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Modification of Immune Markers in Patients With Novel Coronavirus Infection Covid-19 Sars-cov-2

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Abstract

Patients with moderate Covid-19 severity were divided into three groups - with a complete absence of IL-6 in the circulation, with a low and rather high (17.75 - 99.5 pg / ml) content of IL-6. It was revealed that at the lowest concentration of IL-6, patients had a mild variant of the moderate course of Covid-19 and recorded minimal levels of leukocytes, neutrophils, lymphocytes, monocytes, total T-lymphocytes and T-helpers, B- lymphocytes, immune indices "neutrophils/ lymphocytes", CD4+/CD8+. However, with greater severity of the course of Covid-19 of moderate severity and a high content of IL-6, all these indicators significantly increased except for natural killer-effectors, the changes of which were of an opposite nature. It is suggested that a sufficiently high level of IL-6 in the case of moderately severe Covid-19 may be necessary to implement a balanced immune response, in contrast to a milder course, which in this sense may be insufficient. It can be assumed that the level of IL-6 can to a certain extent predict the severity of Covid-19.

Keywords: Covid-19, interleukin-6, immune markers, infection severity

1. Introduction

Numerous studies of COVID-19 show that there are multiple ambiguities in the interpretation of the behavior of the immune system during this infection. Many authors report "crazy" immune responses [1]. Currently, there is information about a number of facts of an inflammatory and immune nature, reflecting the severity of the course of the disease [2]. Moreover, it is indicated that many systems and organs are affected, and even in the event of an end to the course of an acute infection, lesions of various systems and organs of a recovered person remain [2,3].

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Apparently, this problem has arisen since the advent of multicellular organisms, since the interaction of viruses and multicellular organisms has been going on since the creation of our world.

It is essential that the mechanism of transformation of an acute viral infection into a latent form is still an unsolved scientific problem. It is known that when a virus enters in vivo, the host's immune response recognizes and presents the virus to CD4+ T-helper cells, which subsequently stimulate CD8+ T-killer cells and B- cells and trigger the synthesis of virus-specific antibodies. However, SARS-CoV-2 "endangers" the host's immune system, contributes to the development of lymphopenia, a significant reduction in lymphocytes, and an exacerbation of inflammation of a yet "unknown" etiology. It is still believed [1,3,4,5] that the decrease in the number of lymphocytes in SARS-CoV-2 infections is a mandatory act in their activation by strategies "intrinsic" of the virus to "avoid" the host immune response, such as suppression of IFN-I, which disrupts the activation of dendritic cells, differentiation and expansion of T-lymphocytes [1].

In people with asymptomatic and mild infection, the immune system is able to control the infection and minimize the damage caused by the inflammatory response, which is characterized by high numbers of activated CD4+ and CD8+ T-cells, follicular T-cells, Ab-secreting cells (antibody), and minimal levels of pro-inflammatory cytokines and chemokines [1]. Apparently, it is quite enough to control the replication of the virus and not enhance the hyperimmune response to activate an effective immune response.

While COVID-19 was initially recognized as a “pulmonary” disease followed by a “storm” of pro-inflammatory cytokines leading to acute respiratory distress syndrome, acquired immune deficiency syndrome and death, recent reports indicate [6] that this disease is systemic, affecting many organ systems, including the kidneys, gastrointestinal tract, liver, nervous system, and skin. The mechanism of these systemic effects is not yet clear, but many of them may be related to or mediated by cytokine action and immune system dysregulation.

The cytokine "storm" as the concept of "madness" of the immune system, aimed at destroying one's body, was discovered a long time ago [3,7]. But the understanding that each pathogen of a viral or bacterial nature "prescribes its own script" comes only today. Elevated levels of circulating pro-inflammatory cytokines (IFN-γ, IL-1, IL-6, IL-12) and chemokines (CXCL10, CCL2) are a sign of a severe course.

One might think that the important pro-inflammatory cytokine IL-6 may be responsible for suppressing the activation of normal T-cells, which can cause lymphopenia.

With a constant stimulus caused by a viral infection, these cells continue to produce inflammatory mediators to reduce viral replication, but this process causes tissue damage. And to avoid it, it is necessary to maintain a balance between the immune response and the formation of anti-inflammatory mediators (IL-1)[2].

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In the most severe cases of the disease, this situation, as a rule, does not occur. However, this change will depend on the immune response of the person and on his belonging to the risk group. "Severe" patients have high production of IL-10 and IL-6, patients with moderate changes, respectively, have rather a low production of IL-6 (<100 pg/ml), and in patients with a critically high cytokine concentration (deceased), IL-6 was >100 pg/ml. IL-6 and IL-10 levels were associated with disease severity, as were TNF-α (*Tumor Necrosis Factor*-α), IL-12R (IL-12 receptor), ferritin, lymphocyte counts, neutrophils, eosinophils, and procalcitonin. A decrease in peripheral CD4+ and CD8+ T cells in the blood is observed, but we must not forget that they are locally present in the inflammatory infiltrate in the lungs.

Thus, the key point of the immune response in coronavirus infection is not only the activation factor, but also activation control, maintaining the balance of pro-inflammatory and anti-inflammatory components. And the answer will be effective when this balance is restored, which causes less damage to the body.

It is known that SARS-CoV-2 infects alveolar epithelial cells of type I and II, as well as alveolar macrophages by binding to angiotensin-converting enzyme 2 (ACE2), triggers the synthesis of type I interferon with the release of many proteins [7,8,9,10,11]. These include inflammatory cytokines γ-IFN, IL-1RA (*interleukin-1* receptor antagonist), IL-6, IL-8, IL-19, monocyte chemo attractant protein MCP-1, MCP-2, MCP-3, chemokine ligand with motif CXC - CXCL9, CXCL10, CXCL5, α-TNF. Due to the massive stimulation of T-lymphocytes, their number decreases and all these deviations are associated with the severity of the disease. Importantly, these data support the existence of a unique dysregulated immune response that could prove to be one of the most promising therapeutic approaches for COVID-19 to date [9].

Thus, one could consider an "unsuccessful" immune response to SARS as an "innate immunity stuck", since the transition to adaptive immunity with the formation of antibodies significantly correlates with favorable outcomes.

However, it is important to note that the immune pathogenesis of COVID-19 is still poorly understood, and comparisons of the immune response of patients with COVID-19 and patients with pneumonia of other origins are still insufficient. Thus, with COVID-19, there are still known only emerging variants of immune responses in various types of the course of the disease. We point out that with an evolving pathogen such as SARS-CoV-2, it is still necessary to understand the exact mechanisms that confer immune protection against SARS-CoV-2 and future viral variants.

T-cells are more difficult to study, and therefore less work in relative terms has been focused on this branch of adaptive immunity. T-cells are essential for the formation of effective humoral and cellular immunity. Without them, the host loses the ability to control normal, harmless infections and dies, as indicated in both congenital and acquired immune deficiencies (eg, AIDS). The transition between innate and adaptive immune responses is critical to determining disease

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outcomes from SARS-CoV-2 infections. Early immune responses primarily play a protective role, while dysregulated and hyper inflammatory responses can lead to a failure in virus clearance and worse disease outcomes. Accumulation of pro-inflammatory cytokines, lymphopenia, and deviant T-cell responses suggests that COVID-19 may be an immune-mediated disease.

It is now well established that severe infection induces dysregulation and hyper activation of an immune response similar to a “cytokine storm” accompanied by the impaired antiviral response. Recent data showed that in patients with COVID-19, the peripheral number of CD4+ and CD8+ T cells was significantly reduced, but the patients were generally "hyperactive" in immune status.

Based on recently published immunological data [12.13], patients with COVID-19 have a dysregulated immune response characterized by the release of multiple inflammatory cytokines such as tumor necrosis factor, and interleukins, as well as a decrease in the number of T-cells, B-cells and natural killer. In non-severe patients, T- and B-cells are also reduced.

Another notable sign of SARS-CoV-2 infection, as noted above, is lymphopenia, a common COVID-19 progression phenomenon involving major changes in B-cell composition and profound effects on T-cells. The decrease in the number of T-cells in the blood stream, combined with an increase in immune activation profiles, are likely direct consequences of the emerging antiviral T-cell response spreading in the respiratory tract. The adaptive immune system plays a key role in the clearance of SARS-CoV-2 through activated cytotoxic T-cells, which destroy infected cells, and through B-cells, which produce neutralizing antibodies against virus-specific antigens. Blood lymphopenia is a key feature of COVID-19 with a reduced number of CD4+ T-cells, CD8+ T-cells and B-cells. So, in patients not in the intensive care unit, >70.56% of cases showed a decrease in the total number of CD4+ and CD8+ T-cells. However, in the intensive care group, a total of 95% of patients showed a decrease in both total T-cells and CD4+ T-cells, and most importantly, all patients showed a decrease in CD8+ T-cells. The number of total T-cells, CD4+ and CD8+ T-cells was negatively correlated with the levels of TNF-α, IL-6 and IL-10, respectively [14].

Most of the biomarkers studied in patients with COVID-19, such as C-reactive protein (CRP), interleukin-6, procalcitonin, leukocyte count, neutrophil count, lymphocyte count, neutrophil/lymphocyte ratio, D-dimer, prothrombin time, and activated partial thromboplastin time belong to the immune-inflammatory and coagulation pathways. Other non-specific biomarkers of cellular damage and inflammation include lactate dehydrogenase and transaminases.

“Cytokine storm” in COVID-19 patients is associated with disease severity and complications such as general respiratory distress syndrome. Interleukin-6 is the most abundant cytokine secreted by active macrophages. Protective immunity to viruses depends on the activation and interaction between cytokines and chemokines to enhance/regulate innate or adaptive or both

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effector functions. Experiments in vitro and in vivo have shown that the "chemokine" environment induced by a single pathogen, through specific recruitment of T-cells in infected tissue, plays a decisive role in determining the nature of the immune response.

Coexisting conditions such as hypertension, diabetes, and obesity are associated with more severe cases of COVID-19, possibly due to a pre-existing chronic inflammatory condition or a lower threshold for developing organ dysfunction as a result of the immune response [15-17].
The main objective of this study was to analyze variants of the immune response to the COVID-19 virus. It was important to analyze the course of acute COVID-19 infection in its various immune variants with an assessment of the severity of the disease. In the work, we studied various variants of the immune response in patients with a moderately severe new corona virus infection, paying special attention to their relationship with the content of IL-6 in patients.

2. Method

2.1 Participant (Subject) Characteristics

The work examined 65 patients with a new corona virus infection hospitalized in the provisional hospital of the Budgetary Health Institution of the Voronezh Region "Voronezh Regional Clinical Center for the Prevention and Control of AIDS" in the period from 12.12.2020 to 12.29.2020. The average age of the patients was 53.3±1.5 years, in men - 36, in women - 29 years.

The clinical study was carried out in accordance with the Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, Rules clinical practice in the Russian Federation, approved by the order of the Ministry of Health of Russia dated June 19.06.2003 No. 266. All patients signed a voluntary informed consent to participate in the study.

2.3 Sampling Procedures

All patients on the 1st day of admission underwent the following studies: PCR, computed tomography of the chest, blood oxygen saturation, ECG, biochemical studies, IL-6, immunological and hematological studies.

SARS-CoV-2 was determined by PCR. Complete blood analysis was examined on a hematological analyzer (BC-5150 Mindray, China) and included the following indicators: leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, erythrocytes, hemoglobin.

Biochemical parameters (analysis on BS-120 Mindray, China) included the determination of CRP and ferritin. Ferritin was determined by ELISA (Zenyth 340rt, Biochrom, GB) using Alcorbio kits, Russia; IL-6 by ELISA using Vector Best kits, Russia. Immunophenotyping of lymphocytes was performed on a Navios flow cytometer (Beckman Coulter, USA) using labeled monoclonal antibodies also produced by this company. FITC-conjugated CD3 monoclonal antibodies were used; CD16 and CD56 conjugated with PE (phycoerythrin); CD45 conjugated

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with ECD (phycoerythrin conjugated with Texas red); CD8 conjugated with PC7 (phycoerythrin conjugated with cyanine 7), CD4 and CD19 conjugated with APC (alophycocyanin). The following populations of lymphocytes were determined: CD45+CD3+(T-lymphocytes), CD45+CD3+CD4+(T-helpers), CD45+CD3+CD8+ (T-cytotoxic), CD45+CD3-CD19+(B-lymphocytes), CD45+CD3-CD16+56+ (natural killers-effectors).

2.3.1 Sample Size, Power, and Precision

All patients were divided into three groups depending on the level of IL-6: (1) Low level (0-1 pg/ml), 25 patients; (2) Average level (1-10 pg/ml), 18 patients; (3) High level (10-300 pg/ml), 22 patients.

Our study did not include patients with an IL-6 level of more than 300pg/ml, since our preliminary studies showed that these patients were in the group of severe patients with frequent deaths. The main idea of our study was to study the second largest after the asymptomatic and mild form of patients - patients with moderate infection.

An epidemiological history was collected from all patients. The mean time from onset to hospitalization was 6.2 ± 0.28 days. In the first group 6.46 ± 0.47 days, in the second - 6.73 ± 0.61 days and in the third 5.4 ± 0.40 days.

3. Results

3.1 Recruitment

The work was carried out during 12.12.2020 to 29.12.2020.

3.2 Statistics

Statistical analysis was performed using Microsoft Excel. Descriptive statistics of quantitative traits were presented as the central trend of the median and interquartile range (25th and 75th percentiles). Group medians were compared using the Wilcox on test and the Mann-Whitney test.

3.3 Data Analysis

The most common symptoms were fever, cough, weakness, and shortness of breath. The distribution of the frequency of occurrence by groups is presented in Table No. 1.

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Table1. Frequency of occurrence of symptoms in groups

Symptoms	Number of patients in group 1	% of patients in group 1	Number of patients in group 2	% of patients in group 2	Number of patients in group 3	% of patients in group 3
Temperature > 40^0	0	0	0	0	1	4,6
Temperature 39-40^0	8	32	2	11,1	3	13,6
Temperature 38-39^0	15	60	13	72,2	14	63,6
Temperature < 38^0	2	8	1	5,6	1	4,6
Cough	24	96	15	83,3	19	86,4
Sore throat	2	8	3	16,7	0	0
Weakness	25	100	16	88.9	18	81.8
Dyspnea	5	20	6	33,3	7	31,8
Loss of smell	5	20	2	11,1	2	9,1

In the first group, co morbidities were: hypertension in 28% of patients, diabetes mellitus in 12%, coronary artery disease in 16%. In the second group, co morbidities were: hypertension in 27.8% of patients, diabetes mellitus in 16.7%, coronary artery disease in 5.6%. In the third group, concomitant diseases were: hypertension in 18.2% of patients, diabetes mellitus in 9.1%, coronary artery disease in 9.1% (table No. 2). Transferred to the intensive care unit in the first group 1 patient, in the second 1 and in the third-3.

Table2. Comorbidities in groups

Patients	HT	DM 2	CI	ACA	ChrB	BA
1 group	7	3	4	1	2	1
2 group	5	3	1		2	
3 group	4	2	2	1		1

Note. HT - hypertension, DM 2 - type 2 diabetes mellitus, CI - cardiac ischemia, stroke - acute cerebrovascular accident, Chr. bronchitis - chronic bronchitis, BA - bronchial asthma

Patients received treatment in accordance with the temporary guidelines of the Ministry of Health of the Russian Federation. Etiotropic drugs (hydroxychlorin, coronavir), proactive anti-inflammatory therapy and pathogenetic treatment, including glucocorticosteroids, low molecular weight heparins, preemptive anti-inflammatory therapy with Janus kinase and IL-6 inhibitors (olumiant and monoclonal antibodies tocilizumab [artlegia]) were prescribed.
In the first group, 50% of patients received proactive anti-inflammatory treatment, in the second group, respectively, 4%, and in the third group, 65% of patients.

The mean hospitalization time was 10.5 ± 0.34. However, the third group had the most patients (see above) transferred to the intensive care unit. The volume of lung injury in the first group was 32.7 ± 2.79%, in the second group 26.8 ± 1.98% and in the third group 34.28 ± 3.79% (M ± m).

The specific content of interleukin-6 in the groups was: 1 group – 0 (0–1) pg/ml, group 2–4 (3–6, P<0.001) pg/ml, group 3–32 (17.75–99.5, P<0.001) pg/ml. Thus, the lowest was the content of interleukin in group 1, which differed significantly from the 2nd and 3rd groups of patients. Moreover, the differences between the 2nd and 3rd groups of patients in IL-6 were also significantly different (P<0.001), and the smallest of them was the content of IL-6 in the 2nd group. Thus, the content of IL-6 in the three groups of patients was statistically increasing - 0; 4 and 32 pg/ml - from small to large.

The level of ferritin in the three groups was almost the same and did not differ statistically, and the CRP was higher in the patients of the third group compared to those of the patients of the second group (Table No. 3).

Table 3. The level of the main biomarkers in groups of patients

Index	median	quartile 1 (25%)	quartile 3 (75%)	Interquartile range	Confidence interval	$P_{1,2}$	$P_{1,3}$	$P_{2,3}$
Interleukin-6, pg/ml, 1 gr.	0	0	1	1	0,33	0,00	0,00	0,00
Interleukin-6, pg/ml, 2 gr.	4	3	6	3	1,12			
Interleukin-6, pg/ml, 3 gr.	32	17,75	99,5	81,75	30,78			
Ferritin, ng/ml, 1 gr.	394	179,25	650	420	245,23	0,877	0,217	0,332
Ferritin, ng/ml, 2 gr.	388	146	486	340	159,17			
Ferritin, ng/ml, 3 gr.	326,5	278,75	1260	981,25	348,34			
C-reactive protein, mg/l, 1 gr.	35,5	27,5	60	32,5	13,21	0,098	0,958	0,028
C-reactive protein, mg/l, 2 gr.	32,5	17	43	26	11,47			
C-reactive protein, mg/l, 3 gr.	41	25	68,75	43,75	16,41			

Note. gr - group

It is important to note that when calculating differences in parameters between groups, the median is indicated, and in brackets the values of the 1st and 3rd quartiles, for example, for the absolute values of leukocytes in the 1st group [median] and 1-3 quartiles, the values will be (Table. 4): **$3.57 \cdot 10^9$l (2.82 - 5.68)**. Those, the absolute number of leukocytes in the first group was significantly the lowest, amounting to 3.57 (2.82 - 5.68), in comparison with the values in the 2nd group [5.6 (4.64 – 7.72, P<0.014)] and the highest in 3 [8.29 (6.15 – 9.62, P<0.0001] group. The same pattern persisted for neutrophils: in the first group 2.61 (1, 88 - 3.89); 0.34% (0.27 - 0.55%, P<0.003) and 0.36 (0.29 - 0.57, P<0.0001).The relative content of monocytes also

differed in groups 1 and 2 and 3 - 3.8% (2.59 - 5.41), 5.75% (4.43 - 8.88, P<0.009) and 5.0% (3.97 – 6.7, P<0.039). In the last two cases, the value of cells was the lowest in the first group of patients.

The same pattern persisted for the Lymphocytes\Neutrophils, when the differences were highly significant between groups 1 and 3 of patients (P<0.021 and <0.01), and in group 3 it was the largest, indicating a more severe course of infection. There were no differences in the content of this cell type between groups in terms of platelets (Table No. 4). However, in patients group 1 showed a significant decrease in hemoglobin from 145•10^{12}/l (139-152) to 131 g/l (124-139, P<0.002), erythrocytes from 4.8•10^{12}/l (4.57-5.15) to 4.38•10^{12}/l (4.09-4.57), P<0.004) and hematocrit from 0.45 l/l (0.42-0.47) to 0.39 l/l (0.36-0.42, P<0.0003), which suggests a trend towards the development of anemia in the 3rd group of patients compared with 1-th group.

Table 4. Hematological markers in three groups of patients

Index	median	quartile 1 (25%)	quartile 3 (75%)	Interquartile range	Confidence interval	$P_{1,2}$	$P_{1,3}$	$P_{2,3}$
Leukocytes•10^9/l, 1 gr.	3,57	2,82	5,68	2,85	0,65	0,014	0,000	0,145
Leukocytes•10^9/l, 2 gr.	5,6	4,64	7,71	3,07	1,00			
Leukocytes•10^9/l, 3 gr.	8,29	6,15	9,62	3,47	1,60			
Neutrophils•10^9/l, 1 gr.	2,61	1,88	3,89	1,97	0,41	0,043	0,000	0,157
Neutrophils•10^9/l, 2 gr.	4,01	3,10	5,59	2,49	0,72			
Neutrophils•10^9/l, 3 gr.	6,14	4,15	8,36	4,22	1,82			
Lymphocytes•10^9/l, 1 gr.	0,92	0,71	1,12	0,42	0,14	0,059	0,006	0,486
Lymphocytes•10^9/l, 2 gr.	1,21	0,82	1,61	0,79	0,23			
Lymphocytes•10^9/l, 3 gr.	1,26	1,10	1,51	0,41	0,31			
Neutrophils %, 1 gr.	70,1	64,95	75,67	10,73	3,17	0,059	0,211	0,557
Neutrophils %, 2 gr.	72,7	66,1	80,05	13,95	5,95			
Neutrophils %, 3 gr.	79,15	66,9	85,23	18,325	5,21			
Lymphocytes %, 1 gr.	25,2	19,1	31,25	12,15	3,10	0,170	0,067	0,711
Lymphocytes %, 2 gr.	19,75	12,4	27,05	14,65	4,83			
Lymphocytes %, 3 gr.	15,9	11,48	26	14,53	4,33			
Nph %/Lph%, 1 gr.	2	2	3,75	1,75	0,54	0,131	0,021	0,835
Nph %/Lph%, 2 gr.	3	2	6	4,00	1,27			
Nph %/Lph%, 3 gr.	4	2	7	5,00	1,67			
Nph•10^9/l /Lph•10^9/l, 1 gr.	2	2	3,75	1,75	0,54	0,166	0,010	0,347
Nph•10^9/l /Lph•10^9/l, 2gr.	3	2	6	4,00	1,30			
Nph•10^9/l /Lph•10^9/l, 3 gr.	4,5	2,25	7,75	5,50	1,95			
Monocytes %, 1 gr.	3,80	2,59	5,41	2,82	0,56	0,009	0,039	0,338
Monocytes %, 2 gr.	5,75	4,43	8,88	4,45	1,17			

Monocytes %, 3 gr.	5,00	3,97	6,70	2,73	0,79			
Monocytes •10^9/l, 1 gr.	0,13	0,08	0,24	0,16	0,03	0,003	0,000	0,879
Monocytes •10^9/l, 2 gr.	0,34	0,27	0,55	0,28	0,08			
Monocytes •10^9/l, 3 gr.	0,36	0,29	0,57	0,28	0,06			
Platelets•10^9/l, 1 gr.	168,5	149	198,5	49,50	23,78	0,965	0,077	0,266
Platelets•10^9/l, 2 gr.	176,5	148	194,25	46,25	40,90			
Platelets•10^9/l, 3 gr.	211,5	176	250,75	74,75	35,48			
Hemoglobin, g/l, 1 gr.	145	139	152	13	3,45	0,161	0,002	0,107
Hemoglobin, g/l, 2 gr.	141	132,5	150,5	18	5,97			
Hemoglobin, g/l, 3 gr.	131	124	139	15	4,47			
Erythrocytes • 10^{12} / l, 1 gr.	4,8	4,57	5,15	0,585	0,135	0,301	0,004	0,039
Erythrocytes • 10^{12} / 2, 1 gr.	4,74	4,53	4,96	0,435	0,177			
Erythrocytes • 10^{12} / 3, 1 gr.	4,38	4,09	4,57	0,4775	0,137			
Hematocrit, l/l, 1 gr.	0,45	0,42	0,47	0,05	0,01	0,153	0,0003	0,036
Hematocrit, l/l, 2 gr.	0,43	0,40	0,45	0,05	0,01			
Hematocrit, l/l, 3 gr.	0,39	0,36	0,42	0,056	0,01			

Note. Nph – neutrophils, Lph – lymphocytes, gr - group

Table 5. T-cell immunity scores in these three groups

Index	median	quartile 1 (25%)	quartile 3 (75%)	Interquartile range	Confidence interval	$P_{1,2}$	$P_{1,3}$	$P_{2,3}$
T-lymphocytes ($CD45^+CD3^+$)%, 1gr.	57	45	65,25	20,25	4,88	0,016	0,00	0,124
T-lymphocytes ($CD45^+CD3^+$)%, 2 gr.	70	59,75	73,5	13,75	5,15			
T-lymphocytes ($CD45^+CD3^+$)%, 3grp.	70	64	75,75	11,75	3,58			
T-lymphocytes ($CD45^+CD3^+$)•10^9/l, 1 gr.	0,47	0,36	0,73	0,37	0,08	0,039	0,00	0,420
T-lymphocytes ($CD45^+CD3^+$)•10^9/l, 2 gr.	0,83	0,47	1,12	0,65	0,18			
T-lymphocytes ($CD45^+CD3^+$)•10^9/l, 3 gr.	0,88	0,68	1,08	0,40	0,39			
T-helpers ($CD45^+CD3^+CD4^+$)%, 1 gr.	26	20	34	14,00	3,41	0,01	0,00	0,07
T-helpers ($CD45^+CD3^+CD4^+$)%, 2 gr.	33,5	28,75	46	17,25	4,75			
T-helpers ($CD45^+CD3^+CD4^+$)%, 3 gr.	44,5	39,25	50,75	11,50	3,59			
T-helpers ($CD45^+CD3^+CD4^+$)•10^9/l, 1 gr.	0,25	0,20	0,29	0,10	0,01	0,03	0,00	0,078
T-helpers ($CD45^+CD3^+CD4^+$)•10^9/l, 2 gr.	0,38	0,26	0,59	0,33	0,11			
T-helpers ($CD45^+CD3^+CD4^+$)•10^9/l, 3 gr.	0,54	0,41	0,75	0,34	0,15			
T-cytotoxic lymphocytes($CD45^+CD3^+CD8^+$)%, 1 g.	20,5	16,25	31,75	15,50	3,76	0,938	0,938	0,227
T-cytotoxic	26	17,2	32	14,75	5,73			

lymphocytes($CD45^+CD3^+CD8^+$)%, 2 g.		5						
T-cytotoxic lymphocytes($CD45^+CD3^+CD8^+$)%, 3 g.	21,5	18,25	24	5,75	2,36			
T-cytotoxic lymphocytes ($CD45^+CD3^+CD8^+$)•10^9/l, 1 gr.	0,21	0,12	0,35	0,23	0,05	0,938	0,123	0,983
T-cytotoxic lymphocytes ($CD45^+CD3^+CD8^+$)•10^9/l, 2 gr.	0,2	0,13	0,49	0,36	0,11			
T-cytotoxic lymphocytes ($CD45^+CD3^+CD8^+$)•10^9/l, 3 gr.	0,27	0,19	0,39	0,20	0,08			
CD4/CD8, 1 gr.	1,21	0,77	1,8	1,13	0,29	0,094	0,003	0,184
CD4/CD8, 2 gr.	1,37	1,05	2,53	1,49	0,81			
CD4/CD8, 3 gr.	2,22	1,53	2,83	1,30	0,42			
B-lymphocytes ($CD45^+CD3^-CD19^+$)%, 1 gr.	13,5	10	16,75	6,75	2,16	0,711	0,177	0,463
B-lymphocytes ($CD45^+CD3^-CD19^+$)%, 2 gr.	13,5	11,25	17,5	6,25	2,87			
B-lymphocytes ($CD45^+CD3^-CD19^+$)%, 3 gr.	17,5	11,5	21	9,50	2,30			
B-lymphocytes ($CD45^+CD3^-CD19^+$)•10^9/l, 1 gr.	0,12	0,1	0,17	0,06	0,02	0,349	0,017	0,472
B-lymphocytes ($CD45^+CD3^-CD19^+$)•10^9/l, 2 gr.	0,14	0,10	0,22	0,12	0,05			
B-lymphocytes ($CD45^+CD3^-CD19^+$)•10^9/l, 3 gr.	0,18	0,12	0,29	0,16	0,08			
Natural killers ($CD45^+CD3^-CD16^+56^+$)%, 1 gr.	30	20,75	43,75	23,00	5,40	0,011	0,00	0,021
Natural killers ($CD45^+CD3^-CD16^+56^+$)%, 2 gr.	15	11	25	14,00	4,95			
Natural killers ($CD45^+CD3^-CD16^+56^+$)%, 3 gr.	12,5	7,25	17,75	10,50	2,81			
Natural killers ($CD45^+CD3^-CD16^+56$)•10^9/l, 1 gr.	0,28	0,17	0,41	0,24	0,08	0,007	0,003	0,094
Natural killers ($CD45^+CD3^-CD16^+56$)•10^9/l, 2 gr.	0,17	0,15	0,23	0,08	0,03			
Natural killers ($CD45^+CD3^-CD16^+56$)•10^9/l, 3 gr.	0,15	0,10	0,23	0,13	0,04			

Interesting results were obtained in the analysis of the immune status of patients. They indicate (Table 5) that the relative content of T-lymphocytes in the first group of patients was 57% (45–65.25) and these values were significantly lower than those in the 2nd and 3rd groups, respectively 70% (59.75–73.5, $P<0.016$) and 70% (64–75.5, $P<0.001$). Similar significant differences were also observed in absolute values in groups 1, 2 and 3, respectively - 0.47 cells (0.36–0.73)•10^9/l, 0.83 cells (0.47–1.12, $P<0.039$)•10^9/l and 0.88 cells (0.68–1.08, $P<0.001$)•10^9/l. There was no difference in the content of T- lymphocytes between the 1st, 2nd and 3rd groups.

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Similar results were obtained for the content of patients and T-helpers. Thus, the relative content of these cells was the lowest in patients of group 1 - 26% (20 - 34), significantly different from patients of groups 2 and 3 - 33.5% (28.75 - 46, P<0.039) and 44.5% (39.25 - 50.75, P <0.001). As in the case of T-lymphocytes, similar changes were found in the absolute values of T-helpers in all three groups - 1st = 0.25 cells (0.2 - 0.29)•10^9/l, 2nd = 0.38 cells (0.26 – 0.59, P<0.03) •10^9/l, 3rd = 0.54 cells (0.41 – 0.75, P<0.001) •10^9/l. Thus, the same pattern for the lowest content of T-helpers in the 1st group of patients remained in absolute terms. At the same time, as in relation to T-lymphocytes, there were no significant differences in T-cytotoxic cells between groups of patients (Table 5). It was interesting to determine the immunoregulatory index CD4+\CD8+, which was significantly higher in the 3rd group than in the 1st group of patients (P<0.003). It is very important that this index, like Neutrophils\Lymphocytes (see Tabl.4), also indicates a more severe course of the infection process Covid-19. And this is fully consistent with our data obtained on patients. In principle, the same pattern persisted for B-lymphocytes (CD45+CD3-CD19+, P<0.017), while the trend of changes in natural killer-effectors (CD45+CD3-CD16+CD56+) was completely different, since in the 3rd in the group of patients, their content was the lowest in comparison with the 2nd and 1st groups (P<0.011 and <0.0005).

4. Discussion

Thus, according to the content of IL-6, all patients were conditionally divided into 3 groups with the lowest (1), average (2) and highest (3) cytokine content, and all these groups significantly differed from each other.

Significant differences in the acute phase protein CRP were observed only between groups 2 and 3, wherein in group 3 its amount was significantly higher. According to the absolute content of leukocytes, significant differences were noted between all three groups, where their number was the smallest in the 1st group of patients. The same differences in absolute content were noted in relation to neutrophils, and it was the smallest, as in leukocytes, in the 1st group, however, there were no differences between the groups in the relative number.

It is significant that the absolute content of lymphocytes was the lowest in the 1st group, significantly differing from the third.

It is important that the percentage ratio of Neutrophils\Lymphocytes, as in relation to leukocytes, neutrophils and lymphocytes, was also significantly lower in the 1st group compared to the 3rd group of patients (P<0.021 and <0.01). Note that an increase in this index indicates a more severe course of infection. The same difference persisted for monocytes, when their content in patients of the 1st group was the smallest in comparison with the second and third.

In general, according to immune markers reflecting the state of cellular immunity, the same differences persisted - the content of total T-lymphocytes and T-helpers, respectively, turned out to be statistically significantly lower in the 1st group compared to the other groups of patients,

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while in terms of killer T-cytotoxic lymphocytes differences between the groups were not revealed.

It is significant that immune changes developed almost in unison with a wide panel of different markers. Thus, a significantly lower number of B-lymphocytes was also observed in the 1st group of patients compared to the 3rd, while the dynamics of changes in natural killer-effectors was absolutely opposite, characterized by minimal values in the 3rd group of patients compared to the 2nd and group 1 ($P<0.011$ and <0.0005), where it was the highest. It can be assumed that this is due to the severity of the course of a viral infection, when a large mass of natural killers is "spent" on neutralizing virus-infected cells in the 3rd group of patients, and a milder course of infection occurs precisely because of the activation of the cellular killer system, i.e., antitumor immunity, which is due to the growth of killers.

There were no differences in platelets and ferritin between groups of patients.

An analysis of these data suggested that the level of IL-6, apparently, can have a significant effect on the severity of T-cell immunity, and the higher the content of interleukin-6 in the blood, the higher was the severity of T-cell immunity in patients with new moderate coronavirus infection.

Crucially, IL-6 levels were positively correlated with $CD4^+$ lymphocyte count (Spearman's correlation coefficient, 0.712) and negatively with $CD8^+$ lymphocytes (Spearman's correlation coefficient, 0.576).

Thus, in patients with practically zero levels of interleukin 6 in the blood, adaptive immunity was the least pronounced, and vice versa, in patients with a high level of interleukin 6, it turned out to be the highest.

It is extremely important to note that patients of the 1st group with a practical absence of IL-6 (0-1 pg/ml) had the least pronounced course of coronavirus infection than patients of the 3rd group, in whom the level of IL-6 was significantly higher (17.75 - 99.5 pg/ml) and where the infection was somewhat more severe. This is clearly evidenced by the specific symptoms of the infection: for example, in patients of the 1st group, a temperature of 39 - $>40^0$C was recorded in 8%, in the 3rd group - in 20%, and 38-39^0C - in 15% and 70%. Cough, respectively, in 24% and 95% of patients, weakness - in 25% and 90%, and finally, shortness of breath and loss of smell - in 10% and 45% of patients.

Based on these data, it can be assumed that a sufficiently high level of interleukin-6 is necessary for the implementation of "normal" antiviral immunity with a balanced immune response in patients with a new corona virus infection of a mild form in comparison with patients with an average severity of the infection. Thus, to a certain extent, the level of IL-6 in patients with

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Covid-19 can predict the outcome of the infection with the manifestation of the severity of the infectious process.

5. Conclusion

Information about the relationship between hematological, immune and clinical parameters of patients suffering from moderately severe corona virus infection is presented. It was shown that the level of interleukin 6 correlates with the degree of change in indicators, reflecting the severity of the infectious process to a certain extent. This interesting phenomenon is discussed.

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Conflict of Interest

None declared

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