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ORIGINAL RESEARCH ARTICLE

Sars-Cov2 Induced Biochemical Mechanisms in Liver Damage and Intestinal Lesions

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Abstract

Multiple pathogenic mechanisms are found in SARS-CoV2 systemic inflammation. Oxidative stress, altered proteolysis, hypercoagulation, and metabolic disorders are significant in virus-induced lesions.

The study aimed to investigate the biochemical mechanism of virus-induced disorders and determine the biochemical features in SARS-CoV2-associated liver damage and intestine lesions.

A retrospective case series of ninety-two patients diagnosed with COVID-19 pnemonia. The ACE, α1-proteinase inhibitor, trypsin-like proteinase, and elastase activity were measured. Nitrites level was detected in reaction with Griess reagent. The ELISA kit measured Troponin, C-peptide, leptin, adiponectin, PAR4, and neuropilin level.

It was obtained an increase in ACE activity and nitrites ions content in SARS-CoV2 associated patients. The hyperglycemia and an increase in adipose tissue-derived hormones guided the virus-induced metabolic disorders. Proteolysis activation was revealed in SARS-CoV2 pneumonia patients. The found molecular event was accompanied by hyperglycemia induction. Multiorgan lesions manifest in in cardiac failure, which was detected in patients with ARDS. Moreover, high arterial blood pressure in patients with COVID-19 was associated with the hyperglycemia and increased ACE activity and NO ions level. Liver damage was specific for COVID-19-associated patients with severe ARDS and heart failure. Proteolysis overactivation resulting in vasoactive substances imbalance was detected in patients with the intestinal lesions. The obtained data shows the the neuropilin-dependent axis in damage prevalence in the intestine.

Metabolic disorders resulting in the growth of adipose-derived tissue hormones, nitrites, and neuropilin levels was triggered by prolonged inflammation. So, the impaired metabolism and SARS-CoV2 associated hyperglycemia influence on SARS-CoV2 multiple mechanisms. Gastrointestinal manifestations in SARS-CoV2 infection was found to be related to various biochemical and molecular tools. ACE2 receptors axis is prevalent for liver damage, but NRP-1 protein (neuropilin), NO derivatives, and adipose tissue-derived hormones are essential for intestinal lesions.

Keywords SARS-CoV2 · renin-angiotensin system · troponin · metabolic disorders · proteolysis · neuropilin · liver damage, intestinal lesions

Introduction

COVID-19 is a disease-causing current pandemic, and it prevails in patients with preexisting conditions such as diabetes and hypertension. It is known metabolic changes induced by diabetes, especially hyperglycemia, can directly affect the metabolism and predict the COVID-19 complications [1]. The SARS-CoV2 multilayer pathogenesis is based on the interaction of the protein with components of the innate immune system to evade an anti-viral interferon response [2].

ACE2 is a key and an established component of the renin-angiotensin-aldosterone system (RAS) that opposes angiotensin II (ANG II) pressor and tissue remodeling actions. Acting via the type 1 receptor, Ang II initiates an inflammatory cascade of reduced nicotinamide-adenine dinucleotide phosphate oxidase, reactive oxygen species, and nuclear factor-κB mediates transcription gene expression increases adhesion molecules and chemokines [3]. An excess of ROS

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decreases nitric oxide bioavailability and causes endothelial dysfunction [4].

The RAS has a significant role in developing acute lung injury and respiratory distress syndrome (ARDS), a devastating complication of SARS-CoV-2 infection and virus-induced cardiac failure [5]. The serum ACE activity was evaluated, and there is no association between serum ACE activity and COVID-19. The serum ACE activity did not reflect disease severity [6]. Even though it is found the increased susceptibility to COVID-19 infection in patients with to have the higher intestinal expression of ACE2 [7].

COVID-19 could be more aggressive due to a high "basal" inflammation level with low nitric oxide (NO) levels in hypertensive, diabetic, and obese patients. Interestingly, the "protective" effects of several factors (such as estrogens) may play a role by increasing the formation of endogenous NO [8]. The mechanism of this action is connected with the bradykinin that causes vascular relaxations through the release of endothelial relaxing factors. The ACE inhibition enhances bradykinin release [9].

Activation of the RAS and abnormal adipokine levels are biological alterations that affect metabolism and blood pressure regulation manifesting in hypertension, obesity, and metabolic diseases [10]. The comorbidities follow-up accompanies preexisting chronic inflammation that is associated with proteolysis activation. Implementation of proteolysis in community-acquired pneumonia results from an active infectious process in the lung tissue, leading to systemic inflammation and multiorgan damage [11]. Trypsin-like and elastase-like proteinases are the key proteolytic enzymes of neutrophils and macrophages that ensure the development of the inflammatory response [12]. Currently, much attention is paid to the role of the virus, in particular SARS-CoV2, in the activation of trypsin-like proteinases [13].

Proteolysis overactivation originates from the lack of alpha-1 proteinase inhibitor (α1-PI) [14]. The uncontrolled activation of enzymes that hydrolyze proteins leads to damage to organs and tissues, causing dysfunction of the lungs, heart, and nervous system under the influence of SARS-CoV2 infection [15]. Moreover, the SARS-CoV2 virus penetration happened due to the endogenous proteinases [16].

The activation of inflammation and blood clotting is essential synchronous reactions of the human body associated with each other to an infection of any origin [17]. Thrombin triggers platelet activation through proteinase-activated receptors (PARs). PAR4 and PAR-1 activate the synthesis and secretion of thromboxane A2, pro-inflammatory factors IL-1b [18, 19]. Thrombin also increases the adhesion of platelets to monocytes and neutrophils, causing the formation of neutrophil extracellular traps (NET), thereby enhancing the pro-inflammatory activity of NET and lung damage in COVID-19 [20].

Table 1 Clinical Characteristics of SARS-CoV2 infected patients

Characteristic	Yes, n (%)	No, n (%)
Adverse outcome	8 (8.7%)	84 (91.3%)
ARDS	44 (47.8%)	48 (52.2%)
Diabetes Mellitus Type 2	15 (16.3%)	77 (83.7%)
Arterial Hypertension	41 (44.6%)	51 (55.4%)
Glucocorticoids application in combined therapy	19 (20.7%)	73 (79.3%)
ACE inhibitors	78 (84.8%)	14 (15.2%)

There are other alternative receptors, which include CD147, DPP4 (dipeptidyl peptidase-4), ANPEP (alanyl aminopeptidase), ENPEP (glutamaminopeptidase), and NRP-1 [21]. Neuropilin (NRP-1) is a membrane-bound co-receptor of the tyrosine kinase receptor of vascular endothelial growth factors, providing the processes of angiogenesis, as well as cell proliferation and migration [22]. In addition, a relationship was found between serum protein level and glucose concentration. It is believed the neuropilin rate in the blood can be associated with the risk of metabolic disorders [23].

The study aimed to investigate the biochemical mechanism of virus-induced disorders and determine the biochemical features in SARS-CoV2-associated liver damage and intestine lesions.

Materials and Methods

It is a retrospective observational case series of 92 patients with SARS-CoV2. We included only patients with laboratory-confirmed COVID-19 infection admitted to the Second Medical Hospital, Tomsk, Russia, between March 16, 2020, and June 5, 2021 (Table 1). A confirmed case of COVID-19 was defined by a positive result on reverse-transcriptase polymerase chain reaction (RT-PCR) assay on nasopharyngeal swabs. Demographic data, clinical presentation, evolution, and laboratory and radiologic outcomes were recorded. The ARDS severity was rated using the PaO2/FiO2 ratio (partial pressure of arterial oxygen over the fraction of inspired oxygen). Descriptive analysis is presented as the median and range for continuous variables. All procedures were performed following the Ethics Committee in Siberian State Medical University (protocol code 4; 16.11.2020), and the Declaration of Helsinki. Written informed consent was obtained from the patients.

Data collection. All admission data were obtained from patients' electronic medical records and were reviewed by two physicians (Vladimir Masunov and Yumzhana Dagmaeva) (Table 1). Information extracted included demographic data, exposure history, comorbidities, symptoms,

treatments, in-hospital complications, outcomes, laboratory results, and chest CT images.

The age of the patients ranged from 30 to 80 years. 23 people (25.0%) made up the group < 45 years old; 25 people (27.1%) fell into the age category from 45 to 59; 31 people (33.7%) - in 60–75 years.

Based on the clinical data, patients were divided into DM patients (n = 15) and non-DM patients (n = 77). The median ages were 60.00 (49.50; 68.50) and 72.0 (65.00; 77.00) in the non-diabetic and diabetic groups. The median body mass index (BMI) in patients with or without DM was 24.7 (22.0–26.4) and 23.4 (21.0–26.0), respectively. Arterial hypertension (AH) was diagnosed when systolic blood pressure equals or above 140 mm Hg or diastolic blood pressure is similar to or above 90 mm Hg. Thirty-five patients had no AH (50.00 (40.00; 58.00) years), and 56 patients had a verified diagnosis (66.50 (61.50; 72.50) years). Forty-three patients had no gastrointestinal lesions; SARS-CoV2 associated hepatitis was found in 30 patients and intestinal lesions was detected in 29 ill people.

Outcomes and definitions. ARDS was diagnosed based on the WHO guidance for COVID-19. The primary outcomes included entry into the intensive care unit (ICU) and in-hospital deaths. The secondary outcomes were any in-hospital complications, including SARS-CoV-2-related ARDS, acute cardiac injury, acute kidney injury, and secondary infection.

Leptin, adiponectin, C-peptide, PAR4, neuropilin, and C-peptide detection. ELISA kits were used for leptin, adiponectin, C-peptide, PAR4, neuropilin level detection (Cloud-Clone Corporation, Katy, TX 77,494, USA). Sandwich ELISA Kit was applied to measure C-Peptide in Human Serum (Vector-Best. Novosibirsk, Russia).

ACE activity. The ACE activity was measured in serum by the kinetic of synthetic substrate hydrolysis, FAPGG (Sigma, USA), and measured in μmol /min.

Nitrites content. The methods used the Griess diazotization reaction to detect nitrite formed by spontaneous oxidation under physiological conditions spectrophotometrically. The detection limit for this method is 1.0 μM nitrite. 0.1 mL of serum was added to the 1mL of Griess solution. After 15 min, the absorbance is measured. The nitrite content in micrograms is calculated using a calibration graph or a scale of standard solutions.

Determination of the α1-proteinase inhibitor activity. The α1-proteinase inhibitor activity was determined by inhibiting the arginine-esterase activity of trypsin. The activity was expressed in inhibitory units per 1 ml of serum (IU/ml). N-benzoyl-L-arginine ethyl ether (BAEE) was used as a substrate (Nartikova V.F., 1989).

Determination of the activity of trypsin-like proteinases. The N-benzoyl-L-arginine-ethyl ester (BAEE) hydrolysis was used for determining the trypsin-like proteinase activity (nmol BAEE / min per 1 ml of serum).

Determination of the activity of elastase-like proteinases. The elastase-like proteinases activity was measured by the rate of a ρ-nitrophenyl ester of N-butyloxycarbonyl-L-alanine (BANE) hydrolysis (nmol BANE/min per 1 ml of serum).

Statistical analysis. The statistical analysis was performed using the Statistica 12.0 software package. Verification of normality was performed using the Kolmogorov-Smirnov test. The determination of gene expression results is presented as Me (Q1; Q3). The Mann-Whitney test assessed the significance of differences, and differences were considered significant at $p < 0.05$.

Results

ACE Activity, Nitrites, and Troponin Content in SARS-CoV2 Patients, role in Multiorgan Damage

The ACE activity is an essential change in SARS-CoV2 pathogenesis. It was increased by 1.4 times in patients with SARS-CoV2 community-acquired pneumonia compared to healthy people (Table 2). There is no difference in NO derivatives and troponin levels in patients with SARS-CoV2. But ARDS development was accompanied by the growth in troponin level. The revealed results indicated the correlation of pneumonia with heart failure.

ACE and NO derivatives provoke considerable multiorgan damages. It is known that high ACE activity is related to ischemic stroke and myocardial infarction. The increased ACE activity and nitrites content was revealed in SARS-CoV2 patients with AH. There is no change in the ACE activity and nitrites level in patients with DM.

The role of RAS imbalance in cardiac failure in SARS-CoV2 patients was indicated. The growth in ACE activity, NO ions content, and troponin level in SARS-CoV2 led to adverse outcomes. Complex therapy with ACE inhibitors in patients was associated with a decrease in ACE activity and nitrites content, that needs further investigation.

Metabolic Disorders in SARS-CoV2 Patients

The complex metabolic parameters characterizing adipose and carbohydrate metabolism were studied in patients with SARS-CoV2 infection (Table 3). An increase in the leptin and adiponectin levels of 3.1 and 1.3 times, respectively, accompanied by C-peptide reduction has been shown in SARS-CoV2 patients compared with healthy people.

The relationship between hyperglycemia and the ARDS was confirmed (Table 3). A high glucose level was found in

Table 2 ACE activity, nitrites, and troponin content in SARS-CoV2 patients, (Me (Q1; Q3)

Indicator		ACE activity, μmol /min·L	Nitrites, μmol/L	Troponin, ng/mL
Healthy people		23.00 (20.00; 43.30)	30.00 (24.00; 37.00)	0.00 (0.00; 0.00)
SARS-CoV2 patients		32.30 (20.00; 40.00)#	27.00 (26.00; 27.00)	0.03 (0.00; 0.08)
ARDS	No ARDS PaO2 = 100	33.00 (21.65; 37.50)	30.00 (24.00; 36.00)	30.00 (27.00; 45.00)
	Presence of ARDS PaO2 < 95	30.00 (16.67; 43.30)	0.00 (0.00; 0.07)	0.04 (0.00; 0.09)*
DM	Non-DM	30.00 (18.33; 40.00)	30.00 (24.00; 37.300)	0.04 (0.00; 0.08)
	DM	35.00 (33.00; 45.00)	33.00 (29.25; 40.50)	0.04 (0.00; 0.09)
AH	Non-AH	28.30 (11.60; 40.00)	35.00 (29.00; 37.00	0.001 (0.00; 0.01)
	AH	33.00 (22.50; 41.65)*	28.50 (24.00; 45.00)**	0.01 (0.00; 0.09
Outcome	Favorable outcome	29.65 (16.67; 35.00)	30.00 (24.00; 35.00)	0.00 (0.00; 0.02)
	Adverse outcome	37.50 (33.30; 47.45)***	40.5 (27.00; 54.00)***	0.09 (0.06; 0.10)***
ACE inhibitors	No inhibitors of ACE	33.00 (20.00; 43.30)	30.00 (25.50; 41.00)	0.00 (0.00; 0.09)
	Inhibitors of ACE	10.10 (1.60; 29.30)#	24.00 (21.60; 36.00)##	0.00 (0.00; 0.05)

Note: # - the significance of differences compared to the healthy people, $p < 0.05$; *- the significance of differences compared to the SARS-CoV2 patients with no ARDS, $p < 0.05$;

** - the significance of differences compared to the non-AH SARS-CoV2 patients, $p < 0.05$;

*** - the significance of differences compared to the SARS-CoV2 patients with favorable outcome, $p < 0.05$; ## - the significance of differences compared to the SARS-CoV2 patients with ACE inhibitors application

patients with ARDS. The 1.48 times reduction in adiponectin level was found in patients who received glucocorticoid therapy. At the same time, the 1.56 growth in leptin/adiponectin ratio indicated the severity of metabolic disorders in COVID-19 infection. Glucocorticoid-induced diabetes mellitus is a common drug-induced problem in clinical practice, affecting almost all medical specialties, but is often difficult to detect in clinical settings [30].

Proteolysis Overactivation in SARS-CoV2-induced Pneumonia, role in Multiorgan Damage

α1-PI and proteases overactivation was detected in SARS-CoV2 pneumonia (Table 4). It was obtained the high rate in α1-PI, trypsin-like, and elastase-like proteinase activity in 1.5, 4.8, and 3.5 times, consequently, in SARS-CoV2 pneumonia patients compared to the control group. The 1.25 growth in PAR4 content was detected in patients with COVID-19.

Proteolysis imbalance originates from the virus invasion leading to the ARDS initiation. The activity of trypsin-like proteinases in ARDS was 8.6 times higher than in controls and 2.6 times higher than in patients without ARDS. However, the activity of neutrophilic elastase was equally high and independent on the ARDS initiation. Proteolysis overactivation was found in AH patients. The trypsin-like proteases activity was increased by2.7 times in AH patients with COVID-19 compared to the non-AH ones. The PAR4 content in ARDS patients was 1.75 times and1.4 times higher than in healthy people and in COVID-19 patients without signs of ARDS.

The COVID-19-induced deaths were associated with low PAR4 levels compared to recovered patients. A 9.2 and 3.7 times increase in the trypsin-like proteases and elastase activity was observed in patients with adverse outcomes, consequently, compared to the healthy people. Consequently, a decrease in PAR4, an increase in trypsin-like proteinases, and elastases may be an early sign of the lethal outcome in COVID-19 infection.

> In patients who were subsequently treated with corticosteroids, the PAR4 content was 28% lower than in patients whose treatment did not require hormone therapy. A 2.2 growth in trypsin-like activity was found in patients who received the glucocorticoids in treatment.

Neuropilin Content in SARS-CoV2-induced Pneumonia, role in Multiorgan Damage

The ARDS initiation and respiratory failure result from prolonged inflammation and trigger the activation of multiple cellular axes. NRP-1, the transmembrane protein, being the target in SARS-CoV2 invasion, might provoke multiorgan lesions. The neuropilin content in the serum of SARS-CoV2 community-acquired pneumonia patients was increased 1.9 times compared to healthy individuals (Table 5). A 2.15 and 1.4 times increase was found in SARS-CoV2 patients with ARDS and without signs of ARDS. Additionally, a 1.48

Table 3 Glucose, C-peptide, leptin, adiponectin level in SARS-CoV2 patients, (Me (Q1; Q3)

Indicator		The glucose level at admission, mmol/L	C-peptide, ng/mL	Leptin, ng/mL	Adiponectin, ng/mL
Healthy people		4.3 (2.90; 5.70)	350.05 (90.79; 632.40)	3.27 (1.54; 9.88)	0.18 (0.17; 0.19)
SARS-CoV2 patients		7.40 (6.50; 8.60)#	0.00 (0.00; 372.60)#	10.25 (8.75; 10.37)#	0.24 (0.16; 0.33)#
ARDS	No ARDS PaO2 = 100	5.20 (5.0; 6.50)	0.00 (0.00; 281.80)	10.17 (8.81; 10.35)	0.24 (0.15; 0.30)
	Presence of ARDS PaO2 < 95	5.85 (4.40; 7.30)*	0.00 (0.00; 477.50)	10.27 (8.75; 10.48)	0.24 (0.18; 0.33)
DM	Non-DM	6.15 (5.35; 7.35)	0.00 (0.00; 373.65)	10.23 (8.78; 10.35)	0.24 (0.15; 0.31)
	DM	7.05 (6.00; 8.35)	0.00 (0.00; 147.60)	10.26 (8.28; 10.52)	0.27 (0.18; 0.49)
AH	Non-AH	6.00 (58.20; 6.50)	0.00 (0.00; 277.20)	10.20 (9.04; 10.36)	0.26 (0.17; 0.68)
	AH	6.80 (5.90; 7.90)**	0.00 (0.00; 374.70)	10.2 (8.75; 10.75)	0.24 (0.16; 0.29)
Outcome	Favorable outcome	6.20 (5.400; 7.40)	0.00 (0.00; 374.70)	10.25 (8.75; 10.42)	0.24 (0.16; 0.30)
	Adverse outcome	9.00 (9.00;12.00)***	16.97 (0.00; 128.35)	10.23 (7.05; 10.28)	0.27 (0.22; 1.39)
Gluco-corticoids application	No glucocorticoids	6.25 (5.40; 7.45)	0.00 (0.00; 277.20)	10.18 (8.15; 10.39)	0.26 (0.18; 0.49)
	Glucocorticoids	6.60 (6.00; 8.60)	0.00 (0.00; 1058.00	10.27 (10.24; 10.36)	0.17 (0.12; 0.23)*

Note: # - the significance of differences compared to the healthy people, $p < 0.05$; *- the significance of differences compared to the SARS-CoV2 patients with no ARDS, $p < 0.05$;

** - the significance of differences compared to the non-AH SARS-CoV2 patients, $p < 0.05$;

*** - the significance of differences compared to the SARS-CoV2 patients with favorable outcome, $p < 0.05$; ## - the significance of differences compared to the SARS-CoV2 patients without the inclusion of corticosteroids in treatment, $p < 0.05$;

times decrease in neuropilin level was detected in patients with ARDS than in patients without respiratory failure.

In turn, the highest values of this indicator were observed in patients with a favorable outcome, where the neuropilin level was increased 2.1 times compared to the healthy ones. A 2.7-fold decrease in neuropilin was noted in patients with a fatal outcome compared with patients with a favorable outcome.

The neuropilin level was revealed to be increased by 2.1 times in non-DM patients with SARS-CoV2 compared to healthy people. At the same time, there was a decrease in this indicator by 2.56 times in patients with DM compared to non-DM patients and in 16.9% compared to the healthy ones.

The neuropilin level remained elevated in AH and non-AH patients. Thus, the content of neuropilin in COVID-19 patients with hypertension increased 2.2 times, and in non-AH patients was increased 1.9 times compared to the control group. The glucocorticoids application in the treatment did not affect the NRP-1 level. They remained high in both groups of patients by 2.3 and 2.1 times, respectively, than to its level in the control group.

Liver Damage and Intestinal Lesions in SARS-CoV2 Acquired Pneumonia

The typical features of SARS-CoV2 induced biochemical disorders to impact respiratory failure and multiorgan adverse effects (e.g., multiorgan collapse) triggered by COVID-19-mediated an ARDS and other pathologies. SARS-CoV2 induced liver damage accompanied a 5-fold increase in troponin and a 2.2-fold decrease in neuropilin level. Heart failure with high ACE activity was recorded in patients with virus-induced hepatitis (Table 6).

The highest adiponectin content in SARS-CoV2 patients with intestinal lesions indicated the systemic proteolysis implementation. Additionally, it triggered the trypsin-like proteinases activation and neuropilin elevation. At the same time, intestinal damage was associated with an increase in NO derivatives and an ACE activity decrease.

SARS-CoV2 virus-induced intestinal lesions probably depend on the NRP-1 axis and proteolysis activation. The RAS imbalance leading to vascular complications is observed in SARS-CoV2 patients with intestinal lesions.

Discussion

The SARS-CoV2 community-acquired pneumonia originates from complex interactions between biochemical and molecular factors. Recent studies have found that ACE2 is central in diseases affecting almost all organs and systems, including cardiac, respiratory, renal, and endocrine functions. It is the critical host cellular receptor of SARS-CoV-2. It has been identified in multiple organs, but its cellular distribution in the human heart is not illuminated clearly [1–3].

Table 4 α_1-PI and proteases activity on serum of patients with SARS-CoV2 acquired pneumonia, (Me; Q_1:Q_3)

Indicator		α1-PI, IU/mL	Trypsin-like protease activity, nmol BAEE/min·mL	Neutrophilic elastase, nmol BANE/min·mL	PAR4, ng/mL
Healthy people		30,0 (24,6; 37,2)	63,2 (44,9; 68,8)	68,4 (50,30; 90,25)	0.20 (0.14; 0.44)
SARS-CoV2 patients		41,59 (23,55;57,33)#	300,30 (163,80; 565,11)#	238,90 (197,92; 293,47) #	0.25 (0.19; 0.32)#
ARDS	No ARDS PaO2 = 100	31.22 (20.48; 50.09)	207.48 (117.39; 464.10)*	232.05 (180.18; 300.30)	0.25 (0.19; 0.30)
	Presence of ARDS PaO2 < 95	46.41 (31.40; 58.69)	546.00 (264.81; 655.20)#, *	245.70 (218.40; 286.65)#	0.35 (0.19; 0.41)#, *
DM	Non-DM	41.59 (23.55;57.33)	300.30 (163.80; 565.11)	238.90 (197.92; 293.47)	0.25 (0.18; 0.32)
	DM	43.17 (31.77; 53.37)	518.70 (204.75; 1105.65)	251.16 (225.22; 293.47)	0.23 (0.21; 0.34)
AH	Non-AH	36.51 (23.55; 61.83)	207.48 (117.39; 308.49)	245.70 (204.75; 300.30)	0.25 (0.19; 0.50)
	AH	41.97 (28.39; 55.97)	555.55 (253.89; 655.20)**	238.87 (204.75; 259.35)	0.23 (0.19; 0.32)
Outcome	Favorable outcome	39.24 (23.55; 54.19)	262.08 (144.69; 546.00)	245.70 (197.92; 293.47)	0.25 (0.19; 0.34)
	Adverse outcome	47.94 (34.80; 68.80)	582.85 (505.05; 750.75) ***	238.87 (211.57; 279.82)	0.23 (0.18; 0.25)
Glucocorticoids	No glucocorticoids	41.29 (25.59; 54.19)	282.55 (133.77; 569.20)	245.70 (191.10; 286.65)	0.25 (0.19; 0.32)
	Glucocorticoids	55.97 (31.05; 57.33)	627.90 (518.70; 655.20)##	232.00 (218.40; 300.30)	0.18 (0.15; 0.29)##

Note: # - the significance of differences compared to the healthy people, $p < 0.05$; *- the significance of differences compared to the SARS-CoV2 patients with no ARDS, $p < 0.05$; ** - the significance of differences compared to the non-AH SARS-CoV2 patients, $p < 0.05$; *** - the significance of differences compared to the SARS-CoV2 patients with favorable outcome, $p < 0.05$; ## - the significance of differences compared to the SARS-CoV2 patients with glucocorticoids application

The SARS-CoV-2 infection has several effects on the RAS, and conversely, regulation of this receptor may affect the disease's progression [5, 6]. Increased ACE activity with lungs insufficiency and ARDS provoke the processes of heart damage [8].

We revealed the impaired metabolism in COVID-19 patients. The hyperglycemia with growth in adipose tissue-derived hormones is specific for virus-induced disorders and results in unfavorable outcomes and ARDS initiation. Previous studies have shown the importance of metabolic disorders in determining the outcome. Thus, hyperglycemia may be considered as a biomarker that predicts poor prognosis. It was found only for AH patients and in an in-hospital lethality. Some studies have reported an epidemiological association between a history of cardiac disease and worsened outcome during COVID infection. The COVID patients with cardiac disease history or who acquire new cardiac injury are at an increased risk for in-hospital morbidity and mortality. More studies are needed to address the cardiotoxicity mechanism, that can minimize permanent damage to the cardiovascular system [24]. Microbiota is the promising one. Its signals regulate the immune system and protect different tissues during severe viral respiratory infections. It is found microbiota could help manage the mortality and morbidity rates associated with SARS-CoV-2 infection [23, 25, 32].

The high rate of severe cases among COVID-19 patients is associated with primary cardiovascular diseases. It is known, severe ARDS SARS-CoV-2 infects host cells through ACE2 receptors, leading to virus-induced pneumonia and manifestation of acute and chronic cardiovascular system damage [26]. Bradykinin storm is found to be the origin of RAS imbalance leading to the bradykinin receptors activation [5, 6]. The atypical pattern results in vascular

Table 5 Neuropilin level in SARS-CoV2 patients (Me, Q1, Q3)

Indicator		Neuropilin, ng/mL
Healthy people		234.00 (237.60; 342.80)
SARS-CoV2 patients		466.50 (250.30; 625.35)#
ARDS	No ARDS PaO2 = 100	504.60 (358.60; 638.00)#
	Presence of ARDS PaO2 < 95	340.90 (194.30; 512.40) #,*
DM	Non-DM	497.10 (322.00; 629.30)#
	DM	194.30 (80.80; 462.10) #,**
AH	Non-AH	512.10 (260.00; 631.00) #
	AH	462.10 (194.30; 554.60) #
Outcome	Favorable outcome	184.90 (85.60; 451.20)
	Adverse outcome	496,30 (322,00; 629,30)***
Glucocorticoids	No glucocorticoids	536.30 (396.05; 634.05)#
	Glucocorticoids	497.10 (309.60; 629.30) #

Note: # - the significance of differences compared to the healthy people, $p < 0.05$; *- the significance of differences compared to the SARS-CoV2 patients with no ARDS, $p < 0.05$; ** - the significance of differences compared to the non-DM SARS-CoV2 patients, $p < 0.05$; *** - the significance of differences compared to the SARS-CoV2 patients with favorable outcome, $p < 0.05$;

Table 6 SARS-CoV2-induced mechanisms in intestinal lesions and liver damage

Indicators	No gastro-intestinal lesions	SARS-CoV2 induced hepatitis	SARS-CoV2 induced intestinal lesions
	RAS status and troponin level		
ACE activity, µmol /min·l	30.00 (20.00;40.00)	33.30 (29.30; 51.60)	18.30 (1.60;35.00)#,*
Nitrites, µmol/L	28.50 (24.00; 36.00)	30.00 (28.50; 31.50)	45.00 (33.00; 48.00)#,*
Troponin, ng/mL	0.02 (0.00; 0.06)	0.10 (0.07; 0,11)#	0.03 (0.00; 0.06)*
	Metabolic disorders		
The glucose level at admission, mmol/L	6.35 (5.60; 7.80)	5.75 (5.10; 6.50)	5.55 (5.20; 5.90)
C-peptide, ng/mL	0.00 (0.00; 374.70)	0.00 (0.00;147.60)	22.53 (0.00; 208.83)
Leptin, ng/mL	10.20 (8.75; 10.47)	10.26 (10.22;10.35)	10.36 (10.19; 10.49)
Adiponectin, ng/mL	0.23 (0.17; 0.29)	0.28 (0.24; 1.05)	0.82 (0.26; 2.02) #,*
	Proteolysis		
α1-PI, IU/mL	57.33 (28.39; 420.42)	41.29 (23.89; 54.19)	55.97 (36.51; 64.16)*
Trypsin-like protease activity, nmol BAEE/min·mL	203.79 (40.24; 586.95)	245.70 (144.69; 464.10)	308.49 (122.85; 1228.50)#,*
Neutropil elastase, nmol BANE/min·mL	251.16 (62.11; 354.90)	232.05 (191.10; 259.35)	293.47 (266.17; 316.68)
PAR4, ng/mL	0.25 (0.19; 0.32)	0.23 (0.21; 0.25)	0.30 (0.18; 0.82)
	Neuropilin level		
Neuropilin, ng/mL	513.50 (434.85; 655.90)	231.70 (175.50; 340.90)#	322.00 (260.00; 451.20)#,*

Note: # - the significance of differences compared to the healthy people, $p < 0.05$; *- the significance of differences compared to the SARS-CoV2 patients with no ARDS, $p < 0.05$; ** - the significance of differences compared to the non-DM SARS-CoV2 patients, $p < 0.05$; *** - the significance of differences compared to the SARS-CoV2 patients with favorable outcome, $p < 0.05$;

dilation, permeability, and hypotension. The well-known bradykinin-driven outcomes explain most of the symptoms observed in COVID-19 [2].

It is still unclear role of ACE inhibitors in the SARS-CoV2 dependent complications pathogenesis. The study data verified the protective effect of ACE inhibitors on the outcome of COVID-19 patients. Their application reduces the ACE activity in AH patients [4]. But at the same time, the decrease in nitric ions level shown in the investigation may be considered as a sign of the ACE inhibitor's anti-inflammatory action.

AH is a multifactorial disease caused by environmental, metabolic, and genetic factors. It is little currently known about the complex interplay between the variety of factors and changes in blood pressure [27, 28]. The impaired blood pressure regulation was obtained in AH patients, where the growth in ACE activity was associated with nitrites' low level. The association of ACE and eNOS (endothelial NO synthase) genotype polymorphisms is known with risk of cardiovascular disorders, probably resulting in severe susceptibility AH patients to the COVID-19 infection.

Acute inflammation is a factor for the adverse outcome. The association between the ACE activity, NO ions presence, and troponin level is found. The RAS imbalance induced by the inflammation and accumulation of bradykinin impacts the patent's outcome. Bradykinin is an essential part of the vasopressor system. It causes hypotension and vasodilation and is degraded by ACE, enhanced by the angiotensin produced by ACE2, and active the endothelial NO synthase followed by the NO ions release [5, 6].

Another significant reason is a systemic inflammatory reaction with adipose-derived hormones and hyperglycemia as a specific SARS-COV2 metabolic sign. SARS-CoV2 –associated comorbidities and patient outcomes are connected with the blood glucose level [28]. Poor glycemic control originates inappropriate chemical reactions and glycated proteins producing the inflammatory response,

resulting in the cytokine storm associated with COVID-19 morbidity and mortality. The good glycemic control is a tool for successfully managing COVID-19 infection, particularly challenging.

Furthermore, the post-COVID-19 syndrome has also emerged as a sequela in COVID-19 survivors, increasing the risk of deadly complications and further burdening the health care system. In SARS-Cov-2 infection, patients with hyperglycemia could be considered for a more intensive prophylactic hyperglycemic regimen [28]. Metabolic disorders are found to be accompanied by vascular active peptides imbalance. The high NO ions levels in DM patients with the increased glucose levels were detected in serum, which might be responsible for the activation of endothelial cells to enhance NO levels.

The study revealed an α1-PI, trypsin- and elastase-like proteinases activity increase in SARS-CoV2 patients with pneumonia. The data obtained are probably associated with the virus-induced effect on the proteolysis activation [9, 13] due to molecular mimicry of viral proteins with furin in the epithelial sodium channel [11].

RAS induced hemostasis imbalance is typically associated with high rate in proteolytic enzymes [14], manifesting in COVID-19 adverse course in older people with comorbidities. It is found proteinase-activated receptors belong to the subfamily of seven-transmembrane receptors associated with G-protein are of great importance in down-streaming the proteinases action [29]. Platelets express PAR4, which triggers platelet activation and participates in signaling and modulating cellular responses. The activation of thrombus inflammation is facilitated by neutrophil elastase, which is involved in forming NET (Neutrophil extracellular traps) [16]. Indeed, our studies revealed an increase in elastase activity at the early stages of community-acquired pneumonia in COVID-19.

The increase in PAR4 content may be due to the action of multiple stimuli related to thrombus inflammation, including angiotensin II, thrombin, trypsin-like proteinases, high glucose levels, and oxidative stress. An increase in PAR4 content is probably an early sign of ARDS before the coagulopathy [16], provoking the DIC syndrome (disseminated intravascular coagulation), stroke, and heart failure.

The PAR4 level decrease observed during hospitalization could be associated with the unfavorable course of COVID-19. A decrease in PAR4 content may be associated with the receptor–proteinase complex. It was found that patients with low PAR4 levels had high trypsin-like proteinases and neutrophil elastase activity due to prolonged inflammation.

It is known that NRP-1 is a alternative gateway for the virus [20, 28]. It was revealed a relationship between NRP-1 and glucose levels in DM patients. The obtained results may show the nervous system damage in COVID-19 in post-COVID-19 patients without the t metabolic disorders and DM. We obtained an association between the SARS-CoV2-associated hyperglycemia and NRP-1 level [21, 28, 29].

COVID-19 is a disease being induced by the increased virus transmission and infection rates due to the comprehensive expression of the main infection-related ACE2, TMPRSS2, and CTSB/L human genes in tissues of the respiratory and gastrointestinal tract, as well as by host and probably aggressive inflammation and (due to broad organotropism of SARS-CoV-2) collateral tissue damage and systemic failure [27, 31]. Gastrointestinal manifestations such as diarrhea, vomiting, and abdominal pain are reported in many affected individuals. They may be due to the SARS-CoV-2 tropism for the peptidase angiotensin receptor 2 [30]. Similarly, hepatic impairment patients co-infected with SARS-CoV-2 exhibited overexpression of ACE2 receptors and cytokine storm overwhelming, worsening the hepatic impairment and increasing the mortality rate due to the cardiac failure [24, 25].

Recently, another receptor, NRP-1, has been reported to amplify the viral infection. NRP-1, expressed in nonparenchymal liver cells, plays a significant role in the COVID-19-induced intestinal lesions pathogenesis and enhances the systemic inflammatory responses [31, 32]. It has been observed that SARS-CoV-2 infection promotes liver injury through several pathways that may be influenced by the NRP-1 hepatic expression [30]. The NRP-1 elevated level was found in SARS-CoV2 pneumonia patients with intestinal lesions. Multiple mechanisms of virus-induced action included adipose-derived hormone increase, NO derivatives implementation and proteolysis imbalance. Moreover, many effort were made to verify the microbiota influence on SARS-CoV2 dependent local and systemic inflammation. Microbiota-derived signals could protect different tissues during severe viral respiratory infections [24, 25]. But the prolonged inflammation had siginifcant side effects resulting in NO derivitives excess [21]. In common, we found that the gut microflora could triggers the inflammation. It can signify a more severe systemic pro-inflammatory process with adipose tissue involvement.

Conclusion

SARS-CoV2 infection prevails in patients with preexisting conditions such as DM and AH. It is known metabolic changes induced by virus affect the metabolism and predict the COVID-19 complications. SARS-CoV2 associated hyperglycemia and vascular disorders are found to be an essential trigger in AH patients co-infected with COVID-19, provoking the unfavorable outcomes. Increase in ACE

activity and levels of NO derivatives found in the study is a crucial step, guided the comorbidities manifestation. The most urgent COVID-19 associated disorder is cardiac failure, resulting in the troponin release, which was dependent on the ARDS severity. The impaired metabolism and RAS imbalance are the key triggers in variable mechanisms of virus-induced multiorgan damage.

Proteolysis overactivation was accompined by the α1-PI, trypsin-like, elastase activity, and PAR4 level increase. The obtained data shows the neuropilin-dependent axis prevalence in SARS-CoV2 induced intestinal lesions. Liver damage was found in patients co-infected with COVID-19, who had severe ARDS and heart failure. The intestinal lesions manifestation leads to involvemant of alternative factors as well as adipose-derived tissue hormones, nitrites, and neuropilin levels.

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