INFECTIOUS DISEASES

FEATURES OF PERIPHERAL BLOOD CELLULAR IMMUNITY PARAMETERS IN PATIENTS WITH LUNG DAMAGE UP TO 30 % IN COVID-19

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ABSTRACT

Background. *The stability of human organism for different kind of infection, including SARS-CoV-2 is significantly defined by the immune system. The mechanisms of the cellular immunity to the SARS-CoV-2 are not exactly defined and are under study.*

The aim. *To study the features of cell immunity parameters in patients with lung damage up to 30 % in COVID-19.*

Material and methods. *73 people were examined during the 2020–2021 pandemic. The study group consisted of 31 patients with lung damage up to 30 % with COVID-19, the comparison group consisted of 42 people not infected with SARS-CoV-2. A complete clinical blood count was carried out using a Medonic M20 hematological analyzer (Boule Medical, Sweden), the level of lymphocyte subpopulations was determined using a FACS Calibur cytometer (BD, USA) and FITC- and phycoerythrin-labeled monoclonal antibodies (Sorbent, Russia). Differences were considered statistically significant at $p < 0.05$.*

Results. *Patients with COVID-19 with lung damage according to computed tomography (CT)≤ 30 % before the treatment had a restructuring in the ratio of lymphocyte subpopulations in 67.7 % of cases. Lymphopenia ($< 1.1 \times 10^9$ cells/L) was detected in 34.4 % of patients: a decrease in the absolute count of $CD3^+$ lymphocytes by 30.8 %, $CD3^+CD4^+$ – by 35 %, $CD3^+CD8^+$ – by 6.7 % ($p < 0.05$), $CD16^+CD56^+$ natural killer (NK) cells – by 29.4 % ($p = 0.009$). The level of $CD95^+$ lymphocytes in COVID-19 is 3.2 times higher than in healthy individuals. Elevated levels of $HLA\text{-}DR^+$ (> 20 %) and $CD3^+$ $HLA\text{-}DR^+$ lymphocytes (> 6 %) are recorded in 60 % and 86.7 % of patients, respectively. Elevated levels of $CD19^+$ B lymphocytes (> 17 %) in COVID-19 are 2.6 times more common than in healthy individuals. Correlation dependences of the count of NK cells with a wide range of T lymphocyte subpopulations were revealed.*

Conclusion. *Cellular immunity indicators in COVID-19 have a number of features that can serve as predictors of the progression of the severity of the disease.*

Key words: *COVID-19, new coronavirus infection, immune state, cellular immunity, lymphocytes, SARS-CoV-2*

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ОСОБЕННОСТИ ПОКАЗАТЕЛЕЙ КЛЕТОЧНОГО ИММУНИТЕТА ПЕРИФЕРИЧЕСКОЙ КРОВИ У ПАЦИЕНТОВ С ПОРАЖЕНИЕМ ЛЁГКИХ ДО 30 % ПРИ COVID-19

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РЕЗЮМЕ

Обоснование. *Успех противодействия организма человека инфекциям в значительной мере зависит от иммунной системы. Механизмы реагирования клеточного иммунитета на вирус SARS-CoV-2 ещё точно не определены и изучаются.*

Цель работы. *Исследование особенностей показателей клеточного звена иммунитета у пациентов с поражением лёгких до 30 % при COVID-19.*

Материал и методы. *Обследовано 73 человека в период пандемии 2020–2021 гг. Группу изучения составил 31 пациент с поражением лёгких до 30 % при COVID-19, группу сравнения – 42 человека, не инфицированных SARS-CoV-2. Общий клинический анализ крови проводили с использованием гематологического анализатора Medonic M20 (Boule Medical, Швеция), уровень субпопуляций лимфоцитов определяли с использованием цитометра FACS Calibur (BD, США) и меченных ФИТЦ и фикоэритрином моноклональных антител (Сорбент, Россия). Различия считались статистически значимыми при $p < 0,05$.*

Результаты. *У пациентов с COVID-19 при поражении лёгких по данным компьютерной томографии (КТ) ≤ 30 % до начала лечения отмечается перестройка в соотношении субпопуляций лимфоцитов в 67,7 % случаев. Лимфопения (< $1,1 \times 10^9$ кл./л) выявлена у 34,4 % пациентов: снижение абсолютного содержания $CD3^+$-лимфоцитов – на 30,8 %, $CD3^+CD4^+$ – на 35 %, $CD3^+CD8^+$ – на 6,7 % ($p < 0,05$), $CD16^+CD56^+$ натуральных киллеров (НК) – на 29,4 % ($p = 0,009$). Уровень $CD95^+$-лимфоцитов при COVID-19 в 3,2 раза выше, чем у здоровых лиц. Повышенные уровни $HLA\text{-}DR^+$- (> 20 %) и $CD3^+$ $HLA\text{-}DR^+$-лимфоцитов (> 6 %) регистрируются у 60 % и 86,7 % пациентов соответственно. Повышенный уровень $CD19^+$ В-лимфоцитов (> 17 %) при COVID-19 бывает в 2,6 раза чаще, чем у здоровых лиц. Выявлены корреляционные зависимости содержания НК-клеток с широким спектром субпопуляций Т-лимфоцитов.*

Заключение. *Показатели клеточного иммунитета при COVID-19 имеют ряд характерных особенностей, которые могут служить предиктором прогрессирования тяжести заболевания.*

Ключевые слова: *COVID-19, новая коронавирусная инфекция, иммунный статус, клеточный иммунитет, лимфоциты, SARS-CoV-2*

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OBJECTIVES

Since the beginning of the COVID-19 pandemic, it has been observed that the infection manifests in humans in a variety of ways, ranging from simple infection without clinical manifestations to severe conditions with multi-organ damage. Progressive lung damage was most often the cause of death in patients. Persons over 65 years of age and those with chronic diseases are most susceptible to the severe course [1]. Lymphocytes and their subpopulation structure play an important role in antiviral immune protection [2]. Viral infections lead to changes in the number and activity of the main subpopulations of lymphocytes (T and B lymphocytes) and natural killer (NK) cells involved in humoral and cytotoxic antiviral immune response [3, 4]. Studies conducted during 2020 have shown that SARS-CoV-2 has a unique pathological effect on the immune system compared to other coronaviruses [5, 6]. Typical characteristics of SARS-CoV-2 infection are a dramatic decrease in lymphocyte counts, changes in the ratios of T lymphocyte subpopulations, including $CD4^+$ and $CD8^+$ T lymphocytes [7]. The severity of changes in the T cell component of immune system determines the severity of the disease [2]. An important point to note is that the success of human resistance to SARS-CoV-2 infection, like the success of vaccination, depends to a large extent on the initial state of the immune system [8]. Studying the development of the macro-organism immune system response to SARS-CoV-2 infection is important for understanding the pathogenesis of the disease, as well as for developing therapeutic strategies and preventing the development of severe conditions caused by COVID-19.

THE AIM OF THE STUDY

To study the features of cell immunity parameters in patients with lung damage up to 30 % in COVID-19.

MATERIALS AND METHODS

Study design. We analysed the results of 73 patients during the 2020–2021 pandemic, including 31 patients on the 5th–7th day from the first signs of infection with SARS-CoV-2 virus with up to 30 % lung damage (study group) and 42 patients not infected with SARS-CoV-2 (comparison group). Study participants underwent an examination including assessment of patients' history, complaints, blood tests, computed tomography (CT) of the chest organs, and polymerase chain reaction (PCR) testing for SARS-CoV-2 infection. The survey data were recorded in a standardised questionnaire.

Compliance criteria. Patients in the study group had positive PCR test results for SARS-CoV-2, lung changes on CT ≤ 30 %, blood oxygen saturation > 95 %, and were not taking antibacterial or hormonal drugs at the time of admission to the hospital. The comparison group had an appropriate gender and age structure and included practically healthy individuals with negative levels of antibodies to SARS-CoV-2 virus at the time of medical surveillance.

Procedure situation. All patients underwent general clinical blood examination using Medonic M20 haematological analyser (Boule Medical, Sweden), including determination of absolute number of leukocytes, platelets, lymphocytes, microscopic determination of leukocyte formula. The percentage and absolute content of T lymphocyte subpopulations ($CD4^+$ T helper cells, cytotoxic $CD8^+$ T lymphocytes, $CD16^+$ T lymphocyte killers, HLA-DR^+ activated T lymphocytes), NK lymphocyte subpopulations ($CD16^+CD56^+$, $CD3^-CD8^+$) were determined, B lymphocytes ($CD19^+$), expression level of HLA-DR^+ and $CD95^+$ markers in the total blood lymphocyte pool (BP) by flow cytofluorimetry using FACS Calibur cytometer (BD, USA) and FITC- (Fluorescein IsoThioCyanate) and phycoerythrin-labelled monoclonal antibodies (Sorbent, Russia).

The leukocyte shift index (LSI) according to N.I. Yabuchinsky was determined by the ratio of the number of granulocytes (neutrophils, eosinophils and basophils) to agranulocytes (lymphocytes and monocytes). The leukocyte T cell index (LTI) according to A.M. Zemskov was determined by the ratio of the absolute number of leukocytes to that of $CD3^+$ T lymphocytes. The immunoregulatory index (IRI) was determined by the ratio of the percentage of $CD3^+CD4^+$ lymphocytes to the percentage of $CD3^+CD8^+$ lymphocytes.

The diagnosis of COVID-19 was verified using a PCR test for SARS-CoV-2, the levels of IgG antibodies (At) to the recombinant structural protein S1 spike of the SARS-CoV-2 virus in BP serum were determined using a semi-quantitative immunoenzymometric assay (IEMA) (Euroimmun AG, Germany), levels IgM-At and IgG-At to the recombinant protein SARS-CoV-2 – using high-quality IEMA (Vector-Best, Russia).

All the studied age, anamnestic and laboratory data were documented in the form of a standardised database (state registration certificate No. 2022620741 dated April 5, 2022) [9].

Ethical review. The study was carried out in accordance with the World Medical Association Declaration of Helsinki "Ethical principles of conducting scientific medical research with human participation as a subject". Written informed consent was obtained from each study participant before performing the procedures. The research protocol was approved by the Bioethics Committee of the Samara State Medical University of the Ministry of Health of the Russian Federation (Protocol No. 211 dated October 7, 2020).

Statistical processing was carried out using the SPSS 22.0 software (SPSS Inc., USA). Median (Me), 25th (Q1, first quartile) and 75th (Q3, third quartile) percentiles, Mann – Whitney rank test, Pearson's χ^2 test, Spearman's rank correlation (*R*). The level of statistical significance (*p*) was assumed to be < 0.05.

RESULTS

Among those examined, 66% were female. The average age 50.8 ± 15.4 years. A comparative analysis of the number of white cells and BP platelets showed that in the majority of patients the median values were within the reference values (Table 1).

The percentage and absolute lymphocyte levels were 23 % ($p = 0.003$) and 26.3 % ($p = 0.003$) lower, respectively, in the COVID-19 patient group than in the comparison group (Table 1). Severe lymphopenia (< 1.1×10^9 cells/L) was observed in 34.4 % of patients and was not observed in healthy individuals. In addition, with a higher neutrophil level (by 17.3 %; $p = 0.004$), there is also a 1.5-fold increased LSI relative to the comparison group ($p = 0.001$). In 21 (67,7 %) patients the pronounced rearrangements in the population structure of BP white cells were noted, among which in 12 (57 %) cases the increase of LSI values due to lymphopenia on the background of neutrophilia was noted (against 4 (10,7 %) healthy persons).

The percentage of atypical neutrophils ranged from 1 to 16 % (mean 8 %) and atypical lymphocytes from 2 to 15 % (mean 4.1 %) during microscopic examination of blood smears in patients with COVID-19. No atypical forms were observed in the comparison group.

When assessing the cellular link of immunity, it was obtained that in most cases the content of the examined subpopulations of T and B lymphocytes did not exceed the reference values (Table 2).

Against the background of the decrease in the absolute content of $CD3^+$ lymphocytes by 30.8 % ($p = 0.001$) in patients with COVID-19 relative to the comparison group there is an increase in LTI by 20 % ($p = 0.002$), which may indicate the presence of a deficiency of T lymphocytes (Table 2).

Relative to the indices of the comparison group, the patients with COVID-19 showed statistically significantly lower values of the absolute content of the examined subpopulations of T and NK lymphocytes (Table 2). While the percentage of $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes in patients with COVID-19 and in healthy individuals did not differ statistically significantly, analysis of individual immunograms indicates significant variability in these lymphocyte parameters. Thus, in the group of patients with COVID-19 there were 1.5 times more frequent cases of reduced content (< 35 %) of $CD4^+$ T lymphocytes: 35.5 % ($n = 11$) versus 26.2 % ($n = 11$) in the comparison group (Table 2).

When studying the distribution of $CD19^+$ B lymphocyte percentage content, it was found that elevated levels (> 17 %) were 2.6 times more frequent in patients with COVID-19 than in the comparison group.

When studying the NK cell system, it was found that the indicator of the absolute number of $CD16^+CD56^+$ lymphocytes was 29.4 % ($p = 0.009$) lower than that of the comparison group (Table 2). Absolute $CD16^+CD56^+$ lymphocyte content in patients with COVID-19 is below the reference value by 0.13×10^9 cells/L in 50 % of cases (versus 21.4 % of the comparison group).

No statistically significant changes in the percentage of both $CD3^+CD16^+$ and $CD3^-CD8^+$ lymphocytes were found during the average period in the group of patients with COVID-19 relative to the comparison group (Table 2). Comparative analysis of the distribution of these lymphocyte subpopulations showed that elevated levels of $CD3^+CD16^+$ and $CD3^-CD8^+$ lymphocytes (> 8 %) were 2-fold more frequent in patients with COVID-19: 21.4 % ($n = 3$) versus 9.4 % ($n = 3$), respectively, and 22.2 % ($n = 6$) versus 9.1 % ($n = 3$) among healthy individuals.

TABLE 1

GENERAL CLINICAL BLOOD EXAMINATION VALUES OF PATIENTS WITH NEW CORONAVIRUS INFECTION (STUDY GROUP) AND HEALTHY DONORS (COMPARISON GROUP)

Indicators [reference values]	Comparison group ($n = 42$)		Study group ($n = 31$)		p
	Me	Q1–Q3	Me	Q1–Q3	
Leukocytes, $\times 10^9$ cells/L [4–9]	6	[5–7]	6	[5–7.8]	0.5
Lymphocytes, % [20–50]	35.1	[28.1–43.6]	27	[12.5–34]	0.003*
Lymphocytes, $\times 10^9$ cells/L [1.13–2]	1.9	[1.6–2.4]	1.4	[0.9–1.9]	0.001*
Platelets, $\times 10^9$ cells/L [180–320]	222	[165–248.2]	204	[173–298]	0.6
Neutrophils, % [48.5–84]	56	[49.2–65.2]	65.7	[58.3–81.4]	0.004
Monocytes, % [3–11]	7.7	[6.6–8.75]	7.2	[4.4–9]	0.3
LSI, units [1.46–2.36]	1.26	[0.95–1.9]	1.9	[1.4–4]	0.001*

Note. p is the level of statistical significance according to the Mann – Whitney test ($p < 0.05$).

No statistically significant changes in the content of HLA-DR$^+$ lymphocytes at the average in the group of patients with COVID-19 relative to the comparison group were observed ($p = 0.1$). In the analysis of individual immunograms, it was noted that in patients with COVID-19, elevated (> 20 %) HLA-DR$^+$ lymphocyte content was observed in 18 (60 %) patients (versus 14 (33.3 %) patients in the comparison group; $\chi^2 = 5$; $p = 0.03$). The level of CD3$^+$ HLA-DR$^+$ lymphocytes in patients with COVID-19 is statistically significantly higher than that in the comparison group (Table 2). Elevated (> 6 %) CD3$^+$ HLA-DR$^+$ lymphocyte content was observed in 36 (86.7 %) COVID-19 patients (versus 18 (58.3 %) comparison group individuals).

A three-fold increase in the CD95$^+$ lymphocyte ratio was observed in the average group of patients with COVID-19 relative to the comparison group (Table 2).

The results of the correlation analysis showed that the percentage level of CD3$^+$CD16$^+$ lymphocytes showed a correlation with a wide range of other parameters of cellular immunity: the reverse – with IRI ($R = –0.62$; $p = 0.02$), the absolute content of CD3$^+$ lymphocytes ($R = –0.6$; $p = 0.02$), the absolute content of CD3$^+$CD4$^+$ lymphocytes ($R = –0.7$; $p = 0.005$); direct – with LTI ($R = 0.6$; $p = 0.02$) and CD3$^+$ HLA-DR$^+$ lymphocytes ($R = 0.63$; $p = 0.016$). The existence of multiple negative correlations between the content of NK cells and T-lymphocyte subpopulations may indi-

TABLE 2

CELLULAR IMMUNITY INDICATORS IN PATIENTS WITH COVID-19 (STUDY GROUP) AND HEALTHY DONORS (COMPARISON GROUP)

Indicators [reference values]	Comparison group ($n = 42$)		Study group ($n = 31$)		p
	Me	Q1–Q3	Me	Q1–Q3	
CD3, % [6–85]	71	[64.5–75]	70	[60.5–76]	0.5
CD3, × 10^9 cells/L [0.94–2.1]	1.3	[11.6–7]	0.9	[0.5–1.4]	0.001*
CD3$^+$CD4$^+$, % [35–55]	40.9	[32.5–46.3]	39	[30.3–45.3]	0.4
CD3$^+$CD4$^+$, × 10^9 cells/L [0.58–1.3]	0.77	[0.56–0.9]	0.5	[0.3–0.9]	0.009*
CD3$^+$CD8$^+$, % [19–35]	23.15	[17–34.1]	23.4	[17.3–31.2]	0.7
CD3$^+$CD8$^+$, × 10^9 cells/L [0.37–1]	0.375	[0.3–0.7]	0.35	[0.17–0.48]	0.02*
CD3$^-$CD8$^+$, %	4.2	[3–6.8]	5.75	[2.8–11.1]	0.14
CD3$^-$CD8$^+$, × 10^9 cells/L	0.09	[0.06–0.14]	0.07	[0.03–0.1]	0.13
CD16$^+$CD56$^+$, % [10–23]	10.25	[7.4–14.25]	9.4	[5.1–14.4]	0.4
CD16$^+$CD56$^+$, × 10^9 cells/L [0.13–0.5]	0.17	[0.14–0.24]	0.12	[0.06–0.19]	0.009*
CD3$^+$CD16$^+$, % [5–8]	3.2	[2–5]	3	[1.6–9.6]	0.8
CD19$^+$, % [7–17]	8	[6–11.3]	9	[6.2–13.6]	0.26
CD19$^+$, × 10^9 cells/L [0.1–0.38]	0.15	[0.1–0.23]	0.13	[0.07–0.2]	0.12
CD3$^+$ HLA-DR$^+$, % [1–6]	7	[5–11.3]	10	[7.4–14.8]	0.025*
HLA-DR$^+$, % [7–20]	18.8	[15.35–22.3]	21.55	[17–27.2]	0.1
CD95$^+$, % [5–43]	9.3	[4.3–33.45]	29.6	[13–39.75]	0.05*
LTI [4-7]	4.5	[3.3–5]	5.4	[4.2–14.1]	0.002*
IRI [1.5–2.6]	1.7	[1–2.6]	1.9	[0.9–2.5]	0.81

Note. p is the level of statistical significance according to the Mann – Whitney test ($p < 0.05$).

cate increased cytotoxicity against the background of $CD3^+$ and $CD3^+CD4^+$ lymphocyte deficiency.

In the study of correlations, it was found that the percentage level of $CD16^+CD56^+$ lymphocytes shows an inverse relationship with the percentage of $CD3^+$ and $CD3^+CD4^+$ lymphocytes ($R = –0.7$ and $R = –0.34$, respectively; $p < 0.05$), a direct relationship with the percentage and absolute levels of $CD3^-CD8^+$ lymphocytes ($R = 0.8$ and $R = 0.5$, respectively; $p < 0.05$). A wide range of correlations between the levels of $CD16^+CD56^+$ NK cells and $CD16^+$ T lymphocytes with other T-lymphocyte subpopulations may indicate a conjugated response of different lymphocyte subpopulations in response to SARS-CoV-2 virus infection.

The level of $HLA\text{-}DR^+$ lymphocytes in patients with COVID-19 showed an inverse correlation with the absolute content of $CD3^-CD8^+$ lymphocytes ($R = –0.49$; $p = 0.007$) and a direct correlation with the percentage level of $CD3^+CD16^+$ lymphocytes ($R = 0.63$; $p = 0.016$). An inverse correlation between the index of the percentage level of $HLA\text{-}DR^+$ lymphocytes and the percentage level of $CD3^+$ lymphocytes was observed ($R = –0.37$; $p = 0.05$).

The $CD95^+$-lymphocyte indices showed inverse correlations with the percentage level of $CD3^-CD8^+$ lymphocytes ($R = –0.48$; $p = 0.02$) and direct correlations with $CD3^+CD4^+$ lymphocytes ($R = 0.43$; $p = 0.037$).

DISCUSSION

In patients with COVID-19, according to the results of the general blood analysis, the average leukocyte level in the group does not exceed the reference values [10], while 67.7 % of patients have marked rearrangements of the percentage of granulocytes and agranulocytes in BP, among which 57 % of cases show lymphopenia on the background of neutrophilia. The evidence of the rearrangements that occurred in patients with COVID-19 is confirmed by both a 1.5-fold increase in the group mean LSI value and the presence of opposite correlations of lymphocyte levels ($R = –0.47$; $p = 0.07$) and neutrophils ($R = 0.45$; $p = 0.012$) with 30 % lung lesion according to CT.

The numbers of atypical neutrophils and lymphocytes were as high as 8 % and 4.1 %, respectively, during the microscopic examination of blood smears in COVID-19. Atypical morphological changes of neutrophils and lymphocytes at the early stage of the disease development were also observed by some foreign researchers [11].

Severe lymphopenia ($< 1.1 \times 10^9$ cells/l) was observed in 34.4 % of COVID-19 cases. Statistically significant decrease in the absolute content of the studied subpopulations of T and NK cells in patients with COVID-19, including the absolute content of $CD3^+CD4^+$ lymphocytes (by 35 %; $p = 0.009$) and $CD3^+CD8^+$ lymphocytes (by 6.7 %; $p = 0.02$) may be used to define this general lymphopenia. Many authors consider a decrease in the absolute content of $CD4^+$ T cells and a change in their internal subpopulation structure to be a characteristic feature of COVID-19 [5, 7, 12]. However, no statistically significant changes in the percentage contents of $CD4^+$ and $CD8^+$ T lymphocytes and IRI were obtained. The involvement of T lymphocytes in the pathogenesis of the disease is considered by many authors to be determinant [5].

In our study, no statistically significant changes in $CD19^+$ lymphocyte indices were found in patients with COVID-19 relative to the comparison group. A possible reason for this may be the considerable variability in both percentage and absolute B-lymphocyte content in COVID-19 patients. In particular, 35.5 % of patients have a decreased (< 7 %) level of B lymphocytes and 13 % have an increased (> 17 %) level. An increase in the percentage of $CD19^+$ B lymphocytes in patients with COVID-19 was revealed in the study of F. Wang et al. (2020) [13].

Alterations in cellular immunity have been identified by many authors as a characteristic feature [1, 14]. A typical characteristic of COVID-19 infection is a dramatic decrease in lymphocyte levels and, in particular, absolute levels of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes [12, 15]. Migration of lymphocytes from the BP to the lungs is considered one of the causes of progressive lymphopenia [5, 16, 17]. Direct cytotoxic action of SARS-CoV-2 virus against the immune cells is considered as another cause of lymphopenia by a number of authors. Viral particles and the SARS-CoV-2 genome were not only found in monocytes and lymphocytes, but also proved their ability to replicate intracellularly in the *in vitro* system [1, 18, 19]. Infection of T lymphocytes and macrophages/monocytes located in lymph nodes, lungs and spleen in autopsy specimens has been reported [1, 20, 21].

Some authors note an increase in the content of cytotoxic cells and their functional activity in patients with COVID-19 [13, 14, 22]. A decrease in NK cell content correlating with the severity of the disease in COVID-19 has been observed in the works of other authors [4, 7]. Our study revealed a 29.4 % ($p = 0.09$) decrease in absolute $CD16^+CD56^+$ lymphocytes content in patients with COVID-19 relative to the comparison group. As concerns peculiarities of $CD3^+CD16^+$ and $CD3^-CD8^+$ lymphocytes level indices distribution, no statistically significant changes in their percentage content were revealed at the average in the group of patients with COVID-19 relative to the comparison group. However, a high variability in the level indices of these subpopulations can be observed, which may level out the average group values. In addition, the presence of multiple negative correlation interdependencies of the content of these populations of NK-cells with those of T lymphocyte subpopulations may indicate an increase in the cytotoxic activity of these cells against the background of $CD3^+$ and $CD3^+CD4^+$ lymphocyte deficiency. High correlation coefficient of the percentage level of $CD3^+CD16^+$ lymphocytes with LTI ($R = 0.6$; $p = 0.02$) may serve as evidence of these findings.

The increasing expression level of activation markers in COVID-19, including HLA-DR, has been documented in a number of studies [12, 13, 23, 24]. Studies show increased expression of a variety of activation markers on T lymphocytes triggering a "cytokine storm" in patients with COVID-19 [6, 18]. A dramatic increase in the level of cells expressing HLA-DR against a statistically significant de-

crease in the levels of $CD3^+CD4^+$ lymphocytes and NK cells has been indicated as a sign of excessive activation of cellular immunity [17]. In the absence of statistically significant differences in the percentage of $HLA\text{-}DR^+$ lymphocytes in patients with COVID-19 relative to the comparison group, the analysis of individual immunograms is 1.8 times more likely to have an increased content of $HLA\text{-}DR^+$ lymphocytes (> 20 %) ($\chi^2 = 5$; $p = 0.03$). In addition, patients with COVID-19 showed a statistically significant increase in the content of $CD3^+$ $HLA\text{-}DR^+$ lymphocytes by 42.8 % ($p = 0.025$). Changes in the indicators of cellular and humoral immunity in COVID-19 reconvalescents within 1.5–2.0 months after the infection were also revealed in the studies of other authors [25, 26].

According to our study, in response to infection of the organism with SARS-CoV-2 there is a change in quantitative and functional indices of cellular immunity, the degree of expression of which and the direction of dynamics largely depend on the initial state of the immune system.

CONCLUSION

Consequently, in 67.7 % of the examined patients with COVID-19 with lung lesions according to CT ≤ 30 % before the initiation of treatment there is a rearrangement in the ratio of lymphocyte subpopulations, among which in 57 % of cases there is lymphopenia on the background of neutrophilia, which is expressed in the increase of LSI values.

Pronounced lymphopenia ($< 1.1 \times 10^9$ cells/L) was observed in 34.4 % of patients with COVID-19; it was caused by a decrease in the absolute content of the main subpopulations of both T lymphocytes ($CD3^+$ – by 30.8 %, $CD3^+CD4^+$ – by 35 %, $CD3^+CD8^+$ – by 6.7 %; $p < 0.05$), and NK cells ($CD16^+CD56^+$ – by 29.4 %; $p = 0.009$), which was accompanied by an increase in LTI by 20 % ($p = 0.002$).

The features revealed in COVID-19 patients, such as a 3.2-fold increased fraction of $CD95^+$-lymphocytes (30 %), high (> 20 %) content of $HLA\text{-}DR^+$-lymphocytes registered in 60 % of patients, and increased (> 6 %) content of $CD3^+$ $HLA\text{-}DR^+$ lymphocytes observed in 86.7 % of patients, may be characteristic of the cellular level of the immune system in the course of SARS-CoV-2 infection. The degree of severity and dynamics of changes in quantitative and functional indices of cellular immunity in the course of SARS-CoV-2 infection are defined by the initial type of immune system response.

The use of immunological screening to determine the levels of $CD3^+CD4^+$, $CD3^+CD8^+$, $CD16^+CD56^+$, $CD3^+CD16^+$, $CD3^+$ $HLA\text{-}DR^+$, $CD19^+$, $CD95^+$ lymphocytes allows to assess the specific features of immune response to SARS-CoV-2 infection and can be recommended as an additional to the general clinical blood test.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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Vasneva Zh.P. – statistical processing, discussion of the results, writing the text.

Vdoushkina E.S. – obtaining data for study, discussing the results, writing the text.

Borodulin B.E. – review of publications on the topic of the article, development of study design, discussion of the results.

Povalyaeva L.V. – obtaining data for study, analyzing the data obtained, discussing the results.