Proteolysis and Deficiency of $\alpha_1$-Proteinase Inhibitor in SARS-CoV-2 Infection

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Abstract—The SARS-CoV-2 pandemic had stimulated the emergence of numerous publications on the $\alpha_1$-proteinase inhibitor ($\alpha_1$-PI, $\alpha_1$-antitrypsin), especially when it was found that the regions of high mortality corresponded to the regions with deficient $\alpha_1$-PI alleles. By analogy with the data obtained in the last century, when the first cause of the genetic deficiency of $\alpha_1$-antitrypsin leading to elastase activation in pulmonary emphysema was proven, it can be supposed that proteolysis hyperactivation in COVID-19 may be associated with the impaired functions of $\alpha_1$-PI. The purpose of this review was to systematize the scientific data and critical directions for translational studies on the role of $\alpha_1$-PI in SARS-CoV-2-induced proteolysis hyperactivation as a diagnostic marker and a therapeutic target. This review describes the protease-dependent stages of viral infection: the reception and penetration of the virus into a cell and the imbalance of the plasma aldosterone–angiotensin–renin, kinin, and blood clotting systems. The role of ACE2, TMPRSS, ADAM17, furin, cathepsins, trypsin- and elastase-like serine proteinases in the virus tropism, the activation of proteolytic cascades in blood, and the COVID-19-dependent complications is considered. The scientific reports on $\alpha_1$-PI involvement in the SARS-CoV-2-induced inflammation, the relationship with the severity of infection and comorbidities were analyzed. Particular attention is paid to the acquired $\alpha_1$-PI deficiency in assessing the state of patients with proteolysis overactivation and chronic non-inflammatory diseases, which are accompanied by the risk factors for comorbidity progression and the long-term consequences of COVID-19. Essential data on the search and application of protease inhibitor drugs in the therapy for bronchopulmonary and cardiovascular pathologies were analyzed. The evidence of antiviral, anti-inflammatory, anticoagulant, and anti-apoptotic effects of $\alpha_1$-PI, as well as the prominent data and prospects for its application as a targeted drug in the SARS-CoV-2 acquired pneumonia and related disorders, are presented.

Keywords: SARS-CoV-2, ACE2, TMPRSS, ADAM17, plasma proteases, $\alpha_1$-proteinase inhibitor

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INTRODUCTION

COVID-19 is a scientific, medical and social problem. The complexity of acute respiratory syndrome caused by the SARS-CoV-2 coronavirus is due to an unpredictable clinical course of the infection, severe complications and lethal outcomes. Identification of molecular biomarkers for classifying patients on a risk basis is relevant for choosing the strategies of treatment and prevention of complications [1, 2].

The SARS-CoV-2 coronavirus is an RNA-carrying virus from the genus Betacoronavirus of the family Coronaviridae. The new SARS-CoV-2 infection proved to be the most dangerous one among the previously existing infections: SARS-CoV-1, MERS-CoV [3]. The first SARS-CoV-1 coronavirus infection (2002–2003) was successfully localized. The infection by the Middle-East respiratory syndrome virus MERS-CoV identified for the first time in 2012 was accompanied by high mortality and is currently a regional disease. The SARS-CoV-2 infection, which quickly achieved the status of pandemic, is accompanied by severe pneumonia, gastrointestinal, cardiovascular and neurological disorders, the development of multiple organ failure [4, 5]. The number of SARS-CoV-2 infected people has been steadily increasing since 2019 and, according to the data from the John Hopkins Coronavirus Resource Center (https://coronavirus.jhu.edu/), was 35827267 people on January 25, 2022; 5614675 of them died.

Proteolytic enzymes are essential for coronavirus infection. SARS-CoV-2 uses membrane, intracellular and plasma human proteinases for reception and penetration into cells [6–8]. Comorbidities associated with activation of the renin–angiotensin, coagulation, kallikrein–kinin and complement systems create con-
dions for hyperproteolysis and increased sensitivity to viral infection [9–11]. The inhibitor drugs acting on viral proteinases are being actively sought [12–14]. Endogenous α1-proteinase inhibitor (α1-PI) provides 90% of the inhibitory activity of blood. α1-PI drugs are already used for treating patients with genetic α1-antitrypsin deficiency. The compelling evidence of good prospects of using α1-PI preparations in the treatment for COVID-19 is the fact that persons with genetic deficiency of the inhibitor are more susceptible to the infection [15–17]. α1-PI has antiviral, anti-inflammatory, anticoagulant and antiapoptotic properties [18–20], which makes it a potential drug for targeted therapy and is a basis for translational studies under the conditions of hyperproteolysis, in particular, in case of COVID-19. The present review addresses the significance of proteinases, the advantages and shortcomings of using α1-PI in case of the SARS-CoV-2 infection.

1. ROLE OF PROTEINASES IN THE DEVELOPMENT OF CORONAVIRUS INFECTION

Proteolytic enzymes are very important for penetration of the virus into human cells. For replication, the virus uses its own proteinases such as papain-like protease (PLpro) and chemotrypsin-like protease (3CLpro), forming a replicase–transcriptase complex (RTC). The major enzyme of RTC is an RNA-dependent RNA polymerase (RdRp), which catalyzes the replication of positive mRNA to form the genome of the viral particle [21, 22]. The positive mRNA (+, sense), in contrast to the negative mRNA (−, antisense), does not require transcription and is immediately used for the synthesis of viral proteins. The virus has no its own proteinases for penetration into target cells and uses human proteinases, including cysteine, serine and metalloproteinases. The SARS-CoV-2 virus can penetrate through the membrane of human cells in three ways: with the involvement of cysteine proteinases (cathepsins), membrane trypsin-like proteinases, and extracellular plasma proteinases [6, 7, 20].

1.1. Proteolytic Priming of S Protein

Proteolytic enzymes are needed for the activation (priming) of a spike (S) protein from the “corona” of the virus, which consists of two subunits: S1 and S2; one of them contains a receptor-binding domain (RBD) for cell attachment and the other is required for fusion of the viral and target cell membranes [6–8]. The emergence of new sites for proteinase-catalyzed hydrolysis underlies mutations in the virus [3, 14, 23, 24]. The role of these proteinases is to hydrolyze the spike S protein in the S1–S2 region and then S2', followed by the release of the peptide N-terminus on subunit S2 responsible for fusion with the target cell membrane [7, 14]. The proteinases capable of affecting the S1–S2 site are TMPRSS2 (membrane-bound serine protease 2) and ADAM17 (metalloprotease of the ADAM family); the proteinase for the site in the S2' region is furin [6]. Proteolytic activation of the virus is the key moment for initiating the fusion of the viral envelope with the host cell membrane, penetration of the nucleocapsid into the cytoplasm, and release of the viral genome [15, 19, 26].

After the attachment of the viral S protein to the receptor, i.e., angiotensin-converting enzyme 2 (ACE2) of target cells, the complex being formed undergoes conformational modifications under the influence of pH-dependent cysteine protease, cathepsin L, which results in the fusion of the viral envelope with the endosome wall [27]. The invasion mechanism of the virus depends on a cell type. In the culture of epithelial VeroE6 cells obtained from the kidney of the African green monkey, virions bind ACE2 on the cell surface and then enter the endosomal compartment, where cathepsins L and B mediate the cleavage of S2' and membrane fusion is initiated [28, 29]. For penetration into the epithelium of respiratory airways, the spike protein is hydrolyzed at the S2' site with the involvement of furin [19, 30].

The important role of the spike protein is confirmed by the results of studying the RBD structure in the C-terminal region of subunit S1 of SARS-CoV-2 for binding with the ACE2 receptor [25, 31, 32]. S1 RBD is the most variable part of the SARS-CoV-2 genome, and substitution of the key residues in this region increases the ability of S protein to interact with ACE2 with higher affinity, when the SARS-CoV-2 to ACE2 affinity coefficient increases from 10 to 15 compared to that of SARS-CoV-1 [29, 33], which is accompanied by high morbidity and mortality [14].

1.2. Involvement of TMPRSS2, ADAM17 and Furin in the Proteolysis of S Protein

The TMPRSS2–mediated proteolysis of S protein is very important for penetration of the SARS-CoV-2 virus into a cell [19, 34]. TMPRSS2 is a multi-domain type II transmembrane serine protease, which is involved in the priming of S protein of the SARS-CoV-1, MERS-CoV and SARS-CoV-2 viruses for the viral envelope fusion with the target cell membrane and for infecting humans [35]. In addition to S protein, TMPRSS2 also hydrolyzes ACE2, thereby increasing the capture of the virion through the

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The high contagiousness of coronavirus infection is due to the broad coexpression of TMPRSS2 and ACE2, which result in multiple organ failure: the damage to the lungs, the heart, the cardiovascular system (endothelial cells and smooth muscle cells), the brain, the kidneys, the intestines, the immune cells and the reproductive system [36, 38–41]. The maximum expression of ACE2 is typical of type II pneumocytes, followed by epithelial cells of the mucous membrane of the nose, mouth cavity, and alveolar macrophages [42]. ACE2 is also expressed in renal pericytes and in cardiac myocytes. These data can explain the high damage to renal microvessels and the frequency of heart failure in COVID-19 [43]. The expression of TMPRSS2, similar to ACE2, is widely represented in type II pneumocytes [10]. The TMPRSS2 gene expression in the prostate gland determines the higher risk of the severe form of coronavirus infection in men [44]. Androgenic receptors are supposed to participate in the SARS-CoV-2 infection via the regulation of TMPRSS2 transcription, as well as the cross-talk between COVID-19 and prostate cancer [45]. It has been shown that the androgen deprivation therapy used for prostate cancer plays a protective role against COVID-19.

TMPRSS2 inhibitors (camostat mesylate, nafamostat) are extensively studied as therapeutic agents for the new coronavirus infection [30]. Repositioning of the known drugs: bromhexine, plasminogen activator inhibitor-1, aprotinin and benzamidine, is also used [10]. The possibility of using inhibitors in combination with antiviral drugs is under study [46].

In addition to TMPRSS2, the viral S protein is hydrolyzed by metalloprotease ADAM17. Proteinase ADAM17 also cleaves ACE2, which leads to the release of soluble ACE2 (sACE2) and facilitates the fusion of viral particles with the host cell membrane [40]. Transmembrane protease ADAM17 is expressed in many tissues, including the lungs, muscles, heart, kidneys, small intestine, pancreas, placenta, ovaries and testicles [47]. Causing an imbalance of the renin–angiotensin–aldosterone system, ADAM17 leads to inflammation, increased vascular permeability, pulmonary edema and disseminated intravascular coagulation (DIC) syndrome [34, 48, 49]. ADAM17 has an anti-inflammatory effect as it converts membrane tumor necrosis factor α (TNF-α) into soluble TNF-α and causes the loss of ACE2 by reducing its anti-inflammatory effects [50]. The decrease in the ADAM17 activity induced by proteinase inhibitors may evoke the anti-inflammatory effect.

Enhanced invasion of SARS-CoV-2 compared to other coronaviruses is determined, inter alia, by the presence of a site for priming by furin, a Ca2+-dependent endopeptidase (EC 3.4.21.75) [6]. It is considered that the tropism, transmissivity and universality of infection by the SARS-CoV-2 virus in humans increase due to a broad-range furin expression in cells [25, 51, 52]. Furin is localized mainly in the Golgi complex but can also be transported to the cell surface by the endosomal pathway or secreted to the extracellular space [53]. That is why furin can cleave the S protein in the Golgi complex and in the extracellular space [54]. The increased activity of furin in case of comorbidities such as hypertension, obesity and diabetes mellitus is probably one of the causes of the severe course of COVID-19 [55]. Furin convertase inhibitors—chloromethyl ketone and peptidyl chlromethyl ketones—are the promising agents for treating COVID-19 [56]. The studies of bromhexine and the phytolavonoid luteolin, the furin inhibitors that block the activation of S protein cleavage and membrane fusion, are also continued [14, 57].

1.3. Activation of the Angiotensin—Renin and Kinin Systems

It has been established that the extent of the ACE2 receptor binding to the SARS-CoV-2 virus underlies the pathogenesis of infection and the development of unfavorable outcomes of COVID-19 [21]. ACE is the key activator of the renin–angiotensin–aldosterone system [58]. The serine proteinase renin catalyzes the conversion of angiotensinogen into angiotensin 1, followed by ACE-induced conversion of the latter into angiotensin 2. Angiotensin 2 stimulates the secretion of vasopressin and aldosterone, which leads to an increase in arterial blood pressure. The system performs a protective function in case of hemorrhage, preventing the development of hypovolemia and cardiogenic shock. The effect of the system is balanced by ACE2-induced formation of angiotensins 1–7 and 1–9, which, in contrast to angiotensin 2 (AT2), reduce arterial pressure.

The coronavirus binding to ACE2 blocks this receptor, which leads to an increase in the angiotensin 2/angiotensin 7 ratio. The increase in angiotensin 2 causes vasoconstriction by stimulating the synthesis of leukotrienes and prostaglandins and activates NADPH oxidase in endothelial cells, phagocytes and smooth muscle cells of vessels, which results in the generation of reactive oxygen species (ROS) and...
implementation of their damaging effect in the non-specific inflammatory response [28]. However, the interaction between ACE2 receptor and proteinases TMPRSS, ADAM17 results in hydrolysis and the increased level of soluble ACE2 receptor, which shifts the ratio towards angiotensin 7, causing vasodilation, enhanced vascular permeability, and pulmonary edema [1, 59, 60]. ACE inhibitors and angiotensin 1 (AT1) receptor blockers reduce the level of mortality among COVID-19 patients compared to the patients not administered with the ACE inhibitors. Aldosterone antagonists activate ACE2 by restoring the angiotensin 1/angiotensin 1–7 balance and reduce viral priming [39, 59, 61]. Zoufaly et al. [62] describe the treatment of a 45-year-old female patient with a severe form of COVID-19 using intravenous injection of recombinant soluble sACE2. Administration of this agent at a dose of 0.4 mg/kg weight twice a day for 7 days resulted in reduced concentrations of angiotensin 2, IL–6, IL–8, ferritin, TNF-α, surfactant protein D, and improved clinical condition. The authors believe that the antiviral effect of recombinant sACE2 is implemented via its binding to S protein and neutralization of viral particles and/or regulation of angiotensin synthesis, which promotes a decrease in multiple organ system failure [62].

As it has been shown in the model culture of VeroE6 cells, the introduction of recombinant soluble ACE2 (200 μg/mL) together with the antiviral drug remdesivir (4 μM) has an additive effect at subtoxic concentrations and can improve the effect of remdesivir on SARS-CoV-2 infection [63]. sACE2 underwent a placebo-controlled double blind trial of phase 2b in patients with the severe form of COVID-19, acting as a “molecular trap” for blocking penetration of the virus and as a regulator of the renin–angiotensin system [62, 63].

The activation of the angiotensin–renin system leads to a significant increase in vasoactive peptide bradykinin, which largely explains the underlying mechanism of clinical presentation. The presence of proteinase kallikrein-13 involved in bradykinin formation is important for the development of viral infection [64]. The high concentration of bradykinin formed of high-molecular kininogen under the influence of kallikrein determines vasodilation followed by hypotension, as well as by increased vascular permeability [65]. Bradykinin increases the synthesis of hyaluronic acid, inter alia, in the lungs [66]. Interstitial fluid with hyaluronic acid forms a hydrogel in alveolar lumens, causing respiratory failure and leading to inefficiency of artificial lung ventilation [67].

The increase in bradykinin concentration accounts for the manifestations of dysfunction of the cardiovascular system such as hypotension and cardiac rhythm disturbance [68]. Some data show that bradykinin can increase the concentration of the tissue plasminogen activator, increasing the risk of hemorrhages in COVID-19 patients. It is considered that bradykinin and plasmin are jointly activated in pathological processes such as thrombosis and inflammation [67, 68]. The increased concentration of bradykinin can lead to enhanced blood–brain barrier permeability, contributing to the appearance of neurological symptoms in COVID-19 patients [19, 25]. In general, the vasoactive effect of bradykinin results in enhanced migration of immune cells and more intensive inflammation [69, 70]. Inhibition of the kinin system may have a favorable effect on the cascades of proteolytic systems in blood plasma. Kallistatin is also studied among the inhibitors [71] but, in view of the fact that its inhibitory activity disappears when it binds to heparin, the application of kallistatin for the coronavirus infection is limited.

1.4. Activation of the Coagulation System

The major pathogenetic syndrome in case of COVID-19 is activation of proteinases of the coagulation system, which are associated with complications of the infection. Mattar et al. have analyzed 312 works in order to elucidate the high contagiousness of SARS-CoV-2 [72]. It has been shown that the priming of the spike protein involves, in addition to TMPRSS2 and furin, other proteinases such as trypsin, kallikrein and plasminogen activators. Proteolysis of the S protein of the coronavirus by plasmin, trypsin, cathepsins, elastase, TMPRSS, and other serine proteinases results in enhanced penetration of the SARS-CoV-2 virus into the cells of the bronchial epithelium.

The pathogenesis of SARS-CoV-2 infection is associated with coagulopathy and thromboembolic events: the circulating proteinases involved in blood coagulation can contribute to the activation of the S protein and enhance the infection of human cells [73]. The early stage of COVID-19 is usually limited by local hypercoagulation in pulmonary blood vessels, whereas the severe phase of the disease may be accompanied by systemic dissemination intravascular coagulation [74], stroke and cardiac embolism [75, 76]. It is obvious that the triggering of the coagulation cascade is promoted by the induction of COVID-19-associated “cytokine storm”, the activation of the complement and antiphospholipid autoantibodies, and acute lung injury [77]. The blood coagulation cascade spreads due to the chain reaction of serine proteases, including factor Xa and thrombin, each of them being activated via proteolytic processing [78, 79]. Recently it has been demonstrated that factor Xa and thrombin are able to directly cleave the S protein of
SARS-CoV-2, which leads to enhanced penetration of the virus [80]. According to the suggested hypothesis of the possibility of positive feedback, the infection-induced hypercoagulation increases SARS-CoV-2 infection by stimulating penetration of the virus [81]. Moreover, the activation of coagulation due to plasma proteinases may aggravate the SARS-CoV-2 infection in both TMPRSS2-positive and TMPRSS2-negative target cells and contribute to penetration of the virus into many cells [14].

It is known that, even if patients received anticoagulation therapy, the coronavirus infection was often accompanied by the formation of microthrombi and thrombosis associated with the impaired balance of the coagulation and fibrinolytic systems, which can hardly be corrected by drugs [82, 83]. It is supposed that the molecular mechanisms underlying the development of disseminated intravascular coagulation play an important role in COVID-19 outcomes and determine the range of long-term effects [84].

1.5. Proteinase-Activated Receptors (PARs)

Proteinase-activated receptors PAR-1 and PAR-4 are significant for implementation of the effect of proteinases; they play the key role in the development of thrombophilia and thrombosis. Thrombosis includes the activation of PAR-1 and PAR-4 containing cells: platelets, endothelial cells, alveolar epithelium, fibroblasts, monocytes, macrophages and neutrophils [85]. The process is initiated by an increase in tissue factor 3 (TF3) in case of cell damage. Angiotensin 2 can also induce the synthesis and expression of TF3 in endothelial cells, alveolar epithelial cells, fibroblasts, macrophages and neutrophils. The increase in TF3 via the extrinsic pathway triggers the formation of thrombin, which activates platelet PAR-1 and PAR-4. As a result, platelets demonstrate the increased synthesis and secretion of thromboxane A2 aggregant, proinflammatory factors IL-1β, RANTES, as well as initiation of thrombosis. Platelet activation facilitates coagulation via the intrinsic coagulation pathway due to the release of polyphosphates from platelet granules. Thrombin also initiates the adhesion of platelets to monocytes and neutrophils, causing formation of the networks of neutrophil extracellular traps (NETs) and thereby increasing the proinflammatory activity of NET, blood coagulation and lung injury in COVID-19. The activation of PAR-1 favors the profibrotic phenotype of fibroblasts, alveolar inflammation, and apoptosis with endothelial dysfunction and tissue injury [85]. The activation of thrombosis is promoted by neutrophil elastase involved in the NET formation [86, 87]. The elastase inhibitor (sivelestat) is a potential candidate for limiting lung injury and the development of respiratory distress syndrome [88].

PARs inhibitors can be used to treat the coronavirus infection. For example, PARs-1,4 inhibitors such as atopaxar and vorapaxar are tested as antithrombotic agents in the target therapy for myocardiac ischemia and acute coronary syndrome. The advantage of PARs inhibitors is associated with the low risk of hypocoagulation. The inhibitors of thrombin (e.g., dabigatran and argatroban) and factor Xa (e.g., rivaroxaban and apixaban) are being studied and used. These orally administered drugs usually do not require any thorough monitoring of homeostasis, which is necessary in case of heparin administration. The combined therapy with several anticoagulant/antithrombotic drugs for the inhibition of platelet activation and coagulopathy is considered as the most efficient one [85].

1.6. Hyperinflammation

The peculiar feature of the course of SARS-CoV-2 infection is the development of hyperinflammation [14, 89]. The immune system plays a dual role in the SARS-CoV-2 infection. Most patients (85%) develop an adequate immune response promoting elimination of the virus, and a patient becomes asymptomatic or low symptomatic. However, in 10–15% of cases the immune response becomes too intensive and disproportionate, with the development of the immunopathological phase and severe form of the disease [90, 91]. Pathogenesis of the disease is associated with the development of a strong inflammatory response, the so-called “cytokine storm”, with the increases concentrations of IL-6, IL-1, IL-2, IL-10, TNF-α and IFN-γ. Hyperinflammation causes severe forms of the disease characterized by hypoxemic pneumonia, up to the acute respiratory distress syndrome related to multiple organ failure [14, 89].

The new inflammation marker, pentraxin, induced by proinflammatory cytokines such as TNF-α, IL-1, is synthesized mostly by mononuclear cells, dendritic cells, fibroblasts, and endothelial cells [92]. The level of pentraxin is normally low but quickly increases as the pathological process develops, being accompanied by the unfavorable outcome of the disease [93]. At present, pentraxin-3 is considered as one of the promising agents for the study of markers associated with the fatal outcome in case of inflammatory response accompanying the coronavirus infection [94].

The most frequent symptoms of acute COVID-19 are hyperinflammation localized in the bronchopulmonary system, followed by cough, dyspnea (shortness of breath), pneumonia, the development of acute respiratory distress syndrome (ARDS) and respiratory
failure. The respiratory failure associated with exudative diffuse alveolar damage and massive capillary congestion is the main cause of death in 70% of fatal outcomes of COVID-19 [83]. With respect to pathogenesis, these complications can be a consequence of direct invasion of the virus into tissues, hyperinflammation and “cytokine storm”, immune system disorder, hypercoagulable state, or a combination of these factors. Cytokine IL-6 plays a significant role and its increase in serum correlates with ARDS, respiratory failure and unfavorable clinical outcomes. It has been established that bronchopulmonary system disorders persist in patients after the acute period of coronavirus infection. In the trial involving 55 COVID-19 patients, 35 (64%) patients exhibited persistent symptoms (cough, loss of smell) and 39 (71%) patients demonstrated the thickening of interstitial tissue and the signs of fibrosis 3 months after their discharge from hospital [95, 96].

1.7. Hyperproteolysis in Comorbidities

COVID-19 is significantly aggravated by comorbidities. The major comorbid pathologies are arterial blood hypertension (30%), diabetes mellitus (19%), myocardial ischemia (8%) [97, 98], hepatic and pulmonary diseases [99, 100]. Cardiovascular complications (myocarditis, arrhythmia) are observed both in elderly people with numerous comorbidities and in young, previously healthy patients, including athletes [95, 101]. According to the data obtained by Kamysnyi et al., most of patients suffer from one or several comorbid diseases [98]. The lowest survival rate is observed among elderly persons with the high SOFA score (sequential organ failure, risk of death and sepsis), which is combined by the elevated D-dimer level (more than 1 μg/mL) in patients of intensive care unit [102].

Proteinase activation is a universal response of a body to inflammation [101]. The increased activity of elastase and trypsin results in the injuries to the pulmonary system and gastrointestinal tract, respectively; the increase activity of coagulation proteinases leads to an increase in the thrombotic potential of blood and thrombosis. Precisely these systems are damaged by the SARS-CoV-2 virus [36, 38, 41, 73, 103]. The enhanced proteinase activity is detected in diseases of the bronchopulmonary system (pulmonary emphysema, chronic obstructive pulmonary disease) [104–106], respiratory distress, bronchiectasis, diseases of the cardiovascular system [107], rheumatoid arthritis [108, 109], diseases of the gastrointestinal tract [110–112], hemorrhagic shock [107], migraines, brain tissue injury, the development of depression [113], tumors [114–116]. The damaging effect of elastase in bronchopulmonary system diseases is probably due to the proapoptotic effect of this enzyme. Elastase, in addition to proteolytic effect, inhibits proliferation and induces apoptosis of epithelial cells of the lungs and airways through the binding with PAR-1, the activation of Akt (protein kinase B), and the increase in mitochondrial membrane permeability [117–119]. In addition, elastase decreases the content of tropoelastin mRNA and inhibits the synthesis of elastin [120]. Additional tissue injury is promoted by the bacterial elastase, which hydrolyzes pulmonary surfactant proteins A and D [121]. At the same time, the products of degradation of connective tissue proteins under the influence of elastase stimulate collagen synthesis in fibroblasts and contribute to the development of fibrosis [122, 123]. Among the causes of elastase activation in bronchopulmonary diseases, there are unfavorable environmental factors and smoking [124–126].

If the infection is combined with cancer diseases, the conditions for hyperproteolysis have already been formed in the body. The role of proteolysis in tumor growth is largely associated with the involvement of proteinases in proliferation, invasion and metastasis of tumor cells [127–129]. Kinogenensis, fibrinolysis and thrombin formation are stimulated in the blood of patients. The role of plasminogen activators in neoplastic transformation consists in the change of membrane surface properties of cells and enzyme systems, impaired synthesis of DNA and contact inhibition [114]. The hypercoagulation shift is associated with the formation of thrombogenic factors [130]. Trypsin-like proteinases are expressed in many tumor cells and activate PAR-2, contributing to proliferation, invasion and metastasis of tumor cells [66].

The comorbidities with proteinase activation can obviously contribute to the unfavorable course of the coronavirus infection. According to different data, the percentage of comorbid conditions varies from 32% to 46–50% [131]. The percentage of unfavorable outcomes in comorbid patients is up to 67% [132].

Until now, not a single biomarker has been found that could provide an accurate forecast for COVID-19 [132]. About 30 laboratory indicators discussed in literature are associated with the risk of the severe form of this disease [1]. The mains ones are the low lymphocyte and hemoglobin levels, leukocytosis, elevated transaminase activities, the high levels of creatinine, urea, creatine kinase, troponin, C-reactive protein, IL-6, D-dimer, lactate dehydrogenase, procalcitonin and ESR [102]. Preference is given to the models including a complex of indicators: the levels of neutrophils, lymphocytes, platelets and IL-2 receptor [2]. Generally, the search of the markers for the course of COVID-19, the development of unfavorable out-
comes and long-term effects remains a pressing issue of medicine.

Thus, taking into account that the SARS-CoV-2 virus uses human proteinases (both membrane and extracellular) for penetration into cells, the initial level of proteinases, especially in case of comorbidity, is the decisive factor for the infection and long-term effects.

2. **α₁-Proteinase Inhibitor Under Normal and Pathological Conditions**

The activation of proteinases for penetration of the virus and the development of COVID-19-induced inflammation need a thorough control by the endogenous inhibitors of proteolytic enzymes. The most widespread inhibitor of serine proteinases in human blood plasma is α₁-proteinase inhibitor (α₁-PI, antitrypsin), which provides 90% of the inhibitory potential of blood. In addition to trypsin, α₁-PI inhibits the activities of elastase, thrombin, plasmin, renin, kallikrein, blood coagulation factors Xa and X1a, chymotrypsin, trypsin-like and chymase, and bacterial proteases [133]. The significance of α₁-PI in the control of viral infection is confirmed by the facts of high mortality among persons with genetic deficiency of the inhibitor. It is supposed that the high mortality (37.8%) from COVID-19 in the region of Lombardy (Italy) was due to the genetic deficiency of α₁-PI in its residents [134]. Faria et al. have shown that the genetic deficiency of α₁-PI is widespread in Portugal. The statistical analysis has shown that 1 : 5249 people have a ZZ genotype and 1 : 281 people have a SZ genotype [15]. These data correlated with the geographical distribution of COVID-19, as well as with the frequency of fatal outcomes. A significant positive correlation between the frequency of the SZ genotype and the mortality from COVID-19 in 67 countries was reported [135]. A hypothesis has been put forward that patients with the genetic deficiency of α₁-PI, especially those with the severe forms of α₁-PI (PiZZ and/or low α₁-PI levels in blood serum), can be particularly susceptible to SARS-CoV-2 infection [15, 17].

### 2.1. Structure and Functions of α₁-Proteinase Inhibitor

α₁-PI is a glycoprotein with a molecular weight of 50–55 kDa, which is synthesized in the liver as an inactive precursor. Its processing includes limited proteolysis with the removal of a peptide of 24 amino acid residues and glycosylation at Asp83, which is necessary for the secretion of mature glycoprotein from EPR into blood [136]. The active center of α₁-PI contains a methionine residue; its oxidation leads to inactivation of the inhibitor [137]. The most important physiological functions of α₁-PI are to protect pulmonary tissue from aggressive proteolytic enzymes and ROS [138].

α₁-PI is a product of the synthesis of an autosomal Pi (protease inhibitor) locus localized on the 14q31–32.3 chromosome. The gene contains 12.2 kb and 7 exons and is tightly linked to the IgG heavy chain locus and α₁-antichymotrypsin [139–141]. The α₁-PI gene is characterized by polymorphism. More than 75 alleles have been identified [142]; the products of their synthesis differ in posttranslational processing and electrophoretic mobility: M, medium; F, fast; S, slow; and Z, very slow type. The M allele with a frequency of 86–99% is most widespread among the population [143].

α₁-PI is always present in the blood serum of healthy people (20–52 μmol/L) and its concentration can increase several times during inflammation. α₁-PI is an acute phase protein, exerts immunomodulatory and antiviral effects, stimulates repair, and displays tissue protective properties [144, 145]. It protects endothelial cells of the lung against apoptosis [146]. The main functions of α₁-PI are given in the Table 1.

The anti-inflammatory effect of α₁-PI surpasses the effect of corticosteroids in terms of severity [147]. α₁-PI can reduce acute inflammatory reactions and cell death, inhibit the activity of elastase of neutrophils, the proteinase-dependent stages of immune responses [148]. The anti-inflammatory effect of α₁-PI is related to the increase in stability of mitochondrial membranes, the inhibition of apoptosis, the activation of nuclear factor xB (NF-κxB), the production of TNF-α and matrix metalloproteinase-12, the increased secretion of anti-inflammatory IL-10 in macrophages, as well as contributes to the increase in immunological tolerance [149–151].

α₁-PI also exhibits an antiviral effect against SARS-CoV-2. It has been shown that α₁-PI effectively inhibits TMPRSS2 [18], and impaired inhibition is considered as a potential cause of high mortality from COVID-19 [19]. Wettstein et al. [152] has confirmed the hypothesis that α₁-PI is able to inhibit the penetration of SARS-CoV-2. Under in vitro conditions, the culture of human epithelial colorectal carcinoma Caco2 cells and the culture of human airway epithelial cells infected by spike-containing pseudoparticles and wild type SARS-CoV-2 were treated with α₁-PI at physiological concentrations from 2 mg/mL to 4 mg/mL. It has been shown that α₁-PI promoted an almost complete inhibition of the infection; the effect
Table 1. The role of α₁-proteinase inhibitor under normal and pathological conditions

| Effect                                      | Mechanism of effect                                                                                                                                                                                                 | Reference           |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Serine proteinase inhibitor, serpine        | Inhibits neutrophil elastase, trypsin, thrombin, rennin, plasmin, the complement system. The formed α₁-PI+enzyme complex binds to macroglobulin and is eliminated from blood flow                                                                 | [79, 145, 155, 175] |
| Anti-inflammatory effect of α₁-PI           | Enhanced stability of mitochondrial membranes, inhibition of apoptosis, activation of the nuclear factor kappa B (NFkB), production of tumor necrosis factor alpha, matrix metalloproteinase-12, intensification of interleukin 10 secretion in macrophages, increased immunological tolerance | [147–150, 160, 202] |
| Antiviral effect of α₁-PI                   | Inhibition of the S-protein processing and limitation of extracellular spread and dissemination of SARS-CoV-2, inhibition of ACE2, TMPRSS, ADAM17 involve in reception and penetration of SARS-CoV-2 into target cells | [18, 19, 152, 155]  |
| Anti-apoptotic effect                       | Anti-apoptotic effect on endotheliocytes, prevents the development of endothelial dysfunction. Protection against respiratory infections                                                                                 | [4, 19, 50, 146, 160, 193, 209] |
| “Acute phase” protein                       | Increased content of α₁-PI; is considered as a universal response to a pathological process. Increased levels of this protein have been detected in case of COVID-19                                                                 | [17, 18, 160–162, 164, 202] |
| Oxidative stress marker                    | Increase in α₁-PI correlates with the oxidative stress development                                                                                                                                                    | [168–171]           |
| NET inhibition                              | Inhibition of elastase involved in NET formation, the development of thrombosis                                                                                                                                       | [50, 187, 203–205]  |
| α₁-PI dysfunction                           | Genetic and acquired deficiency of the inhibitor: the damage to the active center during oxidation, the effects of ecologically unfavorable environmental factors, smoking. Degradation by proteinases with the formation of proinflammatory peptides | [171–174, 183, 184] |
| Therapeutic effect of α₁-PI                 | Inhibition of membrane and plasma proteinases. Application of proteinase inhibitors as the tools for targeted therapy. Anticoagulant effect. The study of the anti-COVID effect of α₁-PI                                                                 | [10, 13, 40, 148, 200] |
The ability of α₁-PI to reduce the activity of ADAM17 has been demonstrated [153, 154]. The decrease in ADAM17 concentration, in its turn, is associated with the reduced secretion of IL-6R, TNF-α, FcyRIIib (the low-affinity NK-cell receptor for IgG), the activation of neutrophils, the reduction of the “cytokine storm” and the risk of multiple organ failure and unfavorable outcomes [152, 155]. It is considered that the enhanced risk of unfavorable outcomes of COVID-19 in patients with insulin-dependent diabetes mellitus may be associated with the impairment of α₁-PI function due to glycosylation [156], which leads to an increase in ADAM17 and enhanced susceptibility to the SARS-CoV-2 infection [157]. In addition, the α₁-PI deficiency in patients with type 2 diabetes mellitus was due to vitamin D deficiency, which is a risk factor for COVID-19 [158, 159].

The increase in α₁-PI concentration in case of nonspecific inflammation response is considered as a protective response to excessive activation of proteinases and proteolytic injury to organs and tissues. Recent studies have shown that the increased levels of this protein are observed also for COVID-19 [16, 17]. The α₁-PI increase in case of COVID-19 must exert a protective effect aimed at preventing viral invasion and inhibiting proteolysis activation [18, 148, 160–162], α₁-PI is important for protection of the lungs against respiratory infections, and its degradation plays the key role in pathogenesis of COVID-19 [16, 17, 50]. Since pulmonary alveolar macrophages are activated by SARS-CoV-2, the protective role of α₁-PI against COVID-19 can be mediated by the modulation of IL-8 activity and the binding of TNF-α to TNFR1 and TNFR2 preventing neutrophil chemotaxis and tissue injury [153, 163].

The COVID-19 related increase in α₁-PI correlates with the severity of infection. Azouz et al. [18] have shown that the highest concentration of α₁-PI was observed in the group of patients with the severe form of the disease, compared to medium-severity and mild cases of COVID-19. The α₁-PI concentration positively correlated with the disease severity and with the plasma levels of IL-6 (r = 0.65, p < 0.0001), IL-10 (r = 0.33, p = 0.002) and TNFaα (r = 0.3; p = 0.002) [18]. Cytokines IL-2 and IL-8 from lymphocytes and macrophages increase α₁-PI secretion in epithelial cells of the bowel [164].

2.2. The Causes of Impaired Function of α₁-Proteinase Inhibitor

It should be noted that the role α₁-PI is studied with determination of its concentration but not activity. According to literature data, the increased concentration of α₁-PI as an acute phase protein does not limit the development of the coronavirus infection. We have suggested several possible causes of α₁-PI inefficiency.

The first cause is related to hyperinflammation. It has been shown that implementation of the protective effect of α₁-PI depends on IL-6 concentration. The IL-6/α₁-PI ratio, with IL-6 concentration being predominant over α₁-PI concentration, increases in case of the severe course of COVID-19 and decreases in case of clinical improvement [16]. It is believed that the IL-6/α₁-PI ratio reflects the balance between pro- and anti-inflammatory mechanisms. Probably, the increase in α₁-PI level can be an efficient protective mechanism in case of reduced concentration of IL-6.

One more factor of α₁-PI inefficiency may be the ROS-induced damage to the active center during the development of oxidative stress in COVID-19 [165–167]. Oxidized α₁-PI is considered to be a marker for oxidative stress in pathological processes [168]. Oxidized α₁-PI is able to directly interact with epithelial cells, releasing chemokines IL-8 and MCP-1 (monocyte chemotactic protein-1), which in turn attract macrophages and neutrophils to the airways, stimulating inflammatory responses [169–171].

In addition, α₁-PI can undergo proteinase-induced proteolytic degradation under the conditions of hyperproteolysis. For example, C-31 peptide formed during α₁-PI degradation by matrix proteinases demonstrates proinflammatory properties, stimulates neutrophil chemotaxis and adhesion, degranulation, and ROS generation [171, 172]. α₁-PI can be inactivated with the increase in activity of the enzymes of activated neutrophils (myeloperoxidases, elastases), which also damage the inhibitor [173, 174]. The high concentration of α₁-PI in case of disposition to thrombosis and thromboembolism results in reduction of the fibrinolytic potential [175].

Generally, for assessing the favorable or unfavorable effects of α₁-PI on the pathological process, it would be advisable to measure not only its content but also the activity of the inhibitor, because the increase in concentration may be inefficient due to potential damage to the active center and the loss of inhibitory activity.
2.3. Genetic and Acquired Deficiency of α₁-Proteinase Inhibitor

According to Fregonese and Stolk, the wide occurrence of deficient α₁-PI alleles in West Europe and in the United States is 1:2500 and 1:5000 of newborns, respectively [176]. The S allele has been detected in 28% of the population in the south of Europe and in 1.5% of people in the northeastern Mexico [177]. The Z variant is less frequent and has been identified in only 4% of the population in the north of Europe [141]. There are individuals with a complete absence of α₁-PI synthesis in their organisms [178]. The deficient S allele and the Z variant are characterized by Glu264Val and Glu342Lys substitutions, respectively [141]. The α₁-PI levels in people with genetic deficiency decrease, making 1–1.4 g/L for the SS variant and 0.25–0.7 g/L for ZZ [179, 180].

Some authors believe that the patients with α₁-PI deficiency are more susceptible to COVID-19 [17, 135]. The classical homozygous ZZ deficiency of α₁-PI manifests itself rather rarely and is estimated as 1 : 1700 and 1 : 6000 of the population in Europe and in the world, respectively [179, 181], though there are much more unfavorable outcomes of COVID-19 worldwide. Taking into account that PI deficiency may be asymptomatic, without any clinical manifestations [70, 182], the population screening of patients for α₁-PI deficiency is probably significant for prophylaxis, treatment stratification and prevention of severe forms of COVID-19 [160].

The decrease in α₁-PI may be secondary, not related to genetic deficiency. For example, in case of oxidation of methionine residue in the active center of the inhibitor, the latter will lose its ability to control proteinase activities. In this respect, much attention is focused on the effects of ecologically unfavorable environmental factors. It has been shown that air pollution is closely associated with COVID-19 incidence and mortality in the most affected areas of Italy and China [183, 184]. Moreover, it has been shown that in the northern regions of Italy with the higher level of environmental pollution compared to the southern regions there are more people with genetic α₁-PI deficiency [185]. The active center of α₁-PI is damaged by the toxic products of tobacco smoke; hence, smokers are in the risk group for the unfavorable course of COVID-19 [186, 187].

The decrease in α₁-PI activity may be a result of chronic comorbid diseases against the background of enhanced proteinase activity. When binding proteinases, α₁-PI in complex with α₁-macroglobulin is removed from the bloodstream to EPR for degradation, which leads to insufficient inhibitory activity in blood plasma. It is known that the activity of α₁-PI decreases in the presence of chronic inflammation in 2–5% of patients, which is considered as depletion of the inhibitory potential of blood plasma [188, 189].

In case of α₁-PI deficiency (genetic or acquired), the characteristic diseases of the bronchopulmonary system are chronic bronchitis, emphysema, and chronic obstructive pulmonary disease (COPD). The results of the large cohort study carried out in 15586 COVID-19 patients at a Cleveland clinic have shown that 9.2% of COVID patients suffered from COPD [186, 190]. This fact is in agreement with the data of other authors showing that COPD patients without any other comorbidities run the higher risk of severe COVID-19 [187]. At the same time, the increased expression of ACE2 receptor in case of SARS-CoV-2 infection was not associated with the phenotype of the disease [191, 192]. Probably, the low level of α₁-PI against the activation of neutrophil elastase causing lung tissue injury is more significant for COPD patients than viral tropism.

α₁-PI was used as one of the clinical and biological predictors of COVID-19 in two clinical trials in Italy. It is supposed that the combination of the lower concentration and low activity of α₁-PI is related to pulmonary insufficiency and contributes to the development of acute respiratory distress syndrome [193, 194]. In general, α₁-PI is considered as a protective factor against COVID-19, which not only decreases SARS-CoV-2 penetration but also protects against the major clinical complications such as pneumonia and acute respiratory failure [20, 160, 192]. It is up-to-date to determine the concentration and activity of α₁-PI for predicting the course of COVID-19 and the development of complications.

3. APPLICATION OF α₁-PI FOR TARGETED THERAPY

3.1. Preparations of α₁-Proteinase Inhibitor in Substitution Therapy

α₁-PI drugs are already used in medicine as the substitution therapy for pulmonary and hepatic diseases associated with the genetic deficiency of this inhibitor. Prolastin (α₁-antitrypsin of human blood plasma) is used in the substitution therapy for chronic obstructive pulmonary diseases [195]. The patients with α₁-PI deficiency (0.5 g/L) were administered with 180 mg/kg of the drug every three weeks. Since the half life time was 8.7 days, it is recommended to be introduced every two weeks. The application of serotype 2 adenovirus (AAV2) expressing α₁-PI is investigated; its intramuscular or intravenous introduction
provides stable α₁-PI levels in the blood serum of experimental animals. Intrapleural injection of the AAV5-α₁-PI vector contributes to maintenance of the higher α₁-PI levels in the lungs and blood serum of the animals [196]. The drug is administered both through injection and through inhalation [197]. One more preparation of the inhibitor—aralast (purified α₁-antitrypsin from human blood plasma)—shows a more marked effect compared to prolastin [198]. Generally, the drugs display good medial effects, but their large-scale application is limited by their price and cost efficiency [199].

3.2. Prospects of Using α₁-Proteinase Inhibitor for the Coronavirus Infection

Taking into account the importance of proteinases for the penetration mechanism of the SARS-CoV-2 virus and for the development of COVID-19, proteinase inhibitors are potential candidates as the tools of targeted therapy for COVID-19 [10, 30, 46]. Proteinase inhibitors can prevent SARS-CoV-2 penetration into target cells, inhibiting S protein priming by proteinase TMPRSS2 and extracellular proteinases. Many of the drugs are already used or undergo clinical trials for different indications and are actively studied for COVID-19. They include camostat, nafamostat, bromhexine, ammonium chloride, aprotinin, ulinastatin, heparin, tranexamic acid, chloroquine, etc. [12, 13, 200].

Serine protease inhibitors not only limit the virus penetration into target cells but also can reduce the major clinical manifestations of the infection. For example, trypsin inhibitors can reduce pulmonary edema caused by the activation of PARs. The plasmin and factor X inhibitors may be therapeutically advantageous in case of diffuse pulmonary intravascular coagulopathy, which is one of the major COVID-19-associated pathologies responsible for morbidity and mortality. The indirect cathepsin inhibitors—chloroquine and hydroxychloroquine—have an immunomodulatory effect [10].

Azouz et al. assume that the treatment with extracellular protease inhibitors, separately or in combination with other agents against COVID-19, can be a useful antiviral strategy for controlling COVID-19 [18]. In the treatment for COVID-19, the preference is given to the drugs with a dual (antiviral and anti-inflammatory) activity [148].

α₁-PI possessing both antiviral and anti-inflammatory activities is considered as an ideal candidate drug for COVID-19 [18, 148]. By inhibiting protease TMPRSS2, α₁-PI effectively limits proteinase-mediated cell penetration of the virus, reduces the intercellular spread and dissemination of SARS-CoV-2 [19, 20]. At the same time, the effect of α₁-PI was comparable with the effect of camostat [18]. In addition to reducing the infection by SARS-CoV-2, α₁-PI has an anticoagulant effect and can protect against hyperinflammation, cell death and formation of extracellular neutrophil traps; therefore, this multifunctional protein is considered as a candidate drug for treating COVID-19 [135, 148]. In general, the availability and safety profile of α₁-PI attracts attention to its potential clinical application in the therapy for COVID-19 [18].

At present, α₁-PI in involved in four clinical trials for treating COVID-19 patients in Saudi Arabia (NCT04385836), Spain (NCT04495101), the United States (NCT04547140) and Ireland (EudraCT 2020-001391-15) [135]. The combination of antiviral and anti-inflammatory properties of α₁-PI is of particular significance, as they are expected to make this therapeutic technique most efficient [145, 160]. At present, the clinical double-blind randomized placebo-controlled pilot study of the effect of intravenous administration of prolastin (purified plasma antitrypsin) at a dose of 120 mg/kg of body weight on the course of COVID-19 is performed in Ireland. The secondary goal of the study is to assess the frequency and severity of unfavorable events [201]. In Russia, no such studies are performed, in spite of a fairly high distribution of α₁-PI deficiency alleles. In 2020, the first multicenter population study was performed in Russia to estimate the frequency of heterozygous SERPINA1 alleles of antitrypsin among 1244 people (men, 46%; the average age, 44±12 years) living in the Vologda Region (the typical region with the predominance of ethnic Russians) of the Northwestern Federal District. The frequency of heterozygous α₁-antitrypsin deficiency alleles in the Russian population was 4.90% [202].

It is most urgent to use α₁-PI for treating people with genetic deficiency of the inhibitor. It is supposed that α₁-PI deficient patients are in the SARS-CoV-2 risk group, because TMPRSS2 in these people will be easier activated, allowing SARS-CoV-2 to penetrate cells. Since α₁-PI has an inhibitory effect on thrombin, its deficiency can be associated with the enhanced risk of blood clotting disorder. In addition, the deficiency of inhibitory element increases the risk of activation of proteolysis, inflammation, coagulation, apoptosis of endotheliocytes, and causes the development of severe acute lung injury [135]. It is supposed that the study of occurrence of α₁-PI deficiency in patients who have recovered from COVID-19 may establish the clinical significance of proteinase inhibitors in case of SARS-CoV-2 infection [135].
The ability of $\alpha_1$-PI to interact with TMPRSS2, ADAM17 and immunocompetent cells opens up new prospects for the development of efficient therapeutic methods [161, 203]. Bai et al. have postulated seven reasons for using $\alpha_1$-PI as a proteinase inhibitor in COVID-19 [50]:

1. $\alpha_1$-PI is a universal proteinase inhibitor;
2. $\alpha_1$-PI has an antiviral activity;
3. $\alpha_1$-PI has strong anti-inflammatory properties (partially due to inhibition of the activation of NF-kB and ADAM17) and thereby can weaken COVID-19 induced hyperinflammation;
4. $\alpha_1$-PI prevents the release of ACE2 and consequently can reduce the capillary permeability under the influence of bradykinin;
5. $\alpha_1$-PI inhibits thrombin, preventing thrombosis;
6. $\alpha_1$-PI-induced inhibition of elastase prevents the formation of NET and the development of immunothrombosis. In its turn, neutrophil elastase can be a potential biomarker for severe systemic manifestations of COVID-19 [15, 87, 204] and a target for inhibitory drugs, e.g., sivelestat [205, 206];
7. $\alpha_1$-PI limits endothelial injury due to antiapoptotic effect and reduces the probability of severe acute lung injury and multiple organ failure [50, 207].

Thus, the $\alpha_1$-PI based drugs are the promising tools for increasing the inhibitory potential of blood plasma in case of COVID-19, which is most relevant in the event of reduced inhibitory activity of blood plasma.

**CONCLUSIONS**

Proteinase activation is of the key importance for the development of SARS-CoV-2 infection. The activation of membrane proteinases (ACE2, TMPRSS, ADAM17, furin) of target cells under the influence of SARS-CoV-2 provides membrane fusion and viral entry into cells. Blood plasma proteinases are also able to participate in virus activation. Membrane and plasma proteinases are trypsin-like enzymes controlled by $\alpha_1$-PI; hence, the appropriate functioning of $\alpha_1$-PI is highly important for the regulation of viral tropism toward human cells. The patients with genetic $\alpha_1$-PI deficiency are more susceptible to the SARS-CoV-2 virus, and infection in such patients takes the most unfavorable form. The decrease in the inhibitory potential of $\alpha_1$-PI is promoted by chronic inflammatory diseases. Taking into account that genetic deficiency is a rather rare disorder, it would be more relevant to focus on acquired $\alpha_1$-PI deficiency, which develops in the presence of factors such as oxidative stress and smoking, which lead to oxidation of methionine residue in the active center of the inhibitor and the loss of its functional activity. Depletion of the inhibitory potential is favored by the excessive activation of proteolysis in elderly persons and by the presence of comorbidities. Under the conditions of $\alpha_1$-PI deficiency, either genetic or acquired, proteolysis becomes uncontrolled and is accompanied by tissue injury, thrombophilia, DIC syndrome and multiple organ failure. In addition to the inhibitory effect, $\alpha_1$-PI has an antiviral effect, inter alia, on SARS-CoV-2, anti-inflammatory and anticoagulant effects, inhibits the apoptosis of endothelial cells, the formation of NET and the development of thrombosis. In this context, it is relevant to detect the deficiency of $\alpha_1$-PI and the increased inhibitory potential of blood plasma. According to the literature data, $\alpha_1$-PI is considered as a diagnostic marker for unfavorable course of COVID-19 and a drug for normalization of the inhibitory element of proteolysis.

In spite of the great number of publications devoted to the study of $\alpha_1$-PI under the conditions of viral infection, it is still unclear whether all patients demonstrate the anti-COVID effect of $\alpha_1$-PI, and the relationship between the level of ACE2 and the activity of serine proteinases, which are supposed to play the key role in reception and penetration of the virus into target cells, has not been studied. There are no data on the relationship between $\alpha_1$-PI and the development of complications and prediction of outcomes. All the above determines the urgent need of fundamental and translational studies for further assessment of the role of $\alpha_1$-PI in coronavirus infections.

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**COMPLIANCE WITH ETHICAL STANDARDS**

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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