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Factors affecting the content of Ig G-antibodies to S-protein SARS-CoV-2 in the blood of reconvalescents after new coronaviral infection (COVID-19)

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Abstract

Introduction. Morbidity and mortality of COVID-19 actualizes the identification of groups with the greatest risk of primary and re-infection, persons in need of priority vaccination or revaccination.

Objective. To study the factors affecting the content of IgG antibodies to the S-protein SARS-CoV-2 in convalescents after suffering COVID-19 for 6 months.

Materials and methods. The study of the Military Medical Academy and the Helix Laboratory Service was carried out from 06/01/2020 to 08/01/2021 on the basis of the Military Medical Academy and the Helix centers. The study included 1421 people – both sexes from 18 to 70 years old. 1205 with asymptomatic and mild disease (outpatient group). 216 with moderate or severe form (inpatient group). The outpatient group underwent a quantitative determination of IgG to the spike (S) protein SARS-CoV-2 by immunochemiluminescence analysis at 30, 45, 60, 90, 180 days from diagnosis. The diagnosis was verified by a positive RT-PCR result. The inpatient group underwent an identical study on the 1st, 14th, 45th, 60th, 90th and 180th days from the moment of admission to the hospital. The diagnosis was verified in the same way.

Results. In convalescents, post-infectious immunity is formed from 30 days. Older age was associated with a more pronounced production of IgG to the S-protein SARS-CoV-2, mainly in older women. Moderate and severe course is characterized by higher concentrations of IgG to the SARS-CoV-2 S protein. A high level of IgG to the S-protein SARS-CoV-2 persists for up to 90 days, with a subsequent decrease by 180 days. Body weight, days of oxygen therapy, hyperthermia, the volume of lung tissue lesions and the level of C-reactive protein correlate with the concentration of IgG to the S-protein SARS-CoV-2. The use of glucocorticoids (GCS) is characterized by the presence of a higher concentration of IgG to the S-protein SARS-CoV-2 up to 6 months. There is a dose-dependent effect of using GCS.

Conclusion. The formation and maintenance of the level of neutralizing antibodies for 6 months depends on the severity of the disease, the gender and age of the patients, and the fact of using GCS. This must be taken into account when carrying out therapeutic and preventive measures, planning vaccination.

Keywords: SARS-CoV-2, IgG, S-protein, immunity, antibodies, COVID-19

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INTRODUCTION

A recent SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2) outbreak is a global health emergency because more than 257 million people are currently infected, of which more than 5 million have died [1]. Currently, there are no effective antiviral drugs that reliably suppress SARS-CoV-2 replication at any time point in seeking medical care for infected patients. Therefore, research into immunopathogenetic mechanisms of protection against novel coronavirus disease (COVID-19) is of high priority.

It is known that after infection by any pathogen, innate (inherited) immunity first begins to act. In the case of SARS-CoV-2 and other viruses, innate immunity is represented by an interferon system and other cytokines that can destroy virus-containing cells. Acquired (adaptive) immunity is formed after encountering the virus, so it takes time to form, but it acts directly on the pathogen and neutralises it. Adaptive immunity creates a "memory" of the virus and responds immediately when re-acquainted with it. Adaptive active immunity occurs after a disease or after vaccination (postinfectious or postvaccination). T-cells form a cellular immune response (T-cell immunity), and B-cells form a humoral immune response, producing specific antibodies (B-cell immunity). In case of SARS-CoV-2, these are immunoglobulins (Ig) of classes A, M, G. The time of onset of these antibodies is individualised and ranges from 7 to 14 days. Active B-cell (humoral) immunity is assessed by the presence or absence of IgG antibodies to coronavirus infection [1, 2].

SARS-CoV-2 virus is a β-coronavirus that has four structural proteins: nucleocapsid (N), membrane (M), enveloped (E) and surface glycoprotein-spike (S-Spike), which consists of S1 and S2-subunits [3, 4]. S1-subunit binds to the human angiotensin-converting enzyme receptor (ACE2) for attachment via receptor-binding domain (RBD), and the S2-subunit induces fusion with the target cell surface membrane [5].

The S-protein is known to promote viral entry into the cell and is a major target for recognition and neutralisation by antibodies, and the N-protein determines viral pathogenicity and virulence. S-protein antibodies are expected to neutralise the virus by blocking the binding of ACE2 to RBD. The M-protein is involved in the budding of the virus from the host cell membrane, and E-protein plays a role in the intracellular movement and assembly of proteins [6]. All four SARS-CoV-2 proteins are strong immunogens, but only N- and S-proteins with the highest specificity of S1-subunit are used in the diagnosis [7].

Thus, neutralising antibodies against SARS-CoV-2 prevent the interaction of the virus with ACE2 and prevent virus particles from entering the target cell [8]. Therefore, antibodies resulting from the humoral immune response to the surface S-protein SARS-CoV-2 are neutralising and determine the primary pathway of antiviral protection [9]. Moreover, in people with a more severe course of COVID-19, a stronger neutralising activity of antibodies is observed [10], although no such association was noted in other studies [11]. The humoral immune response against SARS-CoV-2 occurs during the recovery phase, but its duration is not clearly established [12]. The generation of IgG antibodies against SARS-CoV-2 S-protein reaches its maximum at 21-49 days after onset of disease symptoms, with a trend towards gradual decline thereafter. IgG antibodies have a half-life ("life span") of about 21 days, therefore, the observed sustained levels of IgG antibodies in the blood beyond this time are likely supported by their production by long-lived plasma cells in the bone marrow [13]. Early reports suggested that due to the action of short-lived plasmablasts anti-SARS-CoV-2 IgG antibodies persisted in the blood for a short time, about 3 months [14], so there is a possibility of reinfection after this period.

Thus, after infection or immunisation, the initial peak and early decrease in antibody levels are common because most short-lived plasmablasts that secrete antibodies responsible for the early peak of the antibody die by month 3. Long-lived plasma cells provide antibody production for 6 months or more [15]. As a result, an adequate response of immune memory cells (T and B-cells) provides protection against reinfection and is critical for effective protection. Recent data show that IgG to SARS-CoV-2 S-protein can persists for up to 270 days [16] depending on the severity of the infection [17]. The maximum duration of action of protective neutralising antibodies is not yet known [15].

The results of several studies demonstrate that an adequate humoral immune response against SARS-CoV-2 is associated with the severity of infection, comorbidity, gender and age of patients, and the therapy administered [18, 15, 19, 20]. Neutralising antibodies are undoubtedly one of the main correlates of protection [21], but levels associated with protection from reinfection have not been clearly determined and remain controversial. Recent studies suggest that 50% protection against COVID-19 is provided by the level of neutralising antibodies, which comprise approximately 1/5 of the average value of levels in reconvalescents [22].

Corticosteroids (GCSs) are the only immunomodulatory agents that have been shown to reduce mortality in many studies and, accordingly, have been recommended for the treatment of COVID-19 when indicated[1] [23–25]. In addition to the benefits associated with their potent anti-inflammatory properties, their possible negative effects of immunosuppressive action on the course of SARS-CoV-2 infection are to be characterised. Specifically, there is limited information on their effect on the antibody response against most viruses. Short GCS treatments were associated with a decrease in serum IgG and IgA concentrations [26]. Short-term and long-term antibody production decrease may adversely affect virus clearance and reinfection protection. Additionally, data on the effect of GCS on SARS-CoV-2 clearance and neutralizing antibodies levels remain limited and contradictory [27, 28].

Therefore, assessment of humoral immunity following COVID-19 through measuring the level of virus neutralizing IgG antibodies to the SARS-CoV-2 S-protein is an important and relevant task. Understanding the specifics of the formation of post-infectious humoral immunity will guide the decision on vaccination and revaccination and on development of current treatments (monoclonal antibodies and other drugs for COVID-19 treatment and prevention).

Understanding the antibody formation dynamics in different severity COVID-19 cases and investigation the factors affecting the maintenance of a significant level of protective antibodies in people after COVID-19 infection of different severity will contribute to optimisation of preventive and treatment activities.

The present study investigating the specifics of the formation and persistence of viral-specific protective antibodies in the blood in people after COVID-19, depending on gender, age, severity and scope of therapy, is one of the first in Russia.

The objective of the study is to investigate the factors affecting the levels of IgG antibodies to SARS-CoV-2 S-protein in the blood of reconvalescents after a new coronavirus infection (COVID-19) for 6 months.

MATERIALS AND METHODS

The study was conducted from 01/06/2020 to 01/08/2021. In the study were enrolled patients hospitalised at the Military Medical Academy (MMA) clinics: a clinic (therapy, continuous professional development of physicians), clinics on hospital therapy, naval therapy and infectious diseases. Outpatients with asymptomatic and mild disease were enrolled in the study by the laboratory service "Helix" according to the collaboration plan.

As a result, the population met the age inclusion criteria. All patients enrolled in the outpatient group were tested for the content of neutralising IgG antibodies to SARS-CoV-2 S-protein by cemiluminescent immunoassay (LIAISON XL, DiaSorin S.p.A., Italy) in venous blood (herein-

[1] WHO. Corticosteroids for COVID-19. Available at: https://www.who.int/publications-detail-redirect/WHO-2019-nCoV-Corticosteroids-2020.1; EMA endorses use of dexamethasone in COVID-19 patients on oxygen or mechanical ventilation. Available at: https://www.ema.europa.eu/en/news/ema-endorses-use-dexamethasone-covid-19-patients-oxygen-mechanical-ventilation.

after referred to as anti-S-SARS-CoV-2-IgG) on 30, 45, 60, 90, 180 days from diagnosis via positive reverse transcriptase polymerase chain reaction (RT-PCR) for SARS-CoV-2 nucleic acid in biological material.

Patients of the inpatient group were tested identically on days 1, 14, 45, 60, 90 and 180 from the time of admission to the hospital based on the results of positive RT-PCR for SARS-CoV-2 nucleic acid in biological material. At the same time, levels of anti-S-SARS-CoV-2-IgG were measured in the inpatient group at the 1st time point to differentiate and exclude patients who had previously had COVID-19 and who had already developed immunity against SARS-CoV-2.

The results obtained were expressed in the referenced arbitrary units for this analyser and the system test – AU/mL (arbitrary units per millilitre). The quantitative measure of antibody response to S-protein intensity were levels of IgG to S1/S2 within the respective reference ranges for this device: 0.0–12.0 AU/mL – negative (no antibody and consequently humoral immunity), 12.0-15.0 AU/mL – doubtful, over 15 AU/mL – positive. The International Units of BAU (Binding Antibodies Unit) was recalculated according to WHO guidelines, using the formula: result obtained in AU/mL x 2.6 = ... BAU/mL.

Patients of the inpatient group underwent a standard examination in the scope of the requirements of the IMR [Interim Methodological Recommendations] as per the version current at that time [29–31].

The study design is shown in *Fig. 1*. The study included 1421 men and women aged 18 to 70 years (mean age 38.2 ± 1.38 years), of which 216 patients received inpatient treatment for moderate to severe disease (hereinafter referred to as the inpatient group) and 1205 patients received outpatient treatment (hereinafter referred to as the outpatient group) for asymptomatic and mild disease. All subjects completed the original questionnaires approved by the Local Ethics Committee. Subjects who wished to participate in the study were enrolled based on eligibility criteria:

Eligibility Criteria

Outpatient inclusion criteria:

- COVID-19 naive and SARS-COV-2 unvaccinated men and women who have had current COVID-19 confirmed by positive RT-PCR test;
- the date of chemiluminescent immunoassay for anti-SARS-COV-2 virus neutralising antibodies – more than 10 days from the time of clinical recovery in symptomatic study participants and application for participation with a result in the laboratory service "Helix" or more than 14 days after the detection of SARS-COV-2 RNA by RT-PCR in asymptomatic patients;
- age between 18 and 70 years, inclusive;
- absence of pregnancy and lactation period;
- no history of autoimmune (rheumatologic), oncological diseases;
- willingness to participate in the study, which was confirmed by signed consent.

Inpatient inclusion criteria:

- COVID-19 naive and SARS-COV-2 unvaccinated men and women who have had current COVID-19 confirmed by positive RT-PCR test;
- inpatient treatment in the clinic (MMA);
- age between 18 and 70 years, inclusive;
- absence of pregnancy and lactation period;
- no history of autoimmune (rheumatologic), oncological diseases;
- willingness to participate in the study, which was confirmed by signed informed consent.

Study Conduct

Laboratory studies were conducted at the Military Medical Academy clinics, as well as with participation of persons from 22 residential settlements at the facilities of three laboratory complexes of the company "Helix" located in St. Petersburg, Moscow and Yekaterinburg.

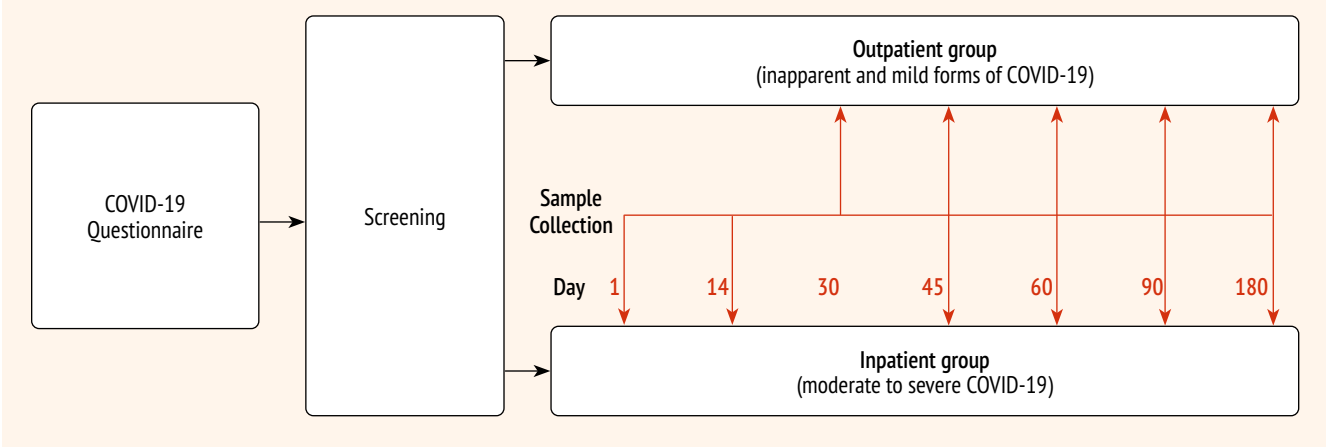
Duration of Study

The joint research study "OTKLIK" was conducted between 01/06/2020 and 01/08/2021.

Study Outcomes

Main study outcome: comprehensive assessment of the dynamics of anti-S-SARS-CoV-2-IgG formation in patients who suffered a new coronavirus infection in various courses of the disease was performed. The specifics of the formation and persisting of protective virus-specific antibodies in the blood were studied depending on the sex, age of patients, severity of COVID-19, and the applied GCS treatment in various doses, and the relationship of various clin-

Figure 1. Study design



ical and instrumental parameters with the level of antibodies was established. The hypothesis that the severity of the disease significantly enhances the humoral immune response was confirmed. The study showed significantly higher serum levels of viral neutralising antibodies and the duration of their blood concentration preservation in the inpatient group. It seems to be important that by 180 days this difference was not significantly different. A phenomenon of dose-dependent increase in anti-S-SARS-CoV-2-IgG production was also noted in patients treated with GCS in the average moderate course of infection. These results characterise the timing and duration of humoral immunity in patients undergoing COVID-19 in asymptomatic, mild, moderate or severe disease, taking into account various factors, and may provide a basis for the development of new treatments, prevention of reinfection with SARS-CoV-2, and timing of vaccination and revaccination against COVID-19.

Subpopulations Analysis

Enrolled patients were stratified by disease severity into two groups: outpatient (asymptomatic and mild) and inpatient (moderate and severe). According to the World Health Organization (WHO) classification, patients were divided into the following age subpopulations: 18–44 years old – young people, 45-59 years old – middle age people; 60-70 years old – elderly people[2].

Ethical Review

All procedures planned for participation of human subjects in the clinical study have been reviewed and approved by the Ethics Committee of the Military Medical Academy named after S.M. Kirov (Minutes No 249 dated 27 April 2021).

Statistic Analysis

Principles of sample size calculation: sample size was not pre-calculated.

Methods of statistical analysis: data statistical analysis was performed using Statistica 12 software (StatSoft, Inc. USA), IBM SPSS Statistics 23 (IBM corporation. SEM). Current predominantly non-parametric methods of statistical analysis (descriptive statistics, comparative analysis of data, rank analysis of variance, regression and correlation analysis) were used.

In all cases, differences, associations and relationships were considered statistically significant at $P \leqslant 0.05$, not significant at $P > 0.10$; in the interim cases ($0.05 < P \leqslant 0.10$), the observed effects were discussed as trends.

RESULTS AND DISCUSSION

Study Subjects (Participants)

Final study results include analysis of data from 1205 male (n = 460) and female (n = 745) patients in the outpatient group aged 18 to 70 years (mean age 38 years). The inpatient group included 216 patients, men (n = 128) and women (n = 88). Outpatient and inpatient groups were representative of sex and age. Both groups received outpatient or inpatient treatment depending on the severity of the disease. The total number of patients allows the use of the necessary statistical methods.

Main Study Results

Anti-S-SARS-CoV-2-IgG formation in outpatient group

In the outpatient group, antibody-free status was determined at Day 30 in 0.9% of young women and 0.8% of men of the same age. During the analysis of the data obtained, it was determined that all subjects developed virus-neutralising antibodies by 45 days from the first positive RT-PCR result. *Fig. 2* shows histograms of the distribution of IgG to SARS-CoV-2 S-protein in the study. The virus neutralising antibody content in patients varied widely, ranging from less than the limit of detection to 400 AU/mL. The distribution of the quantitative trait – anti-S-SARS-CoV-2-IgG concentrations in men and women were abnormal, so non-parametric methods were used in the statistical analysis.

Gender and Age Differences of Anti-S-SARS-CoV-2-IgG Content in the Outpatient Group

Data on the dynamics of anti-S-SARS-CoV-2-IgG levels in men and women of the outpatient group are presented in *Table 1*. The resulting curve reflecting the dynamics of anti-S-SARS-CoV-2-IgG level depending on gender and age, is shown in *Fig. 3*.

As shown in *Fig. 3*, anti-S-SARS-CoV-2-IgG concentrations were higher in elderly men and women than in middle-aged individuals at all time points in the study, and the concentrations in the latter was higher than in young adults. Thus, a clear trend of the dependence of the power of post-infectious antibody response on the age of the COVID-19 patients was established.

In women of all age groups, there is a clear trend towards a gradual increase in the level of anti-S-SARS-CoV-2-IgG over time by 90 days with a sharp rise to 180 days (peak concentration). This trend in antibody level dynamics was most pronounced in women over 60 years of age.

In the Friedman test, the group of women showed statistically significant differences in antibody levels at different times, which appeared stronger than in men: $\chi2(4) = 22.76$; $P < 0.001$. Neutralising antibody levels in dynamics showed that women had an increase in antibody levels by 45 days, which persisted until 60 days, and afterwards increased slightly, and then significantly and by 180 days showed a clear tendency to increase.

The similar tendency of anti-S-SARS-CoV-2-IgG level was recorded in the male group in all age groups by time point. However, at Day 60, differences in the form of multidirectional dynamics were observed in men of different age groups, young and middle-aged men tended to gradually decrease the level of neutralising antibodies during the entire study period, whereas elderly men showed an increase in the level of antibodies from 45 days to 90 (peak antibody concentration), followed by a decrease to approximately baseline level by the end of the study ($p > 0.05$).

Testing of the differences in antibody levels at different times in the Friedman test in the group of men of all age groups revealed their statistically significant differences: $\chi2(4) = 11.91$; $P = 0.018$. Thus, in men, overall non-linear changes of anti-S-SARS-CoV-2-IgG levels were observed: by

[2] World Health Organization. *World report on ageing and health*. 2015. 260 p. Available at: https://apps.who.int/iris/bitstream/handle/10665/186463/9789240694811_eng.pdf.

Figure 2. Distribution of IgG in samples of men and women at different stages of COVID-19 disease

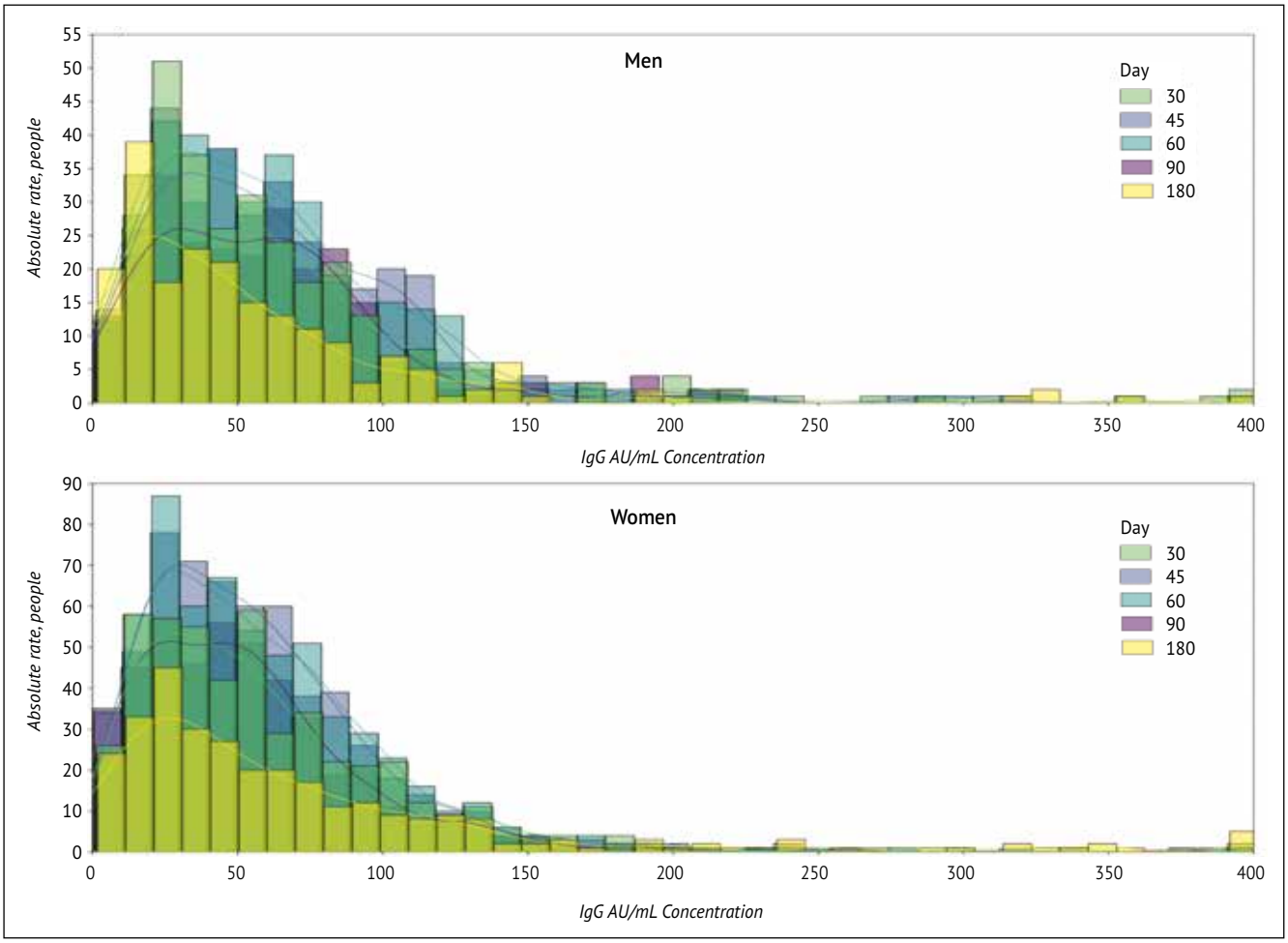
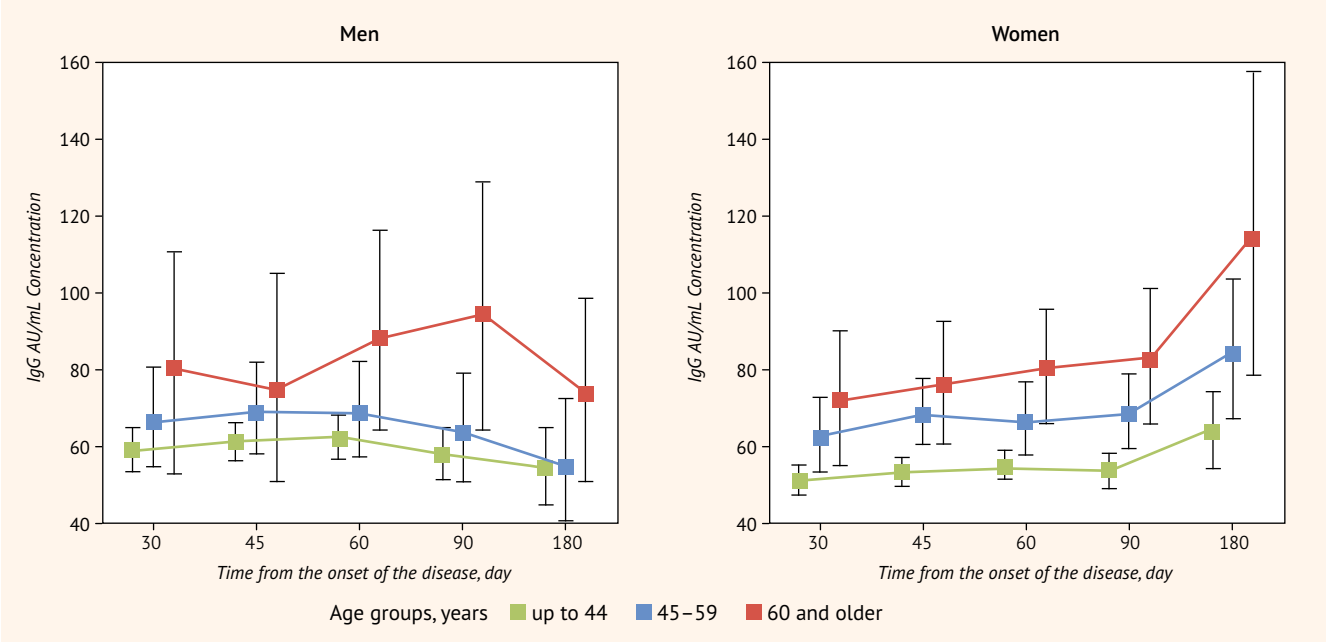


Table 1. Dynamics of changes in the serum level of IgG to the S-protein SARS-CoV-2 (EU/ml) in the outpatient group (average [95% CI])

Age group, years	Time since disease onset, day				
	14–30	45	60	90	180
Men					
Up to 45	58,9 [53,4; 64,6]* n = 194	60,9 [56,1; 65,9]* n = 242	62,2 [56,6; 68,3] n = 258	57,8 [51,2; 65,1] n = 225	54,2 [44,6; 65,1] n = 140
45–59	66,6 [54,3; 80,3] n = 61	68,9 [57,7; 81,5] n = 80	68,8 [57,1; 82,0] n = 84	63,6 [50,7; 79,0] n = 73	54,7* [40,2; 72,2] n = 51
60 and older	80,3 [52,5; 110,5] n = 13	74,9 [50,9; 105,1] n = 12	88,4 [64,2; 116,0] n = 15	94,5 [63,7; 128,8] n = 17	73,9* [50,6; 98,4] n = 11
All groups	61,7 [56,5; 67,0] n = 268	63,3 [58,8; 68,1] n = 334	64,8 [59,7; 70,3] n = 357	61,1 [55,1; 67,6] n = 315	55,4 [47,5; 64,1] n = 202
Women					
Up to45	50,8 [47,0; 54,8] n = 336	53,1 [49,5; 56,9] n = 438	54,7 [51,1; 58,5] n = 424	53,4 [49,0; 58,0] n = 337	63,7 [54,2; 74,0] n = 201
45–59	62,6 [53,4; 73,0] n = 103	68,4 [60,5; 77,3] n = 136	66,5 [57,6; 76,9] n = 141	68,4 [59,2; 78,8] n = 121	84,3 [67,1; 103,4] n = 85
60 and older	71,8 [54,8; 90,0] n = 20	76,1 [60,5; 92,8] n = 25	80,4 [65,6; 95,4] n = 28	82,9 [65,5; 101,1] n = 25	114,3 [78,6; 157,7] n = 21
All groups	54,4 [50,7; 58,2] n = 459	57,6 [54,2; 61,1] n = 599	58,7 [55,2; 62,5] n = 593	58,7 [54,6; 62,9] n = 483	72,8 [64,3; 81,9] n = 307

* – compared to women ($p < 0.05$).

Figure 3. Dynamics of changes in the level of IgG (OU/ml) in the blood of outpatient patients who have undergone COVID-19, taking into account gender and age characteristics



45 days, it increased and remained very close to this value after 60 days, and then decreased slightly by 90 days and very significantly by 180 days.

Young men had significantly higher anti-S-SARS-CoV-2-IgG concentrations at 30 and 45 days post-disease compared to women of similar age, however, in middle-aged group, antibody levels were significantly higher in women by 180 days. In the remaining cases, the anti-S-SARS-CoV-2-IgG concentration in men did not differ significantly by age and measurement time compared to women, as shown in *Table 1*.

In general, in the asymptomatic and mild course of COVID-19, the strength of post-infectious humoral immune response depends on gender, age and timing of measurement.

As age increases from 18 to 69 years, the strength of the postinfectious humoral immune response doubles in both men and women. The main gender difference in the dynamics of anti-S-SARS-CoV-2-IgG level is that after Day 60, the level of antibodies decreases up to 180 days in men, while in women, the level actually increases. In the early reconvalescence period (up to 45 days), a stronger humoral immune response occurs in young men, and in late period (more than 90 days) – in middle-aged women, which is illustrated in *Fig. 4*.

Specifics of the Formation and Maintenance of Anti-S-SARS-CoV-2-IgG Level in Patients of the Inpatient Group

Due to the higher disease severity of patients hospitalised, in addition to the study of the dynamics of the level of anti-S-SARS-CoV-2-IgG depending on gender and age, additional assessment and correlation analysis of their relationship with various markers of disease severity (volume of lung lesions by CT, body mass index (BMI), CRP levels at different periods of the disease, as well as different doses of systemic glucocorticoids (GCS), in the treatment duration of pyrexia and oxygen therapy) have been carried out. This is due to the fact that parameters reflecting the severity of the disease, in opinion of several authors, can affect the expression of the subsequent immune response [23]. The groups of patients were representative of gender and age, the group of men consisted of 128 patients, and the group of women included 88 women.

Effect of Age and Gender on Production of Anti-SARS-CoV-2 IgG in the Inpatient Group

Anti-S-SARS-CoV-2-IgG was not statistically significantly different in the middle-aged and elderly groups of patients over the entire observation period. Anti-S-SARS-CoV-2-IgG was significantly higher in the elderly than in the young at all study time points. Anti-S-SARS-CoV-2-IgG levels in middle-aged individuals were significantly higher compared to younger individuals on Days 14 and 60 only. Data is presented in *Table 2*.

There was no statistically significant difference in anti-S-SARS-CoV-2-IgG levels between men and women during the entire observation period. However, there was a trend in the form of an increase in anti-S-SARS-CoV-2-IgG level (by 14 days in men, by 45 days in women), followed by a period of stable antibody concentration.

There was a trend towards an increase in anti-S-SARS-CoV-2-IgG levels by 60 days in men and women of all ages (*Fig. 5*). Further, immunoglobulin levels decrease by 90 days, with a decrease in anti-S-SARS-CoV-2-IgG level more pronounced but not statistically significant in men. More pronounced but not statistically significant differences were detected by the end of the observation period (180 days). Thus, in elderly men during this period, a clear trend to lower IgG levels was revealed, however, in women of similar age, the trend was towards higher levels (*Fig. 5*). Correlative analysis showed a consistent positive association between neutralising antibody levels from Day 14 to Day 180 and age ($p < 0.005$).

Figure 4. Age-related dynamics of changes in IgG concentration in men and women with COVID-19. Generalized additive model for gamma distribution with 95% confidence limits

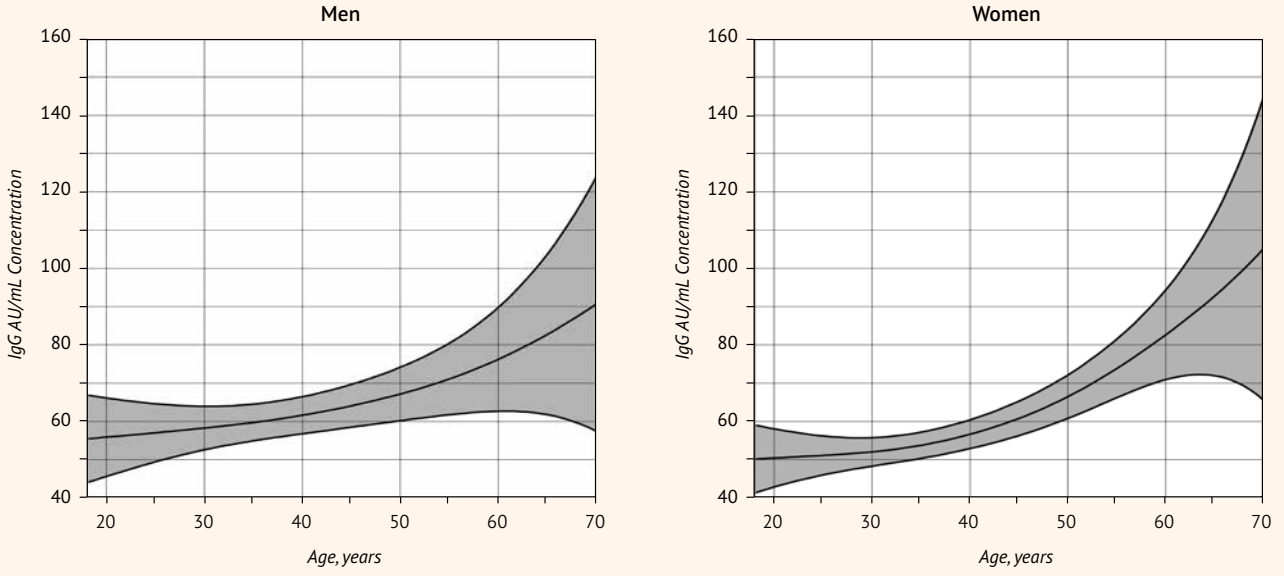


Table 2. Dynamics of blood IgG to the S-protein SARS-CoV-2 (OU/ml) in the stationary group at different ages (Me [Q1; Q3])

Time from disease onset, day	Age, years/number of subjects (n), abs.			p1	p2	p3
	⩽ 44	45–59	⩾ 60			
	n = 92	n = 82	n = 42			
14	25,8 [10,7; 89,2]	104,5 [51,4; 187]	91,2 [69,7; 126]	0,016*	0,007*	0,724
45	67,6 [33,5; 93,2]	106,3 [29,8; 150,5]	127 [88,2; 197]	0,308	0,016*	0,282
60	61,6 [29,3; 111]	121 [68,9; 171]	149 [114; 216]	0,022*	0,023*	0,386
90	52,3 [28,4; 88]	96,1 [45,4; 162]	142 [71,7; 236]	0,082*	0,031*	0,421
180	57,4 [20; 95,8]	114 [54,5; 160]	113 [70,2; 156]	0,067*	0,046*	0,979

* p1 – when comparing young and middle age persons; p2 – when comparing young and elderly persons; – when comparing middle and elderly persons

Thus, a study of immunoglobulin levels according to gender did not reveal any significant differences (*Fig. 5*). However, there is a clear trend towards an effect of age on the content of neutralising anti-S-SARS-CoV-2-IgG, in the form of an increase in their level in the elderly.

Changes in Antibody Levels in Patients Depending on Severity (comparison of anti-S-SARS-CoV-2-IgG levels in outpatients and inpatients)

When comparing patients by severity and neutralising antibody content, there were clear significant differences. Antibodies were significantly higher in the severe and moderate group compared to mild and asymptomatic patients over 3 months. By 180 days of the study, statistically significant differences were eliminated. The data is presented in *Table 3*, graphical display of dependence is presented in Figure 6. The study populations were representative of gender and age.

Effect of GCS Therapy on Anti-S-SARS-CoV-2-IgG Content in Inpatients

Patients treated with GCS were representative of gender, age and severity. Based on GCS dose, the patients were divided into 3 subgroups receiving small, medium or high doses. GCS doses corresponding to 28–42 mg methylprednisolone / 5.3-8 mg dexamethasone were considered low. Average doses were 64-256 mg of methylprednisolone /12-48 mg of dexamethasone and high GCS doses were ⩾512 mg of methylprednisolone / 96 mg of dexamethasone. Statistical analysis showed that anti-S-SARS-CoV-2-IgG levels were significantly lower in patients treated with low doses of GCS compared to patients treated with medium and high doses during the entire observation period.

On Day 45, the group of patients receiving the average doses showed the highest level of anti-S-SARS-CoV-2-IgG. This group was statistically significantly different from the low and high dose groups, where the readings were lower. Data is shown in *Table 4* and *Fig. 7*.

On Day 180, anti-S-SARS-CoV-2-IgG levels were significantly higher in patients in the GCS high-dose group than in the small- and medium-dose groups.

Thus, a clear dose-response effect of GCS on anti-S-SARS-CoV-2-IgG level was observed in the study groups.

Anti-S-SARS-CoV-2-IgG was significantly higher (2-fold) in patients with moderate course (CT-2) who received aver-

Figure 5. Influence of gender and age on the dynamics of anti-S-SARS-CoV-2-IgG titer in inpatients

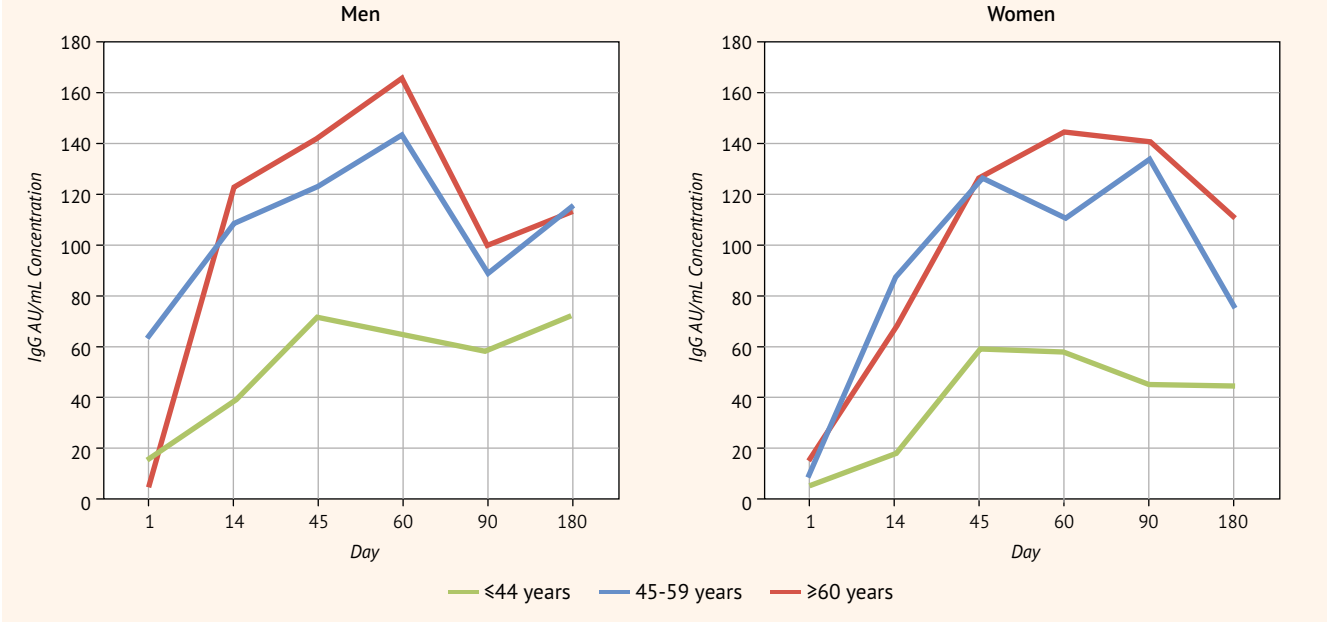
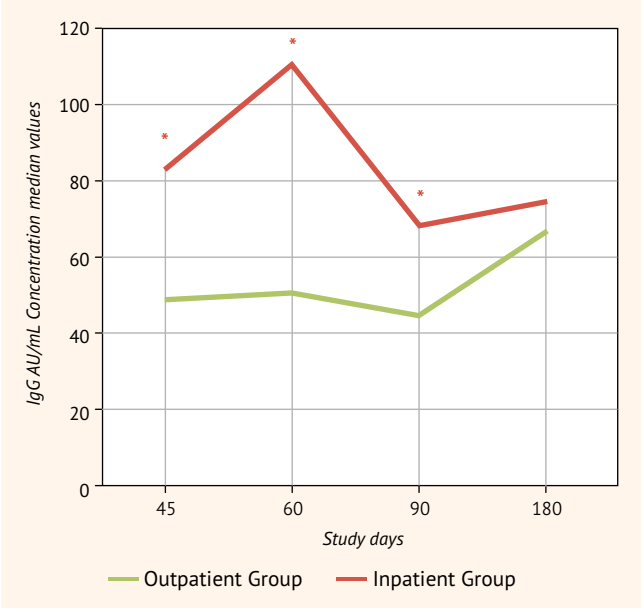


Table 3. The content of anti-S-SARS-CoV-2-IgG (OU/ml) in the blood of outpatients and inpatients (Me [Q1; Q3])

Time from disease onset, day	Outpatients Me [Q1; Q3] IgG (AU/mL)	Inpatients Me [Q1; Q3] IgG (AU/mL)	Mann-Whitney p-value
45	49,50 [27,6; 78,0]	82,90 [41,3; 134,0]	0,0001*
60	50,90 [28,6; 78,1]	111,00 [51,9; 156,0]	0,0001*
90	45,60 [24,2; 74,4]	68,75 [39,0; 143,0]	0,001*
180	66,85 [24,1; 94,1]	74,55 [39,5; 130,0]	0,71

* compared to the measurement times at the level of $p < 0.05$.

Figure 6. Dynamics of anti-S-SARS-CoV-2-IgG (OU/ml) in outpatient and inpatient groups



* – compared to the outpatient group ($p < 0.05$).

age doses of GCS (64–250 mg of methylprednisolone or 12–48 mg of dexamethasone) than in patients who did not receive GCS during the observation period from Day 14 to 90 (*Table 5*). By the end of the 180-day study, this difference was not significant.

In general, it can be concluded that the use of GCSs has a positive effect on the level of neutralising antibodies in the moderate course of COVID-19.

Factors Affecting the Level of Antibodies in the Inpatient Group

The study revealed a significant positive correlation of lung involvement volume based on CT data with anti-S-SARS-CoV-2-IgG level, which manifested from Day 14 and persisted throughout the observation period ($r = 0.3$; $p < 0.001$).

In addition, there was a significant positive correlation of BMI with anti-S-SARS-CoV-2-IgG levels from Day 14 through the end of the study (14-day $r = 0.3$ $p < 0.001$; 45-day $r = 0.06$ $p < 0.06$; 60-day $r = 0.3$ $p < 0.03$; 90-day $r = 0.2$ $p < 0.05$; 180-day $r = 0.3$ $p < 0.01$).

There was also a significant positive correlation of baseline CRP with anti-S-SARS-CoV-2-IgG content established between 14 days and 90 days (14-day $r = 0.6$ $p < 0.001$; 45-day $r = 0.3$ $p < 0.042$; 60-day $r = 0.4$ $p < 0.002$; 90-day $r = 0.4$ $p < 0.024$.)

A similar trend of correlation was noted between oxygen therapy and anti-S-SARS-CoV-2-IgG level starting from day 14 (14-day $r = 0.4$ $p < 0.005$; 45-day $r = 0.4$ $p < 0.002$; 60-day $r = 0.6$ $p < 0.005$; 90-day $r = 0.5$ $p < 0.003$).

A similar correlation was found between the number of hyperthermia days and the anti-S-SARS-CoV-2-IgG value from Day 14 to Day 90 (14-day $r = 0.4$ $p < 0.005$; 45-day $r = 0.5$ $p < 0.003$; 60-day $r = 0.4$ $p < 0.005$; 90-day $r = 0.3$ $p < 0.002$).

Adverse Events

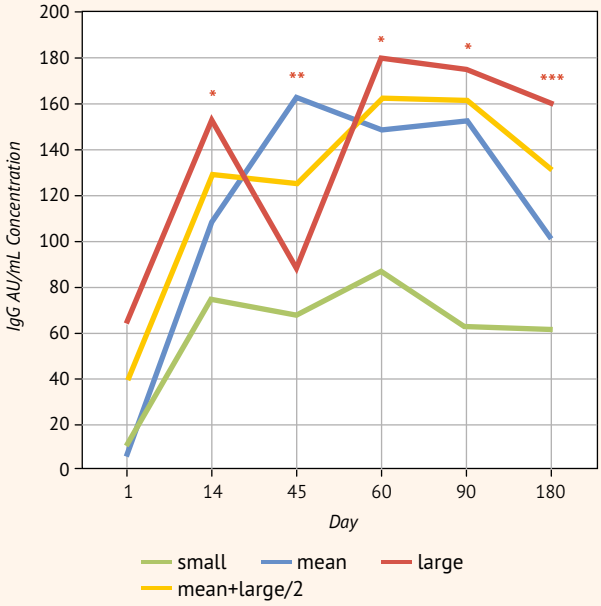
No adverse events were reported.

Table 4. The dynamics of the titer of anti-S-SARS-CoV-2-IgG (OU/ml) in the blood of patients receiving GCS (Me [Q1; Q3])

Time from disease onset, day	GCS doses/number of patients (n), abs.		
	small	mean	large
	n = 42	n = 56	n = 18
14	75,3* [47,8; 117]	108 [84,6; 187]	152 [87,5; 222]
45	68,4* [66,6; 126]	163** [83,4; 231]	88,2 [24,2; 134]
60	86,6* [40,7; 140,5]	149 [97; 205]	180 [158,5; 205,5]
90	63,3* [40,5; 140]	153 [96,1; 176]	175 [175; 175]
180	62,4* [39,5; 125]	101,2 [57,6; 145]	160*** [114; 309]

* – in comparison of the small dose group with the group of medium and high doses of GCS ($p < 0.05$); ** – in comparison of the group of medium doses with the groups of small and large doses of GCS ($p < 0.05$); *** – in comparison of the group of large doses with the group of small and medium doses of GCS ($p < 0.05$).

Figure 7. Dynamics of the titer of anti-S-SARS-CoV-2-IgG in patients of the inpatient group depending on the received dose of corticosteroids



* – in comparison of the low-dose group with the group of medium and high-dose of GCS ($p < 0.05$);
** – in comparison of the medium-dose group with the groups of low and high-dose of GCS ($p < 0.05$);
*** – in comparison of the high-dose group with the groups of low and medium-dose of GCS ($p < 0.05$).

Table 5. The dynamics of the titer of anti-S-SARS-CoV-2-IgG (OU/ml) in the blood of patients of moderate severity with CT-2, depending on the use of GCS (Me [Q1; Q3])

Time from disease onset, day	GCS-naïve n = 30	Treated with GCS n = 74	Mann-Whitney p-value
14	51,85 [9,1; 88,2]	92,45 [69,7; 116,0]	0,039*
45	10,60 [7,6; 51,0]	118,5 [82,9; 163,0]	0,007*
60	26,30 [11,4; 114,0]	146,5 [74,3; 184,5]	0,019*
90	28,40 [11,3; 55,2]	134,0 [71,7; 205,5]	0,004*
180	38,90 [10,5; 56,8]	98,30 [54,5; 160,0]	0,686*

* $p < 0,05$.

SUMMARY OF THE MAIN STUDY OUTCOME

As a result of the study, a comprehensive assessment of the dynamics of the formation of IgG to SARS-CoV-2 S-protein was performed in patients who suffered from coronavirus infection in various courses of the disease. The specifics of the formation of the immune response depending on gender, age, severity and GCS therapy at different doses were studied; factors affecting the level of IgG to SARS-CoV-2 S-protein in the blood in COVID-19 patients for 180 days have been identified.

Discussion on the Primary Outcome of the Study

The focus of our study was to determine the nature and duration of the persistence of neutralising antibodies against SARS-CoV-2 S-protein and to identify factors affecting IgG levels at various disease severity in early stage and long-term course of COVID-19. Identification of certain phenotypes by gender, age or other principle, or their associations will help predict the nature of the immune response following a suffered coronavirus infection.

The results of the conducted study strongly suggest that in the outpatient group, women have the advantage in forming a more pronounced and long-lasting humoral immune response in COVID-19, which may be a predictor of the favourable course and outcomes of the disease in the female population. Despite a relatively short observation period (180 days), according to the current trend in our study, an apparently more intensive decrease in antibody level is observed in men compared to women, which is associated with a short persistence of antibodies in men against SARS-CoV-2 according to the literature sources [32]. However, it is evident that the elderly group of both sexes is characterised by a higher level of IgG to SARS-CoV-2 compared with the young and middle age group, probably due to a stronger immune response and slow elimination of antigen-antibody immune complexes in the elderly, which is consistent with the literature [33].

In elderly patients, gender differences are not statistically significant at any time during the disease. However, at 180 days, in the female group, the IgG value is 1.55-fold higher than the initial values, with no statistical differences with male group of the same age. However, with regard to the mean values, differences between men and women in antibody levels do not have statistical differences, but they are present in the combinations of some age groups and the timing of the disease. This indicates that there is an interaction between factors "gender", "age" and "duration of disease", which requires simultaneous consideration when characterising IgG levels and determining tactics for dynamic follow-up of such patients.

This phenomenon should be considered in the planning of clinical studies aimed at enhancing antiviral mechanisms interventions that block the hyperinflammatory response, as well as in studying post-infectious immunity, methods of its activation and prolongation.

Currently, there is a fairly large number of opinions regarding the impact of gender factor on immunological status, which are based on conflicting results from different studies. The association between male gender and adverse

outcomes in COVID-19 is widely known [34], which is supported by studies in which the authors noted clear gender differences in cytokine levels and cellular response [35].

It was shown that women develop a faster and more pronounced humoral response to infections and vaccinations due to sex hormones levels [36, 37]. Experimental studies in mice shown that estrogens stimulate antibody production, and testosterone may suppress it. Clinical trials have found lower IgG production in men with influenza vaccination, especially in men with high testosterone levels during immunization [38]. On the other hand, estrogens are thought to have a protective effect on the duration of antibody circulation, which is indirectly confirmed by a higher incidence of certain autoimmune diseases in the female population. The results of these studies demonstrate an important role of sex hormones in the formation of an immune response to an infectious pathogen. For elderly women, it can be assumed that the highest antibody level over time compared with men of all age groups is associated with increased NK-cells, decreased NK-cell cytotoxicity and decreased number of B-cells and CD4+T-cells compared with premenopausal women [39, 40]. Increased production of inflammatory proteins (e.g., C-reactive protein and GM-CSF) in women compared to men persists in the elderly. Typically, elderly men have a faster decline in B-cell and T-cell counts and activity than women [40]. Teixeira et al. 2020 states that with age, female T-cells produce more IL-10 than male T-cells, which may help counteract the adverse effects of inflammation with age. It is also believed that regardless of age, women tend to show higher antibody levels, higher basal IgG levels, and higher B-cell counts than men. Thus, gender differences in antibody levels are strongly associated with hormonal background [41].

In our study, a significantly higher antibody level was observed in young men compared with young women in the outpatient group on Day 45, however, in middle-aged women, the antibody level was significantly higher in the period from 90 to 180 days compared with men of similar age. In the remaining cases, there were no significant gender differences in antibody levels in outpatient and inpatient groups, but there was a clear trend towards higher antibody levels in women.

Thus, the analysis of the data revealed a complex nature of variation in IgG in patients who suffered COVID-19. It was influenced not so much by the individual factors examined, but by their association, where the age was the strongest factor affecting IgG concentration. Gender differences in IgG levels occur only with age and duration of disease. However, the main gender differences in men and women of the outpatient group are that after 60 days, the level of antibodies in men decreases up to 180 days, while it even slightly increases in women.

In the inpatient group, statistically significant differences between young and elderly persons were found depending on age, which is consistent with the data of another large study [42].

A correlation between age and almost all dependent parameters: lung tissue involvement volume, duration of oxygen therapy and hyperthermia is important. Strong correlation of IgG levels is evident on Day 14 and has significant correlations throughout the observation period. The highest correlation value at Day 60 is 0.321 ($p = 0.022$). In summary, it can be stated that the elderly tend to have an increase in IgG levels due to the nature of the immune system response [43].

In stressful situations, sex hormones are known to have an important regulatory function in mobilizing the body's defences. Thus, the study by Rastrelli G et al. (2020) showed that low testosterone levels were associated with severe manifestations of a new coronavirus infection. At the same time, scientists from Royal College London, University of Liverpool and Zoe Global Limited found a correlation between high estrogen levels and milder COVID-19 infection in women [44].

In addition to gender- and age-related differences, the severity of the process affects the degree of immune response because the greater the antigen enters the body, the greater is the antibody response. The severity is largely determined by the presence of comorbid pathology, including obesity. Our data show a clear relationship between BMI and lung tissue involvement. Notably, BMI was not associated with CRP levels at any time during treatment. BMI is correlated with IgG levels on Day 14 and remains fairly stable throughout the study.

Interesting findings were obtained from the study of the correlation between the extent of lung tissue involvement on CT scan and IgG level in different periods after the suffered infection. There is a statistically significant strong positive correlation between the percentage of lung involvement and the IgG level. The prevalence of lung injury, duration of oxygen support, number of days of hyperthermia, CRP level is directly associated with the severity of the disease, which is confirmed in the work of other researchers who established the dependence of IgG level on the severity of the disease course and the percentage of lung tissue damage [45, 46]. Interestingly, the association of CRP levels at the start of treatment with IgG levels starting from Day 14 was evident and maintained throughout the study period. This phenomenon was reported in SARS-CoV and MERS-CoV and is due to high levels of pro-inflammatory cytokines released from CD4+T-cells, which is accompanied by an increase in CRP.

In general, factors such as excessive body weight, duration of oxygen therapy and duration of hyperthermia can be said to stimulate antibody generation.

The next important section of our study was the assessment of the humoral immune response with GCS. There is conflicting and limited data on the effect of GCS therapy on the nature of humoral immune response in patients who have had COVID-19. Thus, in the study by Başaran, S. et al. (2021), it was found that patients who received tocilizumab, anakinra or prednisolone had a higher level of antibodies than those who did not take these drugs, but a multivariate analysis confirmed a linear dependence of the serum concentration of antibodies on the degree of lung tissue damage established by CT data. The authors concluded that

there was no independent effect of anticytokine and GCS therapy on antibody synthesis [47]. The opposite results were obtained in the study by Masiá, M. et al., who did not find any independent negative effect of GCS use on virus clearance, with antibody levels being significantly higher in patients receiving high doses of GCS compared with patients without GCS therapy [27]. We compared the antibody content in relation to the dose of the drugs (low, medium, high) in patients receiving GCS in the cases indicated. At the same time, all patients receiving GCS were representative by sex, age and severity of the disease and presence of comorbid pathology. The analysis of the results demonstrates that patients treated with low doses of GCS had significantly lower IgG levels at 45, 60, and 180 days than patients treated with medium and high doses of GCS.

Thus, we may confirm a dose-dependent effect of GCS therapy on the level of IgG to SARS-CoV-2 in the treatment of moderate to severe COVID-19.

As the previous studies established independent factors affecting serum antibody levels in patients recovered from COVID-19, in particular, disease severity [9] and area of lung tissue involvement confirmed by CT findings [47], inpatients who had moderate infection and had the same volume of lung tissue involvement (CT-2) were stratified into two subgroups, which received and did not receive GCS therapy. A comparative analysis of the results showed that at the same severity and volume of lesion, the level of antibodies was significantly higher in patients who received GCS during the entire observation period. However, patients in both groups were representative of gender, age and comorbidity. It is extremely important to point out the result of a statistically significant increase in IgG level in the GCS group relative to the group of patients who did not take them, which demonstrates the stimulating effect of GCS therapy on the immune response. The identified stimulatory nature of GCS effect on IgG production in the early and long-term period after COVID-19 requires its understanding, given that GCS has a known immunosuppressive effect, with success in the treatment of autoimmune diseases [48]. It appears that the bimodal effect of GCS on immunity may be assumed: the characteristic suppressive effect in the acute phase of inflammation according to the principle "dose-response" and the delayed positive anti-inflammatory effects, possibly due to the timely "closure" of the cytokine "storm". Hypothetically, this heterogeneity of GCS action in an evolutionary sense provides an advantage for survival in the form of managing the severity of the inflammatory response, with the simultaneous subsequent enhancement of protective immunity against this infection which created an immediate threat to the body's life. However, with regard to COVID-19, it cannot be excluded that endogenous production of GCS in severe infections, which provides an adequate immune response, is insufficient, thus, explaining the proven benefit of using supraphysiological doses of GCS. This is confirmed by the "dose dependency" of the positive effect of GCS on IgG level in the early and long-term period after COVID-19 demonstrated in our study.

"Bimodality" of GCS action against the adaptive immune response is indirectly confirmed by Chinese authors who showed an increase in CD4+T-cells with administration of GCS, which provides additional protective immunity, indirectly further affecting IgG synthesis, in contrast to the use of antibiotics that significantly reduce IgG levels within 90 days of discharge from the hospital after COVID-19 [49].

The direct mechanisms by which GCSs stimulate the synthesis of viral neutralising antibodies remain unclear and require further investigation.

It is important to note that our study is one of the first to establish the stimulant and dose-dependent effect of GCS therapy on the production of specific viral neutralising antibodies in patients who have had COVID-19.

Study limitations

The limitation of the study is the short observation period – up to 180 days, as well as the number of patients receiving GCS therapy, and the possibility of analysis of their effect on IgG production in the long-term compared to COVID-19 therapy without GCS only with the extent of CT-2 lung tissue involvement (for ethical reasons).

CONCLUSION

Factors that affect the blood levels of SARS-CoV-2 S-protein IgG antibodies in reconvalescents after a new coronavirus infection (COVID-19) for six months are the severity of the disease, sex and age of patients, the use of GCS and their dose. The peak of seroconversion of IgG antibodies to SARS-CoV-2 S-protein is noted by 90 days from the onset of the disease, followed by a trend towards a decrease by 180 days.

Significantly higher levels of viral neutralising antibodies were determined in severe disease, in elderly women (over 60 years of age), and in patients treated with GCS, likely due to a stronger immune response and slower elimination of antigen-antibody immune complexes in the elderly, as well as the anti-inflammatory effect of GCS therapy. These circumstances should be taken into account when conducting treatment and preventive measures and when planning vaccination and revaccination.

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