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Clinical and prognostic value of parameters of cytokine regulation in oxygen-dependent patients with the coronavirus disease COVID-19

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Aim of the work is to find out the clinical and prognostic significance of cytokine regulation parameters in oxygen-dependent patients with COVID-19 coronavirus disease in dynamics of the disease.

Material and methods. 78 oxygen-dependent patients with COVID-19 aged from 52 to 84 years were examined. All patients were examined and treated in accordance with the regulations in force at the relevant time. The patients were divided into groups: I group – 38 patients who recovered; II group – 40 patients with fatal outcome of the disease. In the blood serum of patients with COVID-19 and 20 healthy individuals, were determined by enzyme immunoassay the content of interleukin (IL) IL-2 (Elabscience, USA), IL-4 (Affymetrix eBioscience, Austria), IL-6 (Invitrogen, Austria), monocyte chemotactic protein-1 (MCP-1) (Elabscience, USA). Statistical processing was performed in the Statistica 13 for Windows program (StatSoft Inc., No. JPZ804I382130ARCN10-J).

Results. The content of IL-2 in the blood serum of oxygen-dependent patients with COVID-19 of both groups was significantly higher ($p < 0.001$) than in healthy individuals. IL-2 content was higher in patients of II group ($p < 0.001$) than in patients of I group. In dynamics, a further increase in content of IL-2 was noted in patients of the II group ($p < 0.05$). The diagnostic significance has been established of the increased level of IL-2 in assessing the high probability of the development of a fatal outcome of the disease during hospitalization (AUC = 0.698, $p = 0.030$) and in the dynamics of treatment (AUC = 0.745, $p = 0.015$).

The content of IL-6 in the blood serum of oxygen-dependent patients with COVID-19 of both groups was also significantly higher ($p < 0.001$) than in healthy individuals. However, the level of increase of this cytokine at the time of hospitalization did not have prognostic value regarding the risk of fatal outcome (AUC = 0.539, $p = 0.562$). In the dynamics of treatment, the content of IL-6 in blood serum continued to increase in patients of the II group ($p < 0.01$). During this period of observation, a threshold level of increase IL-6 level was established, which indicates a high probability of the development of a fatal outcome in these patients (AUC = 0.850, $p < 0.001$).

The content of chemokine MCP-1 in blood serum at the time of hospitalization in patients of both groups was higher ($p < 0.01$) than in healthy people. At the same time, the content of MCP-1 in patients of the II group was higher ($p < 0.05$) than in the patients of the I group. The diagnostic significance of the increased level of MCP-1 was established, which indicated a high probability of the development of a fatal outcome during hospitalization. In dynamics, there was a tendency ($p > 0.05$) to decrease its content ($p > 0.05$), however, during this period of observation, MCP-1 did not have prognostic value.

IL-4 turned out to be uninformative in prognostic terms for determining the probability of a fatal outcome of COVID-19.

Conclusions. Changes in the parameters of cytokine regulation in patients with COVID-19 during the development of oxygen dependence are characterized by a significant increase content of IL-2, IL-6 and chemokine MCP-1 in blood serum. The level of increase of these cytokines has diagnostic value in determining the high probability of the development of a fatal outcome of the disease at certain stages of observation.

Ключові слова: коронавірусна хвороба, COVID-19, вірусна інфекція, цитокіни, «цитокіновий шторм», прогноз.

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Клініко-прогностичне значення параметрів цитокінової регуляції в кисневозалежних хворих на коронавірусну хворобу COVID-19

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Мета роботи – з'ясувати клініко-прогностичне значення параметрів цитокінової регуляції в кисневозалежних хворих на коронавірусну хворобу COVID-19 у динаміці хвороби.

Матеріали та методи. У дослідження залучили 78 кисневозалежних хворих на COVID-19 віком від 52 до 84 років. Усі хворі обстежені, отримали лікування згідно з чинними на час дослідження протоколами. Хворих поділили на групи: I – 38 пацієнтів, які одужали; II – 40 осіб із летальним наслідком хвороби. У сироватці крові хворих на COVID-19 і 20 здорових осіб методом імуноферментного аналізу визначали вміст інтерлейкіну IL-2 (Elabscience, USA), IL-4 (Affymetrix eBioscience, Austria), IL-6 (Invitrogen, Austria), моноцитарного хемотактичного протеїну-1 (MCP-1) (Elabscience, USA). Статистично результати опрацювали в програмі Statistica 13 for Windows (StatSoft Inc., No. JPZ804I382130ARCN10-J).

Результати. Вміст IL-2 у сироватці крові кисневозалежних хворих на COVID-19 обох груп значно вищий ($p < 0,001$), ніж у здорових. У хворих II групи вміст IL-2 вищий ($p < 0,001$), ніж у пацієнтів I групи. У динаміці в хворих II групи визначали зростання вмісту IL-2 ($p < 0,05$). Встановили діагностичну значущість підвищення IL-2 в оцінці високої ймовірності настання летального наслідку хвороби і при госпіталізації (AUC = 0,698, $p = 0,030$), і в динаміці лікування (AUC = 0,745, $p = 0,015$).

Вміст IL-6 у сироватці крові кисневозалежних хворих на COVID-19 обох груп також значно вищий (p < 0,001), ніж у здорових осіб. Проте рівень підвищення цього цитокіну на час госпіталізації не мав прогностичного значення щодо ризику летального наслідку (AUC = 0,539, p = 0,562). У динаміці лікування у хворих II групи вміст IL-6 в сироватці крові продовжував зростати (p < 0,01). У цей термін спостереження встановили межовий рівень підвищення рівня IL-6, який вказував на високу ймовірність настання летального наслідку в цих хворих (AUC = 0,850, p < 0,001).

Вміст хемокіну MCP-1 у сироватці крові на час госпіталізації в пацієнтів обох груп вищий (p < 0,01), ніж у здорових. Концентрація MCP-1 у хворих II групи вища (p < 0,05), ніж у пацієнтів I групи. Встановили діагностичну значущість підвищення MCP-1, що під час госпіталізації вказувала на високу ймовірність настання летального наслідку. В динаміці встановили тенденцію (p > 0,05) до зниження його вмісту (p > 0,05), але в цей термін спостереження MCP-1 не мав прогностичного значення.

Прогностично неінформативним для визначення ймовірності ризику летального наслідку COVID-19 виявився IL-4.

Висновки. Зміни параметрів цитокінової регуляції хворих на COVID-19 при розвитку кисневої залежності характеризуються значним підвищенням вмісту IL-2, IL-6 і хемокіну MCP-1 у сироватці крові. Рівень підвищення цих цитокінів має діагностичне значення щодо визначення високої ймовірності настання летального наслідку хвороби на певних етапах спостереження.

Today it is known that the clinical course of the coronavirus disease (COVID-19) is characterized by significant polymorphism, from an almost asymptomatic course to the development of an extremely severe acute respiratory distress syndrome. Due to the tropism of SARS-CoV-2 to type II alveocytes, 15 % of patients with COVID-19 develop pneumonia, which is one of the main causes of death in patients with coronavirus disease [1]. Data from modern literature show that the development of acute respiratory distress syndrome in the case of COVID-19 is not so much related to the viral load as to the formation of an excessive immune response, which leads to a significant release of pro-inflammatory cytokines, that it is a development of the so-called "cytokine storm" [1,2].

In addition, the presence of the S-protein in the structure of the virus, which is the main determinant of its virulence, determines tropism of the pathogen SARS-CoV-2 not only to alveocytes of type II, but also to other cells that express the receptor of angiotensin-converting enzyme type 2 on their membrane. These cells also include cells of the intestinal mucosa, epithelial cells of the distal kidney tubules, endothelial cells, etc. [3]. Therefore, acute uncontrolled hyperproduction of pro-inflammatory cytokines has a multisystem character and causes the development of multiple organ insufficiency, which requires hospitalization of such patients in the Department of Anesthesiology and Intensive Care (ICU) [1,4,5].

Today, the research of immunopathogenetic mechanisms, which are the basis of the formation of the "cytokine storm", continues. One of the explanations is the development of virus-induced cell pyroptosis, which is a powerful inflammatory form of lytic-programmed cell death, which is accompanied by the release of pro-inflammatory cytokines and causes the migration of macrophages and lymphocytes [6,7]. Significant migration of lymphocytes into lung tissue explains the development of lymphopenia in severe and critical patients [8]. In addition, there is some evidence of changes in innate immunity, namely a decrease interferon activity during SARS-CoV-2 replication, which explains why macrophages, dendritic cells, and neutrophils initiate an immune response as the primary defense of the body [6,9]. Evidence of this is also significant infiltration by macrophages of lungs and mucous membrane of the bronchi during the pathomorphological examination of the deceased [10]. Modern research works show numerous evidences of the role of high levels of pro-inflammatory cytokines and excessive activation of immunocompetent cells in formation of an unfavorable prognosis of COVID-19 [4,11,12,13].

Therefore, research continues today to clarify the clinical-pathogenetic role of certain immune changes and search for the most informative prognostic immunological parameters in patients with the new coronavirus disease COVID-19.

Aim

The purpose of the work is to find out the clinical and prognostic value of the parameters of cytokine regulation in oxygen-dependent patients with the coronavirus disease COVID-19 in dynamics of the disease.

Material and methods

The research included 78 oxygen-dependent patients with coronavirus disease COVID-19 who were treated at the ICU of the Municipal Non-Profit Enterprise "Regional Infectious Clinical Hospital" Zaporizhzhia Regional Council" during 2020–2021. In all patients, the diagnosis of COVID-19 was confirmed by isolation of RNA-SARS-CoV-2 in nasopharyngeal mucus by polymerase chain reaction method. All patients were not vaccinated against COVID-19. The age of the patients ranged from 52 to 84 years. There were 42 men, 36 women.

All patients were examined and treated in accordance with the regulations in force at the relevant time: Order of the Ministry of Health of Ukraine dated 28.03.2020 No. 722 "Organization of medical care for patients with coronavirus disease (COVID-19)" (as amended by the order of the Ministry of Health of Ukraine dated 17.09.2020 No. 2122 "On amendments to the Standards of medical care "Coronavirus disease (COVID-19)". Order of the Ministry of Health of Ukraine No. 10 dated 07.01.2021 "On approval of amendments to the Standards of medical care "Coronavirus disease (COVID-19)". Order of the Ministry of Health of Ukraine dated April 6, 2021 No. 638 "Protocol for the provision of medical assistance for the treatment of the coronavirus disease (COVID-19)".

In order to find out the clinical and prognostic value of parameters of cytokine regulation, oxygen-depend-

Table 1. Comparison of the cytokine content in the blood serum of oxygen-dependent patients with the coronavirus disease COVID-19 in dynamics, Me [Q_{25}; Q_{75}]

Indicator, units of measurement	Healthy individuals (n = 20)	I group (n = 38)		II group (n = 40)	
		at the time of admission	after 5–7 days	at the time of admission	after 5–7 days
IL-2, pg/ml	16.82 [2.74; 30.90]	95.43 [47.63; 296.97][1]	252.96 [164.00; 332.51][1,3]	237.10 [168.68; 309.45][1,2]	366.62 [328.51; 494.28][1,2,3]
IL-4, pg/ml	0.06 [0.04; 0.08]	0.08 [0.06; 0.16][1]	0.08 [0.06; 0.14]	0.12 [0.06; 0.20][1]	0.09 [0.06; 0.21][1]
IL-6, pg/ml	0.62 [0.24; 0.96]	5.05 [2.74; 16.5][1]	4.39 [1.77; 5.40][1]	5.04 [4.34; 19.77][1]	9.92 [5.98; 25.48][1,2,3]
MCP-1, pg/ml	18.3 [15.9; 19.45]	38.42 [24.55; 51.08][1]	40.45 [23.69; 75.75][1]	53.48 [34.43; 91.71][1,2]	52.05 [19.55; 123.74][1]

1: the difference is significant, compared to healthy individuals ($p < 0.01$); **2:** compared to the I group patients in the corresponding period of observation ($p < 0.05$); **3:** compared with hospitalization of patients of the corresponding group ($p < 0.05$).

ent patients with COVID-19 were divided into groups depending on the outcome of the disease: group I – 38 patients who recovered; group II – 40 patients with a fatal outcome of the disease. The groups of patients did not differ statistically of gender ($p > 0.05$) and age ($p > 0.05$), and at the time of hospitalization to the ICU, they did not statistically differ in the degree of severity of acute respiratory insufficiency ($p > 0.05$) and the frequency of the need for non-invasive lung ventilation at the time of observation ($p > 0.05$).

The research was carried out in compliance with the provisions of the "Rules of Ethical Principles of Conducting Scientific Medical Research with Human Participation" approved by the Declaration of Helsinki and the legislation of Ukraine. All patients were included in the research on a random sign and provided written informed consent.

The content of interleukin (IL) IL-2 (Elabscience, USA), IL-4 (Affymetrix eBioscience, Austria), IL-6 (Invitrogen, Austria), monocyte chemotactic protein-1 (MCP-1) (Elabscience, USA), according to the instructions provided by the manufacturers, was determined in the blood serum of all patients with COVID-19 and 20 healthy individuals of the control group using the enzyme immunoassay method. Enzyme immunoassays were conducted on basis of the Educational Medical Laboratory center of Zaporizhzhia State Medical and Pharmaceutical University (scientific consultant – PhD, DSc, R. O. Shcherbina).

Statistical processing was performed in the Statistica 13 for Windows program (StatSoft Inc., No. JPZ804I382130ARCN10-J). Non-parametric statistical methods were used in the work, the investigated quantitative parameters are presented in the form of Me [Q_{25}; Q_{75}]. The Mann–Whitney test was used to assess the reliability of differences between quantitative traits in independent samples. The Wilcoxon test was used to assess the reliability of differences between quantitative traits in dependent samples. ROC analysis was used to cut off the threshold level of the indicator.

Results

Based on the results of the research, it was established that in oxygen-dependent patients with COVID-19, changes in cytokine regulation system were characterized by a significant increase in the level of pro-inflammatory cytokines. Patients were admitted to ICU in the second week of illness, namely patients of the I group by 9.0 [7.0; 11.0] day of illness and patients of the II group by 8.0 [6.0; 11.0] day of illness ($p > 0.05$).

The analysis of the content of IL-2 in blood serum showed that when patients were hospitalized to the ICU due to the development of oxygen dependence, the level of IL-2 was significantly higher, compared to the level of healthy individuals, as in patients of group I (by 5.7 times, $p < 0.001$), as well as in patients of the II group (by 14.1 times, $p < 0.001$). A comparison of IL-2 content in blood serum showed that at the time of hospitalization, this indicator was statistically significantly higher ($p < 0.05$) in the II group of patients, who later died of the disease, compared to the I group of patients, who later recovered. The analysis of the IL-2 content in blood serum in dynamics after 5–7 days of treatment showed that in patients of the I group this indicator had only a tendency to increase ($p > 0.05$), while in patients of the II group a further statistically significant increase was noted growth ($p < 0.05$) (*Table 1*).

The content of IL-6 in blood serum during hospitalization of oxygen-dependent patients to ICU was 8.1 times higher, compared to the indicators of healthy individuals in patients of group I ($p < 0.001$) and in patients of group II ($p < 0.001$). At the same time, the degree of its increase at the time of hospitalization when comparing patients of the I and II groups did not statistically differ ($p > 0.05$). Analysis of IL-6 content in blood serum in dynamics after 5–7 days of treatment showed that the IL-6 content in blood serum had a tendency ($p > 0.05$) to decrease in the I group patients who subsequently recovered. However, in patients of the II group, conversely, the level of IL-6 continued to increase and was higher ($p < 0.01$), compared to the content of this cytokine during hospitalization of patients of the corresponding group. Comparison of IL-6 content in the blood serum of patients of the investigated groups after 5-7 days of treatment also showed a higher level in patients of the II group, compared to the I group (by 2.3 times, $p < 0.05$) (*Table 1*).

The analysis of the content of IL-4 in the blood serum of patients with COVID-19 showed its moderate increase in patients of both investigated groups at the time of hospitalization, compared to healthy people ($p < 0.05$). In dynamics of patients of the I group, a clear trend towards its decrease was noted ($p > 0.05$), compared to the corresponding indicator at the time of hospitalization, and after 5–7 days of treatment, this indicator did not statistically differ from the content of IL-4 in the blood serum of healthy individuals ($p > 0.05$). In patients of the II group, the content of IL-4 in blood serum also had a tendency ($p > 0.05$) to decrease, but after 5–7 days of treatment it remained higher (by 1.5 times, $p < 0.05$) than in healthy individuals (*Table 1*).

When analyzing the content of MCP-1 in blood serum, which is a chemokine, it was established that at the time of hospitalization to the ICU due to the appearance of

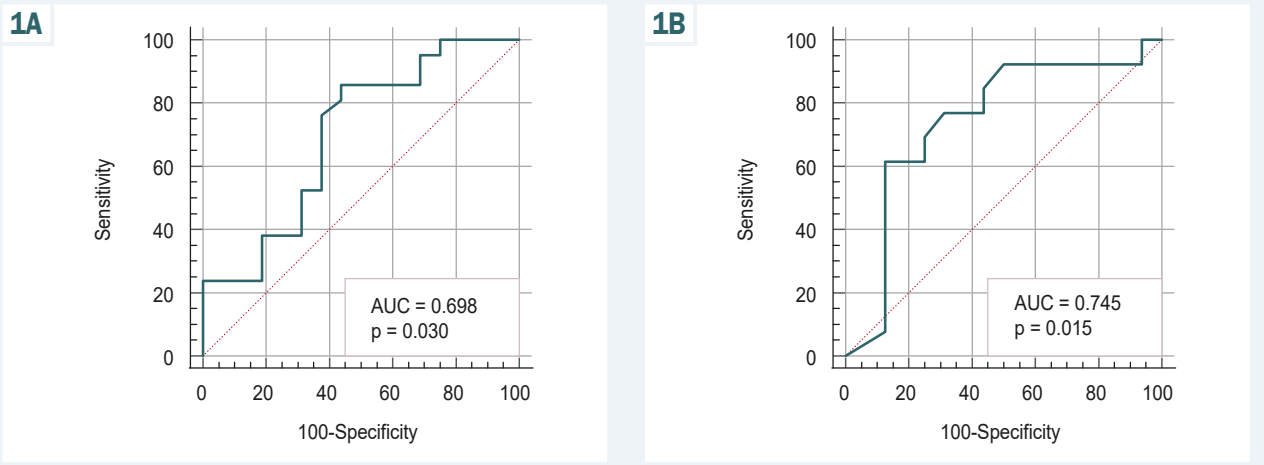

Fig. 1. Prediction of the probability of the development a fatal outcome of the disease in oxygen-dependent patients with COVID-19 according to the level of increased IL-2 in blood serum in the dynamics of observation.

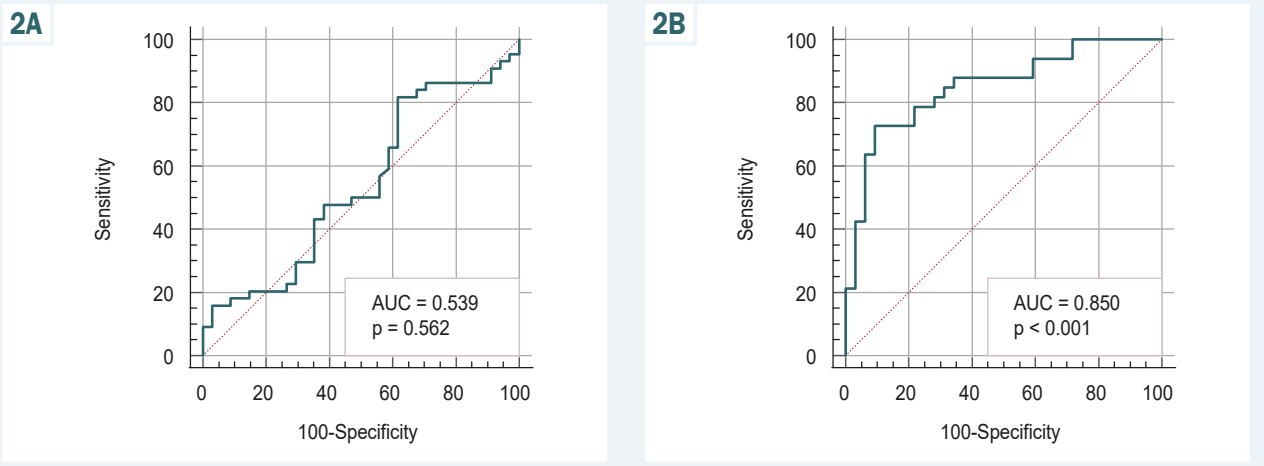

Fig. 2. Prediction of the probability of the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 according to the level of increased IL-6 in blood serum in the dynamics of observation.

oxygen dependence, this indicator was higher in patients of both investigated groups than in healthy people: in patients of the I group by 2.1 times ($p < 0.01$), in patients of the II group by 2.9 times ($p < 0.01$). A comparison of the content of MCP-1 in blood serum of patients of investigated groups at the time of hospitalization to the ICU showed a statistically significantly higher ($p < 0.05$) its content in patients of the II group, who later died of the disease, compared to patients of the I group, who later recovered. In dynamics against the background of treatment, a tendency ($p > 0.05$) to decrease the content of MCP-1 in blood serum was noted in patients of both investigated groups, however, this indicator after 5–7 days of treatment remained higher ($p < 0.01$) than in healthy individuals (*Table 1*).

ROC analysis was carried out in the further part of our research, taking into account the established changes in the content of the investigated cytokines to assess their diagnostic significance in assessing the risk of developing an adverse course of the disease of COVID-19.

When assessing the diagnostic significance the content of IL-2 in blood serum at the time of hospitalization of patients with COVID-19 to the ICU with the appearance of oxygen dependence, according to the results of the ROC analysis, it was established that the threshold level of IL-2 in blood serum, which indicates the high probability of the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 was 96.65 pg/ml (AUC = 0.698, p = 0.030) (sensitivity – 85.71 %, specificity – 56.25 %) (*Fig. 1A*). The level of IL-2 in blood serum retained its diagnostic significance in the dynamics of observation. Namely, after 5–7 days of treatment, the threshold level of IL-2 in blood serum, which indicates a high probability of developing a fatal outcome of the disease in these patients, was 338.03 pg/ml (AUC = 0.745, p = 0.015) (sensitivity – 61.54 %, specificity – 87.50 %) (*Fig. 1B*).

Analysis of the diagnostic significance the content of IL-6 in blood serum of patients with COVID-19 showed that at the time of hospitalization of patients to the ICU, the level of increase of this cytokine in relation to the assessment of the prognosis of the fatal outcome of the disease turned out to be uninformative (AUC = 0.539, p = 0.562) (*Fig. 2A*). However, according to the results of the ROC analysis, after 5–7 days of treatment of oxygen-dependent patients, a threshold level of increase of this cytokine

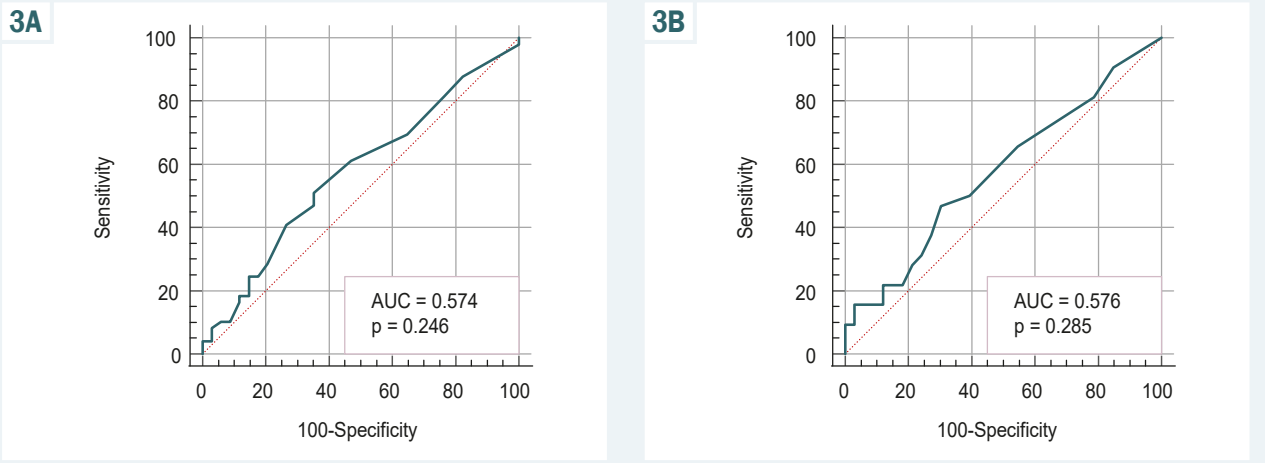

Fig. 3. Prediction of the probability of the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 according to the level of increased IL-4 in blood serum in the dynamics of observation.

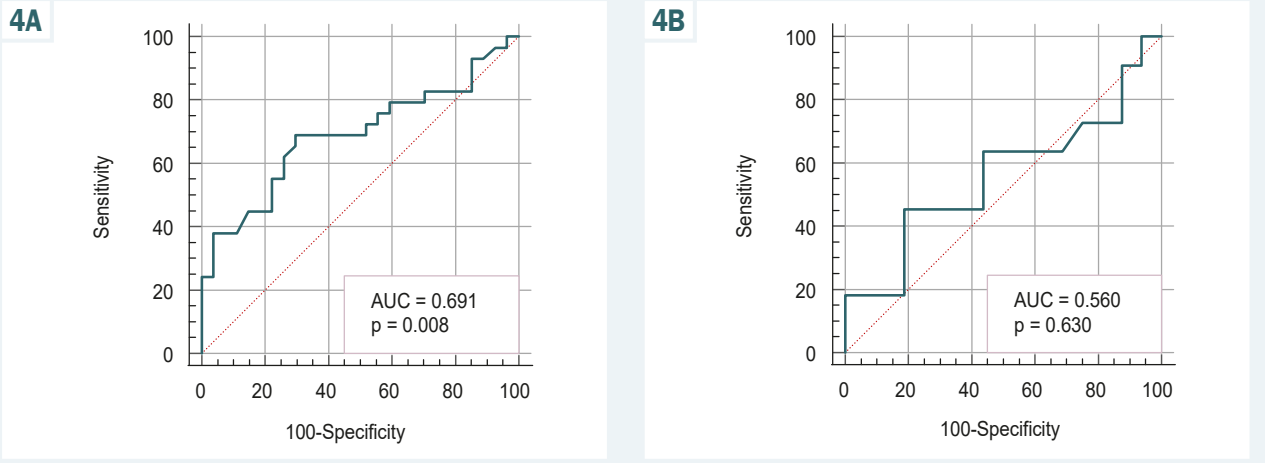

Fig. 4. Prediction of the probability of the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 according to the level of increased MCP-1 in blood serum in the dynamics of observation.

of 7.34 pg/ml was established, which indicates a high probability of the development of a fatal outcome in these patients (AUC = 0.850, p < 0.001) (sensitivity – 72.73 %, specificity – 90.62 %) (*Fig. 2B*).

When conducting ROC analysis, the diagnostic significance of IL-4 content in blood serum for predicting the development of a fatal outcome in oxygen-dependent patients with the coronavirus disease COVID-19 was not established. The threshold level content of IL-4 in blood serum was not established at the time of hospitalization of patients to the ICU (AUC = 0.574, p = 0.246) (*Fig. 3A*), and in the dynamics after 5–7 days of treatment (AUC = 0.576, p = 0.285) (*Fig. 3B*).

The conducted ROC analysis to establish the diagnostic significance of the MCP-1 content in blood serum for predicting the development of a fatal outcome in oxygen-dependent patients with the coronavirus disease COVID-19 established a threshold level of this cytokine at the time of hospitalization of 46.41 pg/ml, which indicates a high probability development of a fatal outcome in these patients (AUC = 0.691, p = 0.008) (sensitivity – 68.97 %, specificity – 70.37 %) (*Fig. 4A*). However, in the dynamics of observation after 5–7 days of treatment, this indicator no longer had a prognostic value (AUC = 0.560, p = 0.630) (*Fig. 4B*).

Discussion

In research devoted to elucidating the immunopathogenesis of COVID-19, IL-6 attracts special attention. This cytokine is able to produce almost all immune cells, endothelial cells, fibroblasts, etc. The chemical structure of IL-6 is a glycoprotein that can exhibit pro-inflammatory and anti-inflammatory properties [14]. The main immunomodulatory role of IL-6 is related to the development of the pro-inflammatory $CD4^+$ T-cell response. Under the influence of IL-6, the cytotoxic activity of $CD8^+$ lymphocytes is stimulated, IL-6 is able to regulate the activity of T-helper type 17, etc. In addition, IL-6 plays a role in differentiation of B-lymphocytes and the subsequent production of antibodies [15,16]. In research devoted to the study of immunopathogenesis of COVID-19, the relationship between an elevated level of IL-6 and the development of a "cytokine storm" has been proven [9,17,18,19,20]. The highest level of IL-6 is associated with the development of a severe course of COVID-19 and its complications.

Thus, in the research [17] it was demonstrated that high levels of IL-6 in blood serum were recorded in 86.8 % of hospitalized patients, of which 22.3 % had a level of increase of this cytokine more than 10 times higher than that of healthy people. Taking into account the certain prognostic value of this cytokine, proposals have been made for continuous measurement content of IL-6 in patients with COVID-19 [21,22].

According to the results of our research there was demonstrated a significant increase in contents of IL-6 in blood serum of oxygen-dependent patients who were hospitalized to the ICU compared to healthy people ($p < 0.001$). However, it should be noted that at the time of admission of oxygen-dependent patients with COVID-19 to the ICU, the level of increase of this indicator had no prognostic value in determining the risk of a fatal outcome (AUC = 0.539, p = 0.562). In the research [9], a similar pattern was also obtained regarding the content of IL-6 in hospitalized patients. It was demonstrated that the level of its increase in hospitalized patients who needed and did not need intensive therapy did not differ statistically [9]. The data obtained in our research made it possible to demonstrate the prognostic significance of measuring content of IL-6 in the dynamics of treatment of oxygen-dependent patients with COVID-19. Namely, after 5–7 days of treatment in ICU, the threshold level of this cytokine was calculated, which indicated a high probability of a fatal outcome of the disease (AUC = 0.745, p = 0.015).

In the research [23], the authors also pay attention to the peculiarities of the dynamics of the immune response in patients with a severe course of COVID-19. At the end of the second week of the disease that intensity of the immune response increases more than it should, which is accompanied by the release of a significant amount of pro-inflammatory cytokines and is associated with severity of the disease and higher mortality [23]. In the research [17], it was also proven that in hospitalized patients with COVID-19, the number of neutrophils increases, and the number of monocytes in blood decreases in accordance with the increase in the level of IL-6, which indicates the inability of IL-6 to fulfill its protective and regulatory role in cellular structures of the innate immunity of these patients, and the continuation of the acute phase and the accumulation of neutrophils lead to tissue damage.

In research devoted to the study of the features of the "cytokine storm" in patients with SARS-CoV-2 infection, it was noted that during its formation, a wide range of cytokines, including chemokines, are released [24,25]. Chemokines are proteins with low molecular weight and potent chemotactic activity that play a role in the recruitment of immune cells during inflammation. Their chemotactic abilities are caused by binding to receptors that are associated with G-protein, which are expressed on the membranes of leukocytes and endothelial cells [26,27]. One of the leading chemokines is MCP-1, the production of which is induced by products of oxidative stress, other cytokines , or growth factors. Monocytes and macrophages are the main source of MCP-1, which regulates the migration of T-cells and natural killer cells [28]. When studying the immunopathogenesis of COVID-19, an elevated level of MCP-1 was found in bronchoalveolar lavage fluid [29] and postmortem in lung tissue [30], which is a clear evidence of the participation of MCP-1 in the pathogenesis SARS-CoV-2 infection. In the research [31] it was proven that the expression of MCP-1 increases rapidly in early phase of infection and then progressively decreases. This gave the reason to the researchers to claim that monitoring the level of MCP-1 and therapeutic response to increasing the level of this chemokine may be a promising strategy to prevent the progression of COVID-19 from a mild to severe course.

According to the results of our research, an increase in the level of MCP-1 in blood serum of oxygen-dependent patients with COVID-19 at the time of hospitalization to the ICU was established, compared to healthy people. At the same time, the MCP-1 content in blood serum was statistically significantly higher in patients who subsequently died of the disease than in patients who subsequently recovered. We established a threshold level of MCP-1 elevation, which indicated a high probability of a fatal outcome of the disease (AUC = 0.691, p = 0.008) precisely at the time of admission to the ICU. The regularity established by us is also confirmed by the results of research by other authors, who found that the level of MCP-1 increase in blood serum is higher in those who required hospitalization to the ICU, which indicates a connection between chemokines and lung damage and, accordingly, the severity of the disease [9]. However, it should be noted that some researchers report that MCP-1 content in blood serum is also elevated in patients with a mild form of the disease of COVID-19 [32], so the question remains open regarding the diagnostic and prognostic value of this indicator for patients with a mild course of COVID-19.

The production of pro-inflammatory cytokines in significant quantities during the development of a «cytokine storm» indicates the hyperactivity of the immune system, which causes the migration of immunocompetent cells into the lung tissue, primarily monocytes and T-lymphocytes. This leads to the formation of inflammatory infiltrates in interstitium of the lungs with a predominance of mononuclear lymphocytes, which is combined with the development of significant lymphopenia with the circulation of hyperactive T-lymphocytes in peripheral blood [4,33]. "Cytokine storm" is characterized by an increase in the level of many pro-inflammatory cytokines IL-1, IL-6, IL-7, tumor necrosis factor-α, interferon-γ, in particular IL-2 [1,4,19,20]. It is believed that IL-2, using the IL-2R-JAK-STAT5 signaling pathway, induces the differentiation of $CD4^+$ T-lymphocytes, $CD8^+$ T-lymphocytes, and normal killer cells [34]. This explains the detection of a high level of IL-2 and its soluble receptors in the severe course of COVID-19 [11]. However, the role of IL-2 in the immunopathogenesis of COVID-19 remains insufficiently elucidated. There are also isolated research that demonstrate, conversely, a decrease IL-2 content during a severe course of COVID-19 [35].

The results of our research demonstrate a significant increase in the level of IL-2 in blood serum of oxygen-dependent patients with COVID-19 upon admission to the ICU and a further increase its level in the dynamics of treatment after 5–7 days. We established a certain diagnostic significance content of IL-2 in blood serum at the time of hospitalization (AUC = 0.698, p = 0.030) and in the dynamics of treatment (AUC = 0.745, p = 0.015).

We set the corresponding threshold levels IL-2 in blood serum indicates a high probability of the development of a fatal outcome of the disease in these patients. The results of our research coincide with the results of most other researchers, who also confirm the association of a high level of IL-2 in blood serum with severe and critical course of COVID-19 [1,4,11,19,20]. A research [9] also demonstrated that the level of increased IL-2 in blood serum is higher in those who required hospitalization for ICU, suggesting a link between this cytokine and lung damage and, accordingly, disease severity. Today, it is suggested that one of the causes of lymphopenia, the progression of which is considered to be an unfavorable prognostic sign [36,37], should be considered lymphocyte apoptosis, which is precisely associated with hypercytokinemia [38,39].

Much fewer works are devoted to clarifying the role of anti-inflammatory cytokines in the immunopathogenesis of COVID-19. However, in the research [9] attention was drawn to the fact that SARS-CoV-2 infection initiated a much more pronounced secretion of T-helper type 2 cytokines, in particular IL-4 and IL-10, in contradistinction to SARS-CoV infection, in which significant increase the level of these cytokines was not observed [40]. It is considered that SARS-CoV-2 antigens contribute to the release of interferons of types I and III, IL-1β, IL-4, IL-6 and interferon-γ [41]. However, when assessing the cytokine profile in patients with a severe course of COVID-19, according to researchers [42], low concentrations of type I interferons and increased levels of tumor necrosis factor-α, interferon-γ, IL-1β, IL-4, IL-6, IL-7 and chemokines were detected. The results of our research also demonstrate an increase the levels of the studied cytokines, however, the level of increase pro-inflammatory cytokines was significantly higher than IL-4. In addition, we did not establish the diagnostic significance of the increased level of IL-4 in assessing the risk of an adverse outcome of the disease when patients are admitted to the intensive care unit and in the dynamics after 5–7 days of treatment.

Conclusions

1. In oxygen-dependent patients with COVID-19, the content of pro-inflammatory cytokines IL-2 and IL-6 is significantly increased at the time of hospitalization ($p < 0.01$), compared to healthy people. Diagnostic value in determining the high probability of death and the development of a fatal outcome COVID-19 at the time of hospitalization has an increased level of IL-2 (AUC = 0.698, $p = 0.030$), and in the dynamics of treatment as IL-2 (AUC = 0.745, $p = 0.015$), and IL-6 (AUC = 0.850, $p < 0.001$).

2. At the time of admission of oxygen-dependent patients with COVID-19, the content of chemokine MCP-1 in blood serum is higher than in healthy individuals ($p < 0.001$), and the level of its increase during this period of observation has diagnostic value in determining a high probability of the development of a fatal outcome of the disease (AUC = 0.691, $p = 0.008$). In the dynamics of treatment, the content of this cytokine tends to decrease ($p > 0.05$), but its level no longer has a prognostic value.

3. The content of anti-inflammatory cytokine IL-4 in blood serum of oxygen-dependent patients with COVID-19 during hospitalization is slightly increased ($p < 0.05$), compared to healthy patients. The level of increased IL-4 has no diagnostic value in terms of determining the probable stage and development of the fatal outcome of the disease at all stages of observation.

Prospects for further research. In our opinion, a promising direction for further research is to establish the prognostic significance of cytokine regulation parameters in patients with a severe and critical course of COVID-19 when various options of immunotropic treatment are used.

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