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THE DURATION AND SPECIFICITY OF THE HUMORAL IMMUNE RESPONSE TO SARS-CoV-2

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***Background**. Our assumption that immunity after COVID-19 will persist has been fully confirmed in the researches already conducted. Our work is a continuation of research that demonstrates the results obtained 12 months of determining the humoral response in patients after COVID-19. **Materials and methods**. The research involved 42 individuals. All subjects had a positive PCR test for COVID-19. At certain intervals, from 40 to 240 days, individuals in the group were tested for IgG SARS-CoV-2. The last step was to check the level of IgG to the COVID-19 nucleocapsid and spike protein in the research group for 360 days from the onset of the disease. A private certified laboratory in Kyiv, the "DNA Laboratory", was involved. Patients were tested for antibodies to COVID-19 by ELISA using serology COVID-19 test systems VitroTest (Ukraine). The immunological laboratory of the Institute of Pediatrics, Obstetrics and Gynecology was used in parallel for interlaboratory quality control. The results of the research coincided. **Results and discussion**. The level of class G immunoglobulins to nucleocapsid in the subjects has gradually decreased over 8 months. It is noteworthy that in the period from 40 to 150 days in all 42 patients (100 %) antibodies did not disappear. Decreasing of antibodies occurred between 150 and 240 days. However, the data obtained for 360 days significantly changed the picture. In a certain part of the subjects, who had low or even negative levels of antibodies for 8 months, as of 12 months, the level of immunoglobulin (Ig) class G again rose above the threshold value. Thus, we see that from the group of 42 people 92.8 % have positive antibodies to the nucleocapsid, and 7.2 %. **Conclusions**. The data obtained illustrate that in the study group within 12 months after SARS-CoV-2, the vast majority of individuals remain with specific antibodies to the nucleocapsid and spike-protein.*

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

Introduction. The issues of immune reactivity after suffering SARS-CoV-2 are among the most urgent at this moment. The strategy of preventing the spread of the virus in the population and the protection of the population is a priority for each country. Our assumption that immunity after a coronavirus infection will persist for a long time has been fully confirmed in the studies already conducted [1, 4, 5]. However, the question of how long immunity can exist after SARS-CoV-2 remains open.

Numerous research groups around the world are also publishing their data on the researches of the duration of immunity. Thus, at the Instituto de Medicina Molecular, Lisbon, Portugal, a group of authors quantified immunoglobulin (Ig) class M, G and A antibodies that recognize the SARS-CoV-2 (RBD) receptor-binding domain within 6 months after the onset of COVID-19 in more than 300 hospital patients and healthcare workers with COVID-19 and 198 volunteers who had COVID-19. The report claims a consistent level of circulating neutralizing antibodies in most people with confirmed SARS-CoV-2 [6].

Another published large-scale research by collaborative teams from California and New York on the diversity and duration of the immune response over 6–8 months after COVID-19 showed similar data. The research involved 188 volunteers who recovered from COVID-19, including 43 samples obtained 6–8 months after infec-

tion. Fifty-one research participants provided longitudinal blood samples that allowed for both cross-sectional and longitudinal analysis of SARS-CoV-2-specific immune memory. According to the results, IgA spike was still present in the vast majority of subjects 6–8 months after infection. The proportion of subjects positive for CD4 + T cells (92 %) also remained high 6–8 months after infection [3].

Researchers from the Key Laboratory of Immunology in Chronic Diseases and Research Laboratory of Clinical Virology National Clinical Research Center for Infectious Diseases (China) also provided their data for 6 months: 159 blood samples were collected from 52 recovered patients with COVID-19 over a period of six months after symptom onset for longitudinal serological tests. The positive proportion of IgG and IgM antibodies was 92.3 % and 90.4 % in the first month after the onset of symptoms, and the seropositivity of IgG antibodies remained high at all points of observation, while the seropositivity of IgM antibodies decreased to 22.73 % due to six months after the onset of symptoms [8].

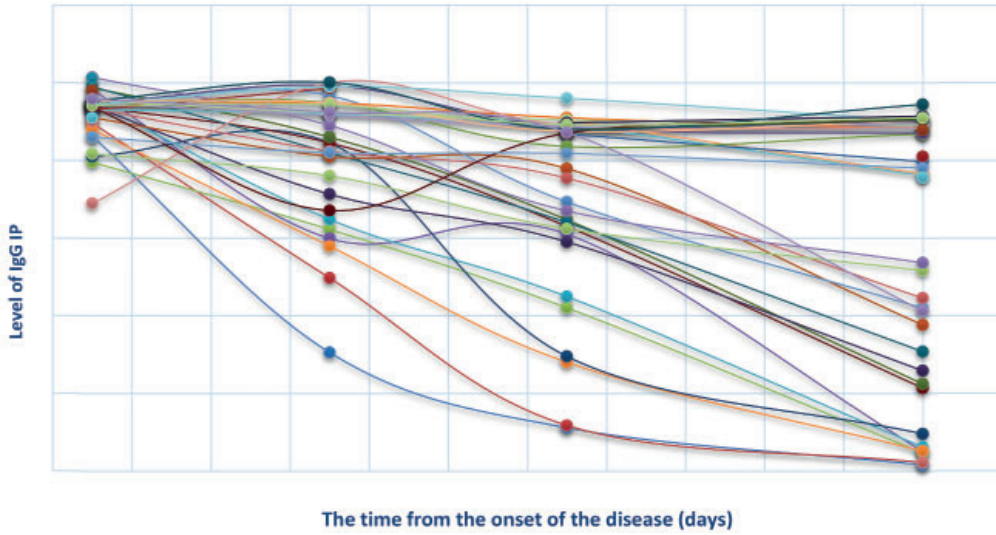
Scientists from Israel Anat Achiron and Michael Gurevich also provided a report on their studies: 9 months, the level of antibodies, although it decreased by 50 %, stabilized after 6 months and maintained a protective level for up to 9 months. Repeated paired analyses of the level of IgG antibodies to SARS-COV-2 showed that after 6 and 9 months, only 15.3 % (9 of 59) and 15.8 % (3 of 19) of subjects became seronegative to SARS-COV-2 IgG, respectively. It is noteworthy that all those seronegative showed low levels of antibodies as early as 3 months after the disease [2]. Similar data can be found in other reports [7, 9, 11].

Taking into account that very often the coronavirus disease proceeds in a latent form, the urgent task was to determine which of the diagnostic methods is the most specific and indicative for screening diagnostics of the population. According to the findings provided by a group of Chinese scientists led by Huaqing Shu from the intensive care unit of Union Hospital in Wuhan, it was shown that the sensitivity of IgG ELISA (92.5 %) was significantly higher than that of IgM (70.8 %). IgG is a sensitive indicator for retrospective diagnosis and contact tracing, while IgM remains an indicator of early infection [10]. This is confirmed by other researchers [5].

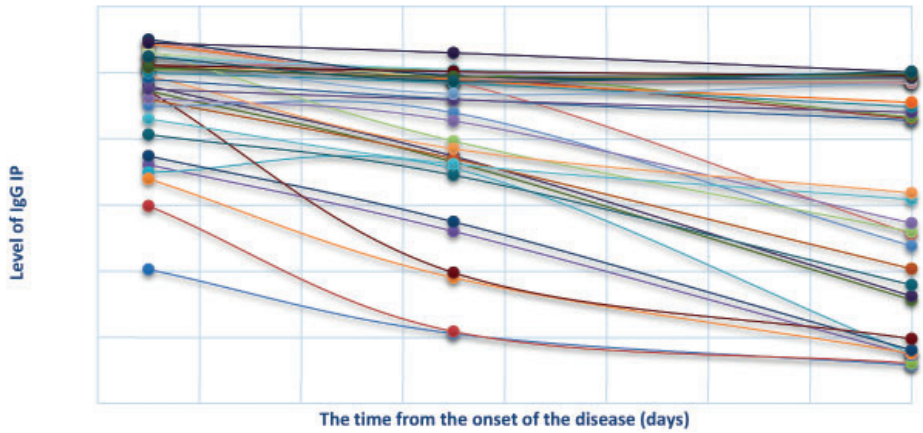
Our work is a continuation of a research that demonstrates the results obtained at 12 months of determining the humoral response in patients during the next stage of testing the immune status in groups of subjects.

Materials and methods. The research involved 42 patients who had a laboratory-confirmed COVID-19 a year ago and sometime later (18–40 days) a positive level of antibodies to COVID-19. At certain intervals, namely 60, 90, 150 and 240 days, all individuals in the group were tested for IgG SARS-CoV-2. The last step was to check the level of specific class G immunoglobulins to the nucleocapsid and spike protein in all of the research groups for 360 days from the onset of the disease. The study was approved by the Research Ethics Committee and all patients signed an informed consent to participate in the study.

Patients were divided into two groups by age. The first group consisted of 14 individuals of 18 to 39 years of age. The second group consisted of 28 individuals from 40 to 65 years. According to the severity the patients were divided into three groups – mild, moderate and severe. Some of the patients during the acute course of the disease were hospitalized in the KMKL No 4 in Kyiv in the infectious department, the other part was treated on an outpatient basis or was asymptomatic and was examined in connection with direct contact exposure to patients. Without exception, all subjects received a positive result for COVID-19 by polymerase chain reaction. A private certified laboratory in Kyiv, the DNA Laboratory, was involved in the laboratory determination of specific immunoglobulins. All patients were tested



a



IP index of positivity

b

Fig. 1. Dynamics of IgG level on SARS-CoV-2 from 40 to 240 days (*a*) and 150 to 240 days (*b*)

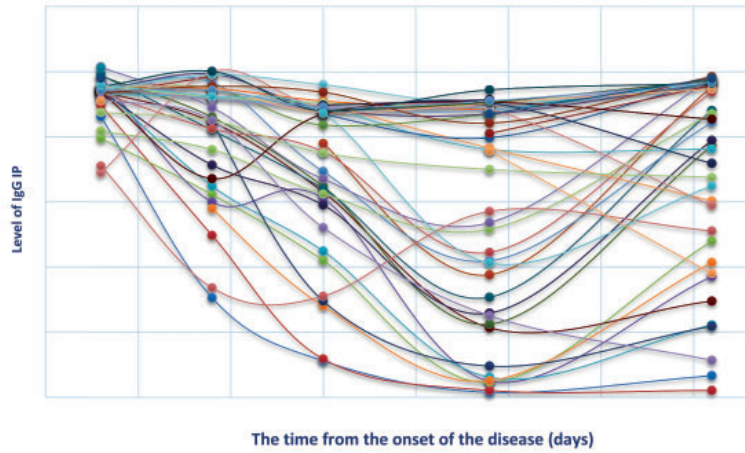


Fig. 2. Dynamics of antibody levels to SARS-CoV-2 during the year

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 systems on IgG SARS-CoV-2 VitroTest (Ukraine). Result calculated in IP (index of positivity) (0–0.9 negative, 1.1 and more as positive) according manufacture instruction. The immunological laboratory of the Institute of Pediatrics, Obstetrics and Gynecology was used in parallel for interlaboratory quality control of antibody detection in serum. The results of the research coincided.

Results. By the beginning of the last stage, the subjects gradually decreased the level of specific IgG to the COVID-19 nucleocapsid over 8 months. If we pay attention to the intermediate points, when control tests of antibody levels were performed, we can see that in the period from 40 to 150 days in all 42 patients (100 %) antibodies did not disappear (Fig. 1, *a*). Although it should be noted that in 2 patients the IgG level decreased to the threshold value. Then it was clearly recorded when exactly IgG disappeared in some patients. This occurred between 150 and 240 days (Fig. 1, *b*).

During this period, in 20 % (n = 7) of the 42 examined subjects a decrease in specific antibodies below the threshold value was recorded [4].

However, the data obtained for 360 days significantly changed the picture. In a certain part of the subjects, who had low or even negative levels of antibodies for 8 months, as of 12 months, the level of IgG again rose above the threshold value (Fig. 2).

Thus, we see that from the group of 42 people 92.8 % (n = 39) have positive antibodies to the nucleocapsid, and 7.2 % (n = 3) do not. The average value of the level of antibodies at the last control point clearly increased compared to the previous value, but did not reach the initial level (Fig. 3).

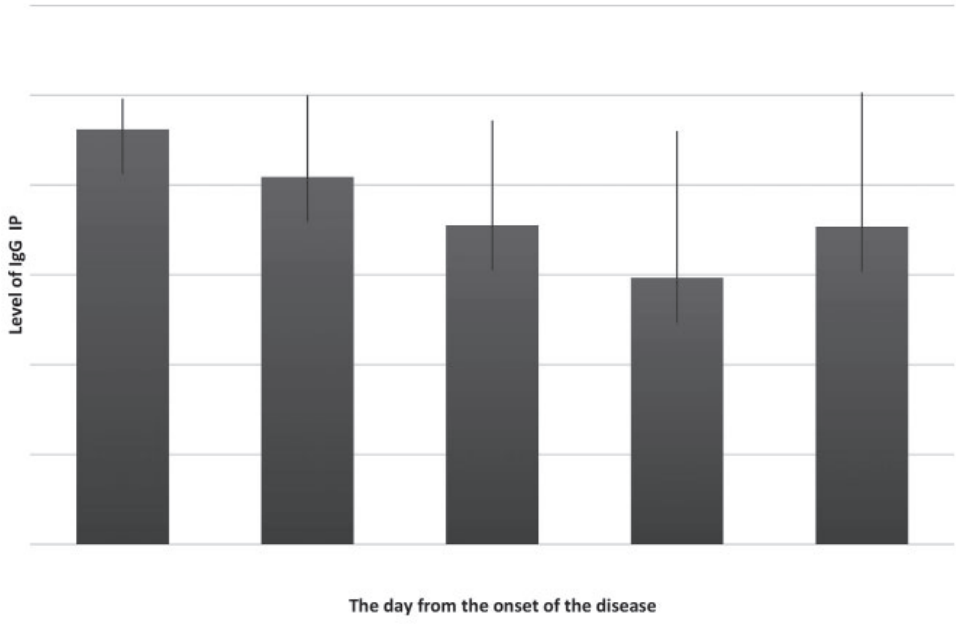


Fig. 3. The average value of antibody levels at control points during the year

When comparing the level of the obtained immunoglobulins of different types, it was possible to make a correlation analysis. The data obtained illustrate the high correlation between the level of immunoglobulins to the nucleocapsid and the spike protein (Fig. 4).

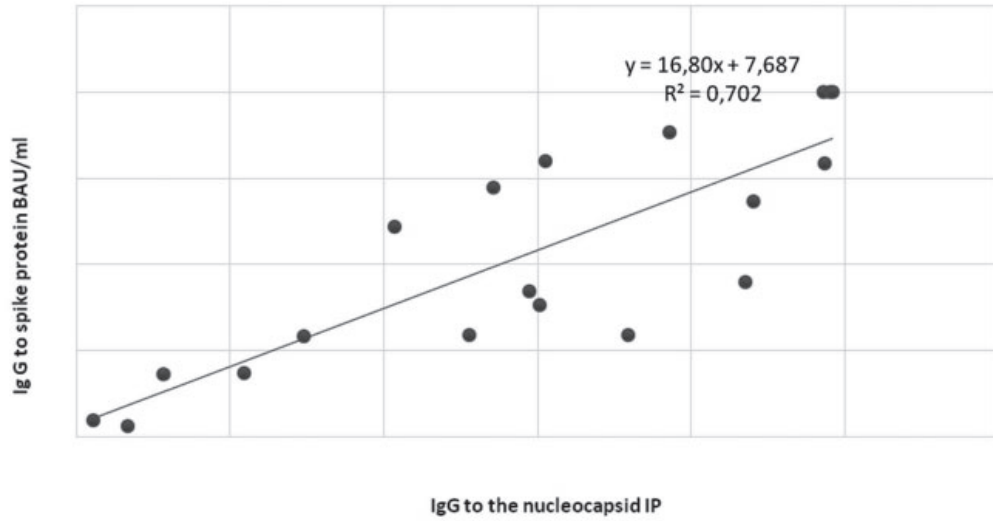


Fig. 4. Relationship between IgG to spike protein and nucleocapsid BAU/ml (binding antibody units/ml 0–25 negative, > 25 positive)

Conclusions. The obtained data illustrated that in the group of subjects for 12 months after the SARS-COV-2 in the vast majority (92.8 %) antibodies remain present in the serum. From the very beginning of the research, which lasted a whole year, a gradual decrease in the level of specific immunoglobulins was observed in a certain group of individuals. But the fact that after the 8th month many again demonstrated an increase in antibody titer is very interesting. It can be assumed that this is due to the contact of these individuals with patients with COVID-19 and the so-called boosterization. However, this cannot be fully stated with certainty, as the effect on the level of antibodies of the improving quality and sensitivity of test systems, which are constantly progressing over time, cannot be ruled out. However, the phenomenon of a prolonged humoral response to SARS-CoV-2 in the research group, in the form of class G immunoglobulins to nuclear and adhesion proteins, has been proven. The obtained results are relevant today and necessary for understanding the processes that occur during immunization of the population naturally.

Conflict of interest. The authors declare no conflict of interest.

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ТРИВАЛІСТЬ ТА СПЕЦИФІЧНІСТЬ ГУМОРАЛЬНОЇ ІМУННОЇ ВІДПОВІДІ НА SARS-COV-2

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Актуальність. Наше припущення, що імунітет після коронавірусної інфекції зберігатиметься протягом тривалого часу, було повністю підтверджено у вже проведених дослідженнях. Дана робота є продовженням дослідження, яке демонструє результати, отримані через 12 міс визначення гуморальної відповіді у пацієнтів на наступному етапі тестування імунного статусу у групі досліджуваних. **Матеріали і методи**. У дослідженні взяли участь 42 пацієнти, які перехворіли на COVID-19 рік тому. Всі без винятку пацієнти мали позитивний аналіз на COVID-19 методом ПЛР. Через певні проміжки часу, а саме на 40-, 60-, 90-, 150-ту та 240-ву добу, усіх осіб у групі тестували на IgG SARS-CoV-2. На останньому етапі було перевірено рівень специфічних імуноглобулінів (Ig) класу G до нуклеокапсиду COVID-19 та спайк-протеїну в дослідницькій групі на 360-й день від початку захворювання. Для визначення специфічних IgG на SARS-CoV-2 була залучена приватна сертифікована лабораторія в Києві – «ДНК-лабораторія». Всіх пацієнтів тестували на антитіла до COVID-19 методом імуноферментного аналізу (ІФА) із використанням серологічних тест-систем VitroTest (Україна). В рамках міжлабораторного контролю якості паралельно було задіяно імунологічну лабораторію Інституту педіатрії, акушерства та гінекології. Результати дослідження співпали. **Результати та їх обговорення.** У досліджуваних протягом 8 міс поступово спостерігалось зниження рівня IgG до нуклеокапсиду. Цікавим є те, що в період від 40 до 150 днів у всіх 42 пацієнтів (100 %) антитіла не зникали. Зниження антитіл відбувалося, в основному, в період між 150 і 240 днями. Однак дані, отримані за 360 днів, суттєво змінили картину. У частини досліджуваних, які мали низький або негативний рівень антитіл через 8 міс, станом на 12 міс рівень імуноглобуліну (Ig) класу G знову піднявся вище порогового значення. Таким чином, ми бачимо, що з групи 42 осіб 92,8 % мають антитіла до нуклеокапсиду, а 7,2 % не мають. **Висновки**. Отримані дані ілюструють, що у групі досліджуваних протягом 12 міс після перенесеного SARS-CoV-2 переважна більшість осіб залишається з специфічними антитілами як до нуклеокапсиду, так й до спайк-протеїну.

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

ПРОДОЛЖИТЕЛЬНОСТЬ И СПЕЦИФИЧНОСТЬ ГУМОРАЛЬНОГО ИММУННОГО ОТВЕТА НА SARS-COV-2

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Актуальность. Наше предположение, что иммунитет после коронавирусной инфекции сохраняется в течение длительного времени, было полностью подтверждено в уже прове-

дённых исследованиях. Данная работа является продолжением исследования и демонстрирует результаты, полученные на 12-й месяц определения гуморального ответа у пациентов в группе испытуемых. **Материалы и методы.** В исследовании приняли участие 42 пациента, переболевших COVID-19 год назад. Все без исключения пациенты имели положительный анализ на COVID-19 методом ПЦР. Через определённые промежутки времени, а именно на 40-, 60-, 90-, 150- и 240-е сутки, всех лиц в группе тестировали на IgG SARS-CoV-2. На последнем этапе был проверен уровень специфических иммуноглобулинов (Ig) класса G к нуклеокапсиду COVID-19 и отдельно на спайк-протеин к COVID-19 у этой же группы на 360-й день от начала заболевания. Определение специфических IgG на SARS-CoV-2 было осуществлено с помощью сертифицированной лаборатории в Киеве – «ДНК-лаборатории». Всех пациентов тестировали на антитела к COVID-19 методом иммуноферментного анализа (ИФА) с использованием серологических тест-систем VitroTest (Украина). В рамках межлабораторного контроля качества параллельно была задействована иммунологическая лаборатория Института педиатрии, акушерства и гинекологии (Киев). Результаты исследования совпали. **Результаты и их обсуждение.** У исследуемых в течение 8 мес постепенно наблюдалось снижение уровня IgG к нуклеокапсиду SARS-CoV-2. Примечательно, что в период от 40 до 150 дня у всех 42 пациентов (100 %) антитела не исчезали. Снижение ниже порогового значения антител происходило, в основном, в период между 150 и 240 днями. Однако данные, полученные на 360-й день, существенно изменили картину. У части исследуемых, имевших низкий или отрицательный уровень антител через 8 мес, на 12-й месяц уровень иммуноглобулина (Ig) класса G снова поднялся выше порогового значения. Таким образом, мы видим, что из группы 42 человек 92,8 % имеют антитела к нуклеокапсиду, а 7,2 % не имеют. **Выводы**. Полученные данные свидетельствуют о том, что в группе исследуемых в течение 12 мес после перенесённого SARS-CoV-2 подавляющее большинство лиц остаются со специфическими антителами как к нуклеокапсиду, так и к спайк-протеину.

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