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**DYNAMICS OF COVID-19 SPECIFIC G-CLASS
IMMUNOGLOBULINS LEVELS IN PATIENTS AFTER
THE RECOVERY**

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Introduction. *This article is devoted to determining the level of protective antibodies of the patients at the early and further stages after being suffered from COVID-19.*
Materials and methods. *On the first stage of the research 66 patients of different age and gender were examined. All patients, with no exception, had positive PCR results on COVID-19. All patients were relatively divided into two age groups: from 18 to 39 (n = 39) and from 40 to 65 (n = 27). The light form of disease was detected in 40 patients, the moderate form – in 15 and the severe one – in other 3 patients. 8 people were ill without symptoms. There were 11 patients hospitalized at the department of infectious diseases of the hospital. Afterwards the level of the antibodies was controlled on the 60th, 90th and 150th day after the onset. All patients were tested for antibodies to COVID-19 by enzyme-linked immunosorbent assay (ELISA) performed on the equipment: enzyme-linked immunosorbent assay Tecan (Austria); PW 40 Microplate Washer (France). ELISA set for serology COVID-19 test system IgG SARS-CoV-2 VitroTest (Ukraine).*
Results. *Analyzing the data obtained via laboratory research, it can be seen that the immune response to COVID-19 was formed in 82 % of the examined patients. The maximum level of antibodies, which remains as such up to 40th day.*
Results. *IgG increases from 12 to 40 days and then has not changed significantly for 60; 90 and 150 days. Only 4 patients had a noticeable decrease on day 150 (however, they still had a positive level).*
Conclusion. *IgG levels in patients with Sars-Cov2 remain high for at least 150 days. The formation of antibodies is not affected by the age difference, neither depends on the severity of the disease.*

Key words: COVID-19; immunoglobulin G-class, SARS-CoV-2; antibody; humoral immunity.

Introduction. The coronavirus-induced epidemic has caused an unprecedented resonance in the world. Human sacrifice, tens or hundreds of thousands of patients in almost every country in the world, combined with the wild panic in population – is the result of a new strain of the virus. Thanks to numerous studies and observations, it has become clear that the most important answers relate to immunological aspects. One such question is: whether humoral immunity is formed after COVID-19 is transferred and for how long, this question is extremely important for understanding future forecasts. This problem is already being considered by many scientists from around the world. There are similar works in which research data are published. In the United States, a mass survey of 279 emergency workers for antibodies to SARS-CoV-2 was introduced in Salt Lake City, Utah. According to the results of the survey, about 5.6 % of seropositive individuals were recorded, which is estimated as a fairly large number, considering the absence of symptoms of respiratory disease [1]. The Journal of Infectious Diseases (JID) published a retrospective research of medical records of 112 patients diagnosed with COVID-19 who were admitted to Renmin Hospital at Wuhan University between February 1 and February 29, 2020. According to the published results, IgM and IgG were detected in 51.79 %, only IgG was detected in 41.07 % of the subjects, and 6.25 % had no antibodies. An-

tibodies to IgG were produced 10 days after infection and lasted a long time [3]. A research published in “Clinical Infectious Diseases” on the 2d of May 2020 also illustrates similar data – the average level of antibodies increased during hospitalization and after discharge for two weeks. Antibodies to SARS-CoV-2 have also been detected in asymptomatic patients [5]. A large-scale research published in “Clinical & Translational Immunology” on the 6 of May 2020 states that immunoglobulins G-class (IgG) was detected after 1 week and were kept at a high level for a long period [8].

The launch of a targeted research sets a goal to answer the question: when class G antibodies are formed after COVID-19 transmission and to determine their level at an early and long term. The research also sets a goal to determine whether there is a relationship between the quantitative level of antibodies and the severity of the disease, age, sex and recovery time.

Materials and methods. For the first stage of the study, 66 patients of different ages and genders were examined. Some patients were treated in Kyiv in KMKL № 4 in the infectious department, the other part had an ambulatory treatment. Some patients who suffered from the disease in a very mild form did not see a doctor, but were tested as contact persons. All patients, without exception, had a positive PCR result for COVID-19. The result was issued by the Central Laboratory of the Ministry of Health of Ukraine. A certified private laboratory in Kyiv, the DNA Laboratory, which has all the permits and equipment to operate at this level, was involved in the laboratory test. All patients were tested for antibodies to COVID-19 by enzyme-linked immunosorbent assay (ELISA) performed on the equipment: enzyme-linked immunosorbent assay Tecan (Austria); PW 40 Microplate Washer (France). ELISA set for serology COVID-19 test system IgG SARS-CoV-2 Vitro Test (Ukraine). The results were calculated in international units (IU).

All patients can be divided into two age groups – from 18 to 39 ($n = 39$) and the second from 40 to 65 ($n = 27$). Among the examined patients, 8 people were asymptomatic. Mild course of the disease was observed in 40 people, moderate – in 15 people, severe – 3 people. 11 patients were hospitalized in an infectious hospital. The second stage of the study was to determine the level of antibodies at a longer time, such as 60–80 days and 130–150 days. The control group consisted of 41 individuals.

Results. According to the results of enzyme-linked immunosorbent assay, the presence of class G antibodies to COVID-19 was detected in 54/66 patients (82 %). 12 individuals (21 %) did not develop antibodies.

The quantitative level of antibodies in the subjects ranged from 4.32 to 10.17 (IU). In eight patients the level of antibodies is below the threshold level, in four more patients the result is doubtful.

Subsequent follow-up of a control group of 40 patients found that over the next 30 days after the first antibody control (day 60), antibody levels increased predominantly, except in a single case (patient X., fig. 1).

On the 90 day, all subjects had positive antibody levels that exceeded cutoff. In 4 patients out of 40 subjects had a drop in antibodies from 1.5 to 2.8 times (fig. 2). On the 150th day, all patients had positive antibody levels, but two had more than a 2.5-times drop and their levels were close to the positive limit. 5 patients had a decrease in antibody levels in the range of 2–1.5 times. The rate of decline of antibody levels was quite individual (fig. 2).

It must be mentioned that within 150 days after the disease there was no change of seroconversion from the positive to the negative level.

All patients were divided into two age groups. We find that young patients in contrast to older one have moderate or low levels of IgG anti COVID-19 (fig. 3).

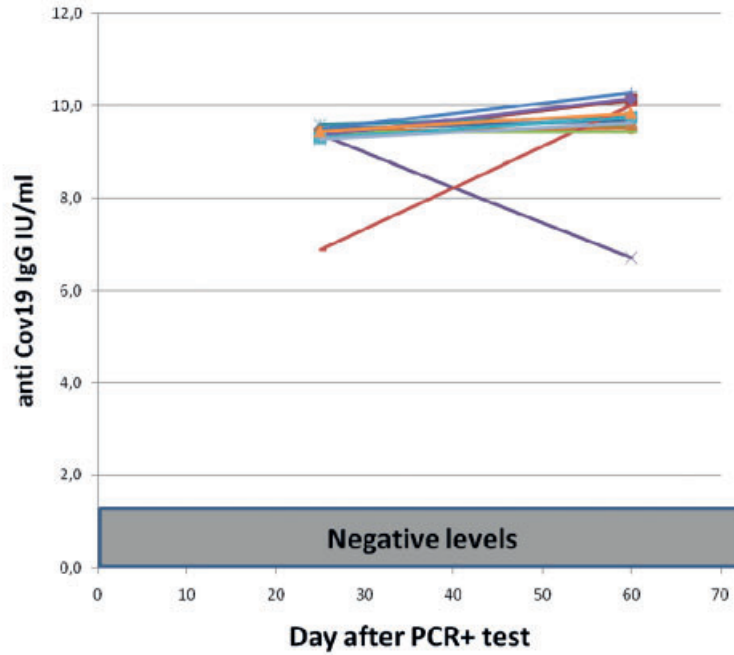


Fig. 1. Levels of IgG anti COVID-19 on 20th and 60th days after PCR+ test

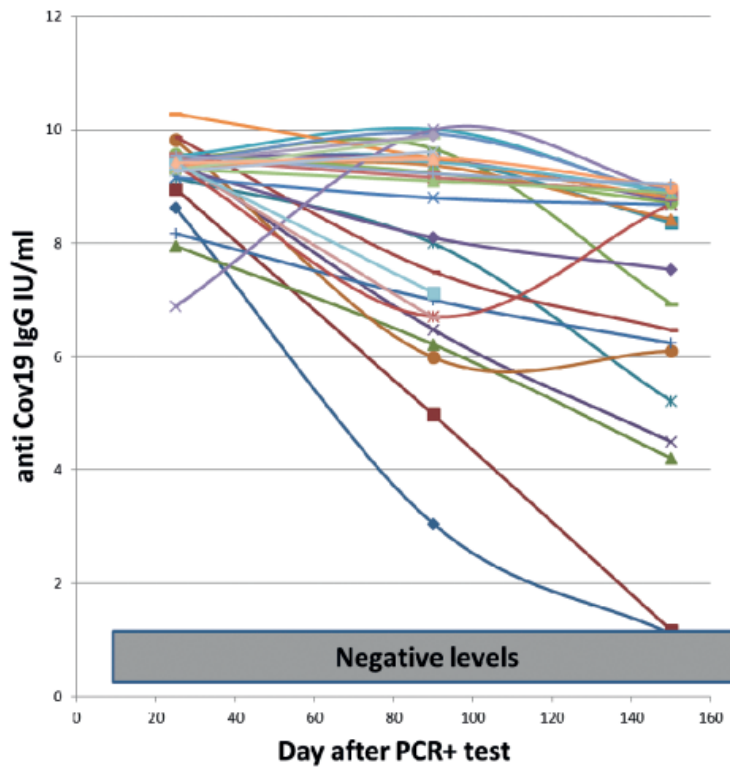


Fig. 2. Levels of IgG anti COVID-19 on 20th, 60th, 90th and 150th days after PCR+ test

We also not fined any associations between IgG levels and severity of the disease. Both mild and severe forms of diseases produce similar anti-COVID-19 antibody levels.

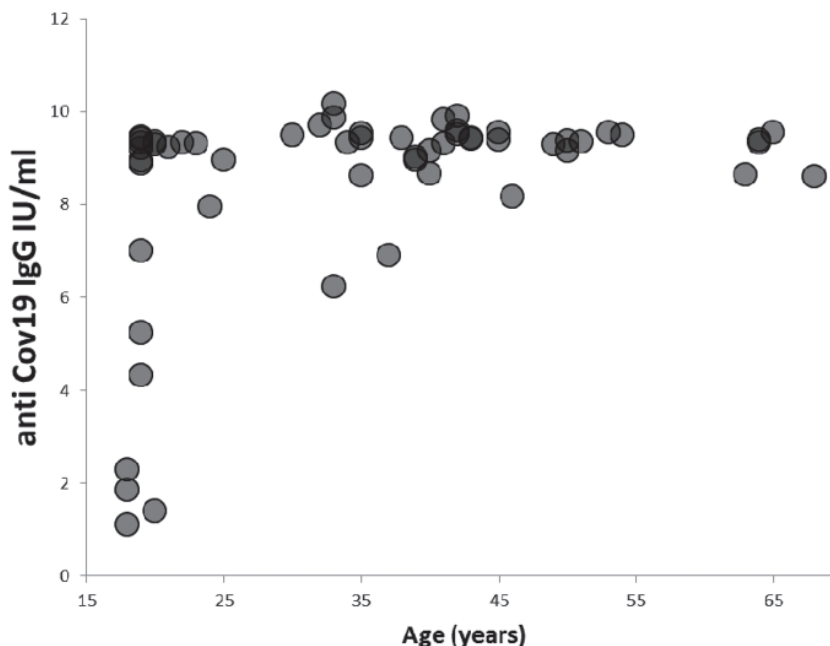


Fig. 3. Association of patients age with anti-COVID-19 IgG levels on 20 day after PCR+ test

Discussion. Unequivocally, most people develop a specific humoral immunity after suffering from COVID-19 disease. Already on the 12th day, a fairly high level of IgG is diagnosed, which remains so for a long time. Such data do not contradict the research conducted in China [1, 3, 5, 8]. On average, the maximum level of antibodies was recorded at 16–20 days from the begin of the disease.

It should be noted that the results illustrate: both mild and severe, equally characterized by the appearance of high levels of antibodies. These results coincide with the results of a large-scale survey in February 2020 from Tongji Hospital, Wuhan, China. Severe and critical cases had higher levels of IgM than mild, while IgG levels in critical cases were lower than in mild and severe cases. This may be due to high disease activity and / or impaired immune response in critical cases [8].

A group of subjects (10 people) who did not develop immunity, suffered from the disease in mild or erased form.

However, there are patients (two people) who had a fever for a month, but humoral immunity was not formed. Although similar cases have been reported by other researchers [4], this suggests some assumptions. First, the possibility of false-positive PCR determinations should not be ruled out. Second, patients may have certain genetic features of the antibody response to the virus. The spectrum of the humoral response may not include antibodies to a particular peptide to which the test system is sharpened. But the main role is still given to the natural resistance and a strong level of barrier function of the mucous membranes. Innate immunity can still be strong enough to eliminate SARS-CoV-2 [4]. These issues remain debatable and require further research and evidence.

Analyzing the obtained data, it can be seen that 79 % of patients developed an immune response to COVID-19. Among those examined by ELISA, specific antibodies to COVID-19 are reliably detected at an early stage. There is an assumption, illustrated by the obtained data, that the level of formed antibodies is not affected by the age. We can also see that the level of the formed antibodies does not depend on the severity and course of the disease. Antibodies have a fairly high level on the 12th day after the onset. From 16–18 days it is already possible to diagnose the

maximum level of antibodies, which remains as such up to the 90th day. On the 150th day, there is a noticeable tendency to a gradual decrease in antibody levels, but even on the 5th month, this decrease is still insignificant. The presence of comorbidities such as hypertension and diabetes did not affect the formation of immunity. The obtained data do not contradict the research in China and USA, viewing the fact that the results are similar [1–7].

Low levels of antibodies in young people are probably caused by high natural resistance to respiratory diseases. It is also possible to participate in the formation of immune defense by powerful T cells, which does not lead to the full formation of a humoral response. It should be taken, into account that all those who did not give a confident humoral immunity were ill in a mild form.

The issue about those patients who have not developed immune response (18 %) remains open. Additional examinations and separate monitoring for this group of patients are required.

Gratitude. We thank the administration, doctors and nurses of Kyiv Clinical Hospital №4 for their help and care when working with the patients. We also thank the DNA Laboratory for its cooperation and organization of the serological examination, which made possible to do this research.

Conflict of interests. There is no conflict of interests to disclose for each author.

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ДИНАМІКА РІВНЯ СПЕЦИФІЧНИХ ІМУНОГЛОБУЛІНІВ КЛАСУ G ДО COVID-19 У ПАЦІЄНТІВ ПІСЛЯ ОДУЖАННЯ

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Вступ. Робота присвячена визначенню рівня захисних антитіл, що перехворіли на COVID-19, на ранніх та більш віддалених строках. **Матеріали та методи.** Для першого етапу дослідження було обстежено 66 пацієнта різного віку та статі. Всі пацієнти мали обов'язково позитивний результат на COVID-19 методом ПЛР. Частина пацієнтів знаходилась на лікуванні в інфекційному відділенні КМКЛ № 4, інші лікувались амбулаторно. Всіх пацієнтів за віком умовно розділили на дві групи: I (39 осіб) – від 18 до 39 років; II (27

осіб) – від 40 до 65. Серед обстежених 8 осіб хворіли безсимптомно. Легкий перебіг відмічався у 40 осіб, середньоважкий – у 15 осіб, важкий – у 3 осіб. Госпіталізовані в інфекційний стаціонар 11 пацієнтів. Надалі контроль рівня антитіл було проведено на 60-ту, 90-ту та 150-ту добу від початку захворювання. Всім пацієнтам було проведено дослідження на антитіла на COVID-19 методом імуноферментного аналізу (ІФА), виконаному на обладнанні: імуноферментний аналізатор Tecan (Австрія); PW 40 Microplate Washer (Франція); набір ELISA для серології COVID-19 тест системами на IgG SARS-CoV-2 VitroTest (Україна). **Результати.** Аналізуючи отримані дані, можна побачити, що у 82 % обстежених сформувалась імунна відповідь на COVID-19. IgG збільшувався з 12 до 40-ту добу, а потім суттєво не змінювався на 60-ту, 90-ту та 150-ту добу. У 4 пацієнтів спостерігалось помітне зменшення на 150-ту добу (однак вони все ще мали позитивний рівень). **Висновок.** Рівень IgG у пацієнтів, що перехворіли на Sars-Cov2, залишається високим щонайменше 150 днів. Ми помітили, що на формування гуморальної відповіді вік та тяжкість перебігу захворювання не впливає.

Ключові слова: COVID-19; імуноглобулін G; SARS-CoV-2; антитіла; гуморальний імунітет.

ДИНАМИКА УРОВНЯ СПЕЦИФИЧЕСКИХ ИММУНОГЛОБУЛИНОВ КЛАССА G К COVID-19 У ПАЦИЕНТОВ ПОСЛЕ ВЫЗДОРОВЛЕНИЯ

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Введение. Данная работа посвящена определению уровня защитных антител на ранних сроках у пациентов, переболевших COVID-19. **Материалы и методы.** В первом этапе исследования было обследовано 66 пациентов разного возраста и пола. Все пациенты имели положительный результат на COVID-19 методом ПЦР. Всех пациентов по возрасту условно разделили на две группы: I ($n = 39$) – от 18 до 39 лет, II ($n = 27$) – от 40 до 65 лет. Среди обследованных 8 человек болели бессимптомно. Лёгкая форма заболевания отмечалась у 40 человек, средней тяжести – у 13, тяжёлая – у 3 человека. Госпитализированы в инфекционный стационар 11 пациентов. Последующий контроль уровня антител был проведён на 60; 90 и 150-й день после начала заболевания. Всем пациентам проведено исследование на антитела на COVID-19 методом иммуноферментного анализа (ИФА), выполненном на оборудовании: иммуноферментный анализатор Tecan (Австрия) PW 40 Microplate Washer (Франция); набор ELISA для серологии COVID-19 тест системами на IgG SARS-CoV-2 VitroTest (Украина). **Результаты.** Анализируя полученные данные, выявлено, что у 82 % обследованных сформировался иммунный ответ на COVID-19. Титр IgG нарастал с 12- до 40-го дня, а затем существенно не менялся на 60-й, 90-й и 150-й день. Только у 4 пациентов наблюдалось заметное его уменьшение на 150-й день (однако они всё ещё имели положительный уровень). **Вывод.** Уровень IgG у пациентов, переболевших Sars-Cov2, остаётся высоким не менее 150 дней. На формирование гуморального ответа возраст и тяжесть течения заболевания не влияет.

Ключевые слова: COVID-19; иммуноглобулин G; SARS-CoV-2; антитела; гуморальный иммунитет.