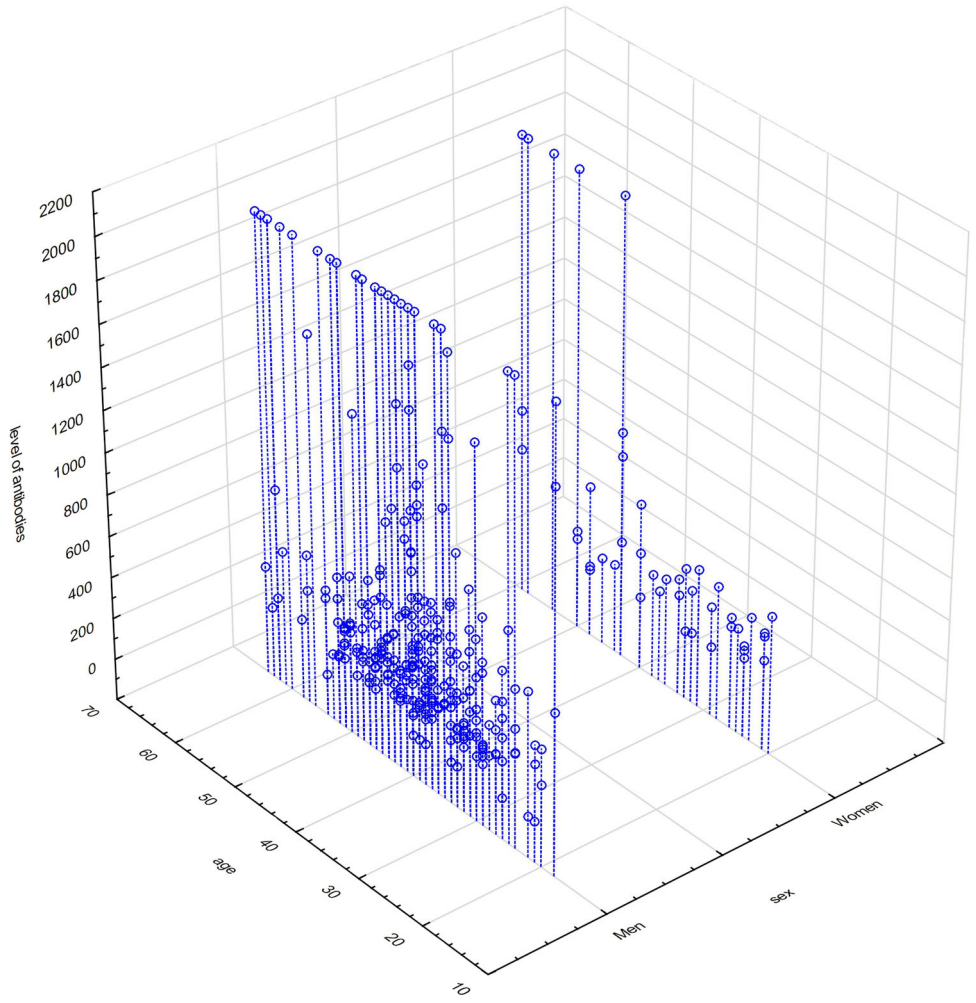

FIGURE 7 The anti-SARS-CoV-2 antibody titers depending on age and sex

most elevated specific IgG antibody titers in convalescents who donated blood over 90 days postrecovery. Klein et al. observed the effectiveness of convalescent plasma, which was assessed in terms of IgG anti-SARS-CoV-2 titer.[8] The poor quality of those antibodies might explain the poor efficacy of treatment using antibodies. It might be due to the titer of these antibodies and the low result of the neutralizing test, and the fact that the donor and recipient are not from the same geographical region. This factor might be of particular significance given the number of region-specific mutations of the SARS-CoV-2 virus. Therefore, plasma obtained from COVID-19 convalescents should be used to treat severely ill patients in the region closest to where the plasma specimen was collected and prepared.

5 | CONCLUSIONS

The anti-SARS-CoV-2 antibody titers increased together with time since infection. The later the convalescent donates blood, the greater the antibody titer. Therefore, the optimal plasma donors are older convalescents (≥39 years of age) who recovered from severe COVID-19.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

The study protocol was approved by the Regional Bioethics Committee of Gdansk Medical University, Poland (approval nr. NKBBN 199/2021). The patient's written consent was obtained to use clinical data without disclosing personal data. All authors affirm that their study accepted the required ethical clearance and respected all ethical considerations.

AUTHOR CONTRIBUTIONS

Contributed to study concept and design, contributed to acquisition, drafted manuscript, critically revised manuscript, gave final approval: Andrzej Skorek. *Contributed to conception and design, data collection contributed to acquisition, critically revised manuscript gave final approval*: Anna Jaźwińska-Curyłło and Aleksandra Romanowicz. *Contributed to acquisition, analysis,*

and interpretation of the results; drafted manuscript; gave final approval: Krzysztof Kwaśniewski. *Contributed to conception, analysis, and interpretation of the results, critically revised manuscript, gave final approval*: Waldemar Narożny. *Contributed to conception, analysis, and results interpretation, drafted manuscript, critically revised manuscript, gave final approval*: Dmitry Tretiakow.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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