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Interrelation between the parameters of endogenous vascular regulation, oxidative stress and the markers of inflammatory response in COVID-19 patients while on extracorporeal membrane oxygenation

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Abstract

Introduction. *Extracorporeal membrane oxygenation has found wide application in clinical practice during the COVID-19 pandemic. Oxidative stress, endothelial dysfunction, and systemic inflammatory response syndrome play an important role in the pathogenesis of COVID-19. Our research was designed to study correlations in-between those factors and the impact of extracorporeal membrane oxygenation on them.*

Aim. *The study of systemic inflammatory response and endothelial function in patients with COVID-19 during extracorporeal membrane oxygenation.*

Material and methods. *In the course of a prospective study, we examined 100 COVID-19 patients aged 26 to 75 years, median 55 years [47;60],*

who were treated at the N.V. Sklifosovsky Research Institute for Emergency Medicine, using extracorporeal membrane oxygenation. As a control group (normal), 25 practically healthy people whose median age was 32 years [25;39] were examined. The function of the vascular endothelium was assessed by the content of nitric oxide stable metabolites in the blood serum and the level of angiotensin-converting enzyme. Next, the ratio of nitric oxide metabolite to angiotensin-converting enzyme level was calculated, reflecting the imbalance between endothelium-dependent vasodilation and vasoconstriction. To assess the severity of oxidative stress in blood serum, malondialdehyde was determined as a marker of lipid peroxidation. The state of the antioxidant system was assessed in terms of total antioxidant status of blood serum. The presence of an imbalance in the system of lipid peroxidation and the antioxidant system total antioxidant status was judged by the oxidative stress coefficient, i.e. the ratio of malondialdehyde to the total antioxidant activity.

Results. *The analysis showed the presence and progression of endothelial dysfunction, impaired vascular regulation, activation of free radical processes, the presence of an imbalance in the prooxidant/antioxidant system, as well as the progression of the inflammatory process with a decrease in the level of markers of the COVID-19 severity.*

Conclusion. *Further studies of the correlation between endothelial damage and the severity of the systemic inflammatory response syndrome may be of fundamental importance for explaining the pathophysiological mechanisms of COVID-19 course and developing new treatments for such patients.*

Keywords: COVID-19, SARS-CoV-2, extracorporeal membrane oxygenation, endothelium, oxidative stress, inflammation

Conflict of interests Authors declare no conflict of interest

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ACE, angiotensin converting enzyme
AOS, antioxidant system
ARDS, acute respiratory distress syndrome
*C*RP, C-reactive protein
ECMO, Extracorporeal Membrane Oxygenation
LPO, lipid peroxidation
MDA, malondialdehyde
NLR, the ratio of the absolute neutrophil count to the absolute lymphocyte count
NOx, nitric oxide
OS, oxidative stress
PLR, the ratio of the absolute platelet count to the absolute lymphocyte count
ROS, reactive oxygen species
TAS, total antioxidant status
VV ECMO, veno-venous extracorporeal membrane oxygenation

Introduction

Oxidative stress (OS) and endothelial dysfunction play an important role in the pathogenesis of a number of human vascular and metabolic diseases, such as stroke, heart disease, and diabetes [1]. The hallmark of SARS-CoV-2 infection is vascular involvement with severe endothelial injury leading to widespread thrombosis and microangiopathies. In addition, the activation and dysfunction of the pulmonary endothelium are the main causes of acute respiratory distress syndrome (ARDS), respiratory failure in patients with severe COVID-19. The venovenous extracorporeal membrane oxygenation (VV ECMO) technique is able to maintain systemic oxygenation in patients with respiratory failure and is used as a bridge to recovery in COVID-19-associated ARDS [2]. The global spread of COVID-19 has led to an

increased frequency of using the extracorporeal membrane oxygenation (ECMO), despite the incoming data on a rather low patient survival rate: at the beginning of the pandemic, less than 10% of patients managed to be weaned from the extracorporeal circuit [3-5]. One of the reasons for the high mortality in this category of patients is a pronounced systemic inflammatory response to the generalization of SARS-CoV-2 and nosocomial infection, which makes it difficult to evaluate the efficacy of the ECMO procedure [6]. The pathogenetic consequence of septic manifestations of new coronavirus infection is endothelial damage that leads to the multiple organ failure development and worse prognosis [7]. However, if the mechanical ventilation turns ineffective in critically ill patients, the ECMO procedure becomes inevitable [8]. For this reason, the assessment of the systemic inflammatory response and the endothelium condition is an important link in shaping the view of the clinical picture and may allow proper adjustment to the tactics of managing patients with COVID-19 under ECMO.

Objective

The study objective was to investigate the course of the systemic inflammatory response and endothelial function in patients with COVID-19 during extracorporeal membrane oxygenation.

Material and methods

In the course of a prospective study, 100 patients with COVID-19 treated by using ECMO at the N.V. Sklifosovsky Research Institute for Emergency Medicine, were examined.

Criteria for inclusion of patients in the study were as follows: ECMO, confirmed COVID-19 diagnosis, age over 18 years. Exclusion criteria were lack of monitoring for hemostasis parameters, SOFA score

exceeding 12 at the time of connection to ECMO, body mass index over 40, ECMO connection timing from the start of mechanical lung ventilation being more than 7 days.

In accordance with the inclusion and exclusion criteria, the study included 100 patients: 72 men and 28 women, aged 26 to 75 years, median age 55 years [47;60]. In all cases, VV ECMO was performed. The cause of respiratory failure, which required VV ECMO, was COVID-19-associated pneumonia in 100 cases (100%).

ECMO was performed in severe respiratory failure refractory to ongoing intensive care. The principle of management was in line with the interim guidelines of the Russian Federation Ministry of Health "Prevention, diagnosis and treatment of new coronavirus infection (*COVID*-19)", relevant at the time of treatment. ECMO was performed using RotaFlow (Maquet, Germany), Cardiohelp (Maquet, Germany), DeltaStream (Medos, Germany), and Stockert (Sorin, USA) devices. For VV ECMO, cannulation of the right or left femoral vein was performed (cannulas sized Fr 21, 23, 25 were used) for blood sampling from the system of inferior vena cava and the right or left jugular vein (cannulas sized Fr 15, 17, 19, 21, 23 were used) to return blood.

Patients received anticoagulant therapy with unfractionated heparin, which dose was selected by monitoring the activated partial thromboplastin time every 6–8 hours.

As a control group (normal), 25 practically healthy people were examined whose median age was 32 [25;39] years, and the male/female ratio was 17/8.

Investigations of the hemostasis system status were performed on an automatic *ACL Top 700* coagulometer, Instrumentation Laboratory (USA). Complete blood count (hematology testing) was performed on a hematological *ADVIA 2120i* analyzer, Bayer. The following coefficients

reflecting the severity of the inflammatory process were calculated: NLR, the ratio of the absolute neutrophil count to the absolute lymphocyte count (Neutr./Lymph., abs.counts); PLR, the ratio of the absolute platelet count to the absolute lymphocyte count (Platelet./Lymph., abs. counts); Systemic Inflammation Index, the ratio of the absolute platelet count multiplied by the absolute neutrophil counts to the absolute lymphocyte count (Platelet.*Neutr., abs.counts /Lymph., abs.counts).

The function of the vascular endothelium was assessed by the content of stable metabolites of nitric oxide (NOx) in the blood serum and the level of angiotensin-converting enzyme (ACE). NOx was measured by the method according to which cadmium in the presence of zinc reduces nitrate to nitrite. The ACE concentration was assessed by the photometric method on an Olympus AU2700 biochemical analyzer (Beckman Coulter, USA) using reagents from Audit Diagnostics (Ireland). Next, the NOx/ACE coefficient was calculated, which reflected the imbalance between endothelium-dependent vasodilation and vasoconstriction according to the formula:

$$\frac{NOx_s/NOx_c}{ACE_s/ACE_c}$$, where

NOx_s, ACE_s are the values of the parameters in the study group patients and NOx_c, ACE_c are means of the parameters in the control group.

To assess the severity of OS, the blood serum malondialdehyde (MDA) was determined as a lipid peroxidation (LPO) marker [9]. The antioxidant system (AOS) state was assessed by the total antioxidant status (TAS) in blood serum, which was measured by spectrophotometry on an Olympus AU2700 biochemical analyzer (Beckman Coulter, USA) using a TAS kit (Randox, UK). The judgement on the imbalance in the LPO/AOS system was made based on the coefficient of oxidative stress MDA/TAS, which was calculated using the formula:

$$\frac{MDA_s/MDA_c}{TAS_s/TAS_c}$$, where

MDA_s, TAS_s are the values of the parameters in the study group patients and MDA_c, TAS_c are means of the parameters in the control group.

The study continued for the first 7 days of ECMO or less if ECMO was terminated earlier or death occurred.

Statistical analysis of the data obtained during the study was carried out using PC, Windows 10 operating system, "Microsoft Excel 2007" software. Statistical data processing was carried out using the R 3.6.3 software environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria) and the lme4 1.1–21 software package, as well as the STATISTICA 12 software package (Starsoft, USA). The normality of the distribution of quantitative variables was measured using the Shapiro-Wilk test. Since most of the data in the study did not correspond to a normal distribution, a nonparametric Mann-Whitney test was used for comparative analysis of quantitative variables. The results of quantitative data statistical analysis are presented as median (M) and quartiles (25%;75%). The analysis of differences between groups in qualitative variables was performed using the $\chi 2$ test. Correlation analysis was performed using the Spearman's correlation coefficient (ρ). Also, the method of Generalized Estimating Equation (GEE) and Generalized Linear Model (GLMM) was used to analyze the data obtained. The following coefficients were calculated: Δ, the gradient of change or Fold-change (FC). A negative value of Δ coefficient indicates a mean daily decrease in the value of the parameter under study by the corresponding number of units of its measurement, a positive value indicates a daily increase [10, 11]. Linear Mixed Effects Regressions (LMER) models were used for comparisons between the groups. Testing of statistical hypotheses and the presence of statistical significance was established at a p value <0.05.

Results and discussion

The dynamics of endothelial damage and OS markers is presented in Tables 1 and 2. The study revealed a statistically significant decrease in the level of NOx relative to the norm in all periods of study (p_{N-1} <0.00001, p_{N-3}=0.0002, $p_{N-7=}$0.0214) (Table 1).

When using GEE and GLM models, the Δ coefficient of -0.85 [-2.89;1.19], p=0.0173 was obtained, which indicated a mean daily decrease in NOx levels of 0.85 μmol/L (Table 2) [10]. This indicated the development of endothelial dysfunction.

The ACE concentration was also statistically significant, below the norm in all periods of study (p_{N-1}=0.0035, p_{N-3}=0.0002, p_{N-7}=0.0004). There is an inverse relationship between the level of NOx and ACE, which plays an important physiological and pathophysiological role in the regulation of vascular tone. To assess the development of an imbalance between endothelium-dependent vasodilation and vasoconstriction, the NOx/ACE coefficient was calculated. The NOx/ACE ratio when compared to the norm was statistically significantly lower on the 1st and 3rd days of the study observation (p=0.0446), which indicated an impaired vascular regulation in patients with severe COVID-19. Thus, the data obtained indicate an impairment of the regulatory function between the NOx vasodilator and the ACE vasoconstrictor on the 1st and 3rd days of the study observation, which is probably one of the pathogenetic mechanisms leading to a change in microcirculation, and further to the development of multiple organ failure in patients with severe COVID-19 during ECMO.

When examining the OS severity, we found that during the entire period of observation, the blood level of MDA statistically significantly exceeded the normal values during all periods of the study observation (p_{N-1}<0.00001, p_{N-3}<0.00001, p_{N-7}<0.00001) (Table 1).

Total antioxidant status (TAS), which simultaneously reflects the ability of enzymes, proteins, and vitamins to suppress the negative effects of free radicals at the cellular level, decreased statistically significantly on the 3rd and 7th days of the study (Table 1).

Table 1. Dynamics of endothelial damage and oxidative stress markers

Parameter	Control group	Day			p
		1st	3rd	7th	
NOx, µmol/L	18.61 [17.70;23.62]	8.01 [4.82;14.38]*	9.31 [5.08;19.98]*	13.70 [6.38;21.40]*	p_{N-1}<0.00001 p_{N-3}=0.0002 p_{N-7}=0.0214
ACE, µmol/l	45.0 [36.5;55.2]	32.2 [20.9;49.4]*	27.6 [18.8;38.8]*	26.6 [16.4;39.5]*	p_{N-1}=0.0035 p_{N-3}=0.0002 p_{N-7}=0.0004
NOx /ACE Coeff.	1.02 [0.85;1.25]	0.59 [0.27;1.02]*	0.72 [0.39;1.19] *	0.84 [0.50;2.01]	p_{N-1}=0.0024 p_{N-3}=0.0148
MDA, µmol/L	2.27 [2.11;2.47]	3.87 [3.47;4.24]*	3.91 [3.44;4.33]*	3.78 [3.30;4.30]*	p_{N-1}<0.00001 p_{N-3}<0.00001 p_{N-7}<0.00001
TAS, mmol/L	1.61 [1.56;1.68]	1.46 [0.98;1.75]	1.32 [0.85;1.58]*	1.47 [1.17;1.71]*	p_{N-3}=0.0016 p_{N-7}=0.0451
MDA/TAS Coeff.	0.96 [0.91;1.11]	2.01 [1.69;2.49]*	2.16 [1.85;3.47]*	2.16 [1.52;2.71]*	p_{N-1} <0.00001 p_{N-3}<0.00001 p_{N-7}<0.00001

Note: NOx, nitric oxide; ACE, angiotensin-converting enzyme; MDA, malondialdehyde; TAS, total antioxidant status; * – the difference significance level in relation to the norm: p_{N-1}, on the 1st day after ECMO; p_{N-3}, on the 3rd day after ECMO; p_{N-7}, on the 7th day after ECMO.

Also, by using the method of GEE and GLM models, Δcoefficient=-0.16 [-0.30;-0.03], p=0.0053, was obtained which demonstrated that the daily TAS level decreased by the value of 0.16 mmol/L, (Table 2), which indicated the activation of free radical processes. Moreover, the oxidative stress coefficient MDA/TAS that reflects the balance in the prooxidant/antioxidant system, was over twice higher than the normal values (p<0.00001) throughout the study period. The data obtained indicate the presence of an imbalance in the prooxidant/antioxidant system in patients with severe COVID-19 who required ECMO.

OS represents a systemic imbalance between the rate of oxidant formation and the level of antioxidants. Studies report that OS plays an important role in the development of endothelial dysfunction through a decrease in the bioavailability of nitric oxide. Nitric oxide, as an active form of oxygen, reacts with the superoxide anion to form a strong oxidizing agent, peroxynitrite, which is involved in the nitration of tyrosine residues in proteins. Reduced expression of endothelial nitric oxide synthase, lack of substrates for it, its inactivation, and increased degradation of nitric oxide can cause a decrease in its bioavailability [12].

Under physiological conditions, the production of reactive oxygen species (ROS) is controlled by an efficient system of antioxidants, i.e. the molecules capable to neutralize ROS, thereby preventing OS. In our study, there is a statistically significant decrease in TAS and an increase in the blood level of MDA, which indicates the activation of free radical processes in this category of patients and is one of the reasons for the development of endothelial dysfunction in patients with severe COVID-19.

Table 2. Dynamics of markers of endothelial damage and oxidative stress

Parameter	Δ/FC [95%CI]	p
MDA [Δ]	-0.03 [-0.20;0.14]	0.8654
TAS [Δ]	-0.16 [-0.30;-0.03]	0.0053
NOx [Δ]	-0.85 [-2.89;1.19]	0.0173
ACE [Δ]	-5.70 [-11.86;0.46]	0.2178

Note: NOx, nitric oxide; ACE, angiotensin-converting enzyme; MDA, malondialdehyde; TAS, total antioxidant status; Δ, gradient of change; FC, fold-change; p, difference significance level.

According to C. Yan et al. (2003), angiotensin II regulates the expression of nitric oxide synthase and its production, while the nitric oxide regulates the level of angiotensin II through the angiotensin II type 1 receptors in a reverse mechanism. Feedback between nitric oxide and

angiotensin II is the basis for maintaining normal vascular regulation and functions [13]. Break of this relationship is the cause of many vascular diseases [14]. In a previous study of the relationship between the NOx level and the ACE concentration in donors, we obtained a statistically significant positive correlation between NOx and ACE (r=0.655, p=0.0007) [15]. The present study did not reveal any relationship between NOx levels and ACE concentrations in ECMO patients with severe COVID-19 (Table 3). Thus, all the above results indicate an impairment of the associated regulatory function between the NOx vasodilator and the ACE vasoconstrictor, which can contribute to the development of endothelial dysfunction and microcirculation disorders.

Table 3. Correlation of the nitric oxide and angiotensin-converting enzyme parameters

Parameter	Day	ACE
NOx	1st day	-0.12 [-0.34;0.11] p=0.4247
	3rd day	-0.003 [-0.21;0.20] p=0.9808
	7th day	-0.08 [-0.37;0.21] p=0.6238 _

Note: NOx, nitric oxide; ACE, angiotensin-converting enzyme; p, the difference significance level

To assess the severity of the inflammatory response, we analyzed the dynamics of the blood level of *C*-reactive protein (*C*RP), the leukocyte count, lymphocyte count, the neutrophils to leukocytes ratio (NLR), platelets to lymphocytes ratio (PLR), as well as the index of systemic inflammation (Table 4).

In patients connected to ECMO, the *C*RP level was statistically significantly increased during all periods of study observation, while the value of this parameter increased by the 7th day of observation (Table 4). It is possible that the causes for the increase in the *C*RP level can be both the activation of the systemic inflammatory response as a result of blood contact with the non-endothelialized surface of the ECMO highways, the

shear pressure in different parts of the circuit, and the mechanical destruction of the blood formed elements by the moving part of the centrifugal pump, and the addition of secondary bacterial infection in some patients.

Table 4. Dynamics of inflammatory response markers

Parameter	Control group	Day			p
		1st	3rd	7th	
CRP, mg/L	0.7 [0.4;1.7]	11.5 [2.1;49.5]*	44.4 [5.3;90.8]*	76.3 [25.3;162.5]*, **	p_{N-1}<0.0001 p_{N-3}<0.0001 p_{N-7}<0.0001 p_{1-7}<0.0001
Leukocytes, 10^9/L	5.58 [5.18;6.29]	14.90 [8.65;19.88]*	13.13 [8.34;18.18]*	13.75 [8.64;18.38]*	p_{N-1}<0.0001 p_{N-3}<0.0001 p_{N-7}<0.0001
Neutrophils, 10^9/L	3.30 [2.80;4.18]	12.79 [7.85;17.73]*	11.35 [7.70;16.04]*	11.60 [6.58;16.06]*	p_{N-1}<0.0001 p_{N-3}<0.0001 p_{N-7}<0.0001
Eosinophils, 10^9/L	0.13 [0.10;0.19]	0.04 [0.01;0.10]*	0.06 [0.02; 0.17]*	0.06 [0.02;0.15]*	p_{N-1}<0.0001 p_{N-3}=0.0310 p_{N-7}=0.0071
Lymphocytes, 10^9 /L	1.80 [1.60;1.98]	0.48 [0.30;0.86] *	0.54 [0.27;0.89]*	0.68 [0.41;0.92]*	p_{N-1}<0.0001 p_{N-3}<0.0001 p_{N-7}<0.0001
Platelets, 10^9/L	248 [228;274]	149 [104;226]*	96 [65;138]*, **	83 [40; 103]*, **	p_{N-1}<0.0001 p_{N-3}<0.0001 p_{N-7}<0.0001 p_{1-3}=0.0002 p_{1-7}<0.0001
NLR (Neutr./ Lymph., abs. counts)	1.94 [1.43;2.38]	21.96 [13.27;38.43]*	16.78 [11.27;32.85]*	15.93 [10.04;27.03]*	p_{N-1}<0.0001 p_{N-3}<0.0001 p_{N-7}<0.0001
PLR (Platelet./ Lymph., abs. counts)	146.58 [112.89;168.59]	317.95 [159.96;499.55]*	182.00 [96.97;304.17] **	109.17 [82.48;165.99]**	p_{N-1}<0.0001 p_{1-3}=0.0030 p_{1-7}<0.0001
Systemic inflammation index (Platelet.* Neutr./Lymph . abs.counts)	484.56 [381.95;577.46]	3836.00 [1880.71;6383.49]*	2099.25 [905.88;3393.44]*, **	1256.41 [682.44;2716.95]*, **	p_{N-1}<0.0001 p_{N-3}<0.0001 p_{N-7}<0.0001 p_{1-3}=0.0117 p_{1-7}<0.0001

Note : *p<0.05 relative to normal; **p<0.017 relative to the 1st day (intragroup comparison for three time points); the difference significance level relative to the norm: p_{N-1}, on the 1st day after ECMO, p_{N-3}, on the 3rd day after ECMO; p_{N-5}, on the 5th day after ECMO; p_{N-7}, on the 7th day after ECMO; p_{1-3}, the difference between the values on the 1st and 3rd day after ECMO; $p_{1-7,}$ the difference between the values on the 1st and 7th days after ECMO

Also, as can be seen from Table. 4, the total leukocyte count was statistically significantly increased in all periods of study observation (p<0.05). In patients with severe COVID-19 on ECMO, the absolute

number of neutrophils was also statistically significantly increased; and the absolute number of lymphocytes was statistically significantly decreased. Thus, in patients of this group, the development of an inflammatory reaction was observed.

When studying the data of the calculated hematological indices, which are widely used to assess the course of coronavirus infection and predict its outcome, their statistically significant increase was found in all periods of the study ($p<0.05$). In various studies, it was noted that higher values of NLR and PLR were associated with a poor prognosis [16, 17].

In a meta-analysis made by S.A. Chan in 2020, which included 19 studies and 3478 cases, the mean values of the NLR index ranged 3.8–15.0 in severe COVID-19 cases, and from 1.9 to 4.1 in non-severe cases [18]. S. Kazancioglu et al. proposed the optimal cut-off point of 2.47 for the NLR index [19]. In a study by M. Rokni et al. (2020), the NLR index above 5.0 was observed in 93% of deceased patients [20].

The data obtained in our study indicated an extremely severe course of the disease and an unfavorable prognosis. Similar results were obtained when studying the PLR index. M. Rokni et al. (2020) found that a systemic inflammation index above 500 was observed in 92.9% of deceased patients, the mean value of the index in deceased patients was 3532.9±565.3, and it was 1163.5±102.9 in survivors [20]. The data obtained in our study are fully consistent with the data of foreign colleagues. We should also note that in an intragroup comparison, PLR and the systemic inflammation index on the 7th day of the study observation were statistically significantly lower compared to that on the 1st day ($p_{1-7}<0.0001$), which indicated the efficacy of the therapy (see Table 4).

According to the literature data, a vicious circle associated with hyperinflammation and microvascular endothelial damage may be a key

factor in multiple organ failure and even death in patients with severe COVID-19 [21]. In this regard, the study examined the relationship between the inflammatory response and endothelial damage in patients with severe COVID-19 (Table 5).

Table 5. Correlation between markers of endothelial damage and markers of inflammatory response (Spearman's method)

Parameter	Day	*CRP*	Leuk.	NLR	PLR	Systemic inflammation index
NOx	1st day	0.28 [0.02;0.51] p=0.0360	-0.02 [-0.29;0.24] p=0.8651	-0.19 [-0.43;0.08] p=0.1705	-0.21 [-0.45;0.06] p=0.1291	-0.19 [-0.43;0.08] p=0.1610
	3rd day	0.25 [-0.05;0.51] p=0.0986	0.03 [-0.27;0.32] p=0.8545	-0.07 [-0.35;0.23] p=0.6669	-0.22 [-0.48;0.08] p=0.1422	-0.24 [-0.50;0.05] p=0.1051
	7th day	0.16 [-0.18;0.46] p=0.3622	-0.08 [-0.40;0.25] p=0.6222	-0.31 [-0.58;0.02] p=0.0640	-0.45 [-0.68;-0.15] p=0.0059	-0.41 [-0.65;-0.09] p=0.0146
ACE	1st day	-0.15 [-0.41;0.12] p=0.2699	-0.03 [-0.30;0.24] p=0.8446	-0.06 [-0.33;0.21] p=0.6546	0.04 [-0.24;0.30] p=0.7889	0.05[-0.22;0.32] p=0.7096
	3rd day	-0.05 [-0.34;0.25] p=0.7444	-0.19 [-0.46;0.11] p=0.2104	0.02[-0.27;0.31] p=0.8907	0.10 [-0.20;0.38] p=0.5153	-0.03 [-0.32;0.26] p=0.8212
	7th day	-0.22 [-0.51;0.12] p=0.2001	0.19 [-0.15;0.49] p=0.2658	0.22 [-0.12;0.51] p=0.2025	-0.01 [-0.34;0.32] p=0.9649	0.12[-0.22;0.43] p=0.4944
NOx/ACE	1st day	0.29 [0.03;0.52] p=0.0323	-0.03 [-0.30;0.24] p=0.8266	-0.05 [-0.32;0.22] p=0.6967	-0.14 [-0.40;0.13] p=0.3126	-0.16 [-0.41;0.12] p=0.2591
	3rd day	0.29 [-0.00;0.54] p=0.0511	0.14 [-0.16;0.41] p=0.3757	-0.04 [-0.33;0.25] p=0.7823	-0.30 [-0.54;-0.00] p=0.0475	-0.24 [-0.50;0.06] p=0.1176
	7th day	0.25 [-0.09;0.53] p=0.1484	-0.16 [-0.47;0.18] p=0.3455	-0.33 [-0.59;0.00] p=0.0506	-0.32 [-0.58;0.01] p=0.0596	-0.34 [-0.60;-0.01] p=0.0435

Note: CRP, *C*-reactive protein, Leuk, leukocytes, NLR, Neutrophils/Lymphocytes ratio, PLR, Platelets/Lymphocytes ratio, Systemic inflammation index = Platelets x Neutrophils/Lymphocytes ratio, NOx, nitric oxide, ACE, angiotensin-converting enzyme, p, the difference significance level

As one can see from Table 5, a statistically significant weak positive correlation was found between the concentration of *CRP* and NOx on the 1st day: R = 0.28 (95% CI 0.02-0.51), p = 0.036; n = 52. Thus, a relationship was found between the NOx level and the severity of the inflammatory response, which confirms the relationship between the development of endothelial dysfunction and systemic inflammation in patients with severe COVID-19.

A statistically significant negative correlation between the NOx concentration and the PLR index (see Table 5) was noted on the 7th day: R=-0.45 (95% CI -0.68;-0.15), p=0.0059; n=49 and indicated that low NOx values coincided with higher values of the PLR index, implying that that a decrease in the NOx protective function was associated with a more severe course of coronavirus disease, which corresponded to the literature data [22].

This correlation identified in ECMO patients on the 7th day may indicate an increasing role of ECMO during this period to suppress oxidative stress and maintain vasotonic function.

A statistically significant negative correlation between NOx concentration and systemic inflammation index (Table 5) was noted on day 7: R=-0.41 (95% CI -0.65;-0.09), p=0.0146; n=49.

The negative correlation between NOx concentration in blood and the systemic immune inflammation index means that low values of the nitric oxide correspond to higher values of the index, that is, a decrease in the NOx protective function was associated with adverse outcomes [17, 23–25].

Thus, studies of the relationship between endothelial damage and the severity of the systemic inflammatory response may be of fundamental importance for explaining the pathophysiological mechanisms of the COVID-19 course and developing new treatments for such patients. We should emphasize that in our study, the activity of the inflammatory process continued to increase while the level of markers of the COVID-19 course severity decreased, and the dynamics of hematological parameters indicated a developing infectious process. Perhaps the reason for this can be both the activation of the systemic inflammatory response resulted from the blood contact with the non-endothelialized surface of the ECMO system lines, shear pressure in

different parts of the circuit, mechanical destruction of the formed elements of blood by the moving part of the centrifugal pump, and the addition of a secondary bacterial infection. We should also note that absent increases in MDA values and the OS coefficient in dynamics, and the improvement in the endothelium vasotonic function (an increase in the NOx/ACE coefficient in dynamics) indicate the therapy efficacy, which is especially important when performing ECMO, since this methodology involves the use of elevated oxygen concentrations, and, accordingly, might contribute to the activation of oxidative stress. Thus, the absence of aggressive trends in the change of MDA values and OS coefficient indicates the tolerance of homeostasis parameters to the ECMO exposure.

The data obtained confirm that pro-inflammatory mediators cause an endothelial dysfunction (specifically, the vasotonic function impairment), which can be a trigger in the development of multiple organ failure in patients with severe COVID-19 and should be taken into account while making decisions in the treatment process.

When assessing the severity of the systemic inflammatory response in patients with severe COVID-19, including those on extracorporeal membrane oxygenation, the hematological indices should be additionally monitored in dynamics, namely the platelet absolute count to lymphocytes absolute count ratio, and systemic inflammation.

Conclusions

1. In the process of extracorporeal membrane oxygenation in patients with severe COVID-19, an impairment of the endothelium vasotonic function occurs, which on the 1st day of the study is manifested by a statistically significant two-fold decrease in the blood level of nitric

oxide relative to the norm, as well as by deranged associated regulatory function between the nitrogen oxide vasodilator and an angiotensin-converting enzyme vasoconstrictor (a statistically significant 1.7-time decrease in the nitric oxide metabolite to angiotensin-converting enzyme ratio relative to the norm on the 1st day), which contributes to the development of endothelial dysfunction.

2. In the process of extracorporeal membrane oxygenation in patients with severe COVID-19, a correlation was found between the markers of endothelial damage and the severity of the inflammatory response (between the blood level of *C*-reactive protein and the nitric oxide concentration on the 1st day: R=0.28 (95% CI 0.02;0.51), p=0.036; n=52; between the blood concentration of nitric oxide and the platelet absolute count to lymphocyte absolute count ratio on the 7^{th} day: R=-0.45 (95% CI -0.68;-0.15), p=0.0059; n=49; between the blood concentration of nitric oxide and systemic inflammation index on the 7^{th} day: R=-0.41 (95% CI -0.65;-0.09), p=0.0146, n=49).

3. After extracorporeal membrane oxygenation, a statistically significant 1.2-time decrease in total antioxidant activity by the 3^{rd} day of the study, and a 1.7-time increase in the blood level of malondialdehyde as soon as on the 1st day of the study observation were revealed, which indicated the activation of free radical processes in this category of patients and is one of the reasons for the development of endothelial dysfunction in patients with severe COVID-19. However, an absent further increase in these parameters by the 7^{th} day of the study is the evidence against of the shift of redox processes towards peroxidation and, thus, the possibility of safe use of extracorporeal membrane oxygenation.

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