MOLECULAR CHARACTERISTICS OF BLOOD SERUM AFTER COVID-19 VACCINATION IN A REMOTE PERIOD

COVID-19 is a dangerous disease with long-lasting consequences. Vaccination contributes to the accumulation of neutralizing anti-S IgG antibodies, reducing the incidence of COVID-19 and its complications. However, in some individuals, the inflammatory process can persist for an indefinite period and lead to a wide range of dysfunctions. The current task is to investigate molecular markers for their detection. The aim of this study is to examine the levels of anti-S IgG antibodies, lactate, glucose, lactate dehydrogenase, and C-reactive protein in the peripheral blood of individuals who have and have not been affected by COVID-19 after vaccination. The research subject is venous blood. Among 547 employees of the Neurosurgery Institute (481 vaccinated against COVID-19 and 66 unvaccinated individuals), levels of anti-S IgG antibodies were investigated, as well as levels of lactate, lactate dehydrogenase, glucose, and C-reactive protein. At the time of the study, among 372 individuals, 16 months had passed from the first vaccination, and 12 months had passed from the second vaccination; in 21 individuals, 12 months had passed after a single vaccination, and in 88 individuals, 16 months had passed from the first vaccination, 12 months from the second, and 6 months from the third vaccination.

Methods. Quantitative determination of IgG antibodies to the S protein of the SARS-CoV-2 virus. Confirmation of COVID-19 using the RT-PCR method (Allplex 2019-nCoV kit, SeeGene, Korea). Levels of lactate, lactate dehydrogenase, glucose, and C-reactive protein were determined using reagents from BioSystems (Spain). Statistical analysis of the obtained data was performed using Jamovi software (USA) and the following criteria: $\chi^2$ — Kruskal-Wallis, $W$ — Dwass-Steel-Critchlow-Fligner (DSCF), $\chi^2$ — Pearson, $t$ — Student, $rs$ — Spearman, $\tau_b$ — Kendall. A statistically significant difference was considered at $p < 0.05$. Results. The level of anti-S IgG antibodies to the SARS-CoV-2 virus was higher in vaccinated individuals compared to unvaccinated individuals ($\chi^2$ = 14.09; $p < 0.001$). A higher level of antibodies to the S protein of the virus was observed when using the Comirnaty vaccine compared to vaccination with Moderna, AstraZeneca, Pfizer, and CoronaVac ($\chi^2$ = 14.09; $p < 0.001$). A higher level of antibodies to the S protein of the virus was observed when using the Comirnaty vaccine compared to vaccination with Moderna, AstraZeneca, Pfizer, and CoronaVac ($\chi^2$ = 14.09; $p < 0.001$). A higher level of antibodies to the S protein of the virus was observed when using the Comirnaty vaccine compared to vaccination with Moderna, AstraZeneca, Pfizer, and CoronaVac ($\chi^2$ = 14.09; $p < 0.001$). A higher level of antibodies to the S protein of the virus was observed when using the Comirnaty vaccine compared to vaccination with Moderna, AstraZeneca, Pfizer, and CoronaVac ($\chi^2$ = 14.09; $p < 0.001$). A higher level of antibodies to the S protein of the virus was observed when using the Comirnaty vaccine compared to vaccination with Moderna, AstraZeneca, Pfizer, and CoronaVac ($\chi^2$ = 14.09; $p < 0.001$).
Coronaviruses are the most common causative agents of respiratory disease outbreaks (Li et al., 2020). The novel coronavirus pandemic disease of 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is asymptomatic or causes mild symptoms in the majority of individuals, while 5% experience acute respiratory syndrome. There are data on 205 different post-COVID symptoms among a cohort of over 3500 individuals (Davis et al., 2021). The precise mechanisms of primary damage modification and the prolonged metabolic changes leading to long-term post-COVID complications are not sufficiently understood.

The question of the role of vaccination in the spread and course of the disease remains important. When defining the target product for COVID-19 vaccines, the World Health Organization (WHO) proposed a criterion that «a clear demonstration of efficacy (on a population basis) with an approximate estimate of ~50%» would be the minimal requirement for any acceptable COVID-19 vaccine, and that effectiveness could be assessed based on endpoints of «disease, severe disease, transmission» (World Health Organization, 2020.). In Ukraine, the main vaccines used for population vaccination were Pfizer, CoronaVac, Comirnaty, AstraZeneca, and Moderna. In retrospect, it is evident that vaccinated individuals were not 100% protected against the disease. Despite many studies on the question «Does this vaccine work against COVID-19?», it remains relevant (Hodgson et al., 2021).
zyme 2 (ACE2) receptor on almost all types of
cells in the body. The expression of ACE2 in nasal
goblet-like cells, epithelial cells of the oral cavity
and nasopharynx, bronchial epithelial cells, and I
and II types pneumocytes creates conditions for
simultaneous infection of the entire respiratory
system (Hamming et al., 2004; Sungnak et al.,
2020). This explains the observation that in 29.7%
to 54% of asymptomatic individuals with a posi-
tive RT-PCR result, small foci with a «ground-
glass» appearance are detected on CT scans of
the lungs (Inui et al., 2020). This CT pattern is
explained by partial filling of the alveoli with exu-
date and inflammation of the alveolar septa. This
process can spread to all parts of the lungs, but in
most cases, areas with a «ground-glass» appear-
ance regenerate and return to normal lung tissue
on subsequent CT scans (Tian et al., 2020; Shan
et al., 2020; Razek et al., 2021). The widespread
presence of the ACE2 receptor for SARS-CoV-2
in cells throughout the body is the cause of multi-
organ pathology, primarily in the lungs (the main
target organ), heart, kidneys, central nervous sys-
tem, as well as blood vessels, bone marrow, and
spleen (Vasquez-Bonilla et al., 2019; Trougakos et
al., 2021; Donoghue et al., 2000).

Lactate, glucose, C-reactive protein (CRP),
and lactate dehydrogenase are markers of inflam-
mation. In areas of lung tissue where changes are
observed on CT scans as «ground-glass» opaci-
ties, characteristic metabolic processes occur.
After the virus interacts with the ACE2 receptor,
facilitated by proteases (Partridge et al., 2021), vi-
rnal RNA is released into the cytoplasm, and viral
gene replication begins. The viral infection
leads to metabolic reprogramming, including in-
creased glucose uptake and a shift toward aerobic
glycolysis (the Warburg effect), accompanied by
elevated lactate production (Mansour et al., 2020;
Krishnan et al., 2021). The cytopathic effect of the
virus results in cell destruction (Li et al., 2020;
Zhuang et al., 2020), the release of virions, infec-
tion of other cells, and further accumulation of
lactate in the extracellular environment.

Today, lactate is considered a signaling mol-
ecule that can affect the immune system. Under
the influence of lactate in the extracellular space,
immune cells also reprogram their metabolism
through aerobic glycolysis (Brooks, 2018; Ratter
et al., 2018; Bost et al., 2021; Blanco-Melo et al.,
2020). Alveolar macrophages, monocytes/macrophages
recruited from the bloodstream, and epithelial cells
in the early stages of infection are the main sources
of cytokines and chemokines. The form in which
COVID-19 develops depends on the primary ac-
tivation of cytokine and chemokine synthesis. The
urgent activation of interferon synthesis is consid-
ered the most important factor in the mild course
of the disease (Ahmad et al., 2020). The important
role of IL6 is also recognized, particularly in the
activation of CRP gene expression under the infl u-
ence of this cytokine (Sproston & Ashworth, 2018).

The adaptive immune response is also im-
portant: T lymphocytes, together with specific
antibodies, eliminate damaged cells, which in-
terrupts further infection and promotes the res-
toration of metabolism and lactate levels in the
extracellular environment (de Biasi et al., 2020).

Lactate dehydrogenase (LDH) is a recognized
marker of chronic post-COVID cell damage. LDH is a cytosolic enzyme that catalyzes the
formation of lactate from pyruvate and does not
perform enzymatic functions in the extracellu-
lar environment. However, the presence of this
enzyme in the blood indicates cell damage (For-
kasiewicz et al., 2020).

**Long COVID.** A significant portion of patients
suffer from long-lasting dysfunctions ranging from
a few weeks to 2 years after COVID-19, a condi-
tion known as long COVID. Long COVID-19 is
characterized by a reduction in the functional ca-
pacity of various organ systems in the body due to
the cytolytic destruction and immune elimination
of infected cells. The functional capacity of cells is
also decreased due to mitochondrial imbalance,
which particularly affects muscles and the nervous
system, which require a large amount of ATP. In-
fection of ACE2-positive cells leads to a decrease
in the expression of this important functional receptor and, consequently, the disruption of the renin-angiotensin-aldosterone and kallikrein-kinin systems (Cooper et al., 2021; Gupta, 2022).

The aim of this study is to investigate the levels of neutralizing anti-S IgG to SARS-CoV-2 virus and inflammation markers such as C-reactive protein, lactate dehydrogenase, lactate, and glucose in the peripheral blood of vaccinated and unvaccinated individuals who were either affected or unaffected by COVID-19 after vaccination.

Materials and Methods. 547 employees of the Institute of Neurosurgery were studied, 481 of whom voluntarily received vaccination against the COVID-19 pandemic, and 66 were unvaccinated individuals. The first dose was received in April 2021; the second dose in August 2021; and the third dose in February 2022. 372 people received 2 vaccinations (16 months had passed from the first vaccination by the time of the study, and 12 months had passed from the second one); 21 individuals received one vaccination (12 months had passed by the study); 88 individuals received three vaccinations (16 months had passed from the first, 12 months from the second, and 6 months from the third vaccinations). The chosen duration of the study is sufficient for the manifestation of long-term post-Covid symptoms (Davis et al., 2021) and allows for comparison between the groups with one, two, and three vaccinations (comparing the group with three vaccinations has limitations in interpretation: only 6 months had passed by the study).

All individuals voluntarily underwent a study of IgG levels to the SARS-CoV-2 virus S protein in September 2022. Among the study population, 436 were female and 111 were male. Measurements of lactate, lactate dehydrogenase, glucose, and CRP were taken at the participant's discretion.

Comparison groups were formed based on the results of voluntary questionnaire surveys.

The study was conducted at the Department of Neurobiochemistry of the State Institution «Institute of Neurosurgery named after A.P. Romodanov of the National Academy of Medical Sciences of Ukraine» in accordance with the ethical principles of the Helsinki Declaration of the World Medical Association «Ethical Principles for Medical Research Involving Human Subjects».

The object of the study was venous blood obtained by venipuncture.

Comparison groups by indicators:
1. Vaccinated individuals; unvaccinated individuals.
2. Vaccinated individuals who had COVID-19; vaccinated individuals who did not have COVID-19; unvaccinated individuals who had COVID-19; unvaccinated individuals who did not have COVID-19. All episodes of the disease occurred at different times from April 2021 to September 2022.
3. Number of COVID-19 episodes (1, 2, 3).
4. Severity of the disease: mild form (early, mild symptoms such as anosmia, ageusia, which disappeared within 1–2 weeks); medium severity (early symptoms of a wide range, which disappeared within 2–4 weeks); severe form (wide range of symptoms, some of which remained at the time of the study (long COVID-19).
5. Groups vaccinated with different vaccines: Pfizer, CoronaVac, Comirnaty, AstraZeneca, Moderna.
6. Groups with different numbers of vaccinations (1, 2, 3).
7. Groups with chronic and without chronic diseases.

Methods. Quantitative determination of the level of protective antibodies — IgG to the SARS-CoV-2 virus S protein, which is synthesized in the human body as a result of the disease or vaccination, was carried out using test kits for SARS-CoV-2 IgG QuantiSpike enzyme immunoassay (Vitrotest, Ukraine). Measurements were made on a Stat Fax spectrophotometer (Awareness Technology, USA) for microplates.

To confirm COVID-19 infection using the RT PCR method on a CFX96 device (Bio-Rad, USA), the Allplex 2019-nCoV kit (SeeGene, Ko-
Molecular Characteristics of Blood Serum After COVID-19 Vaccination in a Remote Period

Lactate, lactate dehydrogenase, glucose, and CRP were measured in serum using a Respons 920 analyzer (DiaSys, Germany) with BioSystems (Spain) reagents. The methods were performed according to the instructions with consideration of the reference values of BioSystems. The system's precision in the series has a coefficient $\nu < 5\%$. The measurements were carried out by specialists in the field of molecular biology and biochemistry of the A. P. Romodanov Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine. Certificate of measurement capabilities determination No. PT-322/21 dated 28.07.2021 until 27.08.2023.

Blood samples were transported to the laboratory on ice, where they were processed and calibrated according to the manufacturer's instructions for sample collection and intervals.

Lactate. The principle of the lactate oxidase/peroxidase method: lactate present in the sample is oxidized by lactate oxidase to pyruvate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminophenazone and TOOS to form a colored complex, which is measured spectrophotometrically at a wavelength of 600 nm.

Lactate dehydrogenase. The principle of the method: lactate dehydrogenase catalyzes the reduction of pyruvate to NADH to form lactate and NAD+. The activity of lactate dehydrogenase is measured by the rate of decrease in the optical density of NADH at 340 nm.

Glucose. The principle of the method: glucose in serum is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide reacts with phenol and 4-aminophenazone to form a colored quinonimine. The intensity of the color is proportional to the concentration of glucose and is measured spectrophotometrically at a wavelength of 500 nm.

CRP. The principle of the method: turbidimetry. CRP in serum causes agglutination of latex particles coated with antibodies to human CRP. The degree of agglutination of the latex particles is proportional to the concentration of CRP and can be measured using turbidimetry at 540 nm.

Statistical analysis of the obtained data was performed using the publicly available software Jamovi (USA) (The Jamovi project. 2022. https://www.jamovi.org.; R Core Team, 2021).

Multiple comparisons were conducted using the Kruskal-Wallis test, the $\chi^2$ test, and the Dwass-Steel-Critchlow-Fligner (DSCF) method with the W-criterion. The relationship between disease incidence and vaccination was assessed using Pearson's $\chi^2$ test. Comparisons of lactate, lactate dehydrogenase, glucose, and C-reactive protein levels with reference values were carried out using a one-sample t-test (Student's t-test). The search for correlations between samples utilized Spearman's rank correlation coefficient ($r_s$) or Kendall's correlation coefficient ($\tau_b$). A difference between parameters was considered statistically significant at $p < 0.05$.

The research was conducted in accordance with the ethical principles of the Helsinki Declaration of the World Medical Association «Ethical Principles for Medical Research Involving Human Subjects,» the International Conference on Harmonisation of Good Clinical Practice (ICH-GCP), and the design was approved by the bioethics committee of the Romodanov Neurosurgery Institute of the National Academy of Medical Sciences of Ukraine.

Results. A total of 547 individuals participated in the study, including 436 women and 111 men. Among them, 481 participants received vaccination against COVID-19. Of these, 21 (4.4%) received one dose of the vaccine (12 months had passed by the study), 372 (77.3%) received two doses (16 months had passed from the first one by the study, and 12 months had passed from the second one). The 12-month period after vaccination is optimal for comparing antibody levels between different study groups and for analyzing the levels of inflammation markers in the long-term period after COVID-19. 88 (18.3%) received three doses (16 months had passed from the first
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One, 12 months — from the second one, and 6 months — from the third one. The Pfizer, CoronaVac, Comirnaty, AstraZeneca, and Moderna vaccines were used. 239 individuals received the Pfizer vaccine, 87 - CoronaVac, 31 - Comirnaty, 39 - AstraZeneca, and 11 - Moderna. The other 74 individuals were vaccinated with various combinations of vaccines (Table 1). Vaccinated individuals were tested for the level of neutralizing anti-S IgG antibodies against SARS-CoV-2 virus.

Comparison of antibody levels between groups of women and men did not show a statistically significant difference (Kruskal-Wallis $\chi^2 = 2.35; p = 0.125$). However, the level of anti-S IgG antibodies was statistically significantly different between vaccinated (481) and unvaccinated (66) individuals (Kruskal-Wallis $\chi^2 = 14.09; p < 0.001$) (Fig. 1, a). The levels of accumulated anti-S IgG antibodies against SARS-CoV-2 virus after two and three doses of vaccination also differed significantly (Kruskal-Wallis $\chi^2 = 14.78; p < 0.001$), (Fig. 1, b); (DSCF: 1 dose < 2 doses — W 3.101, $p=0.072$; 1 dose < 3 doses — W 4.423, $p = 0.005$; 2 doses < 3 doses — W 4.240, $p = 0.008$).

In the comparative analysis of the effectiveness of accumulating anti-S IgG antibodies according to

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**Fig. 1.** Levels of IgG to the S protein of SARS-CoV-2 depending on (a) vaccination status (1 — unvaccinated individuals, 2 — vaccinated individuals); (b) the number of vaccinations (1 — single dose; 2 — double dose; 3 — triple dose). *$p<0.05$

**Fig. 2.** Levels of IgG to the S protein of the SARS-CoV-2 virus depending on the type of vaccine; Moderna (M), AstraZeneca (A), Pfizer (F), Comirnaty (C), CoronaVac (CV); *$p < 0.05$
the type of vaccine, only cases where the same vaccine was used in repeated vaccinations were taken into account. Different combinations of vaccines used for 66 individuals (15.4%) were excluded from the analysis. The comparison of the dependence of anti-S IgG levels on the type of vaccine showed a statistically significant difference in the indicators ($\chi^2=14.98; p=0.005$). Post-hoc pairwise comparison using the DSCF method showed that significantly more antibodies were accumulated when using the Comirnaty vaccine compared to AstraZeneca, Pfizer, and CoronaVac vaccines ($W_{4.26}=4.26; p=0.002; W_{4.62}=4.62; p=0.010; W_{4.84}=4.84; p=0.006$) (Fig. 2). However, a comparison of COVID-19 incidence rates among individuals vaccinated with different vaccines showed no statistically significant difference ($\chi^2=2.072; p=0.723$) (Table 1). Thus, there was no correlation between the incidence of COVID-19 and the type of vaccine used ($\chi^2=2.072; p=0.723$) (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Effectiveness of vaccination as a factor influencing the incidence of COVID-19</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GROUPS</strong></td>
</tr>
<tr>
<td>All persons were diagnosed for the level of anti-S IgG</td>
</tr>
<tr>
<td>SARS-CoV-2 (N, %)</td>
</tr>
<tr>
<td>Vaccinated persons (N, %)</td>
</tr>
<tr>
<td>Non-vaccinated persons (N, %)</td>
</tr>
</tbody>
</table>

Disease incidence on COVID-19 among non-vaccinated and vaccinated cases *

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Case</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinated persons who didn't get sick with COVID-19</td>
<td>Non-vaccinated persons who didn't get sick COVID-19</td>
</tr>
<tr>
<td>170</td>
<td>16</td>
<td>289</td>
</tr>
<tr>
<td>OR (95% CI) 1.84 (1.02–3.30); $\chi^2=4.129; p=0.043$</td>
<td></td>
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</tbody>
</table>

Dependence of COVID-19 morbidity on the number of vaccinations (N, %)*

<table>
<thead>
<tr>
<th></th>
<th>1 vaccination</th>
<th>2 vaccinations</th>
<th>3 vaccinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated individuals who were not sick with COVID-19</td>
<td>8 (34.8%)</td>
<td>125 (34.1%)</td>
<td>37 (40.7%)</td>
</tr>
<tr>
<td>Persons who received 1, 2, 3 doses of the vaccine</td>
<td>21</td>
<td>352</td>
<td>86</td>
</tr>
<tr>
<td>Vaccinated individuals who were sick with COVID-19</td>
<td>12 (57.1%)</td>
<td>227 (64.4%)</td>
<td>50 (58.1%)</td>
</tr>
<tr>
<td>$\chi^2 = 1.05; df=2; p=0.592$</td>
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</tbody>
</table>

Dependence of the incidence of COVID-19 on the type of vaccine (N, %)*

<table>
<thead>
<tr>
<th></th>
<th>Vaccinated persons</th>
<th>Sick with COVID-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfizer</td>
<td>239</td>
<td>155 (64.9 %)</td>
</tr>
<tr>
<td>CoronaVac</td>
<td>87</td>
<td>55 (63.2 %)</td>
</tr>
<tr>
<td>Comirnaty</td>
<td>31</td>
<td>18 (58.1 %)</td>
</tr>
<tr>
<td>AstraZeneca</td>
<td>39</td>
<td>26 (66.7 %)</td>
</tr>
<tr>
<td>Moderna</td>
<td>11</td>
<td>9 (81.2 %)</td>
</tr>
<tr>
<td>All the others</td>
<td>74 (15.4 %)</td>
<td></td>
</tr>
<tr>
<td>$\chi^2 = 2.077; df=4; p=0.723$</td>
<td></td>
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</tbody>
</table>
Not all vaccinated individuals were protected against COVID-19. When calculating the incidence, only data confirmed by PCR tests for the presence of SARS-CoV-2 viral RNA in biological material were taken into account. Among 481 vaccinated individuals, 170 (37.0%) did not get sick. Vaccination reduces the chance of getting sick by 1.84 times (OR 1.84; CI 1.02–3.30; \( \chi^2 = 4.129; p = 0.043 \)). However, there is no statistically significant relationship between the level of antibodies and the severity of the disease (Kruskal-Wallis \( \chi^2 = 2.356; p = 0.308 \)), as well as the number of episodes of COVID-19 (Kruskal-Wallis \( \chi^2 = 1.867; p = 0.600 \)) (Table 1), which shows that there is no direct relationship between the detected high levels of antibodies in the blood serum and the incidence of COVID-19.

As known from the literature, in most cases, COVID-19 is asymptomatic or with mild symptoms that subside soon (within 2–4 weeks). Among individuals in our group, symptoms of varying degrees of severity were observed: in 211 individuals, there were mild early symptoms (anosmia, ageusia) that soon resolved within two weeks. Among the other 125 individuals, early medium complications (weakness, fatigue, muscle pain, high temperature, cough, dull glass symptoms) were observed in 87 individuals, and distant complaints were noted in 38 individuals (11.3%) (Table 2). That is, the largest number — nearly 2/3 of the individuals — had early mild post-COVID symptoms, complications of medium severity were characteristic for every fourth person who got sick, and long-term complications were found in 1 out of 10 individuals.

### Table 2. The effect of vaccination on the course of COVID-19

<table>
<thead>
<tr>
<th>Dependence of the number of episodes of COVID-19 on the presence and absence of vaccination (N, %)*</th>
<th>1 episode</th>
<th>2 episodes</th>
<th>3 episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated persons</td>
<td>216 (74.7%)</td>
<td>68 (23.5%)</td>
<td>5 (1.7%)</td>
</tr>
<tr>
<td>Non-vaccinated persons</td>
<td>38 (76.0%)</td>
<td>11 (22.0%)</td>
<td>1 (2.0%)</td>
</tr>
<tr>
<td>( \chi^2 = 0.0694; df = 2; p = 0.966 )</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Dependence of complications with COVID-19 on the presence and absence of vaccination (N,%)*</th>
<th>No complications and minor complications</th>
<th>Early complications</th>
<th>Late complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-vaccinated persons</td>
<td>43 (65.2%)</td>
<td>16 (24.2%)</td>
<td>7 (10.6%)</td>
</tr>
<tr>
<td>Vaccinated persons</td>
<td>379 (78.8%)</td>
<td>71 (14.8%)</td>
<td>31 (6.4%)</td>
</tr>
<tr>
<td>( \chi^2 = 6.127; df = 2; p = 0.047 )</td>
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<table>
<thead>
<tr>
<th>Distribution of individuals by type of symptoms in cases of COVID-19 (N,%)*</th>
<th>Early minor complications</th>
<th>Early complications of medium severity</th>
<th>Long-term complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anosmia, ageusia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>211 (62.8%)</td>
<td></td>
<td>87 (25.9%)</td>
<td>38 (11.3%)</td>
</tr>
</tbody>
</table>

*PCR-confirmed COVID-19 positive and COVID-19 negative cases
Weakness is most often noted in combination with other symptoms: joint pain, lung pain, respiratory failure, fatigue, dyspnea, arrhythmia, cough, loss of taste, smell, and vision deterioration (Table 2). Vaccination, in addition to its effective impact on antibody accumulation, has a positive effect on disease progression. Complications of various types are observed more frequently among unvaccinated individuals ($\chi^2 = 6.127; df = 2; p = 0.047$) However, vaccination does not have a statistically significant effect on the number of COVID-19 episodes.

Currently, the reasons for residual negative processes after the disease have not been formulated. Based on the fact that the disease starts with cytopathic damage and destruction of mostly epithelial cells by the SARS-CoV-2 virus, we hypothesized that a reliable indicator of persistent destructive processes (in our study — more than six months) would be the level of the recognized marker of destructive processes, the LDH enzyme, which is released into the intercellular space during cytolysis.

Pairwise comparison using the DSCF method showed that the LDH levels between groups with mild form (early) and severe form (late) complications after COVID-19 tended to increase ($W = 3.27, p = 0.054$). There was also a statistically significant difference in the LDH levels between individuals who did not have and those who had chronic diseases at the time of the study ($\chi^2 = 6.08, p = 0.014$) (Table 3).

The norm of the inflammation marker CRP is < 3 mg/L. An increase in CRP to 7 mg/L indicates a low-intensity inflammation. This can be observed in mild, hidden inflammatory conditions that they do not manifest clinically. Pairwise comparison using the DSCF method showed a statistically significant difference in CRP levels between groups that had early mild

<table>
<thead>
<tr>
<th>Table 3. Study of the level of inflammatory markers of lactate dehydrogenase, CRP, lactate, and glucose in individuals after vaccination and disease of COVID-19</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MARKERS</strong></td>
</tr>
<tr>
<td>Lactate mmol/L</td>
</tr>
<tr>
<td>Glucose mmol/L</td>
</tr>
<tr>
<td>CRP mg/L</td>
</tr>
<tr>
<td>Lactate dehydrogenase units/L</td>
</tr>
</tbody>
</table>

Dependence of the level of inflammatory markers on the presence of chronic diseases in the anamnesis for COVID-19 *

<table>
<thead>
<tr>
<th>MARKERS</th>
<th>Without chronic diseases $M \pm SE$</th>
<th>With existing chronic diseases $M \pm SE$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP mg/L</td>
<td>3.55±0.25</td>
<td>4.38 ± 0.72 Kruskal — Wallis, $\chi^2 = 3.74; p = 0.053$</td>
</tr>
<tr>
<td>Lactate dehydrogenase units/L</td>
<td>359.29±7.45</td>
<td>395.93 ± 18.40 Kruskal — Wallis, $\chi^2 = 6.08; p = 0.014$</td>
</tr>
</tbody>
</table>

*PCR-confirmed COVID-19 positive and COVID-19 negative cases*
complications and early medium complications \( (W = 4.19, p = 0.009) \), and a trend toward an increase in CRP levels in patients with severe (remote) outcomes \( (W = 3.15, p = 0.067) \). There was also a tendency toward higher CRP levels in patients with chronic conditions compared to the group of patients without chronic conditions \( (W = 2.74, p = 0.053) \). Research on the correlation between LDH and CRP levels among individuals vaccinated against the SARS-CoV-2 virus using the Kendall τb method showed the presence of a statistically significant positive correlation \( (τb 0.134, p < 0.001) \) (Fig. 3, a).

Comparison of lactate levels — a signaling molecule that activates the immune response during viral infection and glucose levels among individuals in different groups did not show statistically significant differences. However, it should be noted that the mean lactate level among individuals with mild COVID-19 was 1.98 mmol/L, 2.03 mmol/L in medium cases, and 2.13 mmol/L in severe cases (with distant dysfunction), that is, higher than the reference average level of 1.35 mmol/L \( (\text{one-sample t-test: } t = 5.41, p < 0.001; t = 6.69, p < 0.001; t = 3.34, p = 0.002, \text{ accordingly}) \). The mean glucose level in the same groups was 5.45 mmol/L, 5.50 mmol/L, and 6.06 mmol/L — higher than the reference average level of 5 mmol/L \( (\text{one-sample t-test: } t = 30.11, p < 0.001; t = 44.52, p < 0.001; t = 10.54, p < 0.001, \text{ accordingly}) \). The study of the correlation between lactate and glucose levels among vaccinated individuals using the Kendall-τb method showed a statistically significant positive correlation \( (τb 0.082, p < 0.01) \) (Fig. 3, b).

**Discussion.** It is known that Moderna, Pfizer, and Comirnaty are mRNA vaccines from Pfizer, while AstraZeneca is a vector vaccine from Oxford/AstraZeneca. All of these vaccines lead to the accumulation of neutralizing anti-S IgG antibodies. CoronaVac is a vaccine created based on a weakened virus, so the body produces antibodies to all virus antigens, including anti-S IgG in response to this vaccine.

The study results indicate that the level of anti-S protein antibodies in vaccinated individuals is higher compared to unvaccinated individuals \( (\text{Kruskal-Wallis } χ^2 = 14.09; p < 0.001) \). Anti-S IgG antibodies to the SARS-CoV-2 virus accumulate more effectively with two and three vaccinations \( (\text{Kruskal-Wallis } χ^2 = 14.78; p < 0.001) \). However, the disease frequency does not depend on the number of doses \( (χ^2 = 1.08; \text{df} = 2; p = 0.583) \). The proportion of individuals who
get sick with COVID-19 is almost the same with one (57.1%) or two doses (64.4%) and vaccinated three times (58.13%).

When using the Comirnaty vaccine compared to AstraZeneca, Pfizer, and CoronaVac, the level of accumulated anti-S IgG is higher (Comirnaty compared to AstraZeneca, Pfizer, and CoronaVac, respectively DSCF: W 4.26, p = 0.002; W 4.62, p = 0.010; W 4.84, p = 0.006). Vaccination with Pfizer, CoronaVac, Comirnaty, and AstraZeneca prevented the disease within the 33.3% — 41.9% range. However, it should be noted that no more individuals got sick when vaccinated with Comirnaty (41.9%), and those vaccinated with Moderna got sick most often, at 81.8%, but without statistical significance (Kruskal — Wallis χ² = 2.08; df = 4; p = 0.722).

The level of serum lactate, an important indicator of the inflammatory process, represents a balance between its rate of secretion into the bloodstream and its rate of elimination by the liver and kidneys. In healthy individuals, the lactate content in the blood is maintained within the range of 0.5—1.5 mmol/L. Persistent, mild to medium elevation of lactate levels (2—10 mmol/L) without metabolic acidosis is called hyperlactatemia. Hyperlactatemia can be further classified as mild (2.0—3.9 mmol/L), medium (4.0—9.9 mmol/L), or severe (>10 mmol/L). Studies have shown that hyperlactatemia correlates with increased hospital mortality (Spiegelberg et al., 2022; Bou Chebl et al., 2020). A retrospective cohort study with 3,325 patients with different diagnoses showed that even in the normal (1—1.9 mmol/L) range, lactate is a prognostic criterion for long-term mortality in all patients who were hospitalized. The authors of the study conclude that the lactate level should be used as a risk factor regardless of the pathological process, which can help identify patients who need additional examination and monitoring (Villar et al., 2019). In our study, the lactate levels observed in patients with different degrees of COVID-19 complications were: 1.89 mmol/L for mild early complications, 2.01 mmol/L for medium early complications, and 2.17 mmol/L for late complications, which correspond to the levels of mild hyperlactatemia.

The main cause of hyperlactatemic acidosis in viral infections during the acute phase is the transition of infected cells to aerobic glycolysis. In the cases where viral infections progress, lactate production may increase due to tissue hypoperfusion (increased anaerobic glycolysis) and the inability of the liver and kidneys to maintain balance. The activation of anaerobic and aerobic glycolysis is accompanied by an increase (up to 10 times) in glucose uptake. Analysis of the serum glucose levels in individuals with COVID-19 and varying degrees of complications showed an increase in the mean levels of 5.45 mmol/L, 5.50 mmol/L, and 6.06 mmol/L, respectively, compared to the reference level of 5.00 mmol/L. A positive correlation was observed between glucose and lactate levels (Kendall τ = 0.082, p < 0.01).

CRP is an acute-phase protein whose level increases in response to inflammation. The normal level of CRP, which is typical for most healthy adults, is <3 mg/L, while a level between 3—10 mg/L is considered normal or slightly elevated and may be observed in subacute or hidden inflammatory diseases that do not show clinical symptoms (Cleland & Eranki, 2022). CRP plays a certain role in recognizing and clearing foreign pathogens and damaged cells by binding to phospholipids, histones, chromatin, and fibronectin. Acceleration of removal of damaged cells and foreign pathogens is possible through activation of the classical complement pathway and phagocytic cells via Fc receptors. When the process is activated by autoantibodies, it may become pathological and lead to tissue damage (Jungen et al., 2019; Kramer et al., 2019). According to the results of our study, CRP was elevated among individuals with early mild complications and those with early medium complications (W = 4.19, p = 0.009), with a trend toward an increase in CRP levels in patients with severe (remote) outcomes (DSCF W = 3.15, p = 0.067). There was also a statistically
significant difference in CRP levels between the group with chronic diseases and the group without chronic diseases (DSCF: W = 2.74, p = 0.053).

LDH is a recognized multiorgan marker of inflammation — a cytoplasmic enzyme presents in almost all cells of the body. The presence of LDH in biological fluids indicates cell destruction. The normal range of LDH is usually between 140 and 280 U/L, but the clinical interpretation depends on the patient’s signs and symptoms (Henry et al., 2020). According to the results of our study, there was a high level of significance in the difference in LDH levels between groups with early moderate complications and separate complications after COVID-19 (DSCF: W = 3.27, p = 0.054). There was also a statistically significant difference in LDH levels between individuals who did not have and those who had chronic diseases at the time of the study (χ² = 6.08, p = 0.014).

A study of the correlation between LDH levels and CRP among individuals vaccinated against SARS-CoV-2 using the Kendall-τb method showed the presence of a statistically significant positive correlation (τb 0.134; p < 0.001).

Conclusions. The study of antibody levels to the S protein of SARS-CoV-2 in 481 individuals (372 received 2 vaccinations: 16 months had passed from the first vaccination and 12 months — from the second vaccination; 21 individuals received one vaccination, with 12 months having passed before the study; 88 individuals received three vaccinations: 16 months had passed from the first vaccination, 12 months — from the second vaccination, and 6 months — from the third vaccination) revealed that vaccination dose-dependently contributes to an increase in the IgG levels to the S protein of SARS-CoV-2 (DSCF: 1 dose < 2 doses — W 3.10, p = 0.072; 1 dose < 3 doses — W 4.42, p = 0.005; 2 doses < 3 doses — W 4.24, p = 0.008).

Vaccination reduces the chance of contracting COVID-19 (OR 1.84; CI 1.02-3.30; χ² = 4.13; p = 0.043).

The incidence of the disease does not depend on the type of vaccine used, AstraZeneca, Comirnaty CoronaVac, Moderna, Pfizer (χ² = 2.07; p = 0.723), and the presence of antibody titers in the serum of vaccinated persons.

Regardless of the type of vaccines used, the presence of complications is observed in the long-term period after COVID-19. However, among individuals who received vaccination, the number of individuals without complications or with minimal complications is greater than in the group of individuals who did not receive vaccination, while the number of individuals with early complications and severe complications is lower (χ² = 6.13; df = 2; p = 0.047).

Higher level of C-reactive protein (DSCF: W = 4.19, p = 0.009), trend to rise lactate dehydrogenase (DSCF: W = 3.27, p = 0.054), elevated levels of lactate (2.17+1.23, t = 3.34; p = 0.002) and glucose (6.06+0.048, t = 10.54; p<0.001) indicate that after COVID-19, regardless the type of vaccines used, in individuals with distant symptoms there are metabolic changes that are signs of a chronic inflammatory process.

Individuals with existing chronic diseases show a trend to rise in levels of inflammatory marker C-reactive protein (χ² = 3.74; p = 0.053) and lactate dehydrogenase (χ² = 6.08; p = 0.014), compared to those without chronic diseases.

The obtained data indicate that molecular markers of inflammation, including lactate, glucose, C-reactive protein, and lactate dehydrogenase levels are informative for identifying individuals with an inflammatory process in the long-term period after COVID-19.

Conflict of interest. The authors declare that there are no conflicts of interest.
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МОЛЕКУЛЯРНІ МАРКЕРИ СИРОВАТКИ КРОВІ ОСІБ ПІСЛЯ ВАКЦИНАЦІЇ ПРОТИ COVID-19 У ВІДДАЛЕНОМУ ПЕРІОДІ

COVID-19 — небезпечна хвороба з довготривалими наслідками. Вакцинація сприяє накопиченню нейтралізуючих антитіл анти-S IgG, зниженню кількості захворювань на COVID-19 та ускладнень. Однак у деяких осіб запальний процес може продовжуватись невизначений час та викликати широкий спектр дисфункцій. Завданням сьогодення є дослідження молекулярних маркерів для їх виявлення.

Мета даної роботи — дослідити рівні анти-S IgG, лактату, глюкози, лактатдегідрогенази та С-реактивного білка у периферійній крові осіб, що хворіли та не хворіли на COVID-19 після вакцинації. Об'єкт дослідження — венозна кров. У 547 співробітників Інституту нейрохірургії (481 вакцинованих проти COVID-19 та 66 невакцинованих осіб) було досліджено рівні анти-S IgG, а також рівні лактату, лактатдегідрогенази, глюкози та С-реактивного білка. На момент дослідження рівня анти-S IgG у 372 осіб після першої вакцинації пройшло 16 місяців та 12 місяців після другої вакцинації; у 21 особи після однієї вакцинації пройшло 12 місяців та у 88 осіб — 16 місяців після першої вакцинації, 12 місяців після другої та 6 місяців після третьої...
Молекулярні характеристики крові після вакцинації COVID-19 у діагностичному періоді.


Результати. Рівень анти-S IgG до вірусу SARS-CoV-2 у вакцинованих осіб у порівнянні з такими у невакцинованих був вищим (Крускал-Уолліс χ² = 14.09; p < 0.001). Вищий рівень антитіл до S білка вірусу спостерігався при використанні вакцин Comirnaty в порівнянні з такими за вакцинації Moderna, AstraZeneca, Pfizer, CoronaVac (ДСКФ: W = 4.26, p = 0.002; W = 4.62, p = 0.010; W = 4.84, p = 0.006 відповідно). Вакцинація 1.84 рази знижує ймовірність захворіти (відношення шансів (ВШ) 1.84; довірчий інтервал (ДІ) 95% 1.02—3.30; χ² = 4.29; p = 0.043). Проте статистично значущої залежності запобігання захворюваності на COVID-19 від виду застосованих вакцин не виявлено (Крускал-Уолліс, χ² = 2.072; p = 0.72). Статистично значуща відмінність рівнів С-реактивного білка спостерігається між групами з ранніми легкими ускладненнями та ранніми середньою важкості ускладненнями (ДСКФ: W = 4.193, p = 0.009). Відмічається статистично значуща різниця в рівнях ЛДГ між особами, які не мали хронічних захворювань, і тимчасом, які на момент дослідження мали хронічні захворювання (Крускал-Уолліс χ² = 6.08, p = 0.014). У осіб, вакцинованих проти вірусу SARS-CoV-2, виявлено позитивний кореляційний зв’язок між рівнями С-реактивного білка та лактатдегідрогенази (Кендалл — τb 0.134, p < 0.001). Середні значення рівня лактату серед осіб, що хворіли на COVID-19 в легкій, середньої і тяжкій формі, вищі від референтного значення; також і середні значення глюкози у цих же групах вищі від референтного середнього значення. Між рівнями лактату та С-реактивного білка спостерігаються різні ускладнення. Проте серед вакцинованих кількість осіб без ускладнень або з мінімальними ускладненнями більша, ніж у групі без вакцинації, а кількість осіб з ранніми ускладненнями та важкими ускладненнями менша (Крускал-Уолліс χ² = 6.127; p = 0.047). Високий рівень С-реактивного білка (ДСКФ: W = 4.19, p = 0.009) та рівень лактату (Крускал-Уолліс χ² = 2.072; p = 0.723) рівня анти-S IgG до вірусу SARS-CoV-2, існує позитивний кореляційний зв’язок (Кендалл — τb 0.134, p < 0.001). Високий рівень С-реактивного білка (ДСКФ: W = 4.193, p = 0.009) та рівень лактату (Крускал-Уолліс χ² = 6.127; p = 0.047) збільшують ймовірність захворювання на COVID-19 (ВШ 1.84; ДІ 95% 1.02—3.30; χ² = 4.19; p = 0.043). Частота захворюваності на COVID-19 залежить від типу використаної вакцини: AstraZeneca, Comirnaty, Moderna, Pfizer, CoronaVac (ДСКФ: W = 4.26, p = 0.002; W = 4.62, p = 0.010; W = 4.84, p = 0.006 відповідно). Частота захворюваності на COVID-19 залежить від типу використаної вакцини: AstraZeneca, Comirnaty, Moderna, Pfizer, CoronaVac (ДСКФ: W = 4.26, p = 0.002; W = 4.62, p = 0.010; W = 4.84, p = 0.006 відповідно). Загородження від COVID-19 залежать від типу використаної вакцини: AstraZeneca, Comirnaty, Moderna, Pfizer, CoronaVac (ДСКФ: W = 4.26, p = 0.002; W = 4.62, p = 0.010; W = 4.84, p = 0.006 відповідно).

Висновки. Вакцинація сприяє підвищенню рівня анти-S IgG до вірусу SARS-CoV-2. Пік зниження рівня лактату та лактатдегідрогенази відбувається через 1.84 рази після третьої вакцинації (Кендалл — τb 0.134, p < 0.001). Вищий рівень анти-S IgG до вірусу SARS-CoV-2 зазначається при використанні вакцин Comirnaty в порівнянні з такими за вакцинації Moderna, AstraZeneca, Pfizer, CoronaVac (ДСКФ: W = 4.19; p = 0.009). Серед осіб, що хворіли на COVID-19 в легкій, середньої і тяжкій формі, вищі від референтного значення; також і середні значення глюкози у цих же групах вищі від референтного середнього значення. Між рівнями С-реактивного білка та лактату існує позитивний кореляційний зв’язок (Кендалл — τb 0.134, p < 0.001). Серед осіб, що хворіли на COVID-19 в легкій, середньої і тяжкій формі, вищі від референтного значення; також і середні значення глюкози у цих же групах вищі від референтного середнього значення. Між рівнями С-реактивного білка та лактату існує позитивний кореляційний зв’язок (Кендалл — τb 0.134, p < 0.001). Серед осіб, що хворіли на COVID-19 в легкій, середньої і тяжкій формі, вищі від референтного значення; також і середні значення глюкози у цих же групах вищі від референтного середнього значення. Серед осіб, що хворіли на COVID-19 в легкій, середньої і тяжкій формі, вищі від референтного значення; також і середні значення глюкози у цих же групах вищі від референтного середнього значення.