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The endothelial NO synthase content in the blood serum of patients with coronavirus disease (COVID-19) with pneumonia in association with hemostatic parameters depending on the clinical course and its prognostic significance

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Aim: to investigate the content of eNOS in the blood serum of patients with coronavirus disease (COVID-19) with pneumonia in association with hemostatic parameters and to determine its prognostic value in assessing the risk of oxygen dependence and death.

Material and methods. There were 123 patients with COVID-19 with pneumonia under observation. All patients were examined and treated in accordance with the Order of the Ministry of Health of Ukraine No. 722 dated 28.03.2020. The eNOS content in the patients' serum was determined by enzyme-linked immunosorbent assay.

Results. It was found that the content of eNOS in the blood serum of patients with COVID-19 with pneumonia at the time of hospitalization was 9.0 [7.0; 12.0] days lower ($p < 0.001$) than in healthy subjects. The development of oxygen dependence in patients with COVID-19 with pneumonia was accompanied by worsening of endothelial dysfunction and procoagulant changes, which was confirmed by a decrease in the content of eNOS in the blood serum ($p < 0.001$), an increase in the level of fibrinogen ($p < 0.05$) and D-dimer ($p < 0.05$). The threshold level of eNOS in the blood serum ≤327.09 pg/ml (AUC = 0.861, $p < 0.001$) was predictive of the onset of oxygen dependence. In patients with COVID-19 with pneumonia who subsequently died, at the time of hospitalization, eNOS levels were lower ($p < 0.001$) than in patients who recovered, which was combined with a higher level of D-dimer ($p < 0.05$) and its more frequent increase ($p = 0.04$) compared with patients who recovered. The eNOS content in the blood serum of patients with COVID-19 with pneumonia was correlated ($p < 0.05$) not only with the lethal outcome of the disease, but also with the formation of thrombotic complications, which occurred more often in patients with COVID-19 with pneumonia in the event of an adverse outcome ($p = 0.0001$). The threshold level of eNOS in the blood serum ≤201.75 pg/ml (AUC = 0.892, $p < 0.001$) was indicative of a high probability of death.

Conclusion. The eNOS content in the blood serum of patients with COVID-19 pneumonia at the time of hospitalization is lower ($p < 0.001$) than in healthy individuals, and the degree of its decrease depends on the severity of the disease, the development of oxygen dependence and the subsequent outcome of this disease. Limiting levels of eNOS in the blood serum of patients with COVID-19 pneumonia, which are important for predicting the risk of developing oxygen dependence and fatal outcome of the disease, have been established.

Ключові слова: коронавірусна хвороба, COVID-19, пневмонія, ендотеліальна дисфункція, гемостаз, діагностика, прогноз.

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Вміст ендотеліальної NO-синтази в сироватці крові хворих на коронавірусну хворобу (COVID-19) із пневмонією у взаємозв'язку з параметрами гемостазу залежно від клінічного перебігу та її прогностичне значення

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Мета роботи – дослідити вміст eNOS у сироватці крові хворих на коронавірусну хворобу (COVID-19) із пневмонією у взаємозв'язку з параметрами гемостазу та з'ясувати її прогностичне значення під час оцінювання ризику розвитку кисневої залежності та летального наслідку.

Матеріали і методи. Під спостереженням перебували 123 хворих на COVID-19 із пневмонією. Всі пацієнти обстежені та одержали лікування відповідно до Наказу МОЗ України від 28.03.2020 р. № 722. У сироватці крові хворих визначено вміст eNOS імуноферментним методом.

Результати. Встановлено, що вміст eNOS в сироватці крові хворих на COVID-19 із пневмонією на час госпіталізації (на 9,0 [7,0; 12,0] день хвороби) нижчий ($p < 0,001$), ніж у здорових осіб. Розвиток кисневої залежності у хворих на COVID-19 із пневмонією супроводжувався поглибленням ендотеліальної дисфункції та прокоагулянтними змінами, що підтверджувало зниження вмісту eNOS у сироватці крові ($p < 0,001$), підвищення рівня фібриногену ($p < 0,05$) та D-димеру ($p < 0,05$). Межовий рівень eNOS у сироватці крові ≤327,09 pg/ml (AUC = 0,861, $p < 0,001$) мав прогностичне значення щодо виникнення кисневої залежності. У хворих на COVID-19 із пневмонією, які надалі померли, на час госпіталізації вміст eNOS був нижчим ($p < 0,001$), ніж у пацієнтів, які одужали; це поєднувалося з вищим рівнем D-димеру ($p < 0,05$) і частішим його підвищенням ($p = 0,04$) порівняно із тими, хто одужав. Вміст eNOS у сироватці крові хворих на COVID-19 із пневмонією мав кореляцію ($p < 0,05$) не лише з летальним наслідком хвороби, але і з формуванням тромботичних ускладнень, що частіше у хворих на COVID-19 із пневмонією фіксували в разі несприятливого наслідку ($p = 0,0001$). Межовий рівень eNOS у сироватці крові ≤201,75 pg/ml (AUC = 0,892, $p < 0,001$) свідчив про високу імовірність летального наслідку.

Висновки. Вміст eNOS у сироватці крові хворих на COVID-19 із пневмонією на час госпіталізації нижчий (p < 0,001), ніж у здорових осіб, а ступінь його зниження залежав від тяжкості перебігу хвороби, розвитку кисневої залежності та наслідку перебігу цієї хвороби. Встановлені межові рівні eNOS у сироватці крові хворих на COVID-19 із пневмонією, що мають значення щодо прогнозу ризику розвитку кисневої залежності та летального наслідку хвороби.

In December 2019, a new coronavirus, SARS-CoV-2, was detected, evolving into a pandemic with its own name coronavirus disease-19 (COVID-19) [1]. It soon became clear that the clinical manifestations of COVID-19 ranged from asymptomatic and mild to severe and critical. The main pathogenetic mechanism to explain severe and critical disease courses was considered to be an excessive inflammatory immune response of cells, including endothelial cells, to SARS-CoV-2 infection [2].

Convincing evidence that SARS-CoV-2 infects endothelial cells lining both pulmonary and non-pulmonary vessels is currently available [3,4]. Endothelial cells express the angiotensin-converting enzyme 2 receptor on their membrane, which is required by SARS-CoV-2 for binding to the S1 subunit of the adhesion protein [3]. Morphological examination on biopsies of various organs have shown the expression of both angiotensin-converting enzyme 2 receptor and transmembrane serine protease 2 co-receptor on the endothelial cell membrane, that leads to the virus S-protein priming by proteases of target cells, fusion between the virus envelope and the cellular membrane and virus entry into cells [5,6]. The results of electron microscopy of the lung endothelium have demonstrated the presence of SARS-CoV-2 intracellularly, also confirming the ability of this virus to infect endothelial cells [7].

Today, there is mounting evidence to support the role of endothelial dysfunction as one of the key pathogenetic mechanisms of COVID-19 progression, including the development of acute respiratory distress syndrome (ARDS) SARS-CoV-2 [8,9]. However, most studies on the pathogenetic mechanisms of lung damage induced by COVID-19 have focused on the respiratory epithelium, in particular alveolar type II cells, which are the primary target of the virus. Nowadays, in the context of ongoing circulation of SARS-CoV-2, taking into account its tropism to vascular endothelial cells, there is a need to clarify the pathogenetic mechanisms of endothelial dysfunction and the clinical significance of these changes, especially in patients with SARS-CoV-2 pneumonia [10].

According to researchers [11], endothelial dysfunction and the associated risk of thrombosis are the pathogenetic basis of clinical and paraclinical manifestations of COVID-19. The study on pathogenetic mechanisms and clinical significance of endothelial dysfunction will help to develop proper effective key elements of therapeutic management of COVID-19 patients with pneumonia [11].

Aim

To examine the association between serum eNOS levels and hemostatic parameters in COVID-19 patients with pneumonia and to determine its prognostic value in assessing the risk of oxygen requirements and death.

Material and methods

A total of 123 patients with COVID-19 and pneumonia were followed up. The diagnosis of COVID-19 in all the patients was confirmed by the detection of RNA-SARS-CoV-2 in nasopharyngeal swab specimens by polymerase chain reaction; pneumonia was diagnosed by chest X-ray or computed tomography. All patients enrolled in the study were hospitalized in Municipal Non-Profit Enterprise "Regional Infectious Diseases Clinical Hospital" of Zaporizhzhia Regional Council and received examination and treatment in accordance with the Order of the Ministry of Health of Ukraine No. 722 dated 28.03.2020 "Organization of Medical Care for Patients with Coronavirus Disease (COVID-19)". Informed consent was obtained from each patient before the study.

Serum eNOS levels (Wuhan Fine Biotech Co., Ltd, China) were determined by enzyme-linked immunosorbent assay on the basis of the Training Medical and Laboratory Center of Zaporizhzhia State Medical and Pharmaceutical University (scientific adviser – PhD, DSc, Associate Professor R. O. Shcherbyna). To assess changes in eNOS levels, a control group of 20 healthy individuals was formed and patients with COVID-19 pneumonia were divided into groups, depending on the disease severity, oxygen requirements and consequences: 32 patients with moderate disease severity without oxygen requirements; 45 patients with severe disease and oxygen requirements who recovered; 46 patients who died.

The prognostic significance of eNOS levels was assessed for hemostatic parameters in the development of oxygen requirements by dividing patients into groups: 32 patients with moderate disease severity without oxygen requirements; 91 patients with severe disease and oxygen requirements. The prognostic significance of eNOS levels in relation to hemostasis parameters in the development of a lethal outcome was assessed by dividing patients into groups: 77 patients who recovered; 46 patients who died.

An Excel database of patients enrolled in the study was generated. Statistical processing of the data was performed using the software Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J). The data were checked for normal distribution using the Shapiro–Wilk test; non-parametric methods of statistical data processing were used in cases of non-normal distribution. The results of quantitative data were presented in the form of median and interquartile ranges Me [Q25; Q75]. The Mann–Whitney test was employed for analyzing differences between quantitative features in independent samples, and the χ^2 test was used to determine differences between qualitative features. To identify diagnostic significance of eNOS in predicting the risk of oxygen requirements and death in COVID-19 with pneumonia, a ROC analysis was performed to determine cut-offs. Spearman's correlation was calculated to assess whether the quantitative parameters vary together. The gamma rank correlation coefficient was used to detect the relationships between quantitative and rank characteristics. Differences at a p value <0.05 were considered significant.

Table 1. Serum eNOS levels in COVID-19 patients with pneumonia at the time of hospitalization depending on the clinical course of the disease, Me [Q25; Q75]

Parameter, units of measurement	Healthy people, n = 20	COVID-19 patients with pneumonia		
		without oxygen requirements, n = 32	recovered patients requiring supplemental oxygen support, n = 45	patients requiring supplemental oxygen who died, n = 46
eNOS, pg/ml	2036.29 [1984.49; 2450.09]	883.12 [596.32; 1148.39][1]	595.24 [295.15; 759.74][1,2]	155.08 [105.41; 562.27][1,2,3]

[1]: significant differences compared to healthy people ($p < 0.001$); **[2]:** compared to patients without oxygen requirements ($p < 0.001$); **[3]:** compared to recovered patients with the need for supplemental oxygen ($p < 0.001$).

Table 2. Comparison of serum eNOS and hemostatic parameters in COVID-19 patients with pneumonia at the time of hospitalization depending on the need for supplemental oxygen, Me [Q25; Q75]

Parameter, units of measurement	COVID-19 patients with pneumonia	
	without oxygen requirements, n = 32	with oxygen requirements, n = 91
eNOS, pg/ml	883.12 [596.32; 1148.39]	297.36 [157.89; 601.73]*
Prothrombin index, %	111.4 [102.5; 120.5]	107.1 [94.8; 123.9]
International normalized ratio	0.94 [0.88; 1.01]	0.93 [0.83; 1.05]
Fibrinogen, g/l	4.4 [3.8; 5.7]	5.3 [4.1; 6.4]*
Increased fibrinogen, abs. (%)	21 (65.6 %)	73 (80.2 %)
D-dimer, μg/ml	0.7 [0.1; 0.9]	1.3 [0.7; 2.2]*
Increased D-dimer, % (abs.)	60.0 (3 from 5)	81.5 (44 from 54)
Thrombocytes, $\times 10^9$/л	205.0 [162.5; 243.0]	209.0 [175.0; 265.0]
Thrombocytopenia, abs. (%)	10 (31.3 %)	25 (27.5 %)
Thrombocytosis, abs. (%)	1 (3.1 %)	4 (4.4 %)

*: significant differences compared to patients without the need for supplemental oxygen ($p < 0.05$).

Results

According to the study results, it has been found that the serum eNOS level in patients with COVID-19 and pneumonia was significantly lower ($p < 0.001$) than that in healthy control subjects at the time of hospitalization on day 9.0 [7.0; 12.0] of the disease.

The rate of serum eNOS decrease in COVID-19 patients with pneumonia clearly depended on the disease severity, oxygen therapy requirements and the subsequent disease outcome. The serum eNOS level in recovered patients from severe disease with oxygen requirements was lower ($p < 0.001$) than that in patients with moderate disease without signs of oxygen requirements. The serum eNOS level in patients who died was lower ($p < 0.001$) than that in patients with moderate disease course without signs of oxygen requirements and with severe disease course requiring supplemental oxygen, who subsequently recovered *(Table 1)*.

The role of eNOS in the progression of COVID-19 in patients with pneumonia was confirmed by correlations found between indicators of immune inflammation, hemostasis and biochemical signs characterizing the onset of multiple organ failure. Thus, we have found a relationship between eNOS levels and the development of absolute lymphopenia (gamma 0.29, $p = 0.003$) and the degree of C-reactive protein increase ($r = -0.39$, $p < 0.05$), confirming the role of immune inflammation in endothelial cell damage. Correlations have been found between eNOS levels and hyperfibrinogenemia (gamma 0.22, $p = 0.04$) and a decrease in the international normalized ratio (gamma 0.58, $p < 0.0001$), reaffirming the role of endothelial dysfunction to induce hypercoagulability. In addition, correlations between eNOS levels and a decrease in glomerular filtration rate (gamma 0.34, $p = 0.0001$) and its severity ($r = +0.34$, $p < 0.05$) have been found, supporting the role of endothelial dysfunction in acute kidney injury in COVID-19 patients with pneumonia.

In the next section of our work, we compared serum eNOS levels and hemostatic parameters of patients with COVID-19 pneumonia with the onset of oxygen supplementation and determined the prognostic significance of eNOS in assessing the risk of developing oxygen requirements. It has been revealed that the need for supplemental oxygen in COVID-19 patients with pneumonia was accompanied by decreased serum eNOS levels (3-fold, $p < 0.001$), indicating deteriorated symptoms of endothelial dysfunction and procoagulant changes, as evidenced by higher levels of fibrinogen (1.2-fold, $p < 0.05$) and D-dimer (1.9-fold, $p < 0.05$) *(Table 2)*.

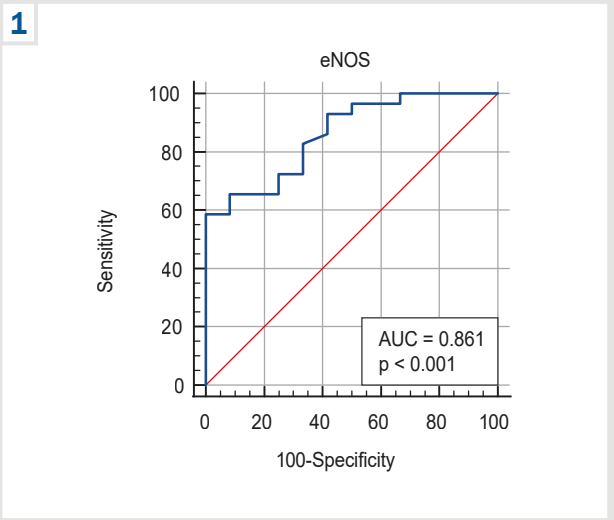
Given the almost threefold lower serum eNOS levels found in patients with COVID-19 pneumonia and oxygen requirements, we performed a ROC analysis to assess the prognostic significance of this indicator in the risk of the need for supplemental oxygen at the time of hospitalization on day 9.0 [7.0; 12.0] of the disease. It has been found that the threshold serum eNOS level ≤327.09 pg/ml (AUC = 0.861, $p < 0.001$) showed a high probability of the need for supplemental oxygen in patients with COVID-19 pneumonia (sensitivity – 58.6 %, specificity – 100.0 %) *(Fig. 1)*.

Comparison of serum eNOS levels and hemostatic parameters in COVID-19 patients with pneumonia, considering the consequences of this disease, has revealed significantly lower (4.5 times, $p < 0.001$) eNOS levels at the time of hospitalization on day 9.0 [7.0; 12.0] of the disease in patients who died, as compared to recovered patients. A significant decrease in eNOS levels in COVID-19 patients with pneumonia who died was associated with a higher level of D-dimer ($p < 0.05$) and a greater frequency of its elevation (90.0 % vs 68.9 %, $\chi^2 = 4.03$, $p = 0.04$)

Table 3. Comparison of serum eNOS and hemostatic parameters in COVID-19 patients with pneumonia at the time of hospitalization depending on the disease outcome, Me [Q25; Q75]

Parameter, units of measurement	COVID-19 patients with pneumonia	
	recovered patients, n = 77	patients who died, n = 46
eNOS, pg/ml	703.46 [327.09; 958.88]	155.08 [105.41; 562.27]*
Prothrombin index, %	110.0 [98.4; 124.0]	107.1 [94.8; 123.6]
International normalized ratio	0.94 [0.87; 1.04]	0.93 [0.83; 1.03]
Fibrinogen, g/l	5.2 [3.9; 5.9]	5.6 [4.3; 6.3]
Increased fibrinogen, abs. (%)	56 (72.7 %)	38 (82.6 %)
D-dimer, μg/ml	0.9 [0.5; 1.8]	1.3 [0.9; 2.3]*
Increased D-dimer, % (abs.)	68.9 (20 from 29)	90.0 (27 from 30)*
Thrombocytes, $\times 10^9$/л	220.0 [181.0; 287.0]	204.0 [158.0; 251.0]
Thrombocytopenia, abs. (%)	19 (24.7 %)	16 (34.8 %)
Thrombocytosis, abs. (%)	4 (5.2 %)	1 (2.2 %)

*: significant differences compared to recovered patients ($p < 0.05$).



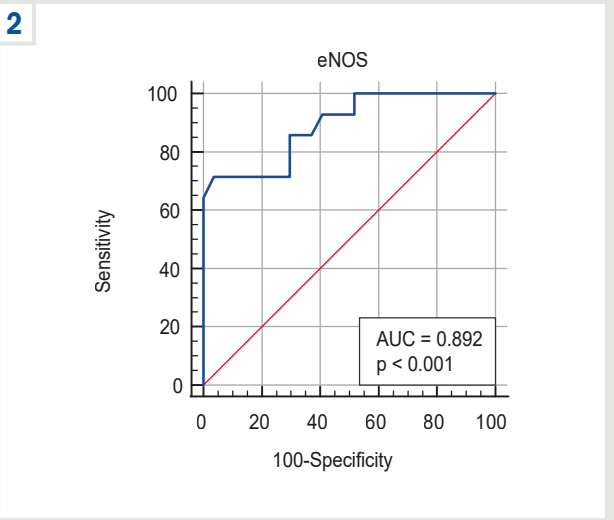


Fig. 1. Prognostic significance of serum eNOS levels in COVID-19 patients with pneumonia at the time of hospitalization in assessing the risk of oxygen requirements.

Fig. 2. Prognostic significance of serum eNOS levels in COVID-19 patients with pneumonia at the time of hospitalization in assessing the risk of death.

compared to patients who recovered, indicating not only the presence of severe endothelial dysfunction but also thrombosis *(Table 3)*. It should be noted that the serum eNOS level in COVID-19 patients with pneumonia was strongly correlated not only with the lethal outcome of the disease (gamma 0.79, $p < 0.05$), but also with thrombotic complications (gamma 0.86, $p < 0.05$). In patients with fatal COVID-19, thrombotic complications (pulmonary embolism, ischemic stroke, myocardial infarction) were significantly more common than in recovered COVID-19 patients with pneumonia, namely 23.9 % (11 of 46) versus 1.3 % (1 of 77) ($\chi^2 = 16.72$, $p = 0.0001$).

Based on the almost 4.5-fold lower serum eNOS levels detected at the time of hospitalization in patients with COVID-19 pneumonia who died, we conducted a ROC analysis to assess the prognostic significance of this indicator in the risk of death. It has been found that the threshold serum eNOS level ≤201.75 pg/ml (AUC = 0.892, $p < 0.001$) indicated a high probability of death from COVID-19 in patients with pneumonia (sensitivity – 71.4 %, specificity – 96.3 %) *(Fig. 2)*.

Discussion

The endothelial barrier integrity is known to be particularly important for the functioning of any organ and strictly regulated to ensure a controlled exchange of oxygen and substances between the circulatory system and tissues [10]. Under physiological conditions, the endothelium has anticoagulant, anti-inflammatory and antioxidant properties. A transition from functional endothelium to dysfunctional one, when the endothelium is rapidly getting prothrombotic, proinflammatory and prooxidant properties, occurs in the development of many pathological conditions. However, a considerable period of time is required to restore endothelial functions in the future [12]. Endothelial cell damage in COVID-19 occurs not only due to the direct effect of SARS-CoV-2, but also due to the development of immune disorders, in particular, a “cytokine storm” [13].

According to the study results, endothelial dysfunction has been revealed in COVID-19 patients with pneumonia in all clinical variants, as evidenced by significantly lower serum eNOS levels compared to healthy people ($p < 0.001$). It should be noted that decreased eNOS levels

were associated with increased severity of COVID-19 with pneumonia, the development of oxygen requirements and further adverse outcomes of the disease. At the time of hospitalization, serum eNOS levels were the lowest in patients who died ($p < 0.001$). The informative value of endothelial assessment in predicting the course of COVID-19 is also evidenced by the results of other researchers. For example, faster detachment of SARS-CoV-2-infected endothelial cells without effective regeneration has been shown [12]. A decrease in the number of endothelial cells on the vascular lumen surface led to a decreased NO production due to a decrease in eNOS, that consequently impaired endothelium-dependent vasodilation [12].

A study [14] has demonstrated that an increase in the number of circulating endothelial cells was an informative predictor of severe COVID-19. Another study has shown the prognostic value of endothelial glycocalyx assessment by the perfusion border region in COVID-19 patients [15]. In patients with critical COVID-19 who were mechanically ventilated, severe glycocalyx damage was detected, and it was the degree of glycocalyx thinning that showed the highest informative value in predicting 60-day mortality in this category of COVID-19 patients [16]. It is believed that shear-induced NO production is largely absent in endothelial cells lacking glycocalyx [17].

Multiorgan failure occurrence in the context of severe and critical COVID-19 is worthy of note. A number of studies have demonstrated immunohistochemical evidence of direct SARS-CoV-2 viral infection of endothelial cells in various locations, including kidney [16] and liver [18]. According to the results of our correlation analysis, we have found a relationship between eNOS levels and a decrease in glomerular filtration rate (gamma 0.34, $p = 0.0001$) and its severity ($r = +0.34$, $p < 0.05$), confirming the role of endothelial dysfunction in acute kidney injury in patients with COVID-19 and pneumonia.

The results of our study have shown that the need for supplemental oxygen in patients with COVID-19 pneumonia, which reflected the progression of ARDS, was accompanied by decreased serum eNOS levels (3-fold, $p < 0.001$), indicating a worsening of endothelial dysfunction and procoagulant changes, as indicated by higher levels of fibrinogen (1.2-fold, $p < 0.05$) and D-dimer (1.9-fold, $p < 0.05$). It is known that eNOS expression is clearly dependent on an increase in the pool of L-arginine, which is synthesized from L-citrulline in the citrulline-NO cycle. The eNOS synthesis cycle is characterized by the presence of arginosuccinate synthase enzyme, which limits the rate of reactions in this cycle and allows controlling NO synthesis induced by eNOS [19]. In the context of ARDS, the availability of L-arginine decreases, which can lead to the cleavage of eNOS, causing oxidative damage to both the pulmonary endothelium and epithelium [2]. The ARDS severity is known to reflect the degree of hypoxemia caused by a mismatch between ventilation and perfusion rates, which is primarily the result of changes in the pulmonary vessels [20].

By results of our study, a comparison of serum eNOS levels and hemostatic parameters in COVID-19 patients with pneumonia depending on the disease outcomes, has shown that at the time of hospitalization, eNOS levels in patients who died were 4.5 times lower ($p < 0.001$) than that in patients who recovered. Significantly decreased eNOS levels in COVID-19 patients with pneumonia who died was associated with a higher level of D-dimer ($p < 0.05$) and a greater frequency of its elevation (90.0 % vs. 68.9 %, $p = 0.04$) compared to patients who recovered, indicating not only the presence of severe endothelial dysfunction but also thrombosis. As is commonly known, diffuse pulmonary inflammation, which occurs in the context of ARDS, stimulates an increase in arginase activity in endothelial cells reducing the L-arginine availability and, accordingly, causing the eNOS destruction and contributing to endothelial dysfunction. These changes explain the formation of an intrapulmonary shunt to areas where gas exchange is compromised, further worsening the mismatch between ventilation and perfusion rates, leading to progressive hypoxemia [2,21].

The results of a study [22] have shown that mortality from COVID-19 may be associated with a decrease in the production and bioavailability of endothelial NO. In addition, vasoconstriction, which progresses as a result of decreased eNOS activity and reduced NO bioavailability, has a certain contribution to thrombotic complications [23]. Based on our study results, the serum eNOS levels in COVID-19 patients with pneumonia were strongly correlated not only with fatal outcomes of the disease (gamma 0.79, $p < 0.05$), but also with thrombotic complications (gamma 0.86, $p < 0.05$). In patients with fatal outcome, thrombotic complications (pulmonary embolism, ischemic stroke, myocardial infarction) were significantly more common than in COVID-19 patients with pneumonia who recovered (23.9 % vs. 1.3 %, $p = 0.0001$).

NO deficiency and reduced eNOS are valuable indicators of endothelial dysfunction and thrombotic events in various pathological conditions [24]. Since endothelial dysfunction causes a deterioration in the endogenous NO availability due to decreased production or increased breakdown, some researchers have suggested that exogenous inhalation of NO can compensate for its deficiency by providing pulmonary vasodilation, direct antiviral action, and antithrombotic effects [25]. ARDS during the epidemic caused by SARS-CoV in 2002–2003, inhaled NO was tested in 6 critically ill patients. The results of the treatment showed a number of positive effects, including a reduction in pulmonary hypertension, improved arterial oxygenation, and a decrease in the intensity of infiltrative changes in the lung parenchyma [26]. Inhaled NO therapy for patients with COVID-19 is currently being investigated in a range of clinical trials [11].

Conclusions

1. The serum eNOS levels in COVID-19 patients with pneumonia at the time of hospitalization on day 9.0 [7.0; 12.0] was lower ($p < 0.001$) than in healthy individuals, and their degree of decrease depends on the disease severity, oxygen requirements and subsequent outcomes of the disease. The role of eNOS in the progression of COVID-19 in patients with pneumonia has been confirmed by correlations ($p < 0.05$) between eNOS levels and the development of absolute lymphopenia, C-reactive protein levels, hyperfibrinogenemia, decreased international normalized ratio, glomerular filtration rate and its severity.

2. The need for supplemental oxygen in COVID-19 patients with pneumonia was accompanied by worsening of endothelial dysfunction and procoagulant changes, confirmed by decreased serum eNOS levels ($p < 0.001$), increased levels of fibrinogen ($p < 0.05$) and D-dimer ($p < 0.05$). The threshold serum level of eNOS ≤327.09 pg/ml (AUC = 0.861, $p < 0.001$) was predictive of oxygen requirements in patients with COVID-19 pneumonia.

3. In patients with COVID-19 pneumonia who died, the admission eNOS levels were lower ($p < 0.001$) than in recovered patients, that was associated with higher levels of D-dimer ($p < 0.05$) and a greater frequency of its elevation ($p = 0.04$) compared to recovered patients. The eNOS levels were strongly correlated not only with the lethal outcome of the disease (gamma 0.79, $p < 0.05$), but also with thrombotic complications (gamma 0.86, $p < 0.05$), which occurred more often in patients with unfavorable outcome (23.9 % vs. 1.3 %, $p = 0.0001$). The threshold serum level of eNOS ≤201.75 pg/ml (AUC = 0.892, $p < 0.001$) indicated a high probability of death from COVID-19 in patients with pneumonia.

Prospects for further research. Prospects for further research in this area, in our opinion, are to determine the diagnostic significance of eNOS levels in assessing the effectiveness of treatment for patients with oxygen requirements and to develop ways of correcting endothelial dysfunction in patients with COVID-19 pneumonia.

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