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Changes in Some Vascular Biomarkers in Patients with Severe COVID-19 with Various Degrees of Pulmonary Hypertension

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The aim of the study was to evaluate the levels of cardiac biomarkers endothelin 1, B-natriuretic peptide (BNP), N-terminal pro-B-type natriuretic peptide (Nt-proBNP), NO_2, and NO_3 in patients with COVID-19 pneumonia and various degrees of pulmonary hypertension. Group 1 included patients with pulmonary artery systolic pressure <25 mm Hg, group 2 with 25-40 mm Hg, and group 3 with 40-60 mm Hg. In the group of patients with pulmonary artery systolic pressure <25 mm Hg, the level of NT-proBNP was higher than in the rest two groups by 41.3% (p=0.015) and 38.2% (p=0.015), respectively. The levels of nitrites and nitrates in group 1 patients were lower: NO_2 was reduced by 31.1% (p=0.026) and 62.8% (p=0.008), and NO_3 was reduced by 28% (p=0.029) and by 54.6% (p=0.006), respectively. No other changes in the parameters in patients receiving oxygen therapy were found. These findings suggest that severe course of COVID-19 in patients with severe pulmonary hypertension is associated with impaired nitrite and nitrate metabolism and reduced levels of Nt-proBNP.

Key Words: *COVID-19; NO; pulmonary hypertension; BNP; NP-proBNP*

Severe COVID-19 (SARS-CoV-2 infection) is currently considered a disorder that produces pronounced damage to the upper airways [14] and alveolar cells, and affecting many other organs and tissues. Multiple organ involvement in COVID-19 is associated with a high incidence of thromboembolic events and the risk of the development of multiple organ failure [6]. Vascular endothelial injury and thrombosis-associated inflammation are new key factors in the COVID-19 pathophysiology [4]. Postmortem examinations of patients who died from COVID-19 demonstrated pronounced endothelial damage [4], and the markers of endothelial cell activation were significantly elevated in patients with a severe disease [7].

Severe COVID-19 is more likely to develop in patients with cardiovascular disorders or those who have risk factors for their development [2]. It is noteworthy that all the factors related to poor prognosis of COVID-19 (*e.g.* cardiovascular disorders, older age, obesity, hypertension, and diabetes) also predispose to endothelial dysfunction.

Endothelial dysfunction together with systemic inflammatory response initiates the impairment of the mechanisms of vascular regulation in the whole cardiovascular system. The pathological process gradually involves the vascular wall of alveolae, pulmonary arteries, and the heart thus increasing the right ventricular load [1]. Hypoxic pulmonary vasoconstriction further aggravates the hemodynamic disorders and also play an important role in the development of pulmonary hypertension [12].

Some serum biomarkers, such as endothelin 1, B-natriuretic peptide (BNP), and N-terminal pro-B-type natriuretic peptide (Nt-proBNP) have been extensively studied in various pathologies over the past years [10]. The frequency of critical conditions in patients with COVID-19 pneumonia can be related to impaired metabolism of these vasoactive substances.

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Our aim was to evaluate the levels of vascular biomarkers in patients with COVID-19-associated pneumonia and pulmonary hypertension of different severity.

MATERIALS AND METHODS

A prospective non-randomized study was conducted in 48 male and female patients aged 40-80 years with community-acquired COVID-19-associated polysegmental viral and bacterial pneumonia who were treated at intensive care units and required respiratory support. The study was approved by the Local Ethics Committee of the Chita State Medical Academy (Protocol No. 102, May 15, 2020), and was conducted in accordance with local treatment protocols. Non-inclusion criteria were confirmed malignancies, severe immunodeficiency, unstable hemodynamics, vasopressor infusions, signs of hypovolemia, systemic vascular disorders, acute cardiovascular disorders and conditions (acute coronary syndrome, myocardial infarction, and pulmonary edema).

The patients were divided into 3 groups depending on the level of pulmonary artery systolic pressure (PASP). Group 1 included 14 patients who had the PASP of <25 mm Hg. Group 2 included 20 patients with PASP of 25-40 mm Hg. Group 3 included 14 patients with PASP of 40-60 mm Hg. All patients had severe chronic pulmonary pathologies (Table 1).

The study design included PASP measurement by an ultrasonic technique and simultaneous single venous blood collection. Blood serum was separated by centrifugation and the levels of endothelin 1, B-natriuretic peptide (BNP), and N-terminal pro-B-type natriuretic peptide (Nt-proBNP) were determined using ELISA kit (Cloud Clone). Nitrites (NO_2) and nitrates (NO_3) were assayed after enzymatic conversion of a nitrate into a nitrite catalyzed by the enzyme nitrate reductase followed by colorimetric measurement of nitrite by azo dye formation in the Griess reaction (Total Nitric Oxide and Nitrate/Nitrite Parameter Assay Kit; Cloud-Clone).

The statistical analysis was carried out using SPSS Statistics 28.0.1.1 (IBM). Given the fact that the sample included less than 50 people, the testing for normality was conducted using the Shapiro–Wilk test. Then we calculated Me (Q1; Q3) of the evaluated parameters. The qualitative parameters were evaluated using the Pearson's χ^2 test with the Yates correction as described in the algorithm [3]. The significance of the differences was evaluated by comparing two independent samples using the Mann–Whitney *U* test. The differences between the median values were considered significant at $p<0.05$.

RESULTS

In group 1 patients (PASP <25 mm Hg), the content of NT-proBNP was higher than in groups 2 and 3 by 41.3% ($p=0.015$) and 38.2% ($p=0.015$), respectively (Table 2). Currently, BNP, NT-proBNP are more related to predicting acute cardiovascular events. Previous studies have shown that the levels of these peptides were within the normal ranges in COVID-19 patients, even in those with severe disease course [9]. This is due to specifics of the metabolism that are closely related to the production of angiotensin-converting enzyme (ACE2) [5,9]. Probably, due to this feature, there is a decrease in the level of not only the BNP, but also NT-pro-BNP. The production of the enzyme is likely to continue decreasing if the disease course gets worse.

Blood levels of nitrites and nitrates in group 1 were lower than in groups 2 and 3: NO_2 was reduced by 31.1% ($p=0.026$) and 62.8% ($p=0.008$), NO_3 was reduced by 28% ($p=0.029$) and 54.6% ($p=0.006$), respectively (Table 2). NO is a short-lived gaseous free radical that controls vascular tone and prevents the development of hypertension in COVID-19 patients [11]. Cardiovascular disorders, such as hypertension, have been shown to be the most common comorbidities in COVID-19 patients. More intensive or impaired NO metabolism can be related to the severity of COVID-19. NO molecule is predominantly synthesized

TABLE 1. Characteristics of Concomitant Disorders and Mortality Rates

Disease	Group 1 (PASP<25 mm Hg; *n*=14)	Group 2 (PASP 25-40 mm Hg; *n*=20)	Group 3 (PASP>40 mm Hg; *n*=14)	Significance df=2
PASP	24 (22; 24)	32.5 (28.25; 35.00)	48 (46; 58)	
Mean age, years	61 (51.50; 62.75)	64 (60.00; 73.25)	68 (63; 72)*	
BMI, kg/m^2	33.38 (27.770; 39.626)	30.625 (28.527; 34.041)	30.725 (26.895; 34.638)	
COPD	2 (14.3%)	4 (20%)	3 (21.4%)	$\chi^2=49.015$, $p<0.001$
Hypertension	10 (71.4%)	20 (100%)	13 (92.9%)	$\chi^2=56.586$, $p<0.001$
Diabetes mellitus	4 (28.6%)	7 (35%)	3 (21.4%)	$\chi^2=49.753$, $p<0.001$
Mortality rate	6 (42.9%)	13 (65.0%)	10 (71.4%)	$\chi^2=51.747$, $p<0.001$

TABLE 2. Changes in the Parameters of Endothelial Damage (Me (Q1; Q3))

Parameter	Group 1 (PASP<25 mm Hg; *n*=14)	Group 2 (PASP 25-40 mm Hg; *n*=20)	Group 3 (PASP>40 mm Hg; *n*=14)
Endothelin 1, pg/ml	187.2 (126.075; 195.4)	174.85 (123.025; 186.220)	145.700 (103.205; 199.850)
BNP, pg/ml	183.4 (143.45; 204.975)	170.400 (134.200; 330.775)	159.500 (124.100; 176.600)
NT-proBNP, pg/ml	60.250 (45.085; 67.465)	35.375 (31.720; 48.842)	37.215 (31.520; 50.435)
	^{+o}p=0.015		
Nitrates (NO_3), μmol/liter	22.695 (19.410; 28.778)	29.070 (24.423; 38.402)	35.085 (28.335; 37.740)
	^{+}p=0.029		
	^{o}p=0.006		
Nitrites (NO_2), μmol/liter	19.518 (16.835; 25.891)	26.178 (23.585; 35.870)	31.786 (26.036; 35.184)
	^{+}p=0.026		
	^{o}p=0.008		

Note. Significance of differences from $^{+}$group 2, ^{o}group 3.

by NO synthases. This reaction requires oxygen and is therefore inhibited in patients with acute respiratory distress syndrome due to hypoxia. There is evidence of the correlation between the pulmonary artery pressure and the disease severity (the severity of lung tissue involvement), which is associated with a higher frequency of complications and poor outcomes [13]. That is why patients with moderate to severe pulmonary hypertension were more likely to require invasive respiratory support [1]. Since the rate of NO formation from nitrite linearly depends on the level of oxygen and pH, the patients suffering from severe hypoxia tended to have higher levels of this parameter [8].

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