ISSN 1990-7508, Biochemistry (Moscow), Supplement Series B: Biomedical Chemistry, 2022, Vol. 16, No. 4, pp. 271–291. © Pleiades Publishing, Ltd., 2022.
Russian Text © The Author(s), 2022, published in Biomeditsinskaya Khimiya.

Proteolysis and Deficiency of α_1-Proteinase Inhibitor in SARS-CoV-2 Infection

O. E. Akbasheva[a], L. V. Spirina[a, b], D. A. Dyakov[a, *], and N. V. Masunova[a]

[a] *Siberian State Medical University, Tomsk, 634050 Russia*

[b] *Cancer Research Institute, Tomsk National Research Medical Center, Tomsk, 634009 Russia*

**e-mail: den66431511@yandex.ru*

Received February 2, 2022; revised March 30, 2022; accepted April 11, 2022

Abstract—The SARS-CoV-2 pandemic had stimulated the emergence of numerous publications on the α_1-proteinase inhibitor (α_1-PI, α_1-antitrypsin), especially when it was found that the regions of high mortality corresponded to the regions with deficient α_1-PI alleles. By analogy with the data obtained in the last century, when the first cause of the genetic deficiency of α_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 α_1-PI. The purpose of this review was to systematize the scientific data and critical directions for translational studies on the role of α_1-PI in SARS-CoV-2-induced proteolysis hyperactivation as a diagnostic marker and a therapeutic target. This review describes the proteinase-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 α_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 α_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 α_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, α_1-proteinase inhibitor

DOI: 10.1134/S1990750822040035

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 358287267 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-

ditions for hyperproteolysis and increased sensitivity to viral infection [9–11]. The inhibitor drugs acting on viral proteinases are being actively sought [12–14]. Endogenous α_1-proteinase inhibitor (α_1-PI) provides 90% of the inhibitory activity of blood. α_1-PI drugs are already used for treating patients with genetic α_1-antitrypsin deficiency. The compelling evidence of good prospects of using α_1-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]. α_1-PI has antiviral, anti-inflammtory, 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 α_1-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 though 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

cathepsin L-dependent pathway [36]. TMPRSS2 cleaves at arginine and lysine residues within amino acid sequence 697–716 of the ACE2 polypeptide [34]. The experiment has shown that the mice with TMPRSS2 deficiency are less sensitive to the infection by SARS-CoV compared to the control mice [18, 37].

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 mesyilate, 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 metalloproteinase 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 proteinase ADAM17 is expressed in many tissues, including the lungs, muscles, heart, kidneys, small intestine, pancreas, placenta, ovaries and testicles [47]. Causing an unbalance 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 Ca^{2+}-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 chloromethyl ketones—are the promising agents for treating COVID-19 [56]. The studies of bromhexine and the phytoflavonoid 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 nonspecific 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 plasmine, 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 thrombine, 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 A_2 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 Kamyshnyi 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]. Kininogenesis, 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. α_1-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 α_1-proteinase inhibitor (α_1-PI, antitrypsin), which provides 90% of the inhibitory potential of blood. In addition to trypsin, α_1-PI inhibits the activities of elastase, thrombin, plasmin, renin, kallikrein, blood coagulation factors Xa and X1a, chymotrypsin, tryptase and chymase, acrosin, and bacterial proteases [133]. The significance of α_1-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 α_1-PI in its residents [134]. Faria et al. have shown that the genetic deficiency of α_1-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 α_1-PI, especially those with the severe forms of α_1-PI (PiZZ and/or low α_1-PI levels in blood serum), can be particularly susceptible to SARS-CoV-2 infection [15, 17].

2.1. Structure and Functions of α_1-Proteinase Inhibitor

α_1-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 α_1-PI contains a methionine residue; its oxidation leads to inactivation of the inhibitor [137]. The most important physiological functions of α_1-PI are to protect pulmonary tissue from aggressive proteolytic enzymes and ROS [138].

α_1-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 α_1-antichymotrypsin [139–141]. The α_1-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].

α_1-PI is always present in the blood serum of healthy people (20–52 μmol/L) and its concentration can increase several times during inflammation. α_1-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 α_1-PI are given in the Table 1.

The anti-inflammatory effect of α_1-PI surpasses the effect of corticosteroids in terms of severity [147]. α_1-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 α_1-PI is related to the increase in stability of mitochondrial membranes, the inhibition of apoptosis, the activation of nuclear factor κB (NF-κB), 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].

α_1-PI also exhibits an antiviral effect against SARS-CoV-2. It has been shown that α_1-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 α_1-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 α_1-PI at physiological concentrations from 2 mg/mL to 4 mg/mL. It has been shown that α_1-PI promoted an almost complete inhibition of the infection; the effect

Table 1. The role of α_1-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 α_1-PI+enzyme complex binds to macroglobulin and is eliminated from blood flow	[79, 145, 155, 175]
Anti-inflammatory effect of α_1-PI	Enhanced stability of mitochondrial membranes, inhibition of apoptosis, activation of the nuclear factor kappa B (NFκB), 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 α_1-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 α_1-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 α_1-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]
α_1-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 α_1-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 α_1-PI	[10, 13, 40, 148, 200]

persisted until high concentrations (8.2 mg/mL) without manifestation of the cytotoxic effect [152].

The ability of α_1-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 α_1-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 α_1-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 α_1-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 α_1-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], α_1-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 α_1-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 α_1-PI correlates with the severity of infection. Azouz et al. [18] have shown that the highest concentration of α_1-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 α_1-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 TNFα ($r = 0.3$; $p = 0.002$) [18]. Cytokines IL-2 and IL-8 from lymphocytes and macrophages increase α_1-PI secretion in epithelial cells of the bowel [164].

2.2. The Causes of Impaired Function of α_1-Proteinase Inhibitor

It should be noted that the role α_1-PI is studied with determination of its concentration but not activity. According to literature data, the increased concentration of α_1-PI as an acute phase protein does not limit the development of the coronavirus infection. We have suggested several possible causes of α_1-PI inefficiency.

The first cause is related to hyperinflammation. It has been shown that implementation of the protective effect of α_1-PI depends on IL-6 concentration. The IL-6/α_1-PI ratio, with IL-6 concentration being predominant over α_1-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/α_1-PI ratio reflects the balance between pro- and anti-inflammatory mechanisms. Probably, the increase in α_1-PI level can be an efficient protective mechanism in case of reduced concentration of IL-6.

One more factor of α_1-PI inefficiency may be the ROS-induced damage to the active center during the development of oxidative stress in COVID-19 [165–167]. Oxidized α_1-PI is considered to be a marker for oxidative stress in pathological processes [168]. Oxidized α_1-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, α_1-PI can undergo proteinase-induced proteolytic degradation under the conditions of hyperproteolysis. For example, C-31 peptide formed during α_1-PI degradation by matrix proteinases demonstrates proinflammatory properties, stimulates neutrophil chemotaxis and adhesion, degranulation, and ROS generation [171, 172]. α_1-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 α_1-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 α_1-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 α_1-Proteinase Inhibitor

According to Fregonese and Stolk, the wide occurrence of deficient α_1-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 α_1-PI synthesis of in their organisms [178]. The deficient S allele and the Z variant are characterized by Glu264Val and Glu342Lys substitutions, respectively [141]. The α_1-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 α_1-PI deficiency are more susceptible to COVID-19 [17, 135]. The classical homozygous ZZ deficiency of α_1-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 α_1-PI deficiency is probably significant for prophylaxis, treatment stratification and prevention of severe forms of COVID-19 [160].

The decrease in α_1-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 α_1-PI deficiency [185]. The active center of α_1-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 α_1-PI activity may be a result of chronic comorbid diseases against the background of enhanced proteinase activity. When binding proteinases, α_1-PI in complex with α_2-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 α_1-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 α_1-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 α_1-PI against the activation of neutrophil elastase causing lung tissue injury is more significant for COPD patients than viral tropism.

α_1-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 α_1-PI is related to pulmonary insufficiency and contributes to the development of acute respiratory distress syndrome [193, 194]. In general, α_1-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 α_1-PI for predicting the course of COVID-19 and the development of complications.

3. APPLICATION OF α_1-PI FOR TARGETED THERAPY

3.1. Preparations of α_1-Proteinase Inhibitor in Substitution Therapy

α_1-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 (α_1-antitrypsin of human blood plasma) is used in the substitution therapy for chronic obstructive pulmonary diseases [195]. The patients with α_1-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 α_1-PI is investigated; its intramuscular or intravenous introduction

provides stable α_1-PI levels in the blood serum of experimental animals. Intrapleural injection of the AAV5-α_1-PI vector contributes to maintenance of the higher α_1-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 α_1-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 α_1-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].

α_1-PI possessing both antiviral and anti-inflammatory activities is considered as an ideal candidate drug for COVID-19 [18, 148]. By inhibiting protease TMPRSS2, α_1-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 α_1-PI was comparable with the effect of camostat [18]. In addition to reducing the infection by SARS-CoV-2, α_1-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 α_1-PI attracts attention to its potential clinical application in the therapy for COVID-19 [18].

At present, α_1-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 α_1-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 α_1-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 α_1-antitrypsin deficiency alleles in the Russian population was 4.90% [2021].

It is most urgent to use α_1-PI for treating people with genetic deficiency of the inhibitor. It is supposed that α_1-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 α_1-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 α_1-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 α_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 α_1-PI as a proteinase inhibitor in COVID-19 [50]:

1. α_1-PI is a universal proteinase inhibitor;

2. α_1-PI has an antiviral activity;

3. α_1-PI has strong anti-inflammatory properties (partially due to inhibition of the activation of NF-κB and ADAM17) and thereby can weaken COVID-19 induced hyperinflammation;

4. α_1-PI prevents the release of ACE2 and consequently can reduce the capillary permeability under the influence of bradykinin;

5. α_1-PI inhibits thrombin, preventing thrombosis;

6. α_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. α_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 α_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 α_1-PI; hence, the appropriate functioning of α_1-PI is highly important for the regulation of viral tropism toward human cells. The patients with genetic α_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 α_1-PI is promoted by chronic inflammatory diseases. Taking into account that genetic deficiency is a rather rare disoder, it would be more relevant to focus on acquired α_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 α_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, α_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 α_1-PI and the increased inhibitory potential of blood plasma. According to the literature data, α_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 α_1-PI under the conditions of viral infection, it is still unclear whether all patients demonstrate the anti-COVID effect of α_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 α_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 α_1-PI in coronavirus infections.

FUNDING

The work was supported by the budgetary funds of the Siberian State Medical University.

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.

REFERENCES

1. Ghahramani, S., Tabrizi, R., Lankarani, K.B., Kashani, S.M., Rezaei, S., Zeidi, N., Akbari, M., Heydari, S.T., Akbari, H., Nowrouzi-Sohrabi, P., and Ahmadizar, F., *Eur. J. Med. Res.*, 2020, vol. 25, no. 1, p. 30.
https://doi.org/doi 10.1186/s40001-020-00432-3

2. Wang, F., Hou, H., Wang, T., Luo, Y., Tang, G., Wu, S., Zhou, H., and Sun, Z., *Travel. Med. Infect. Dis.*, 2020, vol. 36, p. 101782. https://doi.org/10.1016/j.tmaid.2020.101782

3. Abdelrahman, Z., Li, M., and Wang, X., *Front. Immunol.*, 2020, vol. 11, p. 552909. https://doi.org/10.3389/fimmu.2020.552909

4. Azer, S.A., *New Microbes New Infect.*, 2020, vol. 37, p. 100738. https://doi.org/10.1016/j.nmni.2020.100738

5. Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., and Zhang, L., *Lancet*, 2020, vol. 395, no. 10223, pp. 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7

6. Valle, C., de Lamballerie, X., Canard, B., Seidah, N.G., and Decroly, E., *Antiviral Res.*, 2020, vol. 176, p. 104742. https://doi.org/10.1016/j.antiviral.2020.104742

7. Du, L., He, Y., Zhou, Y., Liu, S., Zheng, B.J., and Jiang, S., *Nat. Rev. Microbiol.*, 2009, vol. 7, no. 3, pp. 226–236. https://doi.org/10.1038/nrmicro2090

8. Millet, J.K. and Whittaker, G.R., *Virus Res.*, 2015, vol. 202, pp. 120–134. https://doi.org/10.1016/j.virusres.2014.11.021

9. Hörnich, B.F., Großkopf, A.K., Schlagowski, S., Tenbusch, M., Kleine-Weber, H., Neipel, F., Stahl-Hennig, C., and Hahn, A.S., *J. Virol.*, 2021, vol. 95, no. 9, pp. e00002–21. https://doi.org/10.1128/JVI.00002-21

10. Kaur, U., Chakrabarti, S.S., Ojha, B., Pathak, B.K., Singh, A., Saso, L., and Chakrabarti, S., *Curr. Drug Targets*, 2021, vol. 22, no. 2, pp. 192–201. https://doi.org/10.2174/1389450121666200924113243

11. Senapati, S., Banerjee, P., Bhagavatula, S., Kushwaha, P.P., and Kumar, S., *J. Genet.*, 2021, vol. 100, no. 1, p. 12. https://doi.org/10.1007/s12041-021-01262-w

12. Dai, W., Zhang, B., Jiang, X.M., Su, H., Li, J., Zhao, Y., Xie, X., Jin, Z., Peng, J., Liu, F., Li, C., Li, Y., Bai, F., Wang, H., Cheng, X., Cen, X., Hu, S., Yang, X., Wang, J., Liu, X., Xiao, G., Jiang, H., Rao, Z., Zhang, L.K., Xu, Y., Yang, H., and Liu, H., *Science*, 2020, vol. 368, no. 6497, pp. 1331–1335. https://doi.org/10.1126/science.abb4489

13. Ullrich, S. and Nitsche, C., *Bioorg. Med. Chem. Lett.*, 2020, vol. 30, no. 17, p. 127377. https://doi.org/10.1016/j.bmcl.2020.127377

14. Vianello, A., del Turco, S., Babboni, S., Silvestrini, B., Ragusa, R., Caselli, C., Melani, L., Fanucci, L., and Basta, G., *Biomedicines*, 2021, vol. 9, p. 710. https://doi.org/10.3390/biomedicines9070710

15. Faria, N., Inês Costa, M., Gomes, J., and Sucena, M., *Respir. Med.*, 2021, vol. 181, p. 106387. https://doi.org/10.1016/j.rmed.2021.106387

16. McElvaney, O.J., McEvoy, N.L., McElvaney, O.F., Carroll, T.P., Murphy, M.P., Dunlea, D.M., Choileain, O.N., Clarke, J., O'Connor, E., Hogan, G., Ryan, D., Sulaiman, I., Gunaratnam, C., Branagan, P., O'Brien, M.E., Morgan, R.K., Costello, R.W., Hurley, K., Walsh, S., de Barra, E., McNally, C., McConkey, S., Boland, F., Galvin, S., Kiernan, F., O'Rourke, J., Dwyer, R., Power, M., Geoghegan, P., Larkin, C., O'Leary, R.A., Freeman, J., Gaffney, A., Marsh, B., Curley, G.F., and McElvaney, N.G., *Am. J. Respir. Crit. Care Med.*, 2020, vol. 202, no. 6, pp. 812–821. https://doi.org/10.1164/rccm.202005-1583OC

17. Shapira, G., Shomron, N., and Gurwitz, D., *FASEB J.*, 2020, vol. 34, no. 11, pp. 14160–14165. https://doi.org/10.1096/fj.202002097

18. Azouz, N.P., Klingler, A.M., Callahan, V., Akhrymuk, I.V., Elez, K., Raich, L., Henry, B.M., Benoit, J.L., Benoit, S.W., Noé, F., Kehn-Hall, K., and Rothenberg, M.E., *BioRxiv.*, 2020. https://doi.org/doi 10.1101/2020.05.04.077826

19. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.H., Nitsche, A., Müller, M.A., Drosten, C., and Pöhlmann, S., *Cell*, 2020, vol. 181, no. 2, pp. 271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052

20. Oguntuyo, K.Y., Stevens, C.S., Siddiquey, M.N., Schilke, R.M., Woolard, M.D., Zhang, H., Acklin, J.A., Ikegame, S., Huang, C.T., Lim, J.K., Cross, R.W., Geisbert, T.W., Ivanov, S.S., Kamil, J.P., and Lee, B., *Preprint. BioRxiv.*, 2020. https://doi.org/10.1101/2020.08.14.248880

21. Abd El Hadi, S.R., Zien El-Deen, E.E., Bahaa, M.M., Sadakah, A.A., and Yassin, H.A., *Drug Des. Devel. Ther.*, 2021, vol. 15, pp. 3313–3330. https://doi.org/10.2147/DDDT.S320320

22. Fehr, A.R. and Perlman, S., *Methods Mol. Biol.*, 2015, vol. 1282, pp. 1–23. https://doi.org/10.1007/978-1-4939-2438-7

23. Belen-Apak, F.B. and Sarialioglu, F., *Med. Hypotheses*, 2020, vol. 142, p. 109743. https://doi.org/10.1016/j.mehy.2020.109743

24. Wu, Z., Harrich, D., Li, Z., Hu, D., and Li, D., *Rev. Med. Virol.*, 2021, vol. 31, no. 2, p. e2171. https://doi.org/10.1002/rmv.2171

25. Hoffmann, M., Kleine-Weber, H., and Pöhlmann, S.A., *Mol. Cell*, 2020, vol. 78, no. 4, pp. 779–784.e5. https://doi.org/10.1016/j.molcel.2020.04.022

26. Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., and Li, F., *Proc. Natl. Acad. Sci. USA*, 2020, vol. 117, no. 21, pp. 11727–11734. https://doi.org/10.1073/pnas.2003138117

27. Abuo-Rahma, G.E.-D.A., Mohamed, M.F., Ibrahim, T.S., Shoman, M.E., Samir, E., and Abd El-Baky, R.M., *RSC Adv.*, 2020, vol. 10, pp. 26895–26916.

28. Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W., and Xiao, G., *Cell Res.*, 2020, vol. 30, no. 3, pp. 269–271. https://doi.org/10.1038/s41422-020-0282-0

29. Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K.Y., Zhou, H., and Yan, J., *Cell*, 2020, vol. 181, no. 4, pp. 894–904. e9. https://doi.org/10.1016/j.cell.2020.03.045

30. Li, K., Meyerholz, D.K., Bartlett, J.A., and McCray, P.B., *MBio*, 2021, vol. 12, no. 4, p. e0097021. https://doi.org/10.1128/mBio.00970-21

31. Belouzard, S., Chu, V.C., and Whittaker, G.R., *Proc. Natl. Acad. Sci. USA*, 2009, vol. 106, no. 14, pp. 5871–5876. https://doi.org/10.1073/pnas.0809524106

32. Benton, D.J., Wrobel, A.G., Xu, P., Roustan, C., Martin, S.R., Rosenthal, P.B., Skehel, J.J., and Gamblin, S.J., *Nature*, 2020, vol. 588, no. 7837, pp. 327–330. https://doi.org/10.1038/s41586-020-2772-0

33. Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., and Wang, X., *Nature*, 2020, vol. 581, no. 7807, pp. 215–220. https://doi.org/10.1038/s41586-020-2180-5

34. Heurich, A., Hofmann-Winkler, H., Gierer, S., Liepold, T., Jahn, O., and Pöhlmann, S., *J. Virol.*, 2014, vol. 88, no. 2, pp. 1293–1307. https://doi.org/10.1128/JVI.02202-13

35. Hussain, M., Jabeen, N., Amanullah, A., Baig, A.A., Aziz, B., Shabbir, S., Raza, F., and Uddin, N., *AIMS Microbiol.*, 2020, vol. 6, no. 3, pp. 350–360. https://doi.org/10.3934/microbiol.2020021

36. Fuentes-Prior, P., *J. Biol. Chem.*, 2021, vol. 296, p. 100135. https://doi.org/10.1074/jbc.REV120.015980

37. Iwata-Yoshikawa, N., Okamura, T., Shimizu, Y., Hasegawa, H., Takeda, M., and Nagata, N., *J. Virol.*, 2019, vol. 93, no. 6, pp. e01815–18. https://doi.org/10.1128/JVI.01815-18

38. Fathema, K., Hassan, M.N., Mazumder, M.W., Benzamin, M., Ahmed, M., Islam, M.R., Haque, N., Sutradhar, P.K., Rahman, A.R., and Rukunuzzaman, M., *Mymensingh Med. J.*, 2021, vol. 30, no. 2, pp. 570–579.

39. Gemmati, D., Bramanti, B., Serino, M.L., Secchiero, P., Zauli, G., and Tisato, V., *Int. J. Mol. Sci.*, 2020, vol. 21, no. 10, p. 3474. https://doi.org/10.3390/ijms21103474

40. Guney, C. and Akar, F., *J. Pharm. Sci.*, 2021, vol. 24, pp. 84–93. https://doi.org/10.18433/jpps31455

41. Iba, T., Connors, J.M., and Levy, J.H., *Inflamm. Res.*, 2020, vol. 69, no. 12, pp. 1181–1189. https://doi.org/10.1007/s00011-020-01401-6

42. Xu, H., Zhong, L., Deng, J., Peng, J., Dan, H., Zeng, X., Li, T., and Chen, Q., *Int. J. Oral Sci.*, 2020, vol. 12, no. 1, p. 8. https://doi.org/10.1038/s41368-020-0074-x

43. Guzik, T.J., Mohiddin, S.A., Dimarco, A., Patel, V., Savvatis, K., Marelli-Berg, F.M., Madhur, M.S., Tomaszewski, M., Maffia, P., D'Acquisto, F., Nicklin, S.A., Marian, A.J., Nosalski, R., Murray, E.C., Guzik, B., Berry, C., Touyz, R.M., Kreutz, R., Wang, D.W., Bhella, D., Sagliocco, O., Crea, F., Thomson, E.C., and McInnes, I.B., *Cardiovasc. Res.*, 2020, vol. 116, no. 10, pp. 1666–1687. https://doi.org/10.1093/cvr/cvaa106

44. Dalpiaz, P.L., Lamas, A.Z., Caliman, I.F., Ribeiro, R.F., Abreu, G.R., Moyses, M.R., Andrade, T.U., Gouvea, S.A., Alves, M.F., Carmona, A.K., and Bissoli, N.S., *PLoS One*, 2015, vol. 10, no. 5, p. e0127515. https://doi.org/10.1371/journal.pone.0127515

45. Bahmad, H.F. and Abou-Kheir, W., *Prostate Cancer Prostatic Dis.*, 2020, vol. 23, no. 4, pp. 561–563. https://doi.org/10.1038/s41391-020-0262-y

46. Ianevski, A., Yao, R., Lysvand, H., Grodeland, G., Legrand, N., Oksenych, V., Zusinaite, E., Tenson, T., Bjoras, M., and Kainov, D.E., *Viruses*, 2021, vol. 13, no. 9, p. 1768. https://doi.org/10.3390/v13091768

47. Zipeto, D., Palmeira, J.D.F., Argañaraz, G.A., and Argañaraz, E.R., *Front. Immunol.*, 2020, vol. 11, p. 576745. https://doi.org/10.3389/fimmu.2020.576745

48. Tang, N., Li, D., Wang, X., and Sun, Z., *J. Thromb. Haemost.*, 2020, vol. 18, no. 4, pp. 844–847. https://doi.org/10.1111/jth.14768

49. Hrenak, J. and Simko, F., *Int. J. Mol. Sci.*, 2020, vol. 21, no. 21, p. 8038. https://doi.org/10.3390/ijms21218038

50. Bai, X., Hippensteel, J., Leavitt, A., Maloney, J.P., Beckham, D., Garcia, C., Li, Q., Freed, B.M., Ordway, D., Sandhaus, R.A., and Chan, E.D., *Med. Hypotheses*, 2021, vol. 146, p. 110394. https://doi.org/10.1016/j.mehy.2020.110394

51. Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C., and Garry, R.F., *Nat. Med.*, 2020, vol. 26, no. 4, pp. 450–452. https://doi.org/10.1038/s41591-020-0820-9

52. Seyran, M., Pizzol, D., Adadi, P., El-Aziz, T.M.A., Hassan, S.S., Soares, A., Kandimalla, R., Lundstrom, K., Tambuwala, M., Aljabali, A.A., Lal, A., Azad, G.K., Choudhury, P.P., Uversky, V.N., Sherchan, S.P., Uhal, B.D., Rezaei, N., and Brufsky, A.M., *J. Med. Virol.*, 2021, vol. 93, no. 3, pp. 1204–1206. https://doi.org/10.1002/jmv.26478

53. Oz, M. and Lorke, D.E., *Biomed. Pharmacother.*, 2021, vol. 136, p. 111193. https://doi.org/10.1016/j.biopha.2020.111193

54. Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T., and Veesler, D., *Cell*, 2020, vol. 181, no. 2, pp. 281–292. https://doi.org/10.1016/j.cell.2020.02.058

55. Fitzgerald, K., *Perm. J.*, 2020, vol. 24, p. 20.187. https://doi.org/10.7812/TPP/20.187

56. van Lamvan, T., Ivanova, T., Hardes, K., Heindl, M.R., Morty, R.E., Böttcher-Friebertshäuser, E., Lindberg, I., Than, M.E., Dahms, S.O., and Steinmetzer, T., *ChemMedChem*, 2019, vol. 14, no. 6, pp. 673–685. https://doi.org/10.1002/cmdc.201800807

57. Palit, P., Chattopadhyay, D., Thomas, S., Kundu, A., Kim, H.S., and Rezaei, N., *Phytomedicine*, 2021, vol. 85, p. 153396. https://doi.org/10.1016/j.phymed.2020.153396

58. Shamanaev, A., Emsley, J., and Gailani, D., *J. Thromb. Haemost.*, 2021, vol. 19, no. 2, pp. 330–341. https://doi.org/10.1111/jth.15149

59. Banu, N., Panikar, S.S., Leal, L.R., and Leal, A.R., *Life Sci.*, 2020, vol. 256, p. 117905. https://doi.org/10.1016/j.lfs.2020.117905

60. Ciulla, M.M., *Hypertens. Res.*, 2020, vol. 43, pp. 985–986. https://doi.org/10.1038/s41440-020-0488-z

61. Gheblawi, M., Wang, K., Viveiros, A., Nguyen, Q., Zhong, J.C., Turner, A.J., Raizada, M.K., Grant, M.B., and Oudit, G.Y., *Circ. Res.*, 2020, vol. 126, no. 10, pp. 1456–1474. https://doi.org/10.1161/CIRCRESAHA.120.317015

62. Zoufaly, A., Poglitsch, M., Aberle, J.H., Hoepler, W., Seitz, T., Traugott, M., Grieb, A., Pawelka, E., Laferl, H., Wenisch, C., Neuhold, S., Haider, D., Stiasny, K., Bergthaler, A., Puchhammer-Stoeckl, E., Mirazimi, A., Montserrat, N., Zhang, H., Slutsky, A.S., and Penninger, J.M., *Lancet Respir. Med.*, 2020, vol. 8, no. 11, pp. 1154–1158. https://doi.org/10.1016/S2213-2600(20)30418-5

63. Monteil, V., Dyczynski, M., Lauschke, V.M., Kwon, H., Wirnsberger, G., Youhanna, S., Zhang, H., Slutsky, A.S., Hurtado del Pozo, C., Horn, M., Montserrat, N., Penninger, J.M., and Mirazimi, A., *EMBO Mol. Med.*, 2021, vol. 13, no. 1, p. e13426. https://doi.org/10.15252/emmm.202013426

64. Milewska, A., Falkowski, K., Kulczycka, M., Bielecka, E., Naskalska, A., Mak, Pl., Lesner, A., Ochman, M., Urlik, M., Diamandis, E., Prassas, I., Potempa, J., Kantyka, T., and Pyrc, K., *Sci. Signal.*, 2020, vol. 13, no. 659, p. eaba9902. https://doi.org/10.1126/scisignal.aba9902

65. Ivanov, I., Verhamme, I.M., Sun, M.F., Mohammed, B., Cheng, Q., Matafonov, A., Dickeson, S.K., Joseph, K., Kaplan, A.P., and Gailani, D., *Blood*, 2020, vol. 135, no. 8, pp. 558–567. https://doi.org/10.1182/blood.2019002224

66. Weidmann, H., Heikaus, L., Long, A.T., Naudin, C., Schlüter, H., and Renné, T., *Biochim. Biophys. Acta Mol. Cell Res.*, 2017, vol. 1864, no. 11PtB, pp. 2118–2127. https://doi.org/10.1016/j.bbamcr.2017.07.009

67. Scharfstein, J., Ramos, P., and Barral-Netto, M.G., *Adv. Immunol.*, 2017, vol. 136, pp. 29–84. https://doi.org/10.1016/bs.ai.2017.05.007

68. Talmi-Frank, D., Altboum, Z., Solomonov, I., Udi, Y., Jaitin, D.A., Klepfish, M., David, E., Zhuravlev, A., Keren-Shaul, H., Winter, D.R., Gat-Viks, I., Mandelboim, M., Ziv, T., Amit, I., and Sagi, I., *Cell Host Microbe*, 2016, vol. 20, no. 4, pp. 458–470. https://doi.org/10.1016/j.chom.2016.09.005

69. Dreymueller, D, Uhlig, S., and Ludwig, A., *Am. J. Physiol. Lung Cell Mol. Physiol.*, 2015, vol. 308, no. 4, pp. L325–L343. https://doi.org/10.1152/ajplung.00294.2014

70. Hogarth, D.K. and Rachelefsky, G., *Chest*, 2008, vol. 133, no. 4, pp. 981–988. https://doi.org/10.1378/chest.07-1001

71. Chen, V.C., Chao, L., and Chao, J., *J. Biol. Chem.*, 2000, vol. 275, no. 51, pp. 40371–40377. https://doi.org/10.1074/jbc.M005691200

72. Elrashdy, F., Redwan, E.M., and Uversky, V.N., *Biomolecules*, 2020, vol. 10, no. 9, p. 1312. https://doi.org/10.3390/biom10091312

73. Kastenhuber, E.R., Jaimes, J.A., Johnson, J.L., Mercadante, M., Muecksch, F., Weisblum, Y., Bram, Y.,

Schwartz, R.E., Whittaker, G.R., and Cantley, L.C., *Preprint. BioRxiv*, 2021. https://doi.org/10.1101/2021.03.31.437960

74. McGonagle, D., O'Donnell, J.S., Sharif, K., Emery, P., and Bridgewood, C., *Lancet Rheumatol.*, 2020, vol. 2, no. 7, pp. e437–e445. https://doi.org/10.1016/S2665-9913(20)30121-1
75. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., Gao, H., Guo, L., Xie, J., Wang, G., Jiang, R., Gao, Z., Jin, Q., Wang, J., and Cao, B., *Lancet*, 2020, vol. 395, no. 10223, pp. 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5
76. Kipshidze, N., Dangas, G., White, C.J., Siddiqui, F., Lattimer, C.R., Carter, C.A., and Fareed, J., *Clin. Appl. Thromb. Hemost.*, 2020, vol. 26, p. 1076029620936776. https://doi.org/10.1177/1076029620936776
77. Zuo, Y., Estes, S.K., Ali, R.A., Gandhi, A.A., Yalavarthi, S., Shi, H., Sule, G., Gockman, K., Madison, J.A., Zuo, M., Yadav, V., Wang, J., Woodard, W., Lezak, S.P., Lugogo, N.L., Smith, S.A., Morrissey, J.H., Kanthi, Y., and Knight, J.S., *Sci. Transl. Med.*, 2020, vol. 12, no. 570, p. eabd3876. https://doi.org/10.1126/scitranslmed.abd3876
78. Butenas, S. and Mann, K.G., *Biochem. (Moscow)*, 2002, vol. 67, no. 1, pp. 3–12. https://doi.org/10.1023/a:1013985911759
79. Barkagan, Z.S. and Momot, A.P., *Diagnosis and controlled therapy of hemostasis disorders*, Moscow: NEWDIAMED, 2008, 3rd ed.
80. Frydman, G.H., Streiff, M.B., Connors, J.M., and Piazza, G., *TH Open*, 2020, vol. 4, no. 4, pp. e288–e299. https://doi.org/10.1055/s-0040-1718415
81. Moschonas, I.C. and Tselepis, A.D., *J. Thromb. Thrombolysis*, 2021, vol. 52, no. 1, pp. 111–123. https://doi.org/10.1007/s11239-020-02374-3
82. Janssen, R., Visser, M.P.J., Dofferhoff, A.S.M., Vermeer, C., Janssens, W., and Walk, J., *Br. J. Nutr.*, 2021, vol. 126, no. 2, pp. 191–198. https://doi.org/10.1017/S0007114520003979
83. Menter, T. and Tzankov, A., *Pathobiology*, 2021, vol. 88, no. 1, pp. 11–14. https://doi.org/10.1159/000513602
84. Ponti, G., Maccaferri, M., Ruini, C., Tomasi, A., and Ozben, T., *Crit. Rev. Clin Lab. Sci.*, 2020, vol. 57, no. 6, pp. 389–399. https://doi.org/10.1080/10408363.2020.1770685
85. Sriram, K. and Insel, P.A., *Physiol. Rev.*, 2021, vol. 101, no. 2, pp. 545–567. https://doi.org/10.1152/physrev.00035.2020
86. Ng, H., Havervall, S., Rosell, A., Aguilera, K., Parv, K., von Meijenfeldt, F.A., Lisman, T., Mackman, N., Thålin, C., and Phillipson, M., *Arterioscler. Thromb. Vasc. Biol.*, 2021, vol. 41, no. 2, pp. 988–994. https://doi.org/10.1161/ATVBAHA.120.315267
87. Thierry, A.R. and Roch, B., *J. Clin. Med.*, 2020, vol. 9, no. 9, p. 2942. https://doi.org/10.3390/jcm9092942
88. Hashimoto, S., Okayama, Y., Shime, N., Kimura, A., Funakoshi, Y., Kawabata, K., Ishizaka, A., and Amaya, F., *Respirology*, 2008, vol. 13, no. 4, pp. 581–584. https://doi.org/10.1111/j.1440-1843.2008.01283.x
89. Zhou, Y., Fu, B., Zheng, X, Wang, D., Zhao, C., Qi, Y., Sun, R., Tian, Z., Xu, X., and Wei, H., *Natl. Sci. Rev.*, 2020, vol. 7, no. 6, pp. 998–1002. https://doi.org/10.1093/nsr/nwaa041
90. Kawai, T. and Akira, S., *Nat. Immunol.*, 2011, vol. 11, no. 5, pp. 373–384. https://doi.org/10.1038/ni.1863
91. Shi, Y., Wang, Y., Shao, C., Shi, Y., Wang, Y., Huang, J., Gan, J., Huang, X., Bucci, E., Piacentini, M., Ippolito, G., and Melino, G., *Cell Death Differ.*, 2020, vol. 27, no. 5, pp. 1451–1454. https://doi.org/10.1038/s41418-020-0530-3
92. Kono, H. and Rock, K.L., *Nat. Rev. Immunol.*, 2008, vol. 8, no. 4, pp. 279–289. https://doi.org/10.1038/nri2215
93. Mauri, T., Caironi, P., Tognoni, G., Masson, S., Fumagalli, R., Pesenti, A., Romero, M., Fanizza, C., Caspani, L., Faenza, S., Grasselli, G., Iapichino, G., Antonelli, M., Parrini, V., Fiore, G., Latini, R., and Gattinoni, L., *Eur. J. Clin. Invest.*, 2017, vol. 47, no. 1, pp. 73–83. https://doi.org/10.1111/eci.12704
94. Brunetta, E., Folci, M., Bottazzi, B., de Santis, M., Gritti, G., Protti, A., Mapelli, S.N., Bonovas, S., Piovani, D., Leone, R., My, I., Zanon, V., Spata, G., Bacci, M., Supino, D., Carnevale, S., Sironi, M., Davoudian, S., Peano, C., Landi, F., di Marco, F., Raimondi, F., Gianatti, A., Angelini, C., Rambaldi, A., Garlanda, C., Ciccarelli, M., Cecconi, M., and Mantovani, A., *Nat. Immunol.*, 2021, vol. 22, no. 1, pp. 19–24. https://doi.org/10.1038/s41590-020-00832-x
95. del Rio, C., Collins, L.F., and Malani, P., *JAMA*, 2020, vol. 324, no. 17, pp. 1723–1724. https://doi.org/10.1001/jama.2020.19719
96. Huang, C., Huang, L., Wang, Y., Li, X., Ren, L., Gu, X., Kang, L., Guo, L., Liu, M., Zhou, X., Luo, J., Huang, Z., Tu, S., Zhao, Y., Chen, L., Xu, D., Li, Y., Li, C., Peng, L., Xie, W., Cui, D., Shang, L., Fan, G., Xu, J., Wang, G., Zhong, J., Wang, C., Wang, J.,

Zhang, D., and Cao, B., *Lancet*, 2021, vol. 397, no. 10270, pp. 220–232. https://doi.org/10.1016/S0140-6736(20)32656-8

97. Reynolds, H.R., Adhikari, S., and Iturrate, E., *N. Engl. J. Med.*, 2020, vol. 383, no. 20, pp. 1993–1994. https://doi.org/10.1056/NEJMc2030446
98. Kamyshnyi, A., Krynytska, I., Matskevych, V., Marushchak, M., and Lushchak, O., *Int. J. Hypertens.*, 2020, p. 8019360. https://doi.org/10.1155/2020/8019360
99. Fang, L., Karakiulakis, G., and Roth, M., *Lancet Respir. Med.*, 2020, vol. 8, no. 4, p. e21. https://doi.org/10.1016/S2213-2600(20)30116-8
100. Jordan, R.E., Adab, P., and Cheng, K.K., *BMJ*, 2020, vol. 368, p. m1198. https://doi.org/10.1136/bmj.m1198
101. Ossovskaya, V.S. and Bunnett, N.W., *Physiol. Rev.*, 2004, vol. 84, no. 2, pp. 579–621. https://doi.org/10.1152/physrev.00028.2003
102. Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., and Cao, B., *Lancet*, 2020, vol. 395, no. 10229, pp. 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3
103. del Turco, S., Vianello, A., Ragusa, R., Caselli, C., and Basta, G., *Thromb. Res.*, 2020, vol. 196, pp. 143–151. https://doi.org/10.1016/j.thromres.2020.08.039
104. Abboud, R.T. and Vimalanathan, S., *Int. J. Tuberc. Lung Dis.*, 2008, vol. 12, no. 4, pp. 361–367.
105. Vignola, A., Scichilone, L., Spatafora, N., Bousquet, M., Bonsignore, J., and Bellia, G.V., *Eur. Respir. J.*, 2003, vol. 22, no. 5, pp. 795–801. https://doi.org/10.1183/09031936.03.00112302
106. Chuchalin, A.G., *Pulmonologia*, 2008, vol. 2, pp. 5–14. https://doi.org/10.18093/0869-0189-2008-0-2-5-14
107. Altshuler, A.E., Penn, A.H, Yang, J.A., Kim, G.R., and Schmid-Schönbein, G.W., *PLoS One*, 2012, vol. 7, no. 3, p. e32672. https://doi.org/10.1371/journal.pone.0032672
108. Moore, A.R., Appelboam, A., Kawabata, K., da Silva, J.A., D'Cruz, D., Gowland, G., and Willoughby, D.A., *Ann. Rheum. Dis.*, 1999, vol. 58, no. 2, pp. 109–113. https://doi.org/10.1136/ard.58.2.109
109. Ivanova, S.V., Kirpichenok, L.N., and Kunder, E.V., *Zhurnal Grognenskogo Gosudarstvennogo Medizinskogo Universiteta*, 2009, vol. 4, no. 28, pp. 73–77.
110. de Paula, J.A., Bustos, D., Negri, G., di Carlo, M., Yapur, V., Facente, A., and de Paula, A., *Medicina (B. Aires)*, 1998, vol. 58, no. 3, pp. 262–264.
111. Fischbeck, A., Leucht, K., Frey-Wagner, I., Bentz, S., Pesch, T., Kellermeier, S., Krebs, M., Fried, M., Rogler, G., and Hausmann, M., *Gut*, 2011, vol. 60, no. 1, pp. 55–65. https://doi.org/10.1136/gut.2009.201988
112. Kotlowski, R., Bernstein, C.N., Silverberg, M.S., and Krause, D.O., *Inflamm. Bowel Dis.*, 2008, vol. 14, no. 8, pp. 1112–1117. https://doi.org/10.1002/ibd.20425
113. Tzourio, C., El Amrani, M., Robert, L., and Alpérovitch, A., *Ann. Neurol.*, 2000, vol. 47, no. 5, pp. 648–651.
114. Liotta, L.A. and Schiffmann, E., *Cancer Surv.*, 1988, vol. 7, no. 4, pp. 631–652.
115. Matrisian, L.M., Sledge, G.W., Jr., and Mohla, S., *Cancer Res.*, 2003, vol. 63, no. 19, pp. 6105–6109.
116. Petrosyan, A.M. and Kharchenko, V.Z., *Onkol.*, 2007, vol. 9, no. 4, pp. 303–306.
117. Ginzberg, H.H., Shannon, P.T., Suzuki, T., Hong, O., Vachon, E., Moraes, T., Abreu, M.T., Cherepanov, V., Wang, X., Chow, C.-W., and Downey, G.P., *Am. J. Physiol. Gastrointest. Liver Physiol.*, 2004, vol. 287, no. 1, pp. G286–G298. https://doi.org/10.1152/ajpgi.00350.2003
118. Odaka, C., Mizuochi, T., Yang, J., and Ding, A., *J. Immunol.*, 2003, vol. 171, no. 3, pp. 1507–1514. https://doi.org/10.4049/jimmunol.171.3.1507
119. Suzuki, T., Moraes, T.J., Vachon, E., Ginzberg, H.H., Huang, T.T., Matthay, M.A., Hollenberg, M.D., Marshall, J., McCulloch, C.A., Abreu, M.T., Chow, C.W., and Downey, G.P., *Am. J. Respir. Cell Mol. Biol.*, 2005, vol. 33, no. 3, pp. 231–247. https://doi.org/10.1165/rcmb.2005-0109OC
120. di Camillo, S.J., Carreras, I., Panchenko, M.V., Stone, P.J., Nugent, M.A., Foster, J.A., and Panchenko, M.P., *J. Biol. Chem.*, 2002, vol. 277, no. 21, pp. 18938–18946. https://doi.org/10.1074/jbc.M200243200
121. Alcorn, J.F. and Wright, J.R., *J. Biol. Chem.*, 2004, vol. 279, no. 29, pp. 30871–30879. https://doi.org/10.1074/jbc.M400796200
122. Chua, F., Dunsmore, S.E., Clingen, P.H., Mutsaers, S.E., Shapiro, S.D., Segal, A.W., Roes, J., and Laurent, G.J., *Am. J. Pathol.*, 2007, vol. 170, no. 1, pp. 65–74. https://doi.org/10.2353/ajpath.2007.060352
123. Lucattelli, M., Bartalesi, B., Cavarra, E., Fineschi, S., Lunghi, B., Martorana, P.A., and Lungarella, G., *Re-*

spir. Res., 2005, vol. 6, no. 1, p. 83. https://doi.org/10.1186/1465-9921-6-83

124. Vasilyeva, O.S., *Medizinskaya Sestra*, 2008, vol. 8, pp. 18–20.

125. Viktorova, T.B., *Medizinskaya Genetika*, 2003, vol. 2, no. 2, pp. 77–80.

126. Bukreeva, E.B., *Bull. Sib. Med.*, 2003, vol. 2, no. 1, pp. 75–77.

127. Darmoul, D., Marie, J.C., Devaud, H., Gratio, V., and Laburthe, M., *Br. J. Cancer*, 2001, vol. 85, no. 5, pp. 772–779. https://doi.org/10.1054/bjoc.2001.1976

128. Elumalai, P., Gunadharini, D.N., Senthilkumar, K., Banudevi, S., Arunkumar, R., Benson, C.S., Sharmila, G., and Arunakaran, J., *Toxicol. Lett.*, 2012, vol. 215, no. 2, pp. 131–142. https://doi.org/10.1016/j.toxlet.2012.10.008

129. Sun, Z. and Yang, P., *Lancet Oncol.*, 2004, vol. 5, no. 3, pp. 182–190. https://doi.org/10.1016/S1470-2045(04)01414-7

130. Kovacova, E., Kinova, S., Duris, I., and Remkova, A., *Bratisl. Lek. Listy*, 2009, vol. 110, no. 4, pp. 215–221.

131. Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., Gong, W., Liu, X., Liang, J., Zhao, Q., Huang, H., Yang, B., and Huang, C., *JAMA Cardiol.*, 2020, vol. 5, no. 7, pp. 802–810. https://doi.org/10.1001/jamacardio.2020.0950

132. Aras Atik, E., Özdemir, N., and Demirkan, K., *Turk. J. Pharm. Sci.*, 2020, vol. 17, no. 6, pp. 576–577. https://doi.org/10.4274/tjps.galenos.2020.20727

133. Gooptu, B. and Lomas, D.A., *J. Exp. Med.*, 2008, vol. 205, no. 7, pp. 1529–1534. https://doi.org/10.1084/jem.20072080

134. Vianello, A. and Braccioni, F., *Arch. Bronconeumol.*, 2020, vol. 56, no. 9, pp. 609–610. https://doi.org/10.1016/j.arbres.2020.05.015

135. Yang, C., Chapman, K.R., Wong, A., and Liu, M., *Lancet Respir. Med.*, 2021, vol. 9, no. 4, pp. 337–339. https://doi.org/10.1016/S2213-2600(21)00018-7

136. Braillon, A. and Nguyen-Khac, E., *Am. J. Med.*, 2008, vol. 121, no. 6, pp. e25–e27. https://doi.org/10.1016/j.amjmed.2008.01.042

137. Richardson, D.E., Regino, C.A., Yao, H., and Johnson, J.V., *Free Radic. Biol. Med.*, 2003, vol. 35, no. 12, pp. 1538–1550. https://doi.org/10.1016/j.freeradbiomed.2003.08.019

138. Köhnlein, T. and Welte, T., *Am. J. Med.*, 2008, vol. 121, no. 1, pp. 3–9. https://doi.org/10.1016/j.amjmed.2007.07.025

139. Barlow, I. and Sewell, W.A., *J. Allergy Clin. Immunol.*, 2008, vol. 122, no. 3, p. 658. https://doi.org/10.1016/j.jaci.2008.06.019

140. Crowther, D.C., Belorgey, D., Miranda, E., Kinghorn, K.J., Sharp, L.K., and Lomas, D.A., *Eur. J. Hum. Genet.*, 2004, vol. 12, no. 3, pp. 167–172. https://doi.org/10.1038/sj.ejhg.5201127

141. Parfrey, H., Mahadeva, R., and Lomas, D.A., *Int. J. Biochem. Cell Biol.*, 2003, vol. 35, no. 7, pp. 1009–1014. https://doi.org/10.1016/s1357-2725(02)00250-9

142. de Serres, F.J., Blanco, I., and Fernández-Bustillo, E., *Monaldi Arch. Chest Dis.*, 2007, vol. 67, no. 4, pp. 184–208. https://doi.org/10.4081/monaldi.2007.476

143. Veremeenko, K.N., *Klin. med.*, 1985, vol. 12, pp. 24–29.

144. de Serres, F. and Blanco, I., *J. Intern. Med.*, 2014, vol. 276, no. 4, pp. 311–335. https://doi.org/10.1111/joim.12239

145. Strange, C., *Respir. Care*, 2018, vol. 63, no. 6, pp. 690–698. https://doi.org/10.4187/respcare.05933

146. Aldonyte, R., Hutchinson, T.E., Jin, B., Brantly, M., Block, E., Patel, J., and Zhang, J., *COPD*, 2008, vol. 5, no. 3, pp. 153–162. https://doi.org/10.1080/15412550802092936

147. Schuster, R., Motola-Kalay, N., Baranovski, B.M., Bar, L., Tov, N., Stein, M., Lewis, E.C., Ayalon, M., and Sagiv, Y., *Cell Immunol.*, 2020, vol. 356, p. 104177. https://doi.org/10.1016/j.cellimm.2020.104177

148. Yang, C., Keshavjee, S., and Liu, M., *Front. Pharmacol.*, 2020, vol. 11, p. 615398. https://doi.org/10.3389/fphar.2020.615398

149. Churg, A., Wang, X., Wang, R.D., Meixner, S.C., Pryzdial, E.L., and Wright, J.L., *Am. J. Respir. Cell Mol. Biol.*, 2007, vol. 37, no. 2, pp. 144–151. https://doi.org/10.1165/rcmb.2006-0345OC

150. Janciauskiene, S.M., Nita, I.M., and Stevens, T., *J. Biol. Chem.*, 2007, vol. 282, no. 12, pp. 8573–8582. https://doi.org/10.1074/jbc.M607976200

151. Ozeri, E., Mizrahi, M., Shahaf, G., and Lewis, E.C., *J. Immunol.*, 2012, vol. 189, no. 1, pp. 146–153. https://doi.org/10.4049/jimmunol.1101340

152. Wettstein, L., Weil, T., Conzelmann, C., Müller, J.A., Groß, R., Hirschenberger, M., Seidel, A., Klute, S., Zech, F., Prelli Bozzo, C., Preising, N., Fois, G., Lochbaum, R., Knaff, P.M., Mailänder, V., Ständker, L., Thal, D.R., Schumann, C., Stenger, S., Kleger, A., Lochnit, G., Mayer, B., Ruiz-Blanco, Y.B., Hoffmann, M., Sparrer, K.M.J., Pöhlmann, S., Sanchez-Garcia, E., Kirchhoff, F.,

Frick, M., and Münch, J., *Nat. Commun.*, 2021, vol. 12, no. 1, p. 1726. https://doi.org/10.1038/s41467-021-21972-0

153. Bergin, D.A., Reeves, E.P., Meleady, P., Henry, M., McElvaney, O.J., Carroll, T.P., Condron, C., Chotirmall, S.H., Clynes, M., O'Neill, S.J., and McElvaney, N.G., *J. Clin. Invest.*, 2010, vol. 120, no. 12, pp. 4236–4250. https://doi.org/10.1172/JCI41196

154. Jedicke, N., Struever, N., Aggrawal, N., Welte, T., Manns, M.P., Malek, N.P., Zender, L., Janciauskiene, S., and Wuestefeld, T., *Hepatology*, 2014, vol. 59, no. 6, pp. 2299–2308. https://doi.org/10.1002/hep.27024

155. Jose, R.J. and Manuel, A., *Lancet Respir Med.*, 2020, vol. 8, no. 6, pp. e46–e47. https://doi.org/10.1016/S2213-2600(20)30216-2

156. Hashemi, M., Naderi, M., Rashidi, H., and Ghavami, S., *Diabetes Res. Clin. Pract.*, 2007, vol. 75, no. 2, pp. 246–248. https://doi.org/10.1016/j.diabres.2006.06.020

157. Salem, E.S., Grobe, N., and Elased, K.M., *Am. J. Physiol. Renal. Physiol.*, 2014, vol. 306, no. 6, pp. F629–F639. https://doi.org/10.1152/ajprenal.00516.2013

158. Lindley, V.M., Bhusal, K., Huning, L., Levine, S.N., and Jain, S.K., *J. Am. Coll. Nutr.*, 2020, vol. 40, no. 2, pp. 98–103. https://doi.org/10.1080/07315724.2020.1740629

159. Meltzer, D.O., Best, T.J., Zhang, H., Vokes, T., Arora, V., and Solway, J., *JAMA Netw. Open*, 2020, vol. 3, no. 9, p. e2019722. https://doi.org/10.1001/jamanetworkopen.2020.19722

160. de Loyola, M.B., Dos Reis, T.T.A., de Oliveira, G.X.L.M., da Fonseca Palmeira, J., Argañaraz, G.A., and Argañaraz, E.R., *Rev. Med. Virol.*, 2021, vol. 31, no. 2, p. e2157. https://doi.org/10.1002/rmv.2157

161. Ferrarotti, I., Ottaviani, S., Balderacchi, A.M., Barzon, V., de Silvestri, A., Piloni, D., Mariani, F., and Corsico, A.G., *Respir. Med.*, 2021, vol. 183, p. 106440. https://doi.org/10.1016/j.rmed.2021.106440

162. Lechowicz, U., Rudzinski, S., Jezela-Stanek, A., Janciauskiene, S., and Chorostowska-Wynimko, J., *Int. J. Mol. Sci.*, 2020, vol. 21, no. 23, p. 9187. https://doi.org/10.3390/ijms21239187

163. Bergin, D.A., Reeves, E.P., Hurley, K., Wolfe, R., Jameel, R., Fitzgerald, S., and McElvaney, N.G., *Sci. Transl. Med.*, 2014, vol. 6, no. 217, p. 217ra1. https://doi.org/10.1126/scitranslmed.3007116

164. Faust, D., Raschke, K., Hormann, S., Milovic, V., and Stein, J., *Clin. Exp. Immunol.*, 2002, vol. 128, no. 2, pp. 279–284. https://doi.org/0.1046/j.1365-2249.2002.01843.x

165. Beltrán-García, J., Osca-Verdegal, R., Pallardó, F.V., Ferreres, J., Rodríguez, M., Mulet, S., Sanchis-Gomar, F., Carbonell, N., and García-Giménez, J.L., *Antioxidants (Basel)*, 2020, vol. 9, no. 10, p. 936. https://doi.org/10.3390/antiox9100936

166. Chernyak, B.V., Popova, E.N., Prikhodko, A.S., Grebenchikov, O.A., Zinovkina, L.A., and Zinovkin, R.A., *Biochemistry (Moscow)*, 2020, vol. 85, no. 12, pp. 1543–1553. https://doi.org/10.1134/S0006297920120068

167. Delgado-Roche, L. and Mesta, F., *Arch Med. Res.*, 2020, vol. 51, no. 5, pp. 384–387. https://doi.org/10.1016/j.arcmed.2020.04.019

168. Ueda, M., Mashiba, S., and Uchida, K., *Clin. Chim. Acta*, 2002, vol. 317, nos. 1–2, pp. 125–131. https://doi.org/10.1016/s0009-8981(01)00765-3

169. Moraga, F. and Janciauskiene, S., *J. Biol. Chem.*, 2000, vol. 275, no. 11, pp. 7693–7700. https://doi.org/10.1074/jbc.275.11.7693

170. Yang, P., Sun, Z., and Krowka, M.J., *Arch Intern. Med.*, 2008, vol. 168, no. 10, pp. 1097–1103. https://doi.org/10.1001/archinte.168.10.1097

171. Zelvyte, I., Stevens, T., Westin, U., and Janciauskiene, S., *Cancer Cell Int.*, 2004, vol. 4, no. 1, p. 7. https://doi.org/10.1186/1475-2867-4-7

172. Nie, J. and Pei, D., *Exp. Cell. Res.*, 2004, vol. 296, no. 2, pp. 145–150. https://doi.org/10.1016/j.yexcr.2004.02.008

173. Petropoulou, P., Zhang, Z., Curtis, M.A., Johnson, N.W., Hughes, F.J., and Winyard, P.G., *J. Clin. Periodontol.*, 2003, vol. 30, no. 9, pp. 795–801. https://doi.org/10.1034/j.1600-051x.2003.00369.x

174. Summers, F.A., Morgan, P.E., Davies, M.J., and Hawkins, C.L., *Chem. Res. Toxicol.*, 2008, vol. 21, no. 9, pp. 1832–1840. https://doi.org/10.1021/tx8001719

175. Gombás, J., Kolev, K., Tarján, E., and Machovich, R., *Ann. Hematol.*, 2004, vol. 83, no. 12, pp. 759–763. https://doi.org/10.1007/s00277-004-0928-x

176. Fregonese, L. and Stolk, J., *Orphanet J. Rare Dis.*, 2008, vol. 3, p. 16. https://doi.org/10.1186/1750-1172-3-16

177. Sánchez-Domínguez, C.N., Buenfil-Lozano, J.A., Molina-Guajardo, C.A., Borjas-Almaguer, O.D., Castillo-Lartigue, A., Bustamante-Sáenz, A., Martínez-Rodríguez, H.G., Villarreal Alarcón, M.A., Reyes-López, M.A., and Ortiz-López, R., *Allergy*

Asthma Proc., 2008, vol. 29, pp. 406–410. https://doi.org/10.2500/aap.2008.29.3125

178. Fregonese, L., Stolk, J., Frants, R.R., and Veldhuisen, B., *Respir. Med.*, 2008, vol. 102, no. 6, pp. 876–884. https://doi.org/10.1016/j.rmed.2008.01.009
179. Camelier, A.A., Winter, D.H., Jardim, J.R., Barboza, C.E., Cukier, A., and Miravitlles, M., *J. Bras. Pneumol.*, 2008, vol. 34, no. 7, pp. 514–527. https://doi.org/10.1590/s1806-37132008000700012
180. Averianov, A.V. and Polivanova, A.E., *Pulmonologia*, 2007, vol. 3, pp. 103–109.
181. Khan, H., Salman, K.A., and Ahmed, S., *J. Assoc. Physicians India*, 2002, vol. 50, pp. 579–582.
182. Silverman, E.K., Pierce, J.A., Province, M.A., Rao, D.C., and Campbell, E.J., *Ann. Intern. Med.*, 1989, vol. 111, no. 12, pp. 982–991. https://doi.org/10.7326/0003-4819-111-12-982
183. Conticini, E., Frediani, B., and Caro, D., *Environ Pollut.*, 2020, vol. 261, p. 114465. https://doi.org/10.1016/j.envpol.2020.114465
184. Martelletti, L. and Martelletti, P., *SN Compr. Clin. Med.*, 2020, vol. 15, pp. 1–5. https://doi.org/10.1007/s42399-020-00274-4
185. Massi, G., Cotumaccio, R., and Auconi, P., *Hum. Genet.*, 1982, vol. 61, no. 1, pp. 76–77. https://doi.org/10.1007/BF00291340
186. Alberca, R.W., Lima, J.C., de Oliveira, E.A., Gozzi-Silva, S.C., Ramos, Y.Á.L., de Souza Andrade, M.M., Beserra, D.R., de Mendonça Oliveira, L., Calvielli Castelo Branco, A.C., Pietrobon, A.J., Pereira, N.Z., Teixeira, F.M.E., Fernandes, I.G., Duarte, A.J.S., Benard, G., and Sato, M.N., *Front. Physiol.*, 2021, vol. 11, p. 637627. https://doi.org/10.3389/fphys.2020.637627
187. Tannous, T., Rosso, C., and Keating, M., *Cureus*, 2021, vol. 13, no. 4, p. e14759. https://doi.org/10.7759/cureus.14759
188. Bukreeva, E.B., Akbasheva, O.E., Sukhanova, G.A., Dementieva, E.A., Nestervovich, S.V., Melnik, T.G., Gudkova, L.V., and Ivanchuk, I.I., *Bull. Exp. Biol. Med.*, 2002, vol. 1, pp. 55–58.
189. Gereng, E.A., Sukhodolo, I.V., Pleshko, R.I., Ogorodova, L.M., Akbasheva, O.E., Bukreeva, E.B., Dzyuman, A.N., Kobyakova, O.S., Selivanova, P.A., and Kremis, I.S., *Bull. Sib. Med.*, 2009, vol. 8, no. 3, pp. 11–16.
190. Attaway, A.A., Zein, J., and Hatipoğlu, U.S., *EClinicalMedicine*, 2020, vol. 26, p. 100515. https://doi.org/10.1016/j.eclinm.2020.100515
191. Sen, P., Majumdar, U., Zein, J., Hatipoğlu, U., and Attaway, A.H., *PLoS One*, 2021, vol. 16, no. 6, p. e0252576. https://doi.org/10.1371/journal.pone.0252576
192. Watson, A., Öberg, L., Angermann, B., Spalluto, C.M., Hühn, M., Burke, H., Cellura, D., Freeman, A., Muthas, D., Etal, D., Belfield, G., Karlsson, F., Nordström, K., Ostridge, K., Staples, K.J., and Wilkinson, T., *Respir. Res.*, 2021, vol. 22, no. 1, p. 164. https://doi.org/10.1186/s12931-021-01755-3
193. Ren, Y., He, Q.Y., Fan, J., Jones, B., Zhou, Y., Xie, Y., Cheung, C.Y., Wu, A., Chiu, J.F., Peiris, J.S., and Tam, P.K., *Proteomics*, 2004, vol. 4, no. 11, pp. 3477–3484. https://doi.org/10.1002/pmic.200400897
194. Wang, C., Zhao, P., Sun, S., Teckman, J., and Balch, W.E., *Chronic. Obstr. Pulm. Dis.*, 2020, vol. 7, no. 3, pp. 224–246. https://doi.org/10.15326/jcopdf.7.3.2019.0167
195. Nita, I., Hollander, C., Westin, U., and Janciauskiene, S.M., *Respir. Res.*, 2005, vol. 6, no. 1, p. 12. https://doi.org/10.1186/1465-9921-6-12
196. De, B., Heguy, A., Leopold, P.L., Wasif, N., Korst, R.J., Hackett, N.R., and Crystal, R.G., *Mol. Ther.*, 2004, vol. 10, no. 6, pp. 1003–1010. https://doi.org/10.1016/j.ymthe.2004.08.022
197. Brand, P., Beckmann, H., Maas Enriquez, M., Meyer, T., Müllinger, B., Sommerer, K., Weber, N., Weuthen, T., and Scheuch, G., *Eur. Respir. J.*, 2003, vol. 22, no. 2, pp. 263–267. https://doi.org/10.1183/09031936.03.00096802
198. Molano, R.D., Pileggi, A., Song, S., Zahr, E., San Jose, S., Molina, J., Fort, A., Wasserfall, C., Ricordi, C., Atkinson, M.A., and Inverardi, L., *Transplant. Proc.*, 2008, vol. 40, no. 2, pp. 455–456. https://doi.org/10.1016/j.transproceed.2008.02.009
199. Tonelli, A.R. and Brantly, M.L., *Ther. Adv. Respir. Dis.*, 2010, vol. 4, no. 5, pp. 289–312. https://doi.org/10.1177/1753465810373911
200. Santana, M.V.S. and Silva-Jr, F.P., *BMC Chem.*, 2021, vol. 15, no. 1, p. 8. https://doi.org/10.1186/s13065-021-00737-2
201. McEvoy, N.L., Clarke, J.L., McElvaney, O.J., McElvaney, O.F., Boland, F., Hyland, D., Geoghegan, P., Donnelly, K., Frie, O., Cullen, A., Collins, A.M., Fraughen, D., Martin-Loeches, I., Hennessy, M., Laffey, J.G., McElvaney, N.G., and Curley, G.F., *Trials*, 2021, vol. 22, no. 1, p. 288. https://doi.org/10.1186/s13063-021-05254-0
202. Kiseleva, A.V., Klimushina, M.V., Sotnikova, E.A., Divashuk, M.G., Ershova, A.I., Skirko, O.P., Kurilova, O.V., Zharikova, A.A., Khlebus, E.Yu., Efimova, I.A., Pokrovskaya, M.S., Slominsky, P.A.,

Shalnova, S.A., Meshkov, A.N., and Drapkina, O.M., *J. Pers. Med.*, 2020, vol. 10, no. 3, p. 140. https://doi.org/10.3390/jpm10030140

203. Wichmann, D., Sperhake, J.P., Lütgehetmann, M., Steurer, S., Edler, C., Heinemann, A., Heinrich, F., Mushumba, H., Kniep, I., Schröder, A.S., Burdelski, C., de Heer, G., Nierhaus, A., Frings, D., Pfefferle, S., Becker, H., Bredereke-Wiedling, H., de Weerth, A., Paschen, H.R., Sheikhzadeh-Eggers, S., Stang, A., Schmiedel, S., Bokemeyer, C., Addo, M.M., Aepfelbacher, M., Püschel, K., and Kluge, S., *Ann. Intern. Med.*, 2020, vol. 173, no. 4, pp. 268–277. https://doi.org/10.7326/M20-2003

204. Guéant, J.L., Guéant-Rodriguez, R.M., Fromonot, J., Oussalah, A., Louis, H., Chery, C., Gette, M., Gleye, S., Callet, J., Raso, J., Blanchecotte, F., Lacolley, P., Guieu, R., and Regnault, V., *Allergy*, 2021, vol. 76, no. 6, pp. 1846–1858. https://doi.org/10.1111/all.14746

205. Sahebnasagh, A., Saghafi, F., Safdari, M., Khataminia, M., Sadremomtaz, A., Talaei, Z., Rezai Ghaleno, H., Bagheri, M., Habtemariam, S., and Avan, R., *J. Clin. Pharm. Ther.*, 2020, vol. 45, no. 6, pp. 1515–1519. https://doi.org/10.1111/jcpt.13251

206. Thierry, A.R., *Physiol. Rev.*, 2020, vol. 100, no. 4, p. 1597–1598. https://doi.org/10.1152/physrev.00019.2020

207. Serban, K.A. and Petrache, I., *Ann Am. Thorac. Soc.*, 2016, vol. 13, suppl. 2, pp. S146–S149. https://doi.org/10.1513/AnnalsATS.201505-312KV

Translated by E. Makeeva