DYNAMICS OF HUMORAL IMMUNITY TO SARS-COV-2 IN THE PROFESSIONALLY HOMOGENEOUS GROUP OF PEOPLE OVER A TWO-YEAR PERIOD OF COVID-19 OUTBREAK

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It is important to control the levels of specific IgG against SARS-CoV-2 to ensure the timely monitoring of immunity in patients with COVID-19. Yet it is unclear what antibody levels protect against new infection and how long the protection is maintained. The study was aimed to assess the dynamic changes in the levels of IgG against SARS-CoV-2 by the two-year controlled observation. Healthy individuals (n = 70), COVID-19 survivors (n = 42), and people vaccinated with Sputnik V (n = 43) were enrolled. They were followed-up from April 2020 to April 2022. Serum IgG levels were defined (n = 312) using immunochip and the commercially available test system. Significance of differences was estimated using the Mann–Whitney U test for $p \leq 0.05$. IgG levels in the disease survivors (median 97.1; 95% CI: 80–162 BAU/mL) and vaccinated individuals (103.1; 78–139 BAU/mL) were significantly higher than in healthy people (4.3; 4.1–4.5 BAU/mL). Intensity of immune response significantly increased after vaccination of the disease survivors (up to 1023; 657–1191 BAU/mL) or administration of booster dose to vaccinated individuals (413; 213–545 BAU/mL). In elderly convalescents (60+), IgG levels were significantly higher, and in vaccinated people these were significantly lower, than in people under the age of 60. IgG levels decreased faster in vaccinated individuals (after 3–4 months), than in the disease survivors, and stabilized at <100 BAU/mL in 60% of subjects within 5–9 months. Thus, intensity and duration of immune response in COVID-19 survivors and vaccinated people vary significantly depending on age, observation period, and additional vaccinations/revaccinations. Three cases of infection after full vaccination were reported over the entire follow-up period, including infection in a patient having a history of the disease and subsequent vaccination.

Keywords: COVID-19, IgG, SARS-CoV-2, dynamics of immune response, patients, age, Sputnik V vaccine, immunochip

Acknowledgements: we would like to thank staff members of the State Research Institute of Biological Instrumentation: Kanaeva TA for carrying out immunochip-based analysis, Balaban AS for antigen printing with nanoplotter and immunochip preparation for analysis, Stadnik OB for management of the serum samples acquisition and testing using commercially available immunoassay kit.

Author contribution: Pomelova VG — concept, experimental design, manuscript writing; Bychenkova TA — management of immunochip-based immunochemistry studies, data processing; Bekman NI — statistical analysis, preparation of illustrations; Osin NS — providing technical assistance, data analysis and discussion, manuscript editing; Ishkov YuN, Styazhkin KK — data analysis and discussion, manuscript editing.

Compliance with ethical standards: the study was approved by the Ethics Committee of the State Research Institute of Biological Instrumentation (protocol № 4 dated June 09, 2021). The informed consent was submitted by all subjects.

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Received: 19.05.2022 **Accepted:** 04.06.2022 **Published online:** 16.06.2022

DOI: 10.47183/mes.2022.020

ДИНАМИКА ГУМОРАЛЬНОГО ИММУННОГО ОТВЕТА К SARS-COV-2 В ПРОФЕССИОНАЛЬНО ОДНОРОДНОЙ ГРУППЕ ЛЮДЕЙ ЗА ДВУХЛЕТНИЙ ЭПИДЕМИЧЕСКИЙ ПЕРИОД COVID-19

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Для оперативного мониторинга состояния системы иммунитета при COVID-19 важно контролировать уровень специфичных IgG к SARS-CoV-2. Однако неясно, какой уровень антител и насколько долго может обеспечить защиту от нового заражения. Целью работы было оценить в двухлетнем контролируемом обследовании динамику уровней IgG к SARS-CoV-2. В исследовании участвовали здоровые лица (n = 70), переболевшие COVID-19 (n = 42) и вакцинированные «Спутником V» (n = 43). Период наблюдения: апрель 2020 г. — апрель 2022 г. IgG выявляли в сыворотке крови (n = 312) на иммуночипе и в коммерческом тесте. Достоверность различий оценивали по критерию Манна–Уитни для $p \leq 0,05$. Уровни IgG у переболевших (медиана 97,1; 95% ДИ: 80–162 BAU/мл) и вакцинированных (103,1; 78–139 BAU/мл) были достоверно выше, чем у здоровых людей (4,3; 4,1–4,5 BAU/мл). Напряженность иммунного ответа значительно возрастала после вакцинации переболевших (до 1023; 657–1191 BAU/мл) или введения бустера вакцинированным (413; 213–545 BAU/мл). У реконвалесцентов старшего возраста (60+) уровень IgG достоверно выше, у вакцинированных — достоверно ниже, чем у людей моложе 60. IgG у вакцинированных снижались быстрее (через 3–4 месяца), чем у переболевших, а через 5–9 месяцев стабилизировались на уровне <100 BAU/мл у 60% обследованных. Таким образом, показатели напряженности и продолжительности иммунного ответа у переболевших COVID-19 и вакцинированных людей сильно варьируют в зависимости от возраста, срока наблюдения, дополнительной вакцинации / ревакцинации. За весь период наблюдений отмечено три случая заболевания после полного цикла вакцинации, в том числе у ранее переболевшего (а затем вакцинированного) человека.

Ключевые слова: COVID-19, IgG, SARS-CoV-2, динамика иммунного ответа, пациенты, возраст, вакцина «Спутник V», иммуночип

Благодарности: сотрудникам ФГУП «ГосНИИБП» Т. А. Канаевой — за постановку анализа на иммуночипе; А. С. Балабану — за печать антигенов на наноплоттере и подготовку иммуночипа к работе; О. Б. Стаднику — за организацию сбора и тестирования образцов сывороток в коммерческом тесте.

Вклад авторов: В. Г. Помелова — идея, дизайн экспериментов, подготовка рукописи; Т. А. Быченкова — руководство проведением иммунохимических исследований на иммуночипе, обработка результатов; Н. И. Бекман — статистический анализ, подготовка иллюстративного материала; Н. С. Осин — организация технической части исследований, анализ и обсуждение результатов, корректировка рукописи; Ю. Н. Ишков, К. К. Стяжкин — анализ и обсуждение результатов, корректировка рукописи.

Соблюдение этических стандартов: исследование одобрено этическим отделом ФГУП «ГосНИИБП» (протокол № 4 от 09 июня 2021 г.). Все участники подписали добровольное согласие на участие в исследовании.

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Статья получена: 19.05.2022 **Статья принята к печати:** 04.06.2022 **Опубликована онлайн:** 16.06.2022

DOI: 10.47183/mes.2022.020

The pandemic of novel Coronavirus Disease 2019 (COVID-19), declared by the WHO in March 2020, required a substantial effort on the part of the health systems of different countries, including Russia, to provide morbidity surveillance and the measures to reduce the risk of infection. From an epidemiological standpoint, the most effective protection is ensured by herd immunity developing naturally due to the growing proportion of insusceptible people having a history of infection in the population, or due to vaccination.

It is recommended to control the levels of circulating IgG antibodies to ensure timely monitoring of immunity [1, 2]. For that purpose, it is necessary to use the registered test systems for quantification of antibodies against various coronavirus antigens (S, S1, RBD, N). It is believed that there is a strong correlation between the levels of antibodies against the spike protein S1 receptor-binding domain (RBD) of SARS-CoV-2 and the neutralizing antibody titers measured using neutralization test [3].

However, it is still unclear what antibody levels ensure sufficient protection of the patient against the same and especially against new variants of SARS-CoV-2, and how long the necessary protection is maintained, that results from infection or vaccination [4]. The intensity and duration of immune response vary significantly among patients [5] and are largely dependent on gender, age, and COVID-19 severity [6–9].

The dynamic changes in humoral immunity are studied mainly within 6–8 months after the disease onset or vaccination [5, 8, 9]. That is why a longer follow-up is needed to assess individual characteristics of the developing protective immunity, which provide the basis for forecasting future trends of the pandemic [10], and developing personalized protocols for vaccination [8] and treatment.

It is of interest to obtain data on IgG formation and maintaining the level of IgG antibodies against SARS-CoV-2 over the two-year observation period, the longest-ever reported in the literature.

The study was aimed to assess the dynamic changes in the levels of IgG against SARS-CoV-2 in the two-year controlled observation of the State Research Institute of Biological Instrumentation staff members, and to define the factors affecting the intensity and duration of humoral immunity.

METHODS

Patients

The study was carried out in the molecular diagnostic laboratory of the State Research Institute of Biological Instrumentation from April 2020 to April 2022. A total of 77 research institute staff members and 8 members of their families were enrolled, who had COVID-19 or were vaccinated during the period. Inclusion of family disease history was justified by the possibility of expanding the age range (16–88 years) when assessing IgG levels and dynamic changes in people who had recovered from or were vaccinated against the disease, and by identification of possible disease features in cohabiting family members. Inclusion criteria: well-documented case of COVID-19 or vaccination/revaccination (discharge summary, vaccination certificate). Exclusion criteria: incomplete information about the patient, error in labeling or inadequate appearance of serum samples (hemolysis, drying, microbial germination).

The subjects (a total of 155 people) were divided into three groups (Table 1): control group H that included healthy donors (70 people; serum samples were collected before the pandemic in order to perform another study [11]); group D that included convalescents (COVID-19 survivors, who had not been vaccinated before the disease onset); group V that included vaccinated people (who had no COVID-19 before vaccination and were vaccinated with two doses of Sputnik V).

In group D, 24 out of 42 disease survivors (57.1%) were fully vaccinated with Sputnik V 6–22 months (13.5 on average) after the disease onset; among them 3 individuals were later vaccinated with Sputnik Light (subgroup D + V).

In group V, 14 out of 43 people (32.6%) were revaccinated (RV). They received a booster dose of one of the vaccines (6–9 months after receiving the first dose of Sputnik V): two doses of Sputnik V (8 people) or CoviVac (2 people), and a single dose of Sputnik Light (4 people) (subgroup V + RV).

All the listed above vaccines, Sputnik V, Sputnik Light (N. F. Gamaleya National Research Center for Epidemiology and Microbiology; Russia), CoviVac (Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products; Russia), were registered and approved in the Russian Federation. Vaccination was performed by healthcare professionals in accordance with the manufacturer's instructions.

There were no significant differences between the groups in gender (there were 71 males and 84 females) or age (the average age was 52 years, the age range was 16–88 years).

A total of 1–12 serum samples were obtained from each subject, including before the disease onset (or vaccination) and on various dates after the disease onset (or administration of the first vaccine dose); a total of 312 serum samples were collected (Table 1). Serum aliquots were stored at –20 °C prior to analysis.

Based on medical records (taking into account the length of hospital stay and the extent of lung damage based on the computed tomography results), moderate to severe COVID-19 was diagnosed in 11 out of 42 infected people (26.2%) in the group D; the others had a mild disease.

Assessment of IgG against SARS-CoV-2

In 99 out of 312 serum samples (31.7%), IgG levels were measured by chemiluminescence immunoassay with the ARCHITECT i1000sr immunoassay analyzer (Abbott Laboratories; USA) using the SARS-CoV-2 IgG II Quant Reagent Kit (Abbott Ireland Diagnostic Division; Ireland). Analysis was performed by INVITRO (Moscow).

In 312 serum samples (100%), IgG levels were measured using the experimental immunochip-based test system (State Research Institute of Biological Instrumentation), based on the domestic patented PHOSPHAN phosphorescence analysis technology for identification of the infectious and somatic disease markers [11–13]. Immunoassay was performed in the wells of standard polystyrene microplates by the method similar to the enzyme-linked immunosorbent assay (ELISA). In contrast to ELISA, eight microzones (0.5 mm in diameter each) were printed on the bottom of each well, four microzones per each of two antigens: recombinant SARS-CoV-2 spike protein, RBD, Wuhan variant (catalog number ATMP02479COV; AtaGenix, China), and recombinant SARS-CoV-2 spike protein, RBD (L452R, T478K), variant B.1.617.2, Delta (code YP-009724390.1, catalog number ATMP02527COV; AtaGenix, China).

Serum samples diluted 1 : 100 were injected into the wells of the microplate 50 μL per well. After the 1.5-hour incubation, 50 μL of biotinylated (100 ng/50 μL, 1-hour incubation) monoclonal antibody against human IgG (SORBENT; Russia), and 40 μL of streptavidin (Sigma-Aldrich; USA) conjugated

Table 1. General characteristics of blood sera donors

Group (number) of subjects	Code of the group	Time range of the registered disease cases or vaccination events	Number		Average age (range), years	Number of assessed serum samples
			males	females		
Healthy people (70)	H	No	30	40	50 (20–64)	70
COVID-19 survivors (42)	D	04.2020 – 10.2021	22	20	50 (16–78)*	132
Of them vaccinated with Sputnik V 6–22 months after the disease onset (24)**	D + V	02.2021 – 11.2021	12	12	49 (21–78)*	32
Vaccinated people (43)	V	12.2020 – 09.2021	19	24	55 (26–88)*	110
Of them revaccinated (14)***	V + RV	07.2021 – 01.2022	8	6	61 (38–76)*	14
Total (155)		04.2020 – 01.2022	71	84	52 (16–88)*	312

Note: * — extreme points of the age range (16, 76, 78, 88 years) are represented by relatives of the research institute staff members; ** — including 3 people revaccinated with Sputnik Light about 6 months after receiving the first dose of Sputnik V; *** — booster vaccination with Sputnik V, 2 doses (8 people), Sputnik Light (4 people) or CoviVac, 2 doses (2 people) 6–9 months after receiving the first dose of Sputnik V.

to platinum coproporphyrin (13 ng/40 µL, 30-min incubation) were added to each well. All steps were performed at room temperature with the samples shaken in a shaker. Microplate was three times washed with buffer solution between the steps, and at the final stage it was additionally three times washed with distilled water. Microplate was dried, and then fluorescence intensity was measured with the IPI-05 indicator unit (Immunoscreen, Russia; MA number RZN, January 21, 2022) by time-resolved scanning of the microplate well bottoms.

IgG levels (measured in BAU/mL) were calculated using calibration curves for each of two antigens of the immunochip. Calibration samples were certified based on the first WHO International Standard. The measurement range was 0–10000 BAU/mL. Quality control measurements involved the use of the positive control serum (obtained from the COVID-19 survivor) with the IgG level of about 500 BAU/mL, and the negative control serum (made of serum obtained from healthy donor before the pandemic; according to the commercially available immunoassay, contained no IgG against SARS-CoV-2), which were included in the assay settings. Test results were considered positive (antibody detected) when IgG levels exceeded 10.0 BAU/mL.

Statistical analysis

Statistical processing of the results was performed using the standard Microsoft Office Professional Plus Excel 2013 v. 15.0.4727.1000 (Microsoft; USA) and MedCalc v. 10 (MedCalc Software; Belgium) software using the parametric and non-parametric techniques for data analysis. The Pearson correlation method was used to assess the degree of correlation; significance of differences was estimated using the Mann–Whitney U test for the significance level of 0.05 ($p \leq 0.05$).

RESULTS

There was a high degree of correlation between IgG levels measured by commercially available immunoassay and with the use of immunochip containing SARS-CoV-2 Wuhan variant ($r = 0.928544$; N = 99) or Delta variant ($r = 0.933363$; N = 99); correlation coefficient for the results obtained for two variants of the virus was 0.978057 (N = 312). Against this background, the results of IgG identification are provided only for immunochip containing a Wuhan variant of the virus.

Distribution of the COVID-19 cases among the subjects is presented in Fig. 1. The first cases were reported in April 2020, and the maximum number of infected people was reported in May. The second wave of infection took place from September 2020 to January 2021: 27 out of 42 group D members (64.3%) were infected; of them five people were unvaccinated, and one was a COVID-19 survivor who had been vaccinated with Spitnik V 10 months after recovery.

Vaccination campaign was launched in late December 2020. By April 2021, 30 out of 43 people (69.8%) were fully

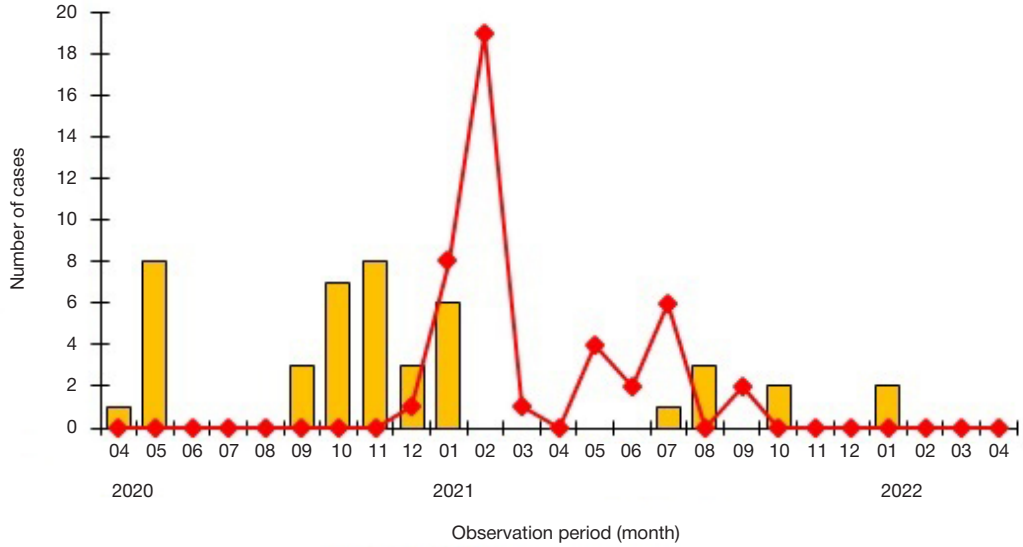

Fig. 1. Monthly distribution of people getting COVID-19 (histograms) and vaccinated with Sputnik V (curve) over the two-year observation period

Table 2. Serum anti-SARS-CoV-2 IgG levels as a function of gender and age

Group	Indicator	Number of samples	Median [95% CI] IgG levels, BAU/mL	*p*
D: COVID-19 survivors	Gender: male female	 44 39	 83,1 [56,5–106,7] 160,1 [85,9–225,7]	0,2099
	Age, years: ≥ 60 < 60	 26 57	 162,8 [95,9–241,2]* 84,4 [55,6–128,5]*	0,0268
V: vaccinated	Gender: male female	 19 33	 79,7 [35,7–143,4] 117,7 [86,6–224,5]	0,1104
	Age, years: ≥ 60 < 60	 22 30	 77,4 [25,9–99,6]* 137,0 [101,8–228,1]*	0,0191

Note: CI — Confidence Interval; * — the difference between the groups is statistically significant.

vaccinated with Spitnik V, and by October 100% of the group V members were vaccinated. Two members of this group were infected in January 2022, six months after administration of the first vaccine dose (Fig. 1).

In elderly COVID-19 survivors (aged 60+), IgG levels were significantly higher ($p = 0.0268$) than in people under the age of 60. On the contrary, significant negative correlation with age was revealed in fully vaccinated people who had no COVID-19 ($p = 0.0191$). The patients' gender had no significant effect on the antibody levels (Table 2).

The majority of infected individuals (73.8%) had a mild disease. Moderate to severe course of infection was reported in 11 people (26.2%). These cases were evenly distributed over the months of the observation period. Males were severely ill more frequently than females (eight out of 22 (36.4%) vs. three out of 20 (15%)); however, the differences were non-significant due to small sample size. The age of individuals with severe COVID-19 was 35–77 years (on average, 58 years). Among eight severely ill males, only three were elderly (70–77 years), and the others were 53 years of age or younger.

High heterogeneity of antibody levels was noted in both COVID-19 survivors (Fig. 2A) and people vaccinated with two doses of Sputnik V (Fig. 2B), especially shortly after the disease onset or administration of the first vaccine dose (Fig. 2A, B).

In vaccinated people, IgG levels gradually declined (Fig. 2C). The maximum value (median 195.3; 95% CI: 45.5–403.2 BAU/mL) was reported on day 37–55 after administration of the first vaccine dose. By month 3–4, antibody levels declined by half (median 108.7; 95% CI: 75.1–147.2 BAU/mL), and by month 7–9 these decreased by four times (median 48.7; 95% CI: 29.8–145.7 BAU/mL). The same trend of the IgG decrease was observed in convalescents (Fig. 2C). The exception were antibody levels observed by month 3–4 after the disease onset.

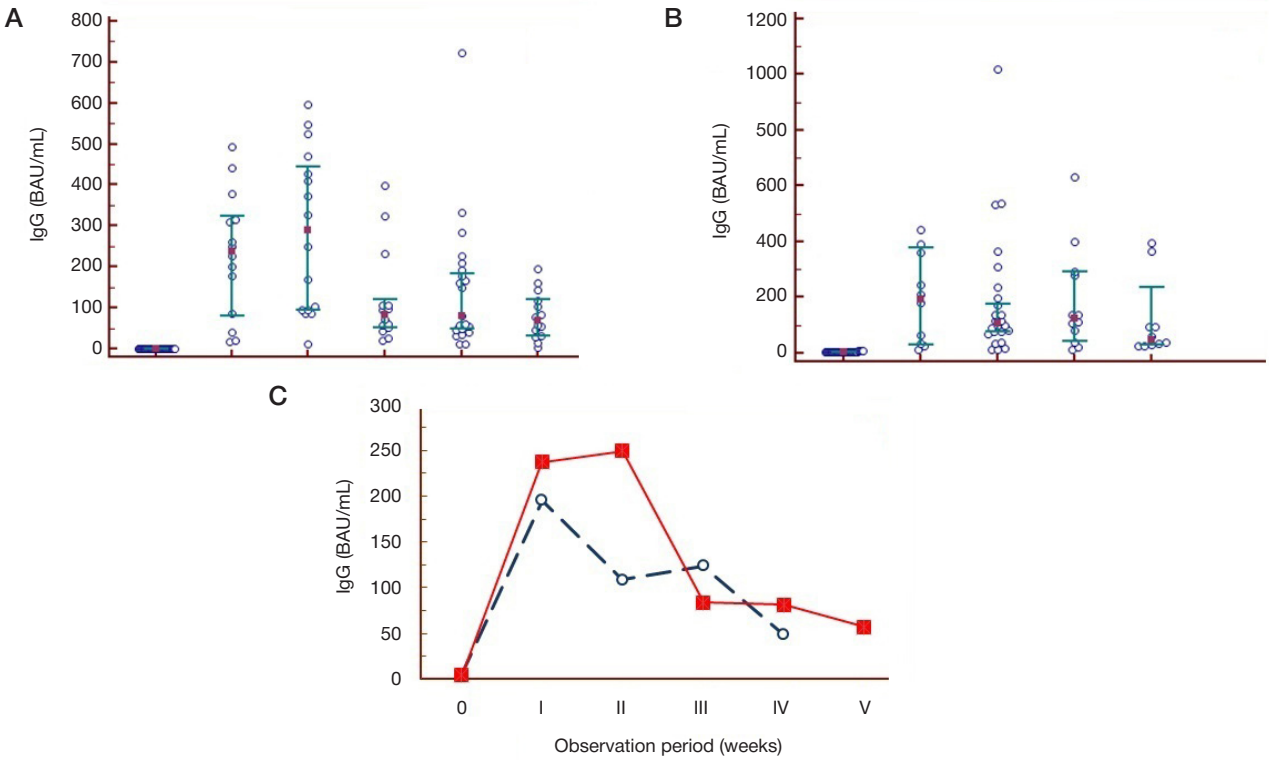

Fig. 2. A. Distribution of the serum levels of antibodies against SARS-CoV-2 in COVID-19 survivors. 0 — before the disease onset ($n = 16$); I — 2–8 ($n = 14$); II — 9–16 ($n = 17$); III — 17–24 ($n = 14$); IV — 25–36 ($n = 22$); V — 37–68 ($n = 15$). **B**. Distribution of the serum levels of antibodies against SARS-CoV-2 in people vaccinated with Sputnik V. 0 — before vaccination ($n = 38$); I — 6–8 ($n = 10$); II — 9–16 ($n = 24$); III — 17–24 ($n = 12$); IV — 25–36 ($n = 12$). Median values with 95% CI are provided for **A** and **B** (*red dot*). **C**. Median IgG levels for the observation period. 0 — before the disease onset or vaccination; I — 2–8 (survivors) or 6–8 (vaccinated individuals); II — 9–16; III — 17–24; IV — 25–36; V — 37–68. Median IgG levels are provided for the disease survivors (*red line*) and vaccinated people (*blue dotted line*)

These values (median 249.8; 95% CI: 94.9–427.5 BAU/mL) were significantly higher ($p = 0.029$) compared to those of vaccinated people observed within the same period after receiving the first vaccine dose. After 5–9 months, antibody levels stabilized at less than 100 BAU/mL (Fig. 2C) in about 60% of the subjects.

In general, antibody levels measured in the group D convalescents (median 97.1; 95% CI: 80–162 BAU/mL) and group V vaccinated individuals (median 103.1; 95% CI: 78–139 BAU/mL) were comparable; these values were significantly higher ($p < 0.0001$) than that of healthy donors (median 4.3; 95% CI: 4.1–4.5 BAU/mL). Vaccination of the disease survivors (subgroup D + V) or administration of booster dose to vaccinated people (subgroup V + RV) resulted in the significantly ($p < 0.0001$) increased immunity: up to median values of 1023 BAU/mL (95% CI: 657–1191) and 413 BAU/mL (95% CI: 213–545 BAU/mL), respectively (Fig. 3).

Three new COVID-19 cases confirmed by positive PCR test results were revealed during the whole observation period (Fig. 1, Table 3). Three women aged 40–43 were infected. Among them two women had no history of COVID-19, and one woman had COVID-19 about 16 months before the new infection. All of them were infected after full vaccination with Sputnik V, about six months after receiving the first vaccine dose; mild course of the disease was observed in all the women.

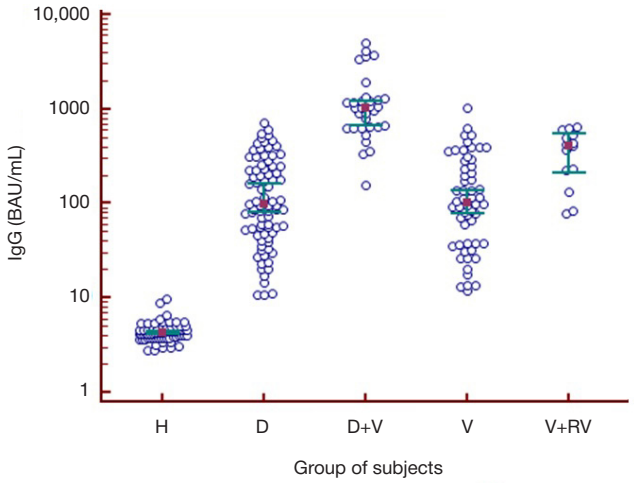

Fig. 3. Distribution of the serum levels of antibodies against SARS-CoV-2 in groups H ($n = 70$), D ($n = 84$), D + V ($n = 32$), V ($n = 58$), and V + RV ($n = 14$). Median values with 95% CI are provided (*red dot*)

DISCUSSION

Significant effects of age on IgG levels were found (Table 2). In elderly convalescents (60+), antibody levels were significantly higher than in people under the age of 60. On the contrary, negative correlation with age was observed in fully vaccinated people who had no history of the disease. Similar patterns were revealed in other studies [6, 7].

No significant effects of gender on the antibody levels in COVID-19 patients could be determined due to small sample size. However, it is obvious that endocrine profile associated with biological differences between men and women can affect the immune response. According to our data, antibody levels in men are almost twice lower compared to those in women (Table 2). Furthermore, men have moderate to severe COVID-19 twice as frequently as women.

These observations are consistent with the results of assessing representative patient samples [6, 14, 15]. According to some reports, men have lower levels of CD4+ T cells and CD19+ B cells, which play a vital part in humoral and cellular immunity providing protection against COVID-19 [6]. This could result in delayed formation of protective antibodies against the coronavirus S1 protein receptor-binding domain. In women, IgG levels dramatically increased and reached peak values during the fourth week after the emergence of clinical symptoms, and in men antibody levels increased gradually and reached peak values during the seventh week [6, 14]. The fact of delayed antibody formation in combination with the clinical and biochemical data set made it possible to consider the patient's male gender a risk factor of more severe COVID-19 and death [6, 9].

We have failed to trace the influence of the disease severity on the protective immunity levels and dynamic changes, despite the availability of evidence of such relationship [7]. Unfortunately, we had only later samples collected mainly not earlier than 7–12 months after the disease onset, because staff members having a history of moderate to severe COVID-19 refused to provide samples shortly after recovery.

Analysis of family history of the disease in three research institute staff members revealed no patterns in the COVID-19 course in their cohabiting relatives (six people), who were infected with an interval of 2–3 days. Two staff members (woman aged 63 and man aged 58) had a mild disease; among their relatives (three people aged 26–78 and two people aged 16 and 42, respectively) only one person (aged 78) was admitted to hospital due to moderate disease. The third female employee (aged 62) was critically ill, however, her husband (aged 65) had a mild disease.

The intensity of humoral immunity was dependent on the time passed since the disease onset or administration of the first vaccine dose. IgG levels in vaccinated people decreased rapidly by month 3–4 of follow-up (Fig. 2B), which was in line with the literature data [8]. Antibody levels observed in convalescents during month 3–4 of follow-up were significantly

Table 3. Characteristics of new COVID-19 cases in staff members having a history of the disease and vaccinated individuals

Name (group)	Gender	Age	Date of previous COVID-19 infection	Vaccination date, vaccine type (dose)	IgG levels, BAU/mL	Date of new COVID-19 infection
RAA (V)	F	43	No	Sputnik V: 02.05.2021 (1) 23.05.2021 (2)	360,5*	15.10.2021
ZhOA (D + V)	F	40	15.10.2020	Sputnik V: 18.07.2021 (1) 08.08.2021 (2)	617,2**	31.01.2022
ZYuN (V)	F	43	No	Sputnik V: 23.07.2021 (1) 07.08.2021 (2)	37,2***	26.01.2022

Note: * — IgG level on day 42 after administration of the first vaccine dose; ** — IgG level on day 90 after administration of the first vaccine dose; *** — IgG level at the time of the disease onset.

higher (p = 0.029) than those observed in vaccinated people (Fig. 2B). This was due to the contribution of sera with high antibody levels obtained from elderly patients of both genders, especially males, who's antibody levels reached maximum values during this period (no data reported). The findings support the conclusion that there is a positive correlation between age and IgG levels in COVID-19 survivors (Table 2) and are probably an indirect evidence of the delayed protective immunity formation in men, as noted earlier [6].

In general, antibody levels decreased by month 5–9 and stabilized at less than 100 BAU/mL (Fig. 2B) in about 60% of surveyed patients, as noted by other researchers [10]. At the same time, significant individual differences in the dynamics of humoral immune response between the study participants were revealed given that at least two serum samples had been collected from each subject. In vaccinated people, IgG levels gradually decreased, as previously reported for the integral indicator of this group (Fig. 2B, 2C), however, in some COVID-19 survivors the levels of protective immunity remained unchanged until the end of the follow-up period (no data reported).

Only three new cases of infection after full vaccination with Sputnik V were revealed over the entire observation period (Fig. 1, Table 3). The subjects were infected and had a mild disease in October 2021 and January 2022, i.e. during the period when the highly contagious Delta and Omicron coronavirus variants predominated in Moscow, and the daily cases increase reached 9,000 and 26,000, respectively [16, 17]. Taking into account the total number (Table 1) of vaccinated people with no history of the disease (43 people) and vaccinated disease survivors (24 people), the prevalence of COVID-19 among vaccinated people was 4.5%. These findings were in line with the available data supporting the fact that Sputnik V vaccine never provided 100% protection [18, 19], but contributed to the milder COVID-19 course in vaccinated people [20].

It should be noted that in one infected female patient out of three, IgG level was low at the time of the disease onset (37.2 BAU/mL). In the other two female patients, antibody levels were 360.5 and 617.2 BAU/mL on days 42 and 90 after receiving the first vaccine dose, respectively (i.e. 3–4.5 months before the disease onset) (Table 3); however, these levels could be reduced in half or more at the time of the new infection, given the dynamics of IgG levels in vaccinated people (Fig. 2B). Although until now there is no clear understanding of what antibody levels are capable of providing sufficient protection against the same and especially against new variants of the SARS-CoV-2 coronavirus [4], the presence of antibodies is clearly not the only factor of protection against COVID-19 [9]. No new cases of infection in 95.5% of the surveyed people, the majority of them having the circulating antibody levels below 100 BAU/mL (Fig. 2C), support the view that the long-term protective immunity is largely ensured by complex interactions between the humoral and cellular immunity factors [5].

An important feature of the study is the use of immunochip allowing us to simultaneously assess the levels of antibodies against the receptor-binding domains of two SARS-CoV-2 variants (Wuhan and Delta). The data for the first variant are provided, since antibody levels measured for both variants are almost identical (r = 0.978057). This suggests that immune response in COVID-19 survivors and people vaccinated with Sputnik V provides effective protection against both variants, as noted earlier [21]. However, such overlapping results were obtained only for sera collected from patients, who had been infected from April 2020 to January 2021, i.e. during the first two waves of infection among staff members (Fig. 1), when the majority of COVID-19 cases were caused by Wuhan (reference) variant and local Russian variants of the virus [22, 23]. People infected in July–October 2021 (the third wave of infection presented in Fig. 1) had at least twice as high levels of antibodies against the Delta variant, that prevailed in the population during this period [17, 23].

The increase in the analysis multiplexity, and, as a consequence, in informativeness is a global trend related to the possibility of combining several tests in a common format by means of miniaturization and development of high-density microarrays. Advanced multiplex technologies based on chemiluminescence methods and flow cytometry (for example, test systems manufactured by Merck, Luminex, etc.) provide an opportunity for simultaneous detection of up to 100 various markers and are best suited to assess the composite immune response when studying various aspects of COVID-19 [24, 25]. However, such tests are expensive; sophisticated equipment and highly qualified operator are required.

In our opinion, more simple and cost-effective tests, such as PHOSPHAN, are more beneficial when used for monitoring. Indeed, the resulting data set (high degree of correlation with the commercially available test, and possibility of detecting specific features of the specific antibody binding to the receptor-binding domains of two variants of novel coronavirus) taking into account the fundamental possibility of constructing multiplex tests of various design depending on the research tasks (for example, the use of a wider range of diagnostically significant antigens, internal positive and negative controls in immunochip) supports the assumption that PHOSPHAN technology platform may be beneficial when used to monitor the levels of circulating antibodies in COVID-19 survivors and vaccinated people. The immunochip developed can make it possible not only to detect specific antibodies, but also to distinguish between variants of the virus based on the significant differences in antibody titers, at least in people with no history of COVID-19 and unvaccinated patients. The detailed analysis of this situation is beyond the current scope of this study and will be reported later.

CONCLUSIONS

1. The intensity and duration of immune response in COVID-19 survivors and vaccinated people varied significantly depending on age, observation period, and additional vaccinations/revaccinations. 2. IgG levels were significantly higher in the following groups: elderly people (60+) having a history of COVID-19 compared to individuals under the age of 60; COVID-19 survivors and individuals vaccinated with Sputnik V compared to people with no disease history and unvaccinated individuals; disease survivors after vaccination and vaccinated individuals after receiving the booster dose. 3. IgG levels were significantly lower in vaccinated elderly people (60+) compared to individuals under the age of 60. 4. IgG levels in vaccinated individuals decreased faster (within 3–4 months), than in COVID-19 survivors; after 5–9 months IgG levels were stabilized at less than 100 BAU/mL in about 60% of subjects. 5. The prevalence of COVID-19 among vaccinated people was 4.5% (three vaccinated people out of 67 were infected). 6. Multiplex immunochip analysis is a promising method for simultaneous quantification of antibodies against two or even more variants of the novel SARS-CoV-2 coronavirus.

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