# SARS-CoV2 Induced Biochemical Mechanisms in Liver Damage and Intestinal Lesions.

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# Abstract

*Introduction.* 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.

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

*Results.* ACE activity and nitrites ions content increase in SARS-CoV2 pneumonia. The hyperglycemia with an increase in adipose tissue-derived hormones is specific for virus-induced disorders and affects the development of unfavorable outcomes. Cardiac failure was detected in patients with ARDS. AH patients with COVID-19 had more pronounced hyperglycemia and increased ACE activity and NO ions level.

Activation of proteolysis was revealed in SARS-CoV2 pneumonia. The found molecular event was accompanied by hyperglycemia induction. Liver damage was specific for patients co-infected with COVID-19 with severe ARDS and heart failure. But the intestinal lesions were associated with the proteolysis activation. The obtained data shows the prevalence of the neuropilin-dependent axis in damage of the intestine with more pronounced inflammation. Growth in adipose tissue hormones, nitrites, and neuropilin levels is triggered by prolonged inflammation.

*Conclusions.* The impaired metabolism, SARS-CoV2 associated hyperglycemia influence on SARS-CoV2 multiple mechanisms of virus invasion. Gastrointestinal manifestations in SARS-CoV2 infection are related to various and varied 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.


# 1. 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]. Multiple pathogenic mechanisms are found in SARS-CoV2 systemic inflammation. Oxidative stress, altered proteolysis, hypercoagulation, and metabolic disorders are significant in virus invasion.

Angiotensin-converting enzyme-2 (ACE2) receptors mediate the entry into the cell of three strains of coronavirus: SARS-CoV, NL63, and SARS-CoV-2. 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 receptors are ubiquitous and widely expressed in the heart, vessels, gut, lung (particularly in type 2 pneumocytes and macrophages), kidney, testis, pancreas, brain, and adipose tissue, responsible for the multiple impairments of the organs and post-COVID complications [3].

ACE2 is 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 [4]. An excess of ROS decreases nitric oxide bioavailability and causes endothelial dysfunction [5].

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 [6]. 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 [7]. But an adverse outcome of COVID-19 was associated with male gender, hypertension, hypercholesterolemia, and the ACE1 genotype. The ACE1-I/D polymorphism might influence COVID-19 severity, but the effect depended on the hypertensive status. This result requires further validation in other large cohorts [8].

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 [9]. 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 [10].

Activation of the RAS and abnormal adipokine levels are biological alterations that affect metabolism blood pressure regulation manifesting in hypertension, obesity, and metabolic diseases [11]. The comorbidities follow-up accompanies preexisted 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 [12]. Trypsin-like and elastase-like proteinases are the key proteolytic enzymes of neutrophils and macrophages that ensure the development of the inflammatory response [13]. Currently, much attention is paid to the role of the virus, in particular SARS-CoV2, in the activation of trypsin-like proteinases [14]. One of the mechanisms is the molecular mimicry of viral proteins with proteinase furin on the α-subunit of the human epithelial sodium channel [15]. The virus-induced complications of the disease may be associated with the characteristics of proteolysis [16, 17].

Proteolysis overactivation originates from the lack of alpha-1 proteinase inhibitor (α1-PI) [18]. 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 [19]. Moreover, the SARS-CoV2 virus penetration happened due to the endogenous proteinases [18, 19].

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 [20]. 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 [21, 22]. 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 [23]. Neutrophil elastase is involved in NET formation and activation of thrombus inflammation, whose role in developing lung diseases is well known [24].

There are other alternative receptors, which include CD147, DPP4 (dipeptidyl peptidase-4), ANPEP (alanyl aminopeptidase), ENPEP (glutamaminopeptidase), and NRP-1 [25]. In particular, the transmembrane protein NRP-1 or neuropilin is widely represented in the central nervous system, mainly in the olfactory tubercles and para-olfactory gyri [26]. Neuropilin 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 [27]. 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 [28]. The gastrointestinal lesions and liver damage in SARS-CoV2 infection determine the prognosis and patient's outcome. But the features in biochemical and molecular mechanisms in these complications are still unknown.

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.

# 2. 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.

Table 1
Clinical Characteristics 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%) |

*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 45–59; -31 (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 ones and intestinal lesions – in 29 ones.

*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.

*C-peptide detection.* Sandwich ELISA Kit was applied to measure C-Peptide in Human Serum (Vector-Best. Novosibirsk, Russia).

*Leptin, adiponectin, C-peptide, PAR4, neuropilin detection*. ELISA kits were used for leptin, adiponectin, C-peptide, PAR$, neuropilin level detection (Cloud-Clone Corporation, Katy, TX 77494, USA).

C-peptide/glucose ratio was calculated using the C-peptide content in serum (ng/mL) and glucose level (mmol/L)

*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 minutes, 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 $p < 0.05$.

# 3. Results

# 3.1. 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 lungs insufficiency with heart failure.

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.

ACE and NO derivatives provoke considerable damages in organs and tissues. 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.

It was indicated the role of RAS imbalance in cardiac failure in SARS-CoV2 patients. The growth in ACE activity, NO ions content, and troponin level in patients with adverse outcomes were obtained.

The impact of ACE inhibitors on the nitric oxides level was widely presented due to the increase in bradykinin content [10]. But the usage of ACE inhibitors resulted in a decrease in ACE activity and nitrites content in serum. It was found the conflicting data according to the nitrites content.

## 3.2. 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 in 3.1 and 1.3 times accompanied by the C-peptide reduction has been shown compared with healthy people.

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) |

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, $p < 0.05$;

| Indicator | | The glucose level at admission, mmol/L | C-peptide, ng/mL | Leptin, ng/mL | Adiponectin, ng/mL |
|---|---|---|---|---|---|
| | 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) |
| Glucocorticoids 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, $p < 0.05$; | | | | | |

The relationship between hyperglycemia and the ARDS was confirmed (Table 3). A high glucose level accompanied the presence of ARDS.

The 1.48 times reduction in adiponectin level was found in patients who received glucocorticoids 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 [29].

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.

# 3.3. 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 of 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.

Table 4

$\alpha_1$-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) |

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.

| Indicator | | α1-PI, IU/mL | Trypsin-like protease activity, nmol BAEE/min·mL | Neutrophilic elastase, nmol BANE/ min·mL | PAR4, ng/mL |
|---|---|---|---|---|---|
| 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.

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, both in the ARDS and in its absence. Overactivation of proteases 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. In general, 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 PAR-4 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.

## 3.4. 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 without ARDS and with signs of ARDS. Additionally, a 1.48 times decrease in neuropilin level was detected in patients with ARDS than patients with respiratory failure.

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, $p < 0.05$;

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 neuropilin level. They remained high in both groups of patients by 2.3 and 2.1 times, respectively, to the indicator in the control group.

## 3.5. 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 complications induction—the 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).

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

| Indicators | No gastrointestinal 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, $p < 0.05$;

The highest adiponectin content in SARS-CoV2 patients with intestinal lesions indicated the implementation of systemic proteolysis. 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.

# 4. 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 [30]. 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 [31]. The SARS-CoV-2 infection has several effects on the RAS, and conversely, regulation of this receptor may affect the disease's progression [32]. Increased ACE activity with lungs insufficiency and ARDS provoke the processes of heart damage [33].

Some studies have reported an epidemiological association between a history of cardiac disease and worsened outcome during COVID infection [Bódi B et al., 2021]. The COVID patients with cardiac disease history or who acquire new cardiac injury are at an increased risk for in-hospital morbidity and mortality [34]. More studies are needed to address the mechanism of cardiotoxicity and the treatments that can minimize permanent damage to the cardiovascular system [35].

The high rate of severe cases among COVID-19 patients with primary cardiovascular disease, and these results also perhaps provide essential re ference to clinical treatment of cardiac injury among severe patients infected by SARS-CoV-2 [30]. Moreover, severe ARDS SARS-CoV-2 infects host cells through ACE2 receptors, leading to coronavirus disease (COVID-19)-related pneumonia while also causing acute myocardial injury and chronic damage to the cardiovascular system [36, 37].

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 [38]. The combined prevalence of AH and DM appears to confer the most significant risk. Arterial hypertension had a substantial impact on inpatient mortality. Social deprivation and ethnicity did not affect the patient once in hospital [39]. 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 [40], 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.

Another significant reason is a systemic inflammatory reaction with adipose-derived hormones and hyperglycemia as a specific SARS-COV2 metabolic sign. SARS-CoV2 complications and patient outcomes are connected with the blood glucose level [40]. An essential characteristic of these conditions is poor glycemic control, which leads to inappropriate chemical reactions and glycated proteins producing the inflammatory response, resulting in the cytokine storm associated with COVID-19 morbidity and mortality [41]. The well-balanced glycemic control is a tool for successfully managing COVID-19 infection, particularly challenging [42].

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 [41]. 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 [43].

The modified ACE expression represents the imbalance in RAS leading to the bradykinin receptors activation [40, 44]. The atypical pattern of the RAS is predicted to elevate bradykinin levels in multiple tissues and systems, resulting in vascular dilation, permeability, and hypotension. The well-known bradykinin-driven outcomes explain most of the symptoms observed in COVID-19 [40] and the critical role of glucocorticoids in the acute and chronic management of inflammatory disease, independent of any effect of RAS [32].

The role of ACE inhibitors in the initiation of SARS-CoV2 dependent vascular complications remains unknown. Their application reduces the ACE activity in AH patients [4]. But at the same time, the decrease in nitric ions level is shown in the investigation may be a sign of the ACE inhibitor's anti-inflammatory action [30]. Found data verified the protective effect of ACE inhibitors on the outcome of COVID-19 patients.

The study revealed an increase in the activity of α1-PI, trypsin- and elastase-like proteinases in the blood plasma of patients with community-acquired pneumonia, especially pronounced during infection with SARS-C0V2. The data obtained are probably associated with the specific effect of the virus on the activation of proteolytic enzymes [13, 17], including due to molecular mimicry of viral proteins with proteinase furin on the α-subunit of the human epithelial sodium channel [15].

Elastase-like proteinases activity is associated with the development of nonspecific inflammation and lung parenchyma damage [14]. The increase in elastase activity is insignificant; in the case of association with DM, it reaches its maximum values, exceeding the control group by 3.4 times.

Trypsin-like proteinases include trypsin, kallikrein, renin, thrombin, plasmin, complement system [16]. An increase in the trypsin-like proteinase activity is associated with the RAS induction hemostasis imbalance, typical for SARS-CoV2 infection [13]. At the same time, comorbidities are also accompanied by proteolysis hyperactivation. It is the reason for the COVID-19 adverse course in older people with comorbidities [45].

SARS-CoV2 acquired pneumonia is associated with the membrane trypsin-like proteinases induction and plasma proteolytic systems activation [46]. Extracellular proteinases, TMPRSS2, and furin can also participate in the coronavirus spike protein priming [22]. An increase in the neutrophil elastase and trypsin-like proteinase activity was found in SARS-CoV2 patients [13, 44]. The trypsin-like proteinase and neutrophil elastase activation occurs in the early stages of COVID-19. At the same time, neutrophil elastase activity was equally high in ARDS patients and patients with adverse outcomes. Thus, the inflammation's high rate is accompanied by an increase in the elastase and trypsin-like proteases activity. It is evidence of the blood plasma proteolysis implementation

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 [47]. Platelets express PAR4, which triggers platelet activation and participates in signaling and modulating cellular responses [46, 48, 49].

Serine, trypsin-like proteinases (thrombin, trypsin) activate PARs receptors present on the membranes of many cells: platelets, monocytes, macrophages, neutrophils, endothelial cells, which promote the activation of these cells [13].

The activation of thrombus inflammation is facilitated by neutrophil elastase, which is involved in forming NET (Neutrophil extracellular traps) [15, 50]. Indeed, our studies revealed an increase in elastase activity at the early stages of community-acquired pneumonia in COVID-19.

Community-acquired pneumonia is associated with the development of ARDS, and a decrease in pO2 most clearly shows the pathogenetic patterns of the disease. An increase in PAR4 content is probably an early sign of ARDS before the coagulopathy [20]. It is logical to assume that the rise in PAR4 reflects the initial stages of thrombus inflammation in the lung tissue. Hypercoagulation leads to DIC syndrome (Disseminated intravascular coagulation) [51], stroke, and heart failure [52, 53].

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 [46, 54, 55].

The PAR4 level decrease observed during hospitalization could be associated with the unfavorable course of COVID-19: patients with low PAR-4 levels had a negative clinical picture during hospitalization and were prescribed glucocorticoids. A low PAR-4 level at the early stages of COVID-19 indicates the development of severe infection. Indeed, in patients with low PAR-4 levels, an unfavorable outcome was

recorded during hospitalization. It manifested at 2–3 weeks of hospitalization. A decrease in PAR4 content may be associated with the receptor–proteinase complex [55].

A comorbid pathology associated with initially overactive proteolysis probably causes an increase in the body's susceptibility to the SARS-CoV2 virus. Patients with low PAR4 levels had high trypsin-like proteinases and neutrophil elastase activity due to prolonged inflammation. Subsequently, the progression of the disease, the ARDS severity, and the multiorgan lesions manifestation led to the DIC syndrome and death.

The rise in neuropilin level was found in patients with community-acquired pneumonia. It is known that neuropilin is a gateway for the virus to disseminate throughout the body [25, 56, 57].

Moin A. S. M. et al. (2021) has revealed a relationship between neuropilin and glucose levels in DM patients [28]. The obtained results may show the signs of nervous system damage in COVID-19, specifically for recovery from the infection without the concomitant metabolic disorders, mainly DM. Similar patterns are shown in single works.

A neuropilin decrease was correlated with the infection severity and adverse outcome. Compensated DM favorably affects the outcome of the disease [58], contributing to a decrease in mortality from concomitant complications. The patients without previously diagnosed metabolic disorders provide unique management. There is an association between the SARS-CoV2-associated hyperglycemia and the neuropilin level. Previous studies by Rizza S. et al. (2021), and Singh AK et al. (2020) showed the role of hyperglycemia in multiorgan lesions [58 59].

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 [Stehura AV, Sirchak YS, 2021]. The increase in ACE activity in COVID-19 patients with signs of heart damage was found.

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 [61]. 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 [61].

Recently, another receptor, NRP-1, has been reported to amplify the viral infection. NRP-1 is expressed in nonparenchymal liver cells. It plays a significant role in the pathogenesis of COVID-19-induced intestinal lesions and enhances the systemic inflammatory responses [62]. It has been observed that SARS-CoV-2 infection promotes liver injury through several pathways that may be influenced by the previous pathological status of the patient and liver expression of NRP-1 [63]. The elevated neuropilin level was

found in patients with severe inflammatory reactions with intestinal lesions. The adipose hormone increase revealed in the gastrointestinal failure, indicating the rate of inflammation. The rise in NO derivatives level, hyperactivation of proteolysis, and α1-PI activity is also seen.

The RAS imbalance results in nitrates increasing due to SARS-CoV2 induced biochemical effects. Its toxic effect on absorptive and secretive functions of the intestinal mucosa is well-known [64]. It can signify a more severe systemic pro-inflammatory process with adipose tissue involvement. The summarise in biochemical mechanisms of SARS-CoV2 infection, role in the liver damage and intestinal lesions is shown in Fig. 1.

# 5. Conclusions

SARS-CoV2 associated hyperglycemia and vascular disorders are found in AH patients suffering from the infection and unfavorable outcomes. Growth in ACE activity and NO derivatives found in the study is a crucial step of the disease, managing the development of vascular complications manifesting in an unfavorable outcome. The most urgent COVID-19 associated disorder is cardiac failure—the troponin release in SARS-CoV2 patients dependent on the ARDS severity and adverse effect. The effective mechanism in SARS-COV2-related complications is impaired metabolism induced by RAS imbalance. Imbalance in active vascular peptides results in impaired regulation of vascular tone, the key trigger in variable mechanisms of multiorgan damage.

SARS-CoV2 disease complicated by pneumonia is accompanied by the high rate of α1-PI, trypsin-like, elastase activity, and PAR4 level in the patients. The PAR4 rate may be an early sign of an unfavorable course of COVID 19. An increase in the neuropilin content in patients with SARS-CoV2-associated pneumonia was revealed. At the same time, the decrease in neuropilin observed in DM patients may be the result of metabolic disorders compensation.

The obtained data shows the prevalence of the neuropilin-dependent axis as a critical molecular event in SARS-CoV2 induced intestinal lesions. Liver damage was found in patients co-infected with COVID-19 with severe ARDS and heart failure. But the intestinal lesions were associated with proteolysis activation, adipose tissue hormones, nitrites, and neuropilin levels.

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# Figures

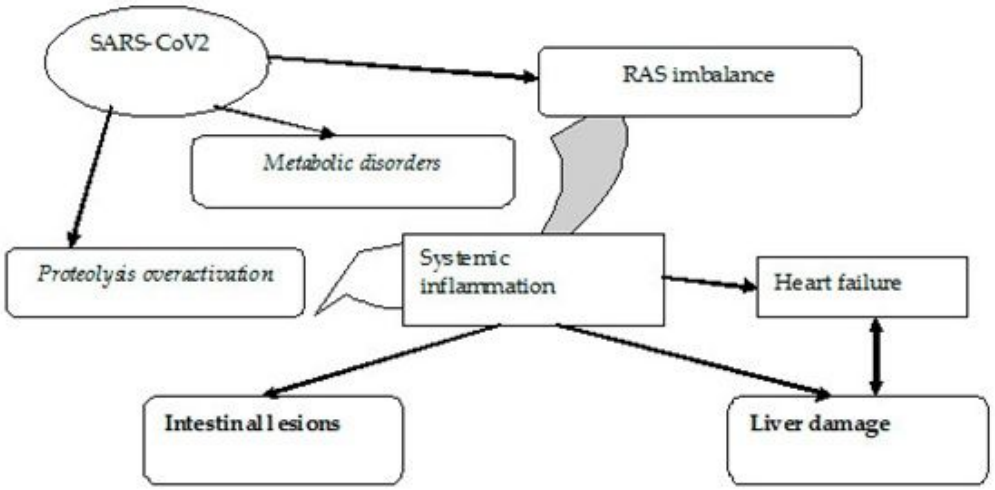


**Figure 1**

Biochemical mechanisms of SARS-CoV2 infection, role in the liver damage and intestinal lesions.

Note: Biochemical mechanisms in SARS-CoV2 infection include RAS imbalance with ACE activity prevalence, metabolic disorders (hyperglycemia and adipose tissue hormone overexpression), and proteolysis activation. The difference in COVID-19-induced mechanism manifestation of liver damage and intestinal lesions is found. 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.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- dataset.xlsx