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Clinical-pathogenetic and prognostic value of the nitrotyrosine level in the blood serum of patients with coronavirus disease (COVID-19) with pneumonia

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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

The aim of the research is to determine the clinical-pathogenetic and prognostic value of nitrotyrosine levels in the blood serum of patients with COVID-19 with pneumonia in the development of oxygen dependence and the risk of fatal outcome.

Materials and methods. 123 patients with COVID-19 with pneumonia were examined, who were examined and treated according to the Order of the Ministry of Health of Ukraine dated March 28, 2020 No. 722. Patients were divided into groups: I group – 32 patients with a moderate course without oxygen dependence; II group – 91 patients with a severe course with the presence of oxygen dependence. Patients in the II group were additionally divided into subgroups: II-A subgroup – 45 patients who recovered; II-B subgroup – 46 patients who died. The content of nitrotyrosine (Hycult Biotech, the Netherlands) was determined in the blood serum by the immunoenzymatic method. Statistical data processing was carried out in the program Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J).

Results. The content of nitrotyrosine in the blood serum of patients with COVID-19 with pneumonia in a severe course with the development of oxygen dependence is higher ($p < 0.001$) than in patients with a moderate course of the disease without signs of oxygen dependence. The level of its increase has an inverse correlation with the oxygen saturation index ($r = -0.53$, $p < 0.05$). When hospitalized for 9.0 [7.0; 12.0] day of the disease, under the condition of nitrotyrosine level >481.97 nmol/ml (AUC = 0.909, $p < 0.001$), the probability of developing oxygen dependence is significant. And under the conditions of nitrotyrosine level >521.96 nmol/ml during this observation period, the probability of a fatal outcome of the disease is significant (AUC = 0.842, $p < 0.001$).

The established correlations confirm the clinical-pathogenetic role of nitrotyrosative stress in the development of the "cytokine storm" and multiorgan failure. The content of nitrotyrosine correlates with the level of C-reactive protein ($r = +0.25$, $p < 0.05$), the ratio of absolute neutrophil count to absolute lymphocyte count ($r = +0.26$, $p < 0.05$), alanine aminotransferase activity ($r = +0.26$, $p < 0.05$) and glomerular filtration rate ($r = -0.27$, $p < 0.05$).

The diagnostic value of determining the level of nitrotyrosine in predicting the course of COVID-19 with pneumonia against the background of treatment after 7 days lies in the possibility of predicting the probability of a fatal outcome of the disease. Namely, the preservation of the level of nitrotyrosine >507.98 nmol/ml (AUC = 0.681, $p < 0.001$) during the specified period of observation indicates a high probability of a fatal outcome of the disease.

Conclusions. In patients with COVID-19 with pneumonia, the level of nitrotyrosine elevation in the blood serum depends on the appearance of oxygen dependence and the outcome of the disease. The highest level of nitrotyrosine is in patients with COVID-19 with pneumonia with a severe course, and the degree of increase of this indicator has diagnostic value in predicting the probability of an unfavorable disease course.

Keywords: coronavirus disease, COVID-19, viral infection, pneumonia, oxidative stress, nitrotyrosine, diagnosis, prognosis.

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Клініко-патогенетичне та прогностичне значення рівня нітротирозину в сироватці крові хворих на коронавірусну хворобу COVID-19 із пневмонією

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Мета роботи – встановити клініко-патогенетичне та прогностичне значення рівня нітротирозину в сироватці крові у хворих на COVID-19 із пневмонією в розвитку кисневої залежності та ризику летального наслідку.

Матеріали і методи. До дослідження залучили 123 хворих на COVID-19 із пневмонією, які обстежені та ліковані згідно з наказом Міністерства охорони здоров'я України від 28.03.2020 р. № 722. Хворих поділили на групи: I – 32 пацієнти з середньотяжким перебігом без кисневої залежності; II – 91 особа з тяжким перебігом і кисневою залежністю. Пацієнтів II групи додатково поділили на підгрупи: II-A – 45 хворих, які одужали; II-B – 46 осіб, які померли. У сироватці крові пацієнтів визначили вміст нітротирозину (Hycult Biotech, Нідерланди) імуноферментним методом. Статистично результати опрацювали в програмі Statistica for Windows 13 (StatSoft Inc., № JPZ804I382130ARCN10-J).

Результати. Вміст нітротирозину в сироватці крові хворих на COVID-19 із пневмонією при тяжкому перебігу з розвитком кисневої залежності вищий ($p < 0,001$), ніж у пацієнтів із середньотяжким перебігом хвороби без ознак кисневої залежності. Рівень його підвищення мав зворотну кореляцію з показником сатурації кисню ($r = -0,53$, $p < 0,05$). Під час госпіталізації на 9,0 [7,0; 12,0] день хвороби, якщо рівень нітротирозину становив >481,97 нмоль/мл (AUC = 0,909, $p < 0,001$), встановлена значуща імовірність виникнення кисневої залежності. Якщо рівень нітротирозину становив >521,96 нмоль/мл у цей період спостереження, то імовірність летального наслідку хвороби значуща (AUC = 0,842, $p < 0,001$).

Ключові слова: коронавірусна хвороба, COVID-19, вірусна інфекція, пневмонія, оксидативний стрес, нітротирозин, діагностика, прогноз.

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Встановлені кореляції підтверджують клініко-патогенетичну роль нітротирозивного стресу у розвитку «цитокінового шторму» та поліорганної недостатності. Вміст нітротирозину корелює з рівнем С-реактивного протеїну (r = +0,25, p < 0,05), показником співвідношення абсолютної кількості нейтрофілів до абсолютної кількості лімфоцитів (r = +0,26, p < 0,05), активністю аланінамінотрансферази (r = +0,26, p < 0,05) та показником швидкості клубочкової фільтрації (r = -0,27, p < 0,05).

Діагностичне значення оцінювання рівня нітротирозину в прогнозуванні перебігу COVID-19 із пневмонією на тлі лікування через 7 діб полягає у можливості прогнозування ймовірності летального наслідку хвороби. Так, збереження рівня нітротирозину >507,98 нмоль/мл (AUC = 0,681, p < 0,001) у цьому терміні спостереження свідчить про високу імовірність настання летального наслідку.

Висновки. У хворих на COVID-19 і пневмонію рівень підвищення нітротирозину в сироватці крові залежить від розвитку кисневої залежності та наслідку хвороби. Найвищий рівень нітротирозину визначено у пацієнтів із COVID-19 і пневмонією з тяжким перебігом. Ступінь підвищення цього показника має діагностичну цінність щодо прогнозування ймовірності несприятливого перебігу хвороби.

The coronavirus disease (COVID-19), the pandemic of which was declared by the World Health Organization at the beginning of 2020, continues to spread significantly [1]. COVID-19 is considered a systemic disease with a wide range of clinical manifestations [2,3]. It is estimated that 80 % of individuals infected with SARS-CoV-2 have a mild or even asymptomatic course of the disease. However, every fifth patient, COVID-19 progresses to pneumonia, which is associated with the development of a hyperinflammatory syndrome, known as the "cytokine storm", which leads to the formation of severe acute respiratory distress syndrome and multiple organ failure [4].

Elucidation of the pathogenetic mechanisms of the formation of COVID-19-associated pneumonia during the development of the "cytokine storm" continues [1]. At the same time, the literature presents two different opinions regarding the understanding of this phenomenon in the case of COVID-19. Researchers [5] suggest that it is the maladaptive excessive immune response that leads to the formation of severe pneumonia, multiple organ failure and, accordingly, a fatal outcome of the disease. However, there are researchers who claim that suppression of the immune response to the pathogen is unwarranted because hypercytokinemia may be necessary for virus elimination [6]. Elucidation of the pathogenetic mechanisms of the progression of pneumonia in COVID-19 is a necessary condition for the creation of effective pathogenetic treatment approaches, since the effectiveness of antiviral drugs in immunopathological phase of SARS-CoV-2 infection is limited [3].

Today, more and more data are accumulating about the clinical-pathogenetic role of oxidative stress in the progression of COVID-19. It is believed that the development of pneumonia and the formation of acute respiratory distress syndrome in the conditions of the "cytokine storm" causes severe hypoxia, which leads to an increase production of reactive oxygen species and consequently, the development of oxidative stress [7,8,9]. Additionally, SARS-CoV-2 infection disrupts the glucose-insulin axis, which further contributes to oxidative stress [10,11,12].

One of the main markers of cell damage under conditions of oxidative stress is nitrotyrosine, which is formed as a result of the reaction between NO and superoxide, leading to the formation of peroxynitrite, which, in turn, nitrates tyrosine residues of proteins [13,14,15]. Therefore, in several research, when determining the level of nitrotyrosine and other components of this reaction, the term nitrosative stress is even used [2,13,14]. Modern research demonstrates a direct connection between hypoxia, oxidative stress and inflammation [16,17]. Pro-inflammatory cytokines, in particular interleukin-6, interleukin-1β, tumor necrosis factor-α are regulated by transcription factors that are highly sensitive to oxidative stress [18,19]. The research [20] also demonstrated the negative impact of increased immunological and nitrosative stress response, the degree of expressiveness of which, along with hematological and hemorheological changes, was significantly higher in hospitalized patients with COVID-19 compared to healthy people.

The aforementioned factors underline, in our view, the necessity to elucidate the clinical-pathogenetic role of nitrosative stress in the formation of clinically significant forms of COVID-19, particularly in the development of pneumonia, and to determine the diagnostic significance of elevated nitrotyrosine levels in predicting the course of the disease.

Aim

The aim of the research is to elucidate the clinical-pathogenetic and prognostic value of nitrotyrosine levels in the blood serum of patients with COVID-19 with pneumonia in the development of oxygen dependence and the risk of a fatal outcome.

Materials and methods

The study included 123 patients with coronavirus disease (COVID-19) with pneumonia, who were treated at the Municipal Non-Profit Enterprise "Regional Infectious Clinical Hospital" of Zaporizhzhia Regional Council. The age of the patients ranged from 29 to 88 years. There were 57 males and 66 females. The diagnosis in all patients was confirmed by the detection of RNA-SARS-CoV-2 from the nasopharyngeal mucus by the polymerase chain reaction method. In all patients included in the research, pneumonia was confirmed by the results of chest X-ray examination or computer tomography.

All patients received examination and treatment in accordance with the Order of the Ministry of Health of Ukraine dated March 28, 2020 No. 722 "Organization of medical care for patients with coronavirus disease (COVID-19)". All patients were included in the research with informed consent.

Depending on the severity of the course, presence of oxygen dependence and consequences, patients with

Table 1. Comparison of the age of patients with coronavirus disease (COVID-19) with pneumonia depending on the presence of oxygen dependence, abs (%)

Indicator, units of measurement	Patients with coronavirus disease (COVID-19) with pneumonia			
	Young age, 25–44 years old	Middle age, 45–59 years old	Elderly age, 60–75 years old	Senile age, 76–90 years old
All patients (n = 123)	8 (6.5)	31 (25.2)	68 (55.3)	16 (13.0)
I group (n = 32)	5 (15.6)	7 (21.9)	20 (62.5)	–
II group (n = 91)	3 (3.3)*	24 (26.4)	48 (52.7)	16 (17.6)

*: the difference is significant, compared to patients of the I group ($p < 0.05$).

COVID-19 with pneumonia were divided into groups: I group – 32 patients with a moderate course without oxygen dependence; II group – 91 patients with a severe course with the presence of oxygen dependence. Patients in the II group were further divided into subgroups: II-A subgroup – 45 patients who recovered; II-B subgroup – 46 patients who died. Patients were monitored throughout the treatment period at the Municipal Non-Profit Enterprise "Regional Infectious Clinical Hospital" of Zaporizhzhia Regional Council.

The content of nitrotyrosine (Hycult Biotech, the Netherlands) was determined in the blood serum of patients by the immunoenzymatic method according to the manufacturer's instructions. Immunoenzymatic research was conducted on the basis of the Educational Medical Laboratory center of the Zaporizhzhia State Medical and Pharmaceutical University (scientific consultant – DSc R. O. Shcherbina). To find out the diagnostic significance of the level of nitrotyrosine in predicting the development of oxygen dependence and the risk of an adverse outcome of the disease, the levels were measured in the blood serum of patients upon admission and after 7 days of treatment.

A database of patients included in the research was formed in Excel program. Statistical data processing was carried out in the program Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J). The normality of the distribution was assessed using the Shapiro-Wilk test, the difference of the distribution of the studied characteristic from the normal law of distribution caused the use of non-parametric methods of statistical data processing. The results quantitative data presented as medians and interquartile ranges Me [Q25; Q75]. The Mann–Whitney criterion was used to determine differences between quantitative characteristics in independent groups, the Wilcoxon criterion was used in dependent groups, and the χ^2 criterion was used between qualitative characteristics. To establish the diagnostic significance of nitrotyrosine in predicting the risk of developing oxygen dependence and the risk of fatal outcome of COVID-19 with pneumonia, ROC-analysis with the determination of the cut-off point was performed. Spearman's correlation was used to establish relationships between quantitative characteristics. Differences at $p < 0.05$ were considered significantly significant.

Results

According to the results of the comparison the groups of patients formed, no statistically significant difference was established by sex ($p > 0.05$) and day of illness at the time of hospitalization ($p > 0.05$). Patients with COVID-19 with pneumonia were hospitalized for 9.0 [7.0; 12.0] day of illness. When comparing, it was noted that the age of patients of the II group was higher ($p < 0.01$) than that of the patients of the I group and was 67.0 [58.0; 73.0] years versus 62.0 [55.5; 65.5] years old. A statistically significantly lower level of oxygen saturation at the time of hospitalization in patients of the II group, compared to the patients of the I group, is explained by the severity of the course and the appearance of oxygen dependence, as predicted when forming groups of patients. Thus, the oxygen saturation index in the air of patients of the II group was 90.0 [86.0; 93.0] % compared to 95.0 [94.0; 97.0] % of patients of the I group ($p < 0.001$).

Taking into account the obtained statistically significant difference in the median age of the patients of investigated groups, we conducted a more detailed analysis of the age structure of patients with coronavirus disease (COVID-19) with pneumonia, included in the research, as well as comparison of nitrotyrosine content in the blood serum depending on age at admission to the hospital. It was established that 80 % (99 out of 123) of patients were middle aged and elderly age, while the frequency of patients of the specified age categories did not have statistically significant differences when comparing the I group and the II group ($p > 0.05$). It should be noted that young patients in both investigated groups were single and accounted for 6.5 % (8 out of 123) of the total number of patients included in the research. Among the patients with COVID-19 with pneumonia of the I group who did not have oxygen dependence, younger patients were registered more often than among the patients of the II group who had oxygen dependence (15.6 % vs. 3.3 %, $\chi^2 = 5.92$, $p = 0.15$). Senile age patients with COVID-19 with pneumonia accounted for 13.0 % (16 out of 123) of the total number of patients, but it should be noted that all these patients had oxygen dependence *(Table 1)*.

Taking into account the mentioned age patterns in patients with coronavirus disease (COVID-19) with pneumonia, we compared the content of nitrotyrosine in the blood serum at the time of admission to the hospital depending on age in different clinical groups. There was no statistically significant difference in the content of nitrotyrosine in the blood serum of patients with COVID-19 with pneumonia without oxygen dependence (group I) when comparing young and middle aged patients with elderly and senile age patients: 312.29 [224.27; 533.93] nmol/ml versus 334.03 [164.98; 507.98] nmol/ml ($p > 0.05$). Similar data were obtained by us in the group of patients with COVID-19 with oxygen-dependent pneumonia (II group) when comparing young and middle aged patients with elderly and senile age patients: the content of nitrotyrosine in the blood serum was 723.83 [509.95; 927.60] nmol/ml versus 741.36 [564.86; 978.04] nmol/ml ($p > 0.05$).

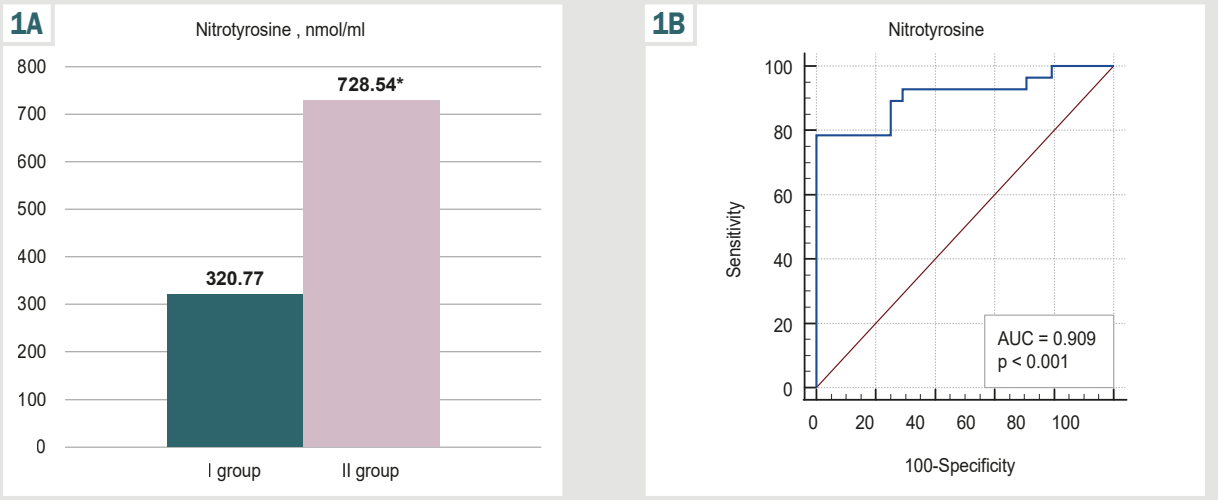

Fig. 1. The level of nitrotyrosine in blood serum (**A**) and its diagnostic significance (**B**) in patients with the coronavirus disease (COVID-19) with pneumonia during hospitalization depending on the development of oxygen dependence. ***:** the difference is significant, compared to the I group of patients ($p < 0.001$).

According to the results of the determination nitrotyrosine levels in the blood serum, it was established that in patients with the coronavirus disease (COVID-19) with pneumonia of all examined groups and in all observation periods, its levels exceeded ($p < 0.001$) those of healthy individuals, which was 9.77 [7.48; 10.50] nmol/ml.

A comparative analysis of nitrotyrosine levels at the time of hospitalization of patients with the coronavirus disease COVID-19 with pneumonia showed a higher ($p < 0.001$) level in the presence of oxygen dependence. Namely, in patients of the II group, who had a severe course with oxygen dependence at the time of hospitalization, the level of nitrotyrosine was 728.54 [519.96; 955.09] nmol/ml compared to 320.77 [281.54; 441.67] nmol/ml in patients of the I group, in whom COVID-19 with pneumonia occurred without oxygen dependence ($p < 0.001$) *(Fig. 1A)*.

To assess the diagnostic significance of nitrotyrosine levels in blood serum in predicting the risk of developing oxygen dependence at the time of hospitalization by 9.0 [7.0; 12.0] day of illness, ROC-analysis was conducted. We established that the threshold level of nitrotyrosine in blood serum >481.97 nmol/ml (AUC = 0.909, $p < 0.001$) indicated a high probability appearance of oxygen dependence in patients with COVID-19 with pneumonia (sensitivity – 78.6 %, specificity – 100.0 %) *(Fig. 1B)*.

In the further part of the research, a comparative analysis of nitrotyrosine levels in the blood serum of patients with COVID-19 with pneumonia was conducted, depending on the severity and consequences of the disease in dynamics. It should be noted that during hospitalization of patients with COVID-19 with pneumonia, the level of nitrotyrosine elevation in the blood serum clearly depended on the severity of the course of the disease, the presence of oxygen dependence and had a certain diagnostic value in assessing the risk of a fatal outcome. Thus, at the time of admission, this indicator was the highest in patients of the II-B subgroup – 776.45 [717.57; 965.07] nmol/ml and patients of the II-A subgroup was 576.85 [414.09; 807.39] nmol/ml, significantly exceeding ($p < 0.001$) the level of nitrotyrosine in blood serum of patients of the I group who did not have oxygen dependence – 320.77 [281.54; 441.67] nmol/ml. Additionally, it should be noted, that during hospitalization the level of nitrotyrosine in the blood serum of the II-B subgroup patients, who subsequently had a fatal outcome, was higher ($p < 0.01$) than in the II-A subgroup patients, who subsequently recovered *(Fig. 2)*.

In the course of treatment, after 7 days the level of nitrotyrosine in the blood serum of patients in all investigated groups decreased ($p < 0.01$), compared with the corresponding indicator at the time of hospitalization, and amounted to 207.29 [114.98; 414.09] nmol/mL in patients of the I group during this period of observation, in the II-A subgroup patients – 310.69 [265.63; 491.53] nmol/ml and in the II-B subgroup patients – 472.21 [258.20; 627.75] nmol /ml. Comparison of this indicator during the specified period of observation established that in oxygen-dependent patients of the II-A subgroup and the II-B subgroup it remained higher ($p < 0.01$) than in patients of the I group who did not have signs of oxygen dependence. However, during the specified period of observation, a statistically significant difference in the level of nitrotyrosine in the blood serum of patients of the II-A subgroup and the II-B subgroup was not detected ($p > 0.05$) *(Fig. 2)*.

In the subsequent part of our research, we conducted ROC-analysis to assess the diagnostic significance of the level of nitrotyrosine in blood serum in predicting the risk of developing a fatal outcome of COVID-19 with pneumonia at different periods of patients observation. This indicator turned out to be informative both at the time of hospitalization of the specified category of patients, and in the dynamics of observation of patients during treatment over 7 days. Thus, at the time of hospitalization of patients with COVID-19 with pneumonia, the threshold level of nitrotyrosine in the blood serum, which indicates high probability of a fatal outcome of the disease was >521.96 nmol/ml (AUC = 0.842, $p < 0.001$) (sensitivity – 92.9 %, specificity – 73.1 %) *(Fig. 3A)*. In the dynamics of treatment of patients with COVID-19 with pneumonia after 7 days of preservation the maintenance of the level

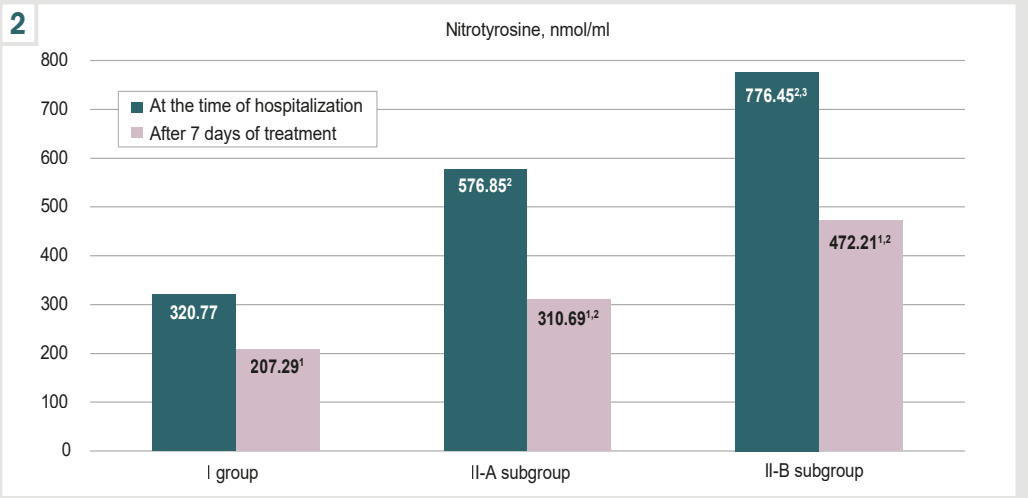

Fig. 2. Comparison of the level of nitrotyrosine (nmol/ml) in the blood serum of patients with COVID-19 with pneumonia depending on the severity and consequences of the disease in the dynamics of treatment.

[1]: the difference is significant, compared to the hospitalization of patients of the corresponding group ($p < 0.001$);
[2]: compared with patients of the I group in the corresponding period of observation ($p < 0.001$);
[3]: compared with the II-A group patients in the corresponding period of observation ($p < 0.01$).

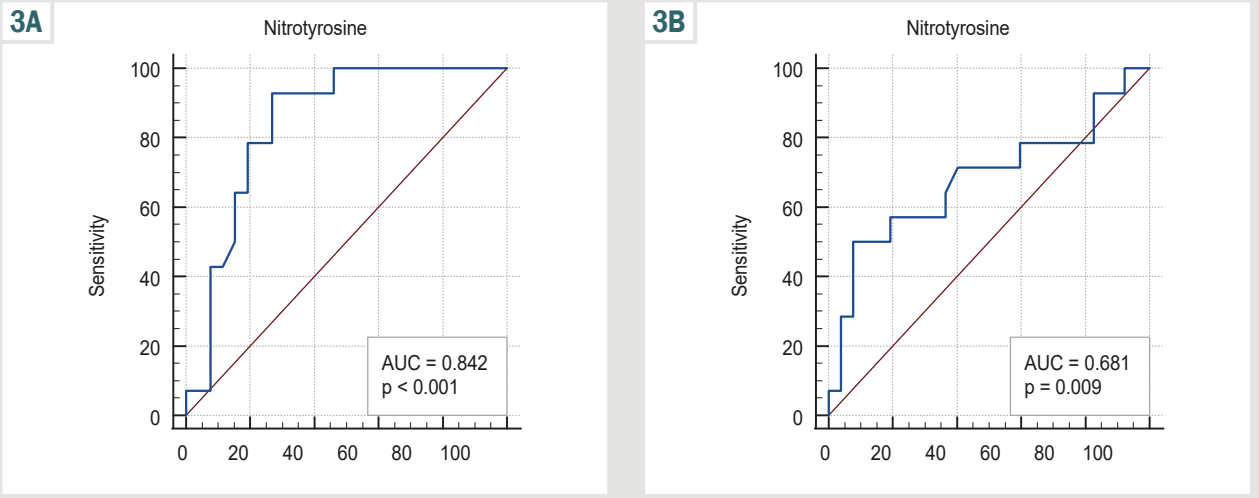

Fig. 3. The diagnostic significance of the level of nitrotyrosine in the blood serum in predicting the probability of a fatal outcome in patients with COVID-19 with pneumonia during hospitalization (**A**) and in the dynamics after 7 days (**B**).

of nitrotyrosine in the blood serum >507.98 nmol/ml (AUC = 0.681, $p < 0.001$) (sensitivity – 50.0 %, specificity – 92.3 %) indicated a high probability of a fatal outcome of the disease *(Fig. 3B)*. The fatal outcome in patients of the II-B subgroup was recorded at 27.0 [22.0; 32.0] days of illness and, respectively, by 18.0 [14.0; 23.0] day of inpatient treatment.

The clinical-pathogenetic role of nitrotyrosine in the course of COVID-19 with pneumonia was confirmed by the results of correlation analysis. The established inverse correlation of nitrotyrosine with the oxygen saturation indicator ($r = -0.53$, $p < 0.05$) confirmed the role of nitrosative stress in the development and progression of oxygen dependence. The presence of statistically significant correlations of the level of nitrotyrosine with indicators of acute inflammatory reactions, namely with the level of C-reactive protein ($r = +0.25$, $p < 0.05$) and the indicator of the ratio of the absolute number of neutrophils to the absolute number of lymphocytes ($r = +0.26$, $p < 0.05$), indicated the involvement of nitrosative stress in the development of "cytokine storm". Additionally, a direct correlation of the level of nitrotyrosine with the indicator of alanine aminotransferase activity ($r = +0.26$, $p < 0.05$) and an inverse correlation with glomerular filtration rate ($r = -0.27$, $p < 0.05$) were confirmed the role of nitrosative stress in the formation of multiorgan failure in patients with COVID-19 with pneumonia.

Discussion

It is believed that the development of COVID-19 with pneumonia is associated with the formation of a hyperinflammatory syndrome, the so-called "cytokine storm", which leads to the formation of severe acute respiratory distress syndrome and multiorgan failure [2,4]. In modern research, attention is drawn to the role of oxidative stress in the progression of pneumonia in COVID-19 and the direct correlation between hypoxia, oxidative stress and inflammation [16,17]. The development of oxidative stress is closely related to the development of endothelial dysfunction. In conditions of hypoxia, which progresses during the development of acute respiratory distress syndrome, the accumulation of oxidative stress leads to a decrease in nitric oxide (NO), as reactive oxygen species inhibit the activity of endothelial NO synthase [21]. In turn, a low level of NO enhances the proliferation of vascular

smooth muscle cells, aggregation of platelets, increased expression of chemokines and proinflammatory cytokines, in particular interleukin-6 [21]. Furthermore, the reduction of NO in conditions of oxidative stress may stimulate the production of metallomatrix proteinase, which leads to lung damage [22].

Our research revealed a clear direct correlation between the severity of nitrosative stress and the degree of lung involvement, which was confirmed by the appearance of oxygen dependence and the risk of a fatal outcome of the disease with an increasing levels of nitrotyrosine in the blood serum. Thus, the content of nitrotyrosine in the blood serum of patients with COVID-19 with oxygen-dependent pneumonia was higher ($p < 0.01$) compared to patients with a moderate disease severity without oxygen dependence at all stages of observation. Additionally, we were able to establish the threshold levels of nitrotyrosine in the blood serum of COVID-19 patients with pneumonia, which have diagnostic value for predicting the probability of developing oxygen dependence and the probability of a fatal outcome of the disease.

The results of our research, regarding the established regularity, coincide with the results of other researchers. Thus, researchers [15] demonstrated an increase in nitrotyrosine levels in the blood of patients with COVID-19, the level of which was higher under conditions of a severe course of the coronavirus disease. Additionally, it should be noted that concomitant with the increase in nitrotyrosine levels, a significant decrease in the total level of NO in the plasma of patients with COVID-19, compared to healthy individuals, was established [15]. The expressiveness of these changes partly explains the development of severe acute respiratory distress syndrome in patients with COVID-19, understanding that NO plays a crucial protective role in limiting the severity of not only cardiovascular diseases, but is also able to act as a selective pulmonary vasodilator, improving pulmonary function in patients with acute and chronic pulmonary hypertension [23].

It is assumed that SARS-CoV-2 sequesters the mitochondrial function of the affected cell and switches it from aerobic to anaerobic metabolism [11,24]. In this state, pyruvate formed from glucose during glycolysis, is oxidized to lactate, leading to an increase in the level of glucose in the cell cytosol and resulting in the formation of a limited amount of adenosine triphosphate [11,25]. Replication of SARS-CoV-2 also consumes large amount of adenosine triphosphate, so it is rapidly depleted. In this state, lactate is not metabolized by gluconeogenesis and accumulates in the blood, which leads to a disruption in glucose metabolism [26]. Furthermore, hyperglycemia, which develops during COVID-19, contributes to hyperproduction of proinflammatory cytokines and prothrombotic shifts [27,28].

SARS-CoV-2 leads to an increase production of reactive oxygen species and reactive nitrogen species, associated with increased activity of inducible NO synthase, nicotinamide adenine dinucleotide phosphate, cyclooxygenase-2 and changes in mitochondrial functions which activate transcription factors, leading to increased production of proinflammatory cytokines [29,30]. An increase in interleukin-6, tumor necrosis factor alpha activates macrophages and neutrophils, leading to the destruction of the alveolar walls, collapse of small airways, hyperpermeability of pulmonary capillaries and pulmonary edema, which, accordingly, further increases hypoxia [29].

Conclusions

1. In patients with COVID-19 and pneumonia with oxygen dependence, the level of nitrotyrosine in the blood serum is higher ($p < 0.001$), compared to patients without oxygen dependence. The role of nitrosative stress in the development and progression of oxygen dependence is confirmed by the inverse correlation between nitrotyrosine and oxygen saturation index ($r = -0.53$, $p < 0.05$). The threshold level of nitrotyrosine in the blood serum indicating a high probability of oxygen dependence in patients with COVID-19 with pneumonia was >481.97 nmol/ml (AUC = 0.909, $p < 0.001$).

2. The dependence of the level of nitrotyrosine increase on the adverse outcome of COVID-19 with pneumonia was established. In patients of the II-B subgroup, the content of nitrotyrosine at the time of hospitalization is higher compared to the I group ($p < 0.001$) and compared to the II-A subgroup of patients ($p < 0.01$). The threshold level of nitrotyrosine in the blood serum was >521.96 nmol/ml, which at the time of hospitalization indicating a high probability of the fatal outcome of the disease (AUC = 0.842, $p < 0.001$).

3. The clinical-pathogenetic role of nitrotyrosative stress in the formation of the development of the "cytokine storm" is confirmed by correlations of nitrotyrosine with indicators of acute inflammatory reactions, specifically the level of C-reactive protein ($r = +0.25$, $p < 0.05$) and the ratio of the absolute number of neutrophils to the absolute number of lymphocytes ($r = +0.26$, $p < 0.05$). The role of nitrosative stress in the formation of multiple organ failure in the conditions of the progression of COVID-19 with pneumonia is confirmed by the correlations of nitrotyrosine with the indicator of alanine aminotransferase activity ($r = +0.26$, $p < 0.05$) and the indicator of the glomerular filtration rate ($r = -0.27$, $p < 0.05$).

4. In the dynamics of treatment of patients with COVID-19 and pneumonia in all studied groups, the level of nitrotyrosine decreases after 7 days ($p < 0.01$), compared to the corresponding indicator at the time of hospitalization, but in patients with oxygen dependence it remains higher ($p < 0.01$), compared to the indicator of patients of the I group. After 7 days of treatment of patients with COVID-19 and pneumonia, preservation of the level of nitrotyrosine in the blood serum >507.98 nmol/ml (AUC = 0.681, $p < 0.001$) indicates a high probability of a fatal outcome of the disease.

Prospects for further research. In our opinion, the promising direction of this research is further clarification of the clinical-pathogenetic role of nitrosative stress in the development of endothelial dysfunction and the risk of developing fatal thrombotic complications in patients with COVID-19 and pneumonia.

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