

The role of clinical and immunological parameters in predicting the effectiveness of additional immunotropic therapy in oxygen-dependent patients with COVID-19 coronavirus disease

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Aim. The purpose of the research is to find out the role of clinical and immunological parameters in predicting the effectiveness of additional immunotropic therapy in oxygen-dependent patients with the coronavirus disease COVID-19.

Material and methods. 79 oxygen-dependent patients with COVID-19 were examined, who received additional therapy with tocilizumab according to current protocols. The patients were divided into groups: I group – 39 patients who recovered; group II – 40 patients with a fatal outcome of the disease. The content of ferritin (Monobind Inc., USA), interleukin (IL) IL-2 (Elabscience, USA), IL-6 (Invitrogen, Austria), monocyte chemotactic protein-1 (MCP-1) (Elabscience, USA) was determined in the blood serum of patients and persons of the control group by the method of immunoenzymatic analysis. Statistical processing of the obtained data was carried out in the program Statistica 13 for Windows (StatSoft Inc., No. JPZ8041382130ARCN10-J).

Results. Threshold levels of immunological parameters at different stages of observation, which have prognostic value regarding the risk of a fatal outcome of COVID-19, have been established. After 5 days of tocilizumab administration, the preservation of the indicator of the absolute number of blood lymphocytes $\leq 1.2 \times 10^9/l$ (AUC = 0.631, p = 0.039), CRP level >67.5 mg/l (AUC = 0.670, p = 0.020), IL-2 >309.45 pg/ml (AUC = 0.761, p = 0.013), IL-6 >4.66 pg/ml (AUC = 0.871, p < 0.001) indicated a high probability of developing a fatal outcome of the disease in patients with COVID-19. The analysis of the diagnostic significance of ferritin showed the informativeness of the level of its increase as at the time of the start of observation >548.02 ng/ml (AUC = 0.718, p = 0.004) and after 5 days the application of additional immunotropic therapy >443.55 ng/ml (AUC = 0.736, p = 0.026).

It has been proven that, in addition to immunological parameters, when assessing the probability of a fatal outcome of COVID-19, such clinical parameters as the presence of febrile fever at the time of the appearance of oxygen dependence (p < 0.05) and the duration of oxygen dependence at the time of additional immunotropic therapy with tocilizumab are informative (p < 0.05). Under the conditions of additional immunotropic therapy with tocilizumab more than 4 days after the onset of oxygen dependence, the probability of ineffectiveness of the specified additional treatment was significant (AUC = 0.756, p < 0.001).

Conclusions. The dynamics of immunological parameters in oxygen-dependent patients with COVID-19 during additional immunotropic therapy with tocilizumab has certain features with different treatment results. When predicting the probable risk of a fatal outcome of the disease, clinical parameters are informative, namely the appearance of febrile fever during the development of oxygen dependence and the duration of oxygen dependence until the moment of tocilizumab administration, and immunological parameters, namely the level of the absolute content of lymphocytes, ferritin, CRP, IL-2, IL-6 in the blood.

Key words:

coronavirus disease, COVID-19, viral infection, oxygen dependence, immunology, cytokines, clinic, diagnosis, treatment, prognosis.

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Роль клінічних та імунологічних параметрів у прогнозуванні ефективності додаткової імуотропної терапії в кисневозалежних хворих на коронавірусну хворобу COVID-19

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Мета роботи – з'ясувати роль клінічних та імунологічних параметрів у прогнозуванні ефективності додаткової імуотропної терапії в кисневозалежних хворих на коронавірусну хворобу COVID-19.

Матеріали та методи. Обстежили 79 кисневозалежних хворих на COVID-19, які отримали додаткову терапію тоцилізумабом, відповідно до чинних протоколів. Пацієнтів поділили на групи: I – 39 осіб, які одужали; II – 40 хворих із летальним наслідком. Методом імуноферментного аналізу в сироватці крові хворих та осіб контрольної групи визначали вміст феритину (Monobind Inc., USA), інтерлейкіну (IL) IL-2 (Elabscience, USA), IL-6 (Invitrogen, Austria), моноцитарного хемотактичного протеїну-1 (MCP-1) (Elabscience, USA). Статистично результати опрацювали в Statistica 13 for Windows (StatSoft Inc., ліцензія № JPZ8041382130ARCN10-J).

Результати. Встановлено межові рівні імунологічних параметрів на різних етапах спостереження, що мають прогностичне значення щодо ризику летального наслідку COVID-19. Через 5 діб після введення тоцилізумабу збереження показника абсолютної кількості лімфоцитів крові $\leq 1,2 \times 10^9/l$ (AUC = 0,631, p = 0,039), рівня CRP $>67,5$ mg/l (AUC = 0,670, p = 0,020), IL-2 $>309,45$ pg/ml (AUC = 0,761, p = 0,013), IL-6 $>4,66$ pg/ml (AUC = 0,871, p < 0,001) свідчило про високу ймовірність настання летального наслідку хвороби в пацієнтів із COVID-19. Аналіз діагностичної значущості феритину

Ключові слова:

коронавірусна хвороба, COVID-19, вірусна інфекція, киснева залежність, імунологія, цитокіни, клініка, діагностика, лікування, прогноз.

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показав інформативність рівня його підвищення і на початку спостереження ($>548,02$ ng/ml; AUC = 0,718, $p = 0,004$), і через 5 діб від застосування додаткової імунотропної терапії ($>443,55$ ng/ml; AUC = 0,736, $p = 0,026$).

Крім імунологічних, під час оцінювання ймовірності летального наслідку COVID-19 інформативними є такі клінічні параметри: наявність фебрильної лихоманки на час виникнення кисневої залежності ($p < 0,05$), тривалість періоду кисневої залежності на час додаткової імунотропної терапії тоцилізумабом ($p < 0,05$). Якщо додаткова імунотропна терапія тоцилізумабом проведена більш ніж через 4 доби від виникнення кисневої залежності, ймовірність неефективності цього додаткового лікування значуща (AUC = 0,756, $p < 0,001$).

Висновки. Динаміка імунологічних параметрів у кисневозалежних хворих на COVID-19 під час додаткової імунотропної терапії тоцилізумабом має певні особливості при різних результатах лікування. Під час прогнозування ймовірного ризику летального наслідку хвороби інформативними є клінічні (виникнення фебрильної лихоманки при розвитку кисневої залежності та тривалість кисневої залежності до введення тоцилізумабу) та імунологічні (рівень абсолютного вмісту лімфоцитів, феритину, CRP, IL-2, IL-6 у крові) параметри.

The coronavirus disease COVID-19 is accompanied by the development of acute respiratory distress syndrome with the appearance of oxygen dependence in almost every fifth patient, which is currently explained by the formation of an excessive immune response with the production of a significant number of pro-inflammatory cytokines [1,2]. Such acute uncontrolled hyperproduction of pro-inflammatory cytokines is called “cytokine storm”, which causes not only lung damage, but also the development of multiple organ insufficiency [1,3]. This explains the role of excessive activation of immunocompetent cells in the formation of adverse consequences of COVID-19 [4,5]. In researches that study the features of the “cytokine storm” in the case of coronavirus disease COVID-19, attention is drawn to a significant increase of IL-6 content [6,7,8]. It is believed that the main role of IL-6 in the immunopathogenesis of COVID-19 is related to the formation of a pro-inflammatory CD4⁺ T-cell response, stimulation of the cytotoxic activity of CD8⁺ lymphocytes, differentiation of B-lymphocytes into plasma cells with subsequent production of antibodies [7,9].

Understanding the leading role of immune-dependent mechanisms in the progression of COVID-19 led to the rapid search for options for effective immunotropic therapy of this disease for oxygen-dependent patients. However, in the conditions of the COVID-19 pandemic, only the repurposing of drugs as a method of developing drugs for the treatment of patients with SARS-CoV-2 infection made it possible to significantly reduce the period of time compared to the creation and research of *de novo* drugs [10].

The first randomized research on the feasibility of using corticosteroids in the treatment of oxygen-dependent patients with COVID-19 made it possible not only to answer this question, but also to determine the minimum dose that has a therapeutic effect [11]. It was proven in the British randomized research RECOVERY that the introduction of dexamethasone at a dose of 6 mg per day, compared to placebo, allowed to reduce the mortality rate (22.9 % vs. 25.7 %) [11]. A subsequent clinical research comparing the efficacy of different doses of dexamethasone in patients with COVID-19 and severe hypoxemia showed that increasing the dose of dexamethasone to 12 mg/day compared to 6 mg/day did not result in a statistically significant mortality reduction when patients were followed for 28 days [12]. Studies that were devoted to finding out the effectiveness of other corticosteroids were stopped immediately after receiving the RECOVERY results. Since that corticosteroids became a standard of

treatment for oxygen-dependent patients with COVID-19 [13,14].

However, on the one hand, insufficient effectiveness of the use of corticosteroids in oxygen-dependent patients, and on the other hand, the appearance of numerous researches, which proved the association of a high level of IL-6 with the development of a severe course of COVID-19 and the risk of a fatal outcome [6,15,16], determined the relevance of the use of cytokine-targeted therapy as an additional treatment by corticosteroids [17]. Therefore, the IL-6 receptor inhibitor (tocilizumab), which is a recombinant humanized monoclonal antibody to IL-6 receptors, was quickly introduced into clinical practice for severe forms of COVID-19, and currently it is used for the treatment of rheumatoid arthritis as an immunobiological drug [18]. However, prescribing tocilizumab for patients with COVID-19 remained outside the instructions for medical use (“off-label”), despite its inclusion in a number of international recommendations [19,20].

In accordance with existing international recommendations, tocilizumab was also included in the Protocol for the provision of medical assistance for the treatment of coronavirus disease (COVID-19) and approved by the Order of the Ministry of Health of Ukraine as an additional therapy to corticosteroids in severe and critical course of the disease, in which rapid decompensation of breathing is noted. This made it possible to use this variant of immunotropic treatment in patients who belong to the risk group due to the presence of comorbid conditions [21,22]. However, the results of various studies regarding the effectiveness of the use of tocilizumab in clinical practice have certain discrepancies. On the one hand, the literature presents research that demonstrate a decrease in the mortality rate among patients with COVID-19 who received additional treatment with tocilizumab [23,24]. On the other hand, according to the results of the research [25], it was proved that there are no advantages of additional treatment with tocilizumab.

Therefore, the above determines the expediency of finding out the dynamics of immunological changes in oxygen-dependent patients with COVID-19 who received additional therapy with tocilizumab, but had different disease outcomes, in order to determine informative prognostic predictors of the effectiveness of such immunotropic treatment. To find out the role of clinical and immunological parameters in predicting the effectiveness of additional immunotropic therapy in oxygen-dependent patients with the coronavirus disease COVID-19.

Material and methods

79 oxygen-dependent patients with COVID-19 were examined, who during 2020–2021 were treated in the Department of Anesthesiology and Intensive Care (ICU) of the Communal Non-Profit Enterprise “Regional Infectious Clinical Hospital of the Zaporizhzhia Regional Council”. The diagnosis of COVID-19 was confirmed by the isolation of RNA-SARS-CoV-2 in nasopharyngeal mucus or sputum by the polymerase chain reaction method. All patients were not vaccinated against COVID-19. The age of the patients was from 28 to 85 years. There were 41 men and 38 women. All patients were examined and treated in accordance with current regulations. Order of the Ukraine Ministry of Health (UMoH) dated 28.03.2020 No. 722 “Organization of medical care for patients with coronavirus disease (COVID-19)” (as amended by the order of the UMoH dated 17.09.2020 No. 2122 “On amendments to the Standards of medical care “Coronavirus disease (COVID-19)”. Order of the UMoH No. 10 dated 07.01.2021 “On approval of Amendments to the Standards of medical care “Coronavirus disease (COVID-19)”. Order of the UMoH from April 6, 2021 No. 638 “Protocol for the provision of medical care for the treatment of coronavirus disease (COVID-19)”.

Taking into account the possibility of additional immunotropic therapy with tocilizumab for oxygen-dependent patients in which a positive effect is not achieved after the appointment of corticosteroids, our research included patients who received additional therapy with tocilizumab at a dose of 800 mg intravenously.

To clarify the role of immunological parameters in predicting the effectiveness of additional immunotropic therapy with tocilizumab in oxygen-dependent patients with the coronavirus disease COVID-19, patients were divided into groups depending on the outcome of the disease: group I – 39 patients who recovered; group II – 40 patients with a fatal outcome of the disease. Charlson's comorbidity index [26] was used to compare groups of patients according to comorbid pathology.

The research was carried out in compliance with the provisions of “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 basis and provided written informed consent.

Special research methods included the determination of ferritin content in the blood serum of all patients with COVID-19 and 20 healthy individuals of the control group by the method of immunoenzymatic analysis of ferritin content (Monobind Inc., USA), interleukin (IL), IL-2 (Elabscience, USA), IL-6 (Invitrogen, Austria), monocyte chemoattractant protein-1 (MCP-1) (Elabscience, USA) according to the instructions provided by the manufacturers. Enzyme immunoassays were conducted on the basis of the Educational Medical Laboratory center of the Zaporizhzhia State Medical and Pharmaceutical University (scientific consultant – PhD, DSc, Associate Professor R. O. Shcherbina).

Statistical processing of the obtained data was carried out in the program Statistica 13 for Windows (StatSoft Inc., No. JPZ804I382130ARCN10-J). Non-parametric statistical methods were used in the work, the investigated

Table 1. Comparison of basic demographic indicators and oxygen status parameters of oxygen-dependent patients with the coronavirus disease COVID-19

| Indicator, units of measurement | Patients with COVID-19 | |
|--|------------------------|-------------------|
| | I group (n = 39) | II group (n = 40) |
| Age, Me [Q 25; Q 75] | 62.0 [55.0; 71.0] | 66.0 [54.0; 71.0] |
| Men, abs (%) | 23 (59.0) | 18 (45.0) |
| Women, abs (%) | 16 (41.0) | 22 (55.0) |
| Charlson comorbidity index | 5.0 [2.0; 5.0] | 5.0 [3.5; 6.0] |
| Hospitalization to ICU, day of illness | 9.0 [8.0; 11.0] | 8.5 [7.0; 10.0] |
| Term of additional use of tocilizumab, day of illness | 12.0 [11.0; 14.0] | 12.5 [10.0; 14.0] |
| Air oxygen saturation index at the time of hospitalization in the intensive care unit, % | 84.0 [80.0; 88.0] | 79.0 [77.5; 84.5] |

quantitative parameters are presented in the form of Me [Q25; Q75]. The χ^2 test was used to assess the reliability of differences between qualitative characteristics, the Mann–Whitney test was used between quantitative characteristics in independent samples, the Wilcoxon test was used between quantitative characteristics in dependent samples, and ROC-analysis was used to cut off the threshold level of the indicator.

Results

Based on the results of the analysis of demographic indicators of oxygen-dependent patients with COVID-19 who received additional immunotropic therapy with tocilizumab, it was established that the patients of the I and II studied groups did not statistically differ in terms of age ($p > 0.05$), gender ($\chi^2 = 1.54$, $p > 0.05$), comorbidities according to the Charlson comorbidity index ($p > 0.05$). In addition, the patients of the research groups at the time of hospitalization to ICU with the appearance of oxygen dependence did not statistically differ in terms of duration of the disease ($p > 0.05$); oxygen saturation in air ($p > 0.05$); the day of the disease, on which additional therapy with tocilizumab was applied ($p > 0.05$) (Table 1). In patients of the II group, the fatal outcome of the disease was recorded at 25.5 [18.0; 29.0] day of illness.

Changes in immunological indicators during the development of oxygen dependence were characterized in patients of both research groups by a decrease in the absolute number of lymphocytes, a significant increase the level of acute-phase indicators (CRP and ferritin), pro-inflammatory interleukins (IL-2 and IL-6) and the chemokine MCP-1. The analysis of the changes in these parameters 5 days after the application of additional immunotropic therapy with tocilizumab showed a clear dependence of the dynamics of the absolute number of lymphocytes, CRP, IL-2 and IL-6 on the outcome of the coronavirus disease COVID-19. Thus, in the I group of patients whose disease ended with recovery, 5 days after additional immunotropic therapy with tocilizumab, an increase the absolute number of blood lymphocytes ($p < 0.01$) and a decrease the level of CRP ($p < 0.01$) in blood serum were noted, compared with the corresponding indicators before the use of tocilizumab. In contrast to the patients who recovered, in patients of the II group, a further increase in the level of IL-2 ($p < 0.05$) and IL-6 ($p < 0.05$) in the blood serum was noted against the background of additional immunotropic treatment, as well

Table 2. Dynamics of immunological parameters of oxygen-dependent patients with COVID-19 against the background of additional immunotropic therapy depending on the outcome of the disease

| Indicator, units of measurement | Healthy individuals (n = 20) | I group (n = 39) | | II group (n = 40) | |
|----------------------------------|------------------------------|------------------------------------|---|--------------------------------------|--|
| | | to additional use of tocilizumab | 5 days after tocilizumab administration | to additional use of tocilizumab | 5 days after tocilizumab administration |
| Lymphocytes, ×10 ⁹ /l | 1.2–3.2 | 0.70 [0.60; 0.90] | 1.20 [0.70; 1.40] ² | 0.80 [0.50; 0.95] | 0.80 [0.60; 1.00] ³ |
| CRP, mg/l | <5.0 | 80.4 [47.3; 176.0] | 27.1 [6.2; 58.3] ² | 97.4 [41.3; 158.2] | 70.2 [11.0; 200.0] ³ |
| Ferritin, ng/ml | <150.0 | 502.7 [404.0; 548.3] | 411.5 [217.3; 546.0] | 549.0 [521.0; 734.0] ³ | 523.0 [483.0; 581.0] ³ |
| IL-2, pg/ml | 16.82 [2.74; 30.90] | 96.70 [45.70; 300.70] ¹ | 250.0 [151.00; 336.50] ^{1,2} | 237.10 [168.70; 309.50] ¹ | 367.57 [329.46; 494.28] ^{1,2,3} |
| IL-6, pg/ml | 0.62 [0.24; 0.96] | 4.28 [2.48; 5.18] ¹ | 4.46 [4.00; 5.00] ¹ | 4.60 [3.50; 4.80] ¹ | 8.49 [5.58; 11.26] ^{1,2,3} |
| MCP-1, pg/ml | 18.3 [15.9; 19.45] | 38.42 [20.05; 48.82] ¹ | 37.65 [20.03; 62.30] ¹ | 53.50 [36.80; 103.90] ¹ | 52.05 [19.55; 123.74] ¹ |

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

as the absence of statistically significant changes in the indicators of the absolute amount lymphocytes and CRP ($p > 0.05$) (Table 2).

Comparison the expressiveness of indicators of immunological changes in patients with COVID-19 showed that with the appearance of oxygen dependence, the absolute content of lymphocytes, the level of CRP, IL-2 and IL-6, the chemokine MCP-1 in patients of the I and II groups did not differ statistically ($p > 0.05$). During this period of observation, only the content of ferritin was higher in patients of the II group ($p < 0.05$) than in the patients of the I group. However, in the dynamics, after 5 days introduction of tocilizumab in patients of the I group, the absolute number of blood lymphocytes was statistically significantly higher ($p < 0.05$), and the content of CRP, ferritin, IL-2 and IL-6 was lower ($p < 0.05$), than in patients of the II group (Table 2).

In our opinion, the peculiarity of the dynamics of changes in immunological parameters in oxygen-dependent patients with COVID-19 with different disease outcomes with the additional use of tocilizumab, determined by us, determined in the further part of the work the expediency of finding out the diagnostic significance of the level of changes in these parameters in predicting the effectiveness of this additional immunotropic therapy in patients at different periods of observation.

When assessing the diagnostic significance of the absolute number of blood lymphocytes at the onset of oxygen dependence in patients with COVID-19, according to the results of the ROC-analysis, it was established that the threshold level of this indicator had no prognostic value (AUC = 0.515, $p = 0.824$) (Fig. 1A). However, in dynamics, after 5 days the introduction of tocilizumab, the threshold level of the absolute number of blood lymphocytes $\leq 1.2 \times 10^9/l$ was established, which indicated a high probability of the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 (AUC = 0.631, $p = 0.039$) (sensitivity – 97.50 %, specificity – 33.33 %) (Fig. 1B).

The level of increased CRP in oxygen-dependent patients at the time of the development of oxygen dependence did not have diagnostic value in predicting the probability of a fatal outcome of the disease (AUC = 0.539, $p = 0.587$) (Fig. 2A), however, after 5 days the use of additional immunotropic therapy with tocilizumab the level of CRP increase, which remains at the level of >67.5 mg/l, had a diagnostic value in predicting the probability of a fatal outcome of the di-

sease (AUC = 0.670, $p = 0.020$) (sensitivity – 51.72 %, specificity – 90.00 %) (Fig. 2B).

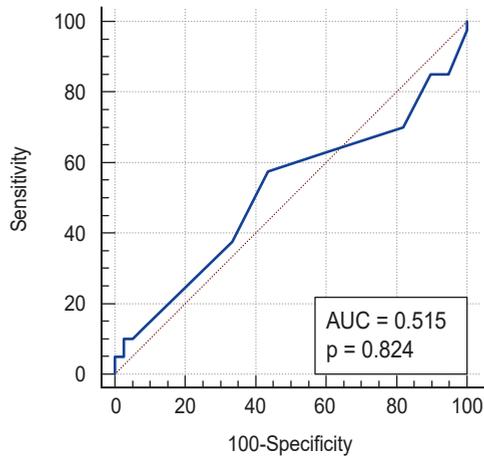
Analysis of the diagnostic significance of another acute-phase indicator, namely ferritin, showed that, unlike CRP, the level of increase of this indicator according to the data of the ROC-analysis had diagnostic significance in terms of predicting the fatal outcome of the disease in patients at the time of observation and 5 days from the moment of application of additional immunotropic therapy. So, with the development of oxygen dependence, the level of ferritin >548.02 ng/ml (AUC = 0.718, $p = 0.004$) (sensitivity – 71.43 %, specificity – 68.00 %) (Fig. 3A), and 5 days after additional use of tocilizumab, the elevated level of ferritin remained >443.55 ng/ml (AUC = 0.736, $p = 0.026$) (sensitivity – 80.00 %, specificity – 71.43 %) (Fig. 3B) indicated to the high probability of a fatal outcome of the disease.

The assessment of diagnostic significance the level of increased IL-2 content in blood serum according to the data of the ROC-analysis demonstrated that at the time of the beginning of the observation, this indicator had no diagnostic significance for assessment the risk of a fatal outcome of the disease (AUC = 0.684, $p = 0.063$) (Fig. 4A). The level of increased IL-2 in blood serum had diagnostic value after 5 days the application of additional immunotropic therapy with tocilizumab, namely, under the conditions of maintaining an elevated IL-2 content in blood serum >309.45 pg/ml, the probability of a fatal outcome was significant (AUC = 0.761, $p = 0.013$) (sensitivity – 83.30 %, specificity – 73.30 %) (Fig. 4B).

A similar pattern was obtained when assessing the diagnostic significance of the increased level of IL-6 in blood serum in dynamics of treatment with a use of additional immunotropic therapy. Thus, at the time of the beginning of observation, this indicator did not have diagnostic significance in terms of assessing the risk of a fatal outcome of the disease (AUC = 0.536, $p = 0.730$) (Fig. 5A). The diagnostic value of the increased level of IL-6 in the blood serum was 5 days after the application of additional immunotropic therapy with tocilizumab, namely, under the conditions of maintaining the elevated content of IL-6 in the blood serum >4.66 pg/ml, the probability of a fatal outcome was significant (AUC = 0.871, $p < 0.001$) (sensitivity – 93.75 %, specificity – 68.75 %) (Fig. 5B).

In addition to directly evaluating changes in immunological parameters and clarifying their diagnostic significance in predicting the probability of a fatal outcome of

1A



1B

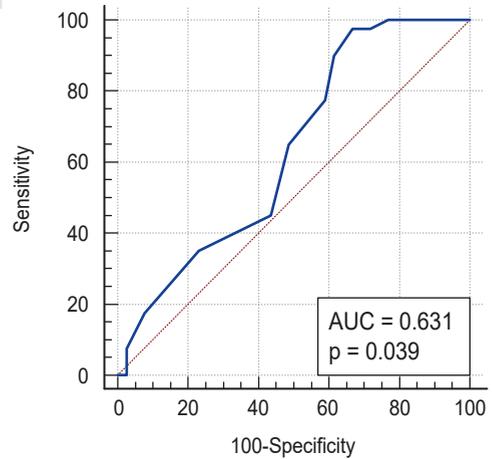
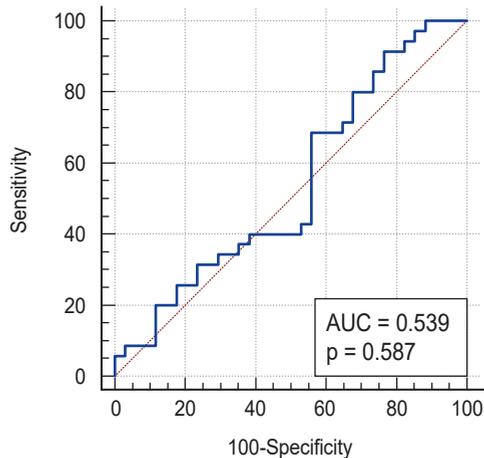


Fig. 1. Prediction of the probability of the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 who received additional immunotropic therapy, based on the indicator of the absolute number of blood lymphocytes in the dynamics of observation.

2A



2B

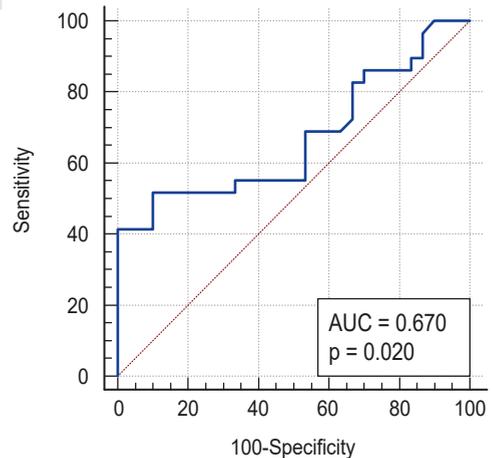
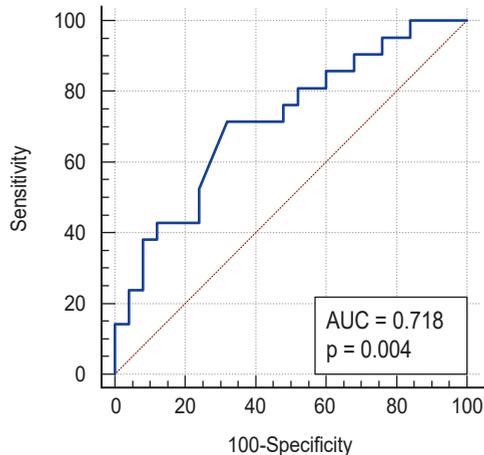


Fig. 2. Prediction of the probability of the development of fatal outcome of the disease in oxygen-dependent patients with COVID-19 who received additional immunotropic therapy, based on the CRP indicator in the blood in dynamics of observation.

3A



3B

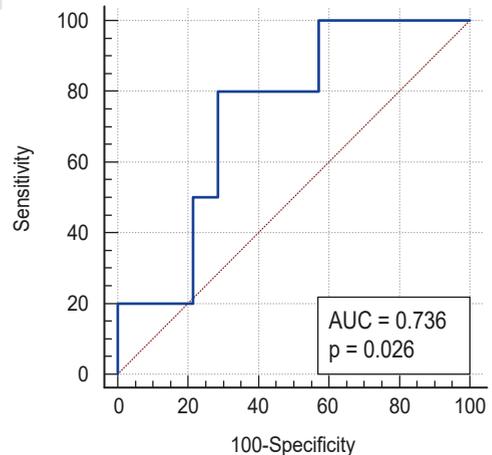


Fig. 3. Prediction of the probability the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 who received additional immunotropic therapy, based on the ferritin indicator in the blood in dynamics of observation.

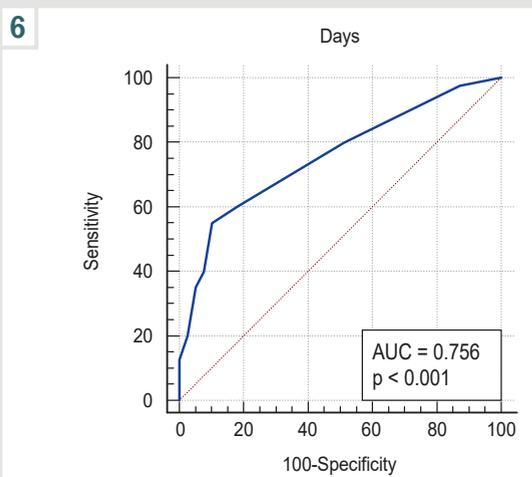
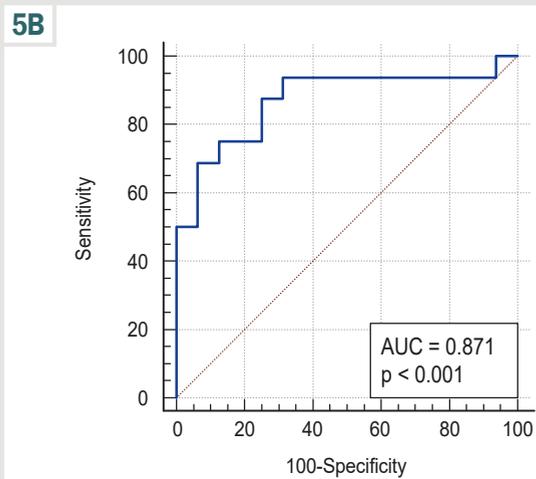
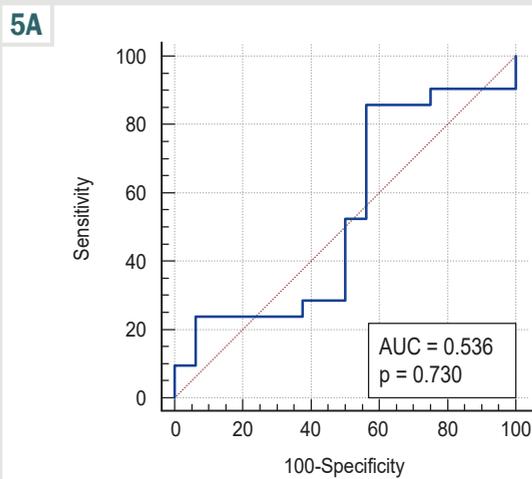
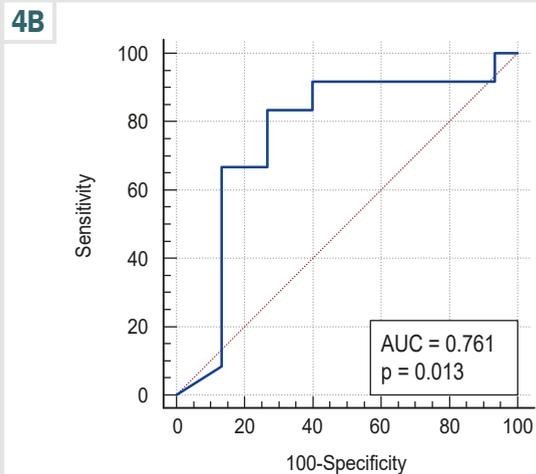
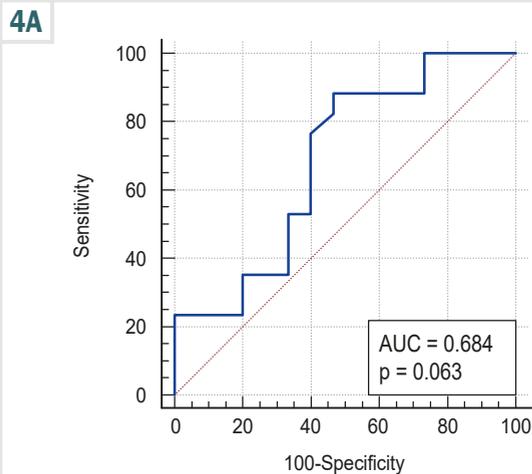


Fig. 4. Prediction of the probability the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 who received additional immunotropic therapy, based on the IL-2 indicator in the blood in dynamics of observation.

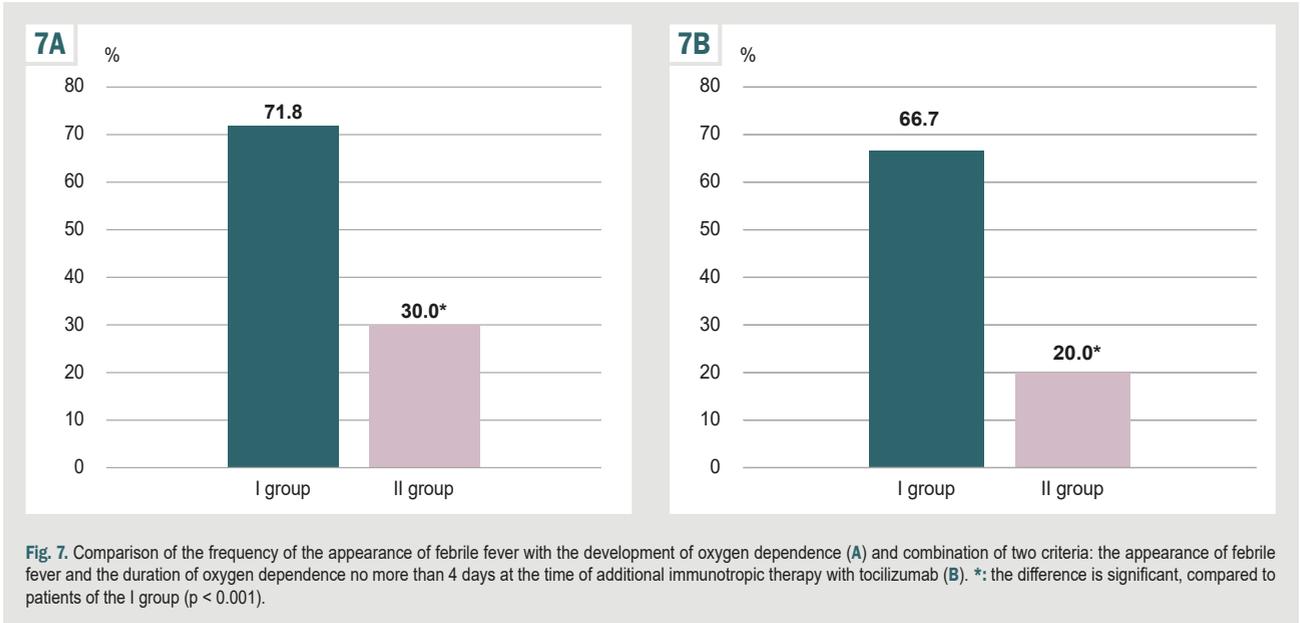
Fig. 5. Prediction of the probability the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 who received additional immunotropic therapy, according to the IL-6 indicator in blood serum in the dynamics of observation.

Fig. 6. Prediction of the probability of ineffectiveness of additional immunotropic therapy with tocilizumab according to the development of the fatal outcome of the disease in oxygen-dependent patients with COVID-19 according to the indicator of the duration of oxygen dependence at the time of application of additional immunotropic treatment.

COVID-19, in our opinion, it is also important to assess the prognostic significance of such clinical components of the “cytokine storm” as the presence of febrile fever at the time of oxygen dependence, the duration time of oxygen dependence at the time of additional immunotropic therapy with tocilizumab.

It should be noted that the patients of both investigated groups did not statistically differ in terms of the day of

illness, on which they were hospitalized to ICU with the appearance of oxygen dependence ($p > 0.05$) (Table 1). However, period of time from the moment of oxygen dependence to the moment of additional immunotropic therapy with tocilizumab in patients of the investigated groups was statistically significantly different. Thus, patients of the I group received additional immunotropic therapy with tocilizumab by 3.0 [2.0; 3.0] day of appea-



range of oxygen dependence against 5.0 [3.0; 7.0] day of patients of the II group ($p < 0.001$).

Taking into account the statistically significant difference regarding duration of additional immunotropic therapy in patients with different disease outcomes, we conducted ROC-analysis to find out the diagnostic significance of this indicator in predicting the effectiveness of specified additional therapy. According to the results of the ROC-analysis, it was established that under the conditions of additional immunotropic therapy with tocilizumab more than 4 days after the onset of oxygen dependence, the probability of ineffectiveness of the indicated additional treatment, that is the fatal outcome of the disease, was significant (AUC = 0.756, $p < 0.001$) (sensitivity – 55.00 %, specificity – 89.74 %) (Fig. 6).

An analysis of frequency of occurrence febrile fever, which accompanied the development of oxygen dependence in patients with COVID-19, and may be one of the clinical components of the “cytokine storm”, showed that its frequency was statistically significantly higher in patients of the I group who recovered ($\chi^2 = 13.8$, $p = 0.0002$) than in patients of the II group, in which the disease ended fatally: in 28 (71.8 %) versus 12 (30.0 %) patients (Fig. 7A). In addition, the frequency of combination of such two indicators as the presence of febrile fever at the time of the appearance of oxygen dependence and the duration of oxygen dependence no more than 4 days at the time of additional immunotropic therapy with tocilizumab in patients of the I group was statistically significantly higher ($\chi^2 = 21.36$, $p = 0.0001$) than in patients of the II group: in 26 (66.7 %) versus 8 (20.0 %) patients (Fig. 7B).

Discussion

According to the data of many researches [6,7,8] and meta-analysis [7], it was proved that the vast majority of patients with severe and critical course of COVID-19 have high concentrations of IL-6 and higher mortality rate [7]. Taking into account this fact, already at the beginning of the pandemic, it was assumed that the use of

IL-6 receptor antagonist (tocilizumab) is a theoretically justified therapeutic strategy to reduce the consequences of the developing “cytokine storm” [23]. Tocilizumab is a recombinant humanized monoclonal antibody that blocks soluble and membrane IL-6 receptors [27]. Tocilizumab is characterized by a non-linear pharmacological profile and has a relatively long half-life of 5 to 12 days [28]. However, despite the theoretical rationale for the use of tocilizumab, the results in clinical practice are ambiguous [23].

Today, the clinical and laboratory evaluation of the effectiveness of such immunotropic treatment remains relevant, in particular the evaluation of the ability of tocilizumab to restore the number of T-lymphocytes in the blood of patients with severe forms of COVID-19 by blocking the transmission of IL-6 signals [29]. In addition, researches continues to clarify the clinical-pathogenetic and prognostic role of various immunological parameters with the additional use of tocilizumab in patients with COVID-19 who receive systemic corticosteroids and require oxygen therapy or artificial lung ventilation [30,31].

According to the results of our research, such parameters as age, sex, comorbidity according to the Charlson comorbidity index did not have a prognostic value when assessing the risk of an adverse outcome of the disease at the time of appointment of additional immunotropic treatment with tocilizumab ($p > 0.05$). The expediency of additional immunotropic therapy with tocilizumab is also evidenced by the research [32], which demonstrated a significant difference in the mortality rate after 28 days of observation in the group of patients who received tocilizumab and the control group (16.1 % vs. 37.2 %) despite the probably higher index Charlson’s comorbidities in the group of patients who received tocilizumab.

It is known that “cytokine storm” is an uncontrolled dysfunctional immune response, the development of which is accompanied by the appearance of oxygen dependence and multiple organ insufficiency in patients with COVID-19 [33,34]. According to the results of our research, changes in immunological indicators during the development of oxygen dependence in patients

with COVID-19 were characterized in patients of both studied groups by decreasing the absolute number of lymphocytes, a significant increase the level of acute phase indicators (CRP and ferritin), pro-inflammatory interleukins IL-2 and IL-6, and chemokine MCP-1. During the “cytokine storm” in COVID-19, there is a rapid production of pro-inflammatory cytokines, which stimulate such cells as hepatocytes, Kupffer cells, and macrophages to produce acute-phase compounds, in particular, ferritin and CRP [35]. At the same time, it is believed that, on the one hand, an increase in acute phase indicators is the result of excessive immune inflammation, and on the other hand, acute phase compounds are able to activate macrophages to secrete proinflammatory cytokines [30,36].

Data from the literature show that high levels of these acute-phase indicators, caused by excessive inflammation due to infection of various etiologies, are associated with hospitalization in ICU and high mortality. Therefore, in various pathological conditions, the determination of these parameters is appropriate for identifying patients with a high risk of an adverse course of the disease, which makes it possible to justify the direction of therapeutic measures to reduce the signs of inflammation [36,37]. According to the results of our research, a significant increase in the level of ferritin and CRP was established during the development of oxygen dependence in patients with COVID-19, compared to reference values. At the time of admission to the ICU, the ferritin content was higher in patients of the II group ($p < 0.05$) than in patients of the I group, and the performed ROC-analysis showed the diagnostic significance of the level of increased ferritin >548.02 ng/ml (AUC = 0.718, $p = 0.004$) to assess the prognosis of the adverse outcome of the disease.

However, the analysis of the dynamics of acute-phase parameters that we studied during additional immunotropic therapy with tocilizumab showed that 5 days after the administration of tocilizumab, only patients of the I group, in which the disease subsequently ended with recovery, showed a decrease in level of CRP ($p < 0.01$) in the absence of statistically significant changes in the content of ferritin ($p > 0.05$) in blood serum, compared with the corresponding indicators before the use of tocilizumab. The peculiarity of the dynamics of these acute-phase indicators established by us with the additional use of tocilizumab is confirmed by the results of other research. Thus, according to the data [38], the ferritin level was the last laboratory indicator that returned to normal, compared to other acute phase parameters, in particular CRP.

Other researchers [39,40] also demonstrated that only the level of CRP, but not ferritin, was significantly reduced in case of successful treatment. However, it should be noted that assessing the prognostic significance of the dynamics of acute-phase indicators during additional treatment with tocilizumab in patients with a severe course of COVID-19 remains a difficult issue. On the one hand, the lower rate of reduction of ferritin limits its prognostic value [30], but on the other hand, even a rapid significant decrease the level of CRP after the additional administration of tocilizumab does not exclude the risk of a fatal outcome of COVID-19 [31].

When evaluating the prognostic role of the dynamics of changes in the content of cytokines during the addi-

tional administration of tocilizumab in oxygen-dependent patients with COVID-19, IL-6 deserves special attention, since when administering tocilizumab, receptors for this cytokine are blocked. According to the results of our research, it was established that at the time of the start of observation at the onset of oxygen dependence, the level of increased IL-6 did not differ statistically when comparing patients of the I and II groups ($p > 0.05$). However, in dynamics after 5 days from the moment of additional use of tocilizumab in patients of the II group, a further increase in the level of IL-6 ($p < 0.05$) in blood serum was noted, in contrast to the patients of the I group who recovered. Exactly in the dynamics of monitoring patients after additional administration of tocilizumab based on the results of ROC-analysis that it was possible to establish the threshold level of increase of this cytokine. Namely, under the conditions of preservation of the increased content of IL-6 in the blood serum >4.66 pg/ml 5 days after the additional use of tocilizumab, the probability of a fatal outcome turned out to be significant (AUC = 0.871, $p < 0.001$).

The regularity we obtained is confirmed by the results of research by other authors. Thus, researchers [31] during the assessment of the dynamics of IL-6 content in blood serum after the administration of tocilizumab in patients with COVID-19 demonstrated an initial increase, and then, under the conditions of a favorable course of the disease, a decrease the level of IL-6 after the administration of tocilizumab, as expected, given the mechanism of action of IL-6 receptor blocking. And according to the results of a meta-analysis with the inclusion of 2120 patients, it was concluded that the determination of the content of IL-6 in blood serum should be available in a planned manner to assess response to tocilizumab administration [41].

Given the understanding that in the conditions of a “cytokine storm” the production of many pro-inflammatory cytokines is uncontrolled, elucidation of the prognostic significance and dynamics of other cytokines during additional immunotropic treatment with tocilizumab is of particular interest. According to the results of our research, at the time of start observation at the onset of oxygen dependence, the content of IL-2 in the blood serum of patients of both groups was significantly increased, with a tendency to a higher level in patients of the II group. However, after additional immunotropic therapy with tocilizumab, the level of IL-2 in blood serum continued to increase, which was statistically significant in patients of the II group. In the dynamics we managed to establish a threshold level of IL-2 in blood serum >309.45 pg/ml, which indicates a high probability of a fatal outcome (AUC = 0.761, $p = 0.013$).

There are isolated researches in the scientific literature that discuss the possible role of soluble IL-2 receptors as markers of immune aggression that continues even after the administration of tocilizumab [42]. In this research, it was established that the level of soluble IL-2 receptors was increased in more than 53.7 % of patients with a severe course and 69.0 % of patients with a critical course of COVID-19, while their level continued to increase after the introduction of tocilizumab [42]. Today, the reasons for the increase the level of soluble IL-2 receptors after the

administration of tocilizumab remain unclear and require further study. However, taking into account the obtained data, it is suggested that the increase of the level of soluble IL-2 receptors after the additional administration of tocilizumab against the background of corticosteroid treatment may indicate the need for combination immunotropic therapy to modulate more than one hyperinflammatory pathway associated with T-lymphocyte dysregulation in COVID-19 [42].

The need for research in this direction is also evidenced by the results of the study of IL-2 dynamics in blood serum of patients with COVID-19, in which the disease progressed without oxygen dependence and, accordingly, the treatment did not involve the use of immunotropic therapy [43]. Namely, in this research, the normalization of the level of IL-2 was demonstrated in all patients during the period of convalescence [43].

To date, the leading role of immunopathogenetic changes in the formation of severe lung damage in patients with COVID-19 has been proven. In patients with a severe and critical course of COVID-19, lower levels of CD4⁺ and CD8⁺ T-lymphocytes in peripheral blood are determined, which are combined with a higher content of pro-inflammatory cytokines, in particular IL-6 and IL-2, which determine the migration of these cells to lung tissue [44,45]. The specified immune changes explain certain changes in the general blood analysis of patients with manifest clinical forms of COVID-19, in particular, the expressiveness of lymphopenia, which correlates with the severity of the disease and the risk of a fatal outcome [46].

According to the results of our research, an increase the absolute number of lymphocytes in the peripheral blood was noted after the use of additional immunotropic treatment in patients of the I group, who subsequently recovered. When observing the dynamics after 5 days the introduction of tocilizumab, the threshold level of the absolute number of blood lymphocytes $\leq 1.2 \times 10^9/l$ was established, which indicated a high probability of the development of a fatal outcome of the disease in oxygen-dependent patients with COVID-19 (AUC = 0.631, $p = 0.039$).

The results of other researchers [29] confirm that the number of clinical and laboratory parameters that allow evaluating the effectiveness of blocking the transmission of IL-6 signals includes the assessment of the ability of tocilizumab to restore the number of lymphocytes in the peripheral blood by stopping their migration to the lung tissue in patients with severe forms of COVID-19.

The analysis of the data of modern scientific literature and the results obtained in the course of our research indicate the significant complexity of predicting the course of COVID-19 in oxygen-dependent patients, in particular, under the conditions use of additional immunotropic therapy with tocilizumab. When predicting the effectiveness of immunotropic treatment in oxygen-dependent patients with COVID-19, several basic clinical and immunological parameters should be taken into account. In particular, taking into account the appearance of febrile fever during the development of a "cytokine storm" in most patients, researchers [31] noted that use of tocilizumab in the treatment of patients with a severe and critical course of COVID-19 contributed to a significant reduction or

normalization of temperature and many inflammatory biomarkers, however, it did not improve clinical outcomes, namely, it did not accelerate clinical improvement and did not reduce the frequency of fatal outcomes [31].

In our research, we analyzed whether the occurrence of febrile fever during the development of oxygen dependence has an effect on the effectiveness of tocilizumab. We established that the frequency of febrile fever in patients of the I group who recovered was statistically significantly higher (71.8 % vs. 30.0 %, $p = 0.0002$) than in patients of the II in which the disease ended fatally.

In the vast majority of researches devoted to finding out the effectiveness of additional immunotropic therapy with tocilizumab [23,27,28,29], the time period from the moment of oxygen dependence to the moment of tocilizumab administration is not taken into account. Only a few studies have taken this information into account. Thus, according to data [47], in a cohort research including 3924 patients with a critical course of COVID-19, a decrease in hospital mortality by 9.6 % was recorded in patients who received tocilizumab treatment in the first 2 days from the moment of admission to the ICU, compared to patients whose treatment did not include early administration of tocilizumab.

The results of our researches also indicate a higher effectiveness of the use of tocilizumab at an earlier time of the appearance of oxygen dependence ($p < 0.001$). According to the results of the ROC-analysis, we established that under the conditions of additional immunotropic therapy with tocilizumab more than 4 days after the onset of oxygen dependence, the probability of ineffectiveness of the specified additional treatment, namely the fatal outcome of the disease, was significant (AUC = 0.756, $p < 0.001$).

Conclusions

1. Certain clinical and immunological parameters should be taken into account in dynamics when predicting the likely effectiveness of additional immunotropic therapy with tocilizumab in oxygen-dependent patients with COVID-19.
2. Under the conditions of the appearance of febrile fever with the development of oxygen dependence and the combination of febrile fever with the duration of oxygen dependence no more than 4 days at the time of additional immunotropic therapy with tocilizumab, the effectiveness of the indicated treatment is higher.
3. At the time of the development of oxygen dependence the level of ferritin >548.02 ng/ml indicated high probability of fatal outcome of the disease (AUC = 0.718, $p = 0.004$). The dynamics of immunological parameters after 5 days from the moment of additional immunotropic therapy with tocilizumab has a diagnostic value in terms of predicting its effectiveness. Preservation of the absolute number of blood lymphocytes $\leq 1.2 \times 10^9/l$ (AUC = 0.631, $p = 0.039$), increased level of CRP >67.5 mg/l (AUC = 0.670, $p = 0.020$), ferritin >443.55 ng/ml (AUC = 0.736, $p = 0.026$), IL-2 content >309.45 pg/ml (AUC = 0.761, $p = 0.013$) and IL-6 >4.66 pg/ml (AUC = 0.871, $p < 0.001$) has diagnostic value in predicting the probability of a fatal outcome of the disease.

Prospects for further research, in our opinion, consist in the further determination of prognostic factors regarding the effectiveness of immunotropic treatment in patients with various comorbid conditions, which will allow further individualization of treatment.

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