TOPICAL ISSUE

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# PERIPHERAL BLOOD CELLULAR IMMUNITY PARAMETERS IN COVID-19

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This article carried material about the results of the investigation of peripheral blood cellular immunity parameters in the COVID-19 patients with ≤30% lung damage hospitalizated in 2020 year. When infected with SARS-CoV-2 on the background of severe lymphopenia and neutrophilia were revealed the multidirectional changing in a cellular immunity parameters, the severity and dynamics of which can be determined by the initial type of immune system response.

**Keywords:** COVID-19; new coronavirus infection; immune state, cellular immunity, lymphocytes, SARS–CoV-2.

**Introduction.** From the very beginning of the COVID-19 pandemic since April 2020, it has been noted that the infection manifests itself in people in different ways: from a simple infection without clinical manifestations to a severe condition with damage to various organs, primarily the lungs. Progressive lung damage was most often the cause of death of patients. Persons over 65 years of age and those with chronic diseases are most susceptible to severe course [7,11,12]. Lymphocytes and their subpopulation structure play an important role in antiviral immune protection [4]. Viral infections lead to dysregulation of the main subpopulations of lymphocytes (T-, B- and natural killer (NK) cells ) involved in the humoral and cytotoxic antiviral immune response [6,8]. Studies conducted during 2020 have shown that SARS-CoV-2 has a unique pathological effect on the immune system compared to other coronaviruses [5,10]. A typical characteristic of SARS-CoV-2 infection is a sharp decrease in the level of lymphocytes, shifts in the T-cell link of immunity, including a decrease in the absolute number of CD3+-, CD4+- and CD8+-T-lymphocytes. The severity of changes in the T-cell link of immunity directly depends on the severity of the course of coronavirus infection. It is important to note that the success of the human body's response to SARS-CoV-2 infection, as well as the success of vaccination, largely depends on the state of the immune system [1,2,3]. The study of the development of the response of the immune system of a macroorganism to infection with the SARS-CoV-2 virus is an important factor both for understanding the pathogenesis of the disease and for developing therapeutic strategies and preventing the development of severe conditions caused by COVID-19.

The aim of the study was to identify the characteristics of the cellular immunity in patients with COVID–19.

**Material and methods.** This study was conducted as part of the implementation of NIOKTR №121051700033-3 "Lung lesion of infectious etiology. Improvement of methods of detection, diagnosis and treatment". The study group consisted of 31 people in the initial period of the COVID-19 pandemic of the 2020 year, the comparison group consisted of 42 people who were not infected with SARS-CoV-2. The study group at the time of admission to the hospital had positive results of a PCR test for SARS-CoV-2, changes on a computed tomogram (CT)≤30%, blood oxygen saturation >95%, antibacterial and hormonal drugs were not taken before hospitalization. The comparison group was of the appropriate gender and age structure, practically healthy, with negative levels of antibodies to the SARS-CoV-2 virus at the time of examination. The examination of the groups included an assessment of anamnesis, complaints, CT of the chest organs, PCR testing for SARS-CoV-2 infection.

The survey data were entered into a standardized questionnaire. All patients underwent a general clinical examination of peripheral blood (PB) using the Medonic M20 hematological analyzer (Boule Medical, Sweden), including determination of the absolute number of leukocytes, platelets and platelet parameters MPV (average platelet volume), PDW (platelet distribution width by volume) and P-LCR (large platelet ratio), lymphocytes, microscopic determination of the leukocyte formula. The percentage and absolute content of T-lymphocyte subpopulations (CD3+, CD3+CD4+-T-helper cells, cytotoxic CD3+CD8+-T-lymphocytes, CD3+16+-T-lymphocytes, CD3+56+-T-lymphocytes, CD3+HLA-DR+), NK subpopulations were determined-cells (CD16+56+, CD3-CD8+), B-lymphocytes (CD19+), HLA-DR+-lymphocytes and CD95+-lymphocytes in PB by flow cytofluorimetry using a FACS Calibur cytometer (BD, USA) and monoclonal antibodies labeled with FITC and phycoerythrin (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. To clarify the etiology of clinical manifestations, the levels of IgG antibodies (At) to the recombinant structural protein S1 of the SARS-CoV-2 virus spike in serum were determined using a semi-quantitative enzyme immunoassay system (EIAS) (EUROIMMUN AG, Ger-

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many), the levels of IgM–At and IgG-At to the recombinant SARS-CoV protein-2 – using high-quality EIAS (Vector-Best, Russia).

Statistical processing was carried out using Software SPSS 22.0 (SPSS Inc.). Median (Me), 25th (Q1-quartile) and 75th (Q3-quartile) percentiles, Mann–Whitney rank criterion, Pearson's criterion χ2 were used. The significance level (p) was taken < 0,05.

**Results.** In the group of patients with COVID-19, 66% of women, 34% of men. The average age is 50,8±15,4 years. In most cases, the average group indicators of the number of white cells and platelets in patients with COVID-19 do not exceed the reference values (Table 1).

In the group of patients with COVID-19, the lymphocytes level was on average lower than in the comparison group, both percentage (by 23%) and absolute (by 26,3%) (Table 1). Severe lymphopenia (<1,1•$10^9$ cells/l) was in 34,4%. With a higher neutrophil level (by 17,3%), there is also a 1,5-fold increased LSI relative to the comparison group. 67,7% (n=21) showed marked rearrangements in the population structure of white PB cells, among which 57% (n=12) showed an increase in LSI values due to lymphopenia on the background of neutrophilia (Table 1). Microscopic examination of blood smears in patients with COVID-19, the percentage of atypical neutrophils varied from 1 to 16% (on average 8%), atypical lymphocytes – from 2 to 15% (on average 4,1%). No atypical forms were observed in the comparison group.

When assessing the cellular link of immunity, the indicators of T- and B-lymphocytes (CD3+, CD3+CD4+-, CD3+CD8+-, CD16+CD56+-, HLA-DR+- and CD3+HLA-DR+-) in most cases do not go beyond the reference values. In patients with COVID-19, relative to the comparison group, against the background of a decrease in the average group absolute level of CD3+-lymphocytes by 30,8%, there is an increase in LTI by 20%, significantly lower average group values of the absolute level of the studied subpopulations of T- and NK-cells (Table 2). Despite the fact that the average group percentage of CD3+CD4+- and CD3+CD8+-lymphocytes in patients with COVID-19 and in healthy donors did not differ significantly, the analysis of individual immunograms indicates the variability of these lymphocytic parameters. In the COVID-19 group, cases of reduced CD4+-T lymphocytes (<35%) were 1,5 times more common (35,5% (n=11) vs 26,2% (n=11) in the comparison group. Elevated levels of CD19+-B lymphocytes (>17%) are 2,6 times more common in the comparison group (Table 2).

When studying the NK-cell system, the average group of the absolute number of CD16+CD56+ lymphocytes is 29,4% lower than the comparison group and in 50% of cases lower than the reference value – 0,13•$10^9$ cells/l (Table 2). On average, there were no significant changes in the % level of both CD3+CD16+- and CD3+CD56+-lymphocytes and CD3-CD8+-lymphocytes in the group of patients with COVID-19 relative to the comparison group (Table 2). However, a comparative analysis of the distribution of these subpopulations of lymphocytes showed elevated levels of CD3+CD16+- and CD3-CD8+ - lymphocytes (> 8%) are noted 2 times more often.

In patients with COVID-19, an in-

**Table 1**

**Blood cells counts of patients with COVID-19 (study group) and healthy donors (comparison group)**

| Parameter | Comparison group (n = 42) | | Study group (n = 31) | | *p* |
|---|---|---|---|---|---|
| Units of measurement [reference value] | *Me* | Q1 – Q3 | *Me* | Q1 – Q3 | |
| Leucocytes, •$10^9$ cells/l [4 – 9] | 6 | 5 – 7.8 | 6 | 5 – 7.8 | 0.5 |
| Lymphocytes, % [20 – 50] | 35.1 | 28.1 – 43.6 | 27 | 12.5 – 34 | 0.003 |
| Lymphocytes, •$10^9$ cells/l [1,13 – 2] | 1.9 | 1.6 – 2.4 | 1.4 | 0.9 – 1.9 | 0.001 |
| Platellet, •$10^9$ cells/l [180 – 320] | 222 | 165 – 248.2 | 204 | 173 – 298 | 0.6 |
| MPV, fl. [9,4 – 12,4] | 8.8 | 8 – 9.4 | 8.6 | 8 – 9.3 | 0.9 |
| PDW, fl. [10 – 20] | 12 | 11 – 13 | 12.1 | 11.2 – 12.8 | 0.9 |
| PLCR, % [13 – 43] | 19.1 | 14.6 – 24.3 | 19.75 | 15.6 – 23.7 | 0.8 |
| 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, unit [1,46 – 2,36] | 1.26 | 0.95 – 1.9 | 1.9 | 1.4 – 4 | 0.001 |

**Table 2**

**Cellular immunity parameters of patients with COVID-19 (study group) and healthy donors (comparison group)**

| Parameter | Comparison group (n = 42) | | Study group (n = 31) | | *p* |
|---|---|---|---|---|---|
| Units of measurement [reference values] | *Me* | Q1 – Q3 | *Me* | Q1 – Q3 | |
| CD3, % [61-85] | 71 | 64.5 – 75 | 70 | 60.5 –- 76 | 0.5 |
| CD3, •$10^9$ cells/l [0,94-2,1] | 1.3 | 1 –1.67 | 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 |
| CD3+CD56+,% [5-8] | 7 | 3.7 – 12.9 | 5.7 | 4 – 8.4 | 0.3 |
| 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, unit [4-7] | 4.5 | 3.3 – 5 | 5.4 | 4.2 – 14.1 | 0.002 |
| IRI, unit [1,5-2,6] | 1.7 | 1 – 2.6 | 1.9 | 0.9– 2.5 | 0.81 |

creased (>20%) level of HLA-DR+ lymphocytes was observed in 60% (n=18) vs 33,3% (n=14) of the comparison group ($\chi 2$ = 5, p=0,03). An increased (>6%) level of CD3+HLA-DR+ lymphocytes was observed in 86,7% (n=36) patients vs 58,3% (n=18) of the comparison group. There was an increase in the CD95+ lymphocyte level by 3,2 times on average in the group of patients with COVID-19 relative to the comparison group (p=0,05) (Table 2).

**Conclusion.** Against the background of severe lymphopenia and neutrophilia during infection with SARS-CoV-2, multidirectional changes in the studied indicators of cellular immunity were revealed, including a decrease in the absolute level of CD3+-, CD3+CD4+–, CD3+CD8+ – and CD16+CD56+-lymphocytes; an increase in the % level of CD3+HLA-DR+ and CD95+ lymphocytes, the severity and dynamics of which can be determined by the initial type of immune system response. It is promising to study the initial "immunological passport" of a person to identify personal predictors of the course of the disease when infected with coronavirus infection.

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# EXCESS MORTALITY IN THE REPUBLIC OF SAKHA (YAKUTIA) DURING THE COVID-19 PANDEMIC (2020-2021)

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Excess mortality is defined as an increase in all-cause mortality over expected mortality (historical baseline for previous years). In the context of COVID-19, excess mortality may reflect the overall impact of the pandemic on mortality, including not only the number of confirmed deaths from COVID-19, but also deaths from COVID-19 when they were not correctly diagnosed and reported, and deaths from other diseases due to pandemic-related causes.

The purpose of the study: to assess the indicators of excess mortality during the COVID-19 pandemic (2020-2021) in the Republic of Sakha (Yakutia). For the analysis, data from the Federal State Statistics Service for 2015-2022 were used. For 2 years of the spread of a new coronavirus infection in the Republic of Sakha (Yakutia), 19556 people died. 7.8% of deaths in 2020 and 21.5% in 2021 were related to COVID-19. The number of all deaths was 22% and 44% respectively higher than the expected number of deaths. The proportion of excess deaths in 2020 was 19% of all deaths, in 2021 - 31%. Of the excess deaths, 42% and 69%, respectively, were related to COVID-19. The excess mortality rate reached 333 per 100,000 population in 2021. The high correlation coefficients (0.94-0.95) between COVID-19-related deaths and additional deaths suggest that excess deaths during the period 2020-2021 will largely be due to the spread of COVID-19.

The decline in mortality underreporting in 2021 against the background of an increase in excess mortality reflects improved diagnosis and correct identification of the causes of death. Research into the causes of excess mortality is needed to assess the impact of the pandemic and other factors on various aspects of mortality in the population.

**Keywords:** new coronavirus infection, COVID-19, pandemic, excess mortality, Republic of Sakha (Yakutia).

**Introduction.** Excess mortality is defined as an increase in all-cause mortality over expected mortality (historical baseline for previous years). The increase in mortality is associated with the emergence of some new factors, emergencies that affect the health of the population. In the context of COVID-19, excess mortality may reflect the overall impact of the pandemic on mortality, including not only the number of confirmed deaths from COVID-19, but also deaths from