Role of endothelial dysfunction in the severity of COVID-19 infection (Review)

TANYA KADIYSKA[1,2], IVAN TOURTOURIKOV[2,3], KRISTIYAN DABCHEV[2], RADOSTINA CHERNEVA[4], NIKOLAY STOYNEV[1], RADKA HADJIOLOVA[1], VANYO MITEV[2], DEMETRIOS A. SPANDIDOS[5], MARIA ADAMAKI[6] and VASSILIS ZOUMPOURLIS[6]

[1]Department of Physiology and Pathophysiology, Medical University, 1413 Sofia; [2]Genetic Medico-Diagnostic Laboratory Genica, 1612 Sofia; [3]Department of Medical Chemistry and Biochemistry, Medical University, 1413 Sofia; [4]University Hospital for Respiratory Diseases St. Sophia, 1413 Sofia, Bulgaria; [5]Laboratory of Clinical Virology, Medical School, University of Crete, Heraklion 71003; [6]Biomedical Applications Unit, Institute Of Chemical Biology, National Hellenic Research Foundation, 11635 Athens, Greece

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Abstract. COVID-19 patients with severe infection have been observed to have elevated auto-antibodies (AAs) against angiotensin II receptor type 1 (AT1R) and endothelin (ET) 1 receptor type A (ETAR), compared with healthy controls and patients with favorable (mild) infection. AT1R and ETAR are G protein-coupled receptors, located on vascular smooth muscle cells, fibroblasts, immune and endothelial cells, and are activated by angiotensin II (Ang II) and ET1 respectively. AAs that are specific for these receptors have a functional role similar to the natural ligands, but with a more prolonged vasoconstrictive effect. They also induce the production of fibroblast collagen, the release of reactive oxygen species and the secretion of proinflammatory cytokines (including IL-6, IL-8 and TNF-α) by immune cells. Despite the presence of AAs in severe COVID-19 infected patients, their contribution and implication in the severity of the disease is still not well understood and further studies are warranted. The present review described the major vascular homeostasis systems [ET and renin-angiotensin-aldosterone system (RAAS)], the vital regulative role of nitric oxide, the AAs, and finally the administration of angiotensin II receptor blockers (ARBs), so as to provide more insight into the interplay that exists among these components and their contribution to the severity, prognosis and possible treatment of COVID-19.

Correspondence to: Dr Tanya Kadiyska, Department of Physiology and Pathophysiology, Medical University, 84 Ami Boue Street, 1413 Sofia, Bulgaria
E-mail: kadiyska_t@abv.bg

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Contents

1. Introduction

The SARS-CoV-2 virus, responsible for COVID-19 disease, is characterized by a broad spectrum of clinical symptoms; these can be separated into mild symptoms, such as fever, dyspnea, coughing, loss of taste and smell, and into more severe symptoms, especially in elderly people with concomitant pathological conditions, including respiratory and severe alveolar insufficiency (1,2). In extremely severe forms of COVID-19, rapidly progressive multi-organ insufficiency may take place, a condition that usually manifests as a series of complications including shock, acute heart damage, disseminated intravascular coagulopathy, Acute Respiratory Distress Syndrome (ARDS) and acute renal impairment (1). Recent studies have shown that respiratory failure from COVID-19 disease is not only due to ARDS, but may also be due to macro- and microvascular involvement (3), in which case vascular endothelial damage plays a central role (4). Recent observations suggest that COVID-19 is an endothelial disease, responsible for the observed manifestations of inflammation, cytokine storm, oxidative stress and coagulopathy (5). This hypothesis is also supported by the fact that patients with endothelial dysfunction due to various concomitant pathologies (obesity, hypertension and type 2 diabetes mellitus), develop more severe forms of COVID-19, as evidenced by additional pathological changes of the already dysfunctional vascular endothelium (4). The endothelial tissue produces a wide variety of molecules that are

associated with homeostasis in the human body. Some of the regulatory mechanisms responsible for vascular homeostasis are antagonistic and are shown in Fig. 1.

Viral entry of SARS-COV2 and other coronaviruses into the host (human) cells is facilitated through the angiotensin converting enzyme 2 (ACE2) acting as a receptor that binds to the spike protein on the viral envelope. ACE2 is a vital component of the renin angiotensin aldosterone system (RAAS) that is responsible for normal cardiovascular function (6). During SARS-CoV-2 infection, in addition to RAAS, the endothelin (ET) 1 pathway, which is known to have vasoconstrictive power, is also affected. The present review provided a thorough discussion of how these systems are implicated in COVID-19 disease and described a novel approach for predicting/assessing SARS-CoV-2 infection severity; the latter includes a detailed description of the functional autoantibodies against the systems regulating normal vascular activity and which exhibit similar functions to the natural ligands. Lastly, the action of nitric oxide (NO) and ARBs are discussed and their implication in COVID-19 infections, as well as their use as a supplement treatment and possible cure.

2. How does vasoconstriction occur?

Contraction of vascular smooth muscles is triggered through stimulation of G-protein coupled receptors via ET1 and Ang II, which causes a Ca^{2+} influx through the receptor-operated Ca^{2+} channels of the plasma membrane and initiates phospholipase C to produce inositol-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 then initiates calcium release from the sarcoplasmic reticulum stores via IP_3 and ryanodine receptors. The increase of intracellular calcium then activates the myosin light chain kinase, leading to myosin phosphorylation, thereby resulting in smooth muscle contraction (7). Furthermore, DAG initiates PKC activation, which induces the phosphorylation and subsequent activation of the protein phosphatase type 1 inhibitor protein CPI-17, causing inhibition of myosin light chain phosphatase (8). This inhibition causes a prolonged myosin phosphorylation for a given Ca^{2+} concentration (7).

3. Endothelin and endothelin receptors

The endothelin family consists of the ET1, ET2 and ET3 vasoconstrictor peptides which, depending on their expression location, are coded by three different genes, with ET1 being the main isoform (9). The promoter of the *EDN1* gene, which codes for the ET1 protein, possesses several regulatory elements that facilitate transcription via different environmental and hormonal stimuli such as ILs, TNFα and Ang II. In humans, the gene product consists of a 212 amino acid (aa) long protein termed preproendothelin-1, which undergoes proteolytic cleavage and gives a 38-aa-long 'big endothelin-1' (big ET-1) (10). This 38-aa peptide can be converted into the active 21-aa form of ET-1 through the actions of endothelin converting enzymes 1, 2 and 3 respectively, even though several other enzymes have also been found to convert ET-1 into its active form (11,12). In addition, even though big ET-1 is relatively inactive, it still possesses low receptor affinity and can protect the final, active ET-1, from proteolysis (13).

Of the two endothelin receptors, ETAR (endothelin type A receptor) is located on vascular smooth muscle cells and ETBR (endothelin type B receptor) is located on both vascular smooth muscle cells and endothelial cells (14). The ETAR has the highest affinity for the ET1 protein, whereas the ETBR has the same affinity for all three ETs, with the two receptors exhibiting the same affinity for ET1 (15). Depending on tissue type, the receptors may have synergistic or opposing functions. For example, each receptor behaves differently upon ET1 binding. When ET1 binds to ETAR, the effect is long lasting due to slow dissociation of ET1 from the receptor (16). On the other hand, when ET1 binds to ETBR, the receptor is targeted to the lysosomes for destruction, suggesting that these receptors play a main role in the clearing of ET1 from the circulation (17). Macrophages also possess ETARs, which release cytokines upon induction (18). By contrast, the activation of ETBR by ET1 induces NO synthesis through endothelial nitric oxide synthase (eNOS), which in turn causes the stimulation of soluble guanylate cyclase acid [cyclic guanosine monophosphate (cGMP)] synthesis, leading to reduced intracellular Ca^{2+} concentration and ultimately to vasodilation (19). Furthermore, impairment of the ETBR has been associated with impaired clearance of ET-1, leading to increased blood pressure (20). This further stratifies the roles of the two receptors and their possible implication in COVID-19 infected patients. Improved understanding of the functions and actions of each receptor and how these may vary depending on the location and tissue type, may help in the introduction of novel treatment approaches with the potential to relieve the severity of COVID-19 (21).

4. RAAS

The RAAS constitutes a natural defense mechanism for maintaining normal blood circulation. Renin release from the juxtaglomerular apparatus is stimulated by renal hypoperfusion. The role of renin is to convert angiotensinogen (Ang) to Ang I, which is further hydrolyzed by angiotensin-converting enzyme (ACE) to Ang II (22). Furthermore, Ang II stimulates the production of aldosterone via the Ang II type 1 receptor (AT1R), resulting in retention of sodium, water reabsorption and ultimately in vasoconstriction (23). ACE2 maintains a balance by converting Ang II to Ang 1-7, which induces antioxidant, anti-inflammatory and vasodilatory effects through binding to the mitochondrial assembly protein 1 (MAS1) receptor (24). In cases where there is insufficient expression of ACE2, Ang II predominantly binds to AT1R and exhibits vasoconstrictive and pro-inflammatory effects (25).

The implication of RAAS in COVID-19 disease has been observed from the very beginning of the pandemic, due to the fact that SARS-CoV-2 enters human cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed mainly in the respiratory epithelium and vascular endothelium (24). In addition, viral cell entry has been suspected to utilize several proteases, such as transmembrane protease serine subtype 2, turin, cathepsin B or L, and basigin (CD147) (26).

ACE2 plays an essential role in the renin-angiotensin system (RAS), being responsible for cardiovascular homeostasis and for the generation of Ang 1-9 and Ang 1-7 through carboxypeptidase activity (27). In addition to being a direct

route for viral cell entry, ACE2 function might also be implicated in the manifestation of disease severity variations (28). A previous study has shown that RAAS-modulating drugs can also modulate ACE2 expression and activity in different ways,specifically, certain ACE inhibitors (ACEIs) have been shown to downregulate ACE2 expression, while angiotensin II receptor blockers (ARBs) and mineralocorticoid receptor antagonists (MRAs) seem to increase the activity of ACE2 (29).

In the alveolar epithelial cells, binding of the viral spike protein to ACE2 reduces the receptor's expression, blocking Ang II conversion to Ang 1-7 and causing Ang II to bind to AT1R, which in turn induces hypokalemia, hyperaldosteronism, proliferation of inflammatory cells, as well as vasoconstriction and fibrosis in severe cases of the disease (30). Notably, loss of ACE2 expression has been shown to result in impaired lung function in mice, via increased vascular permeability, pulmonary edema and neutrophil accumulation (31).

Another possible consequence of COVID-19 infection is thought to be viral neurotropism. Neurons in the circumventricular organs that are implicated in respiratory and cardiovascular regulation express ACE2 receptors and have almost no protection through the blood brain barrier (32). This provides an opportunity to the virus to infect a region representing the central nervous system and to further disrupt the regulation of respiratory and cardiovascular events (33). Excessive Ang II has also been associated with poor outcomes in the COVID-19 setting. Significantly higher Ang II plasma concentrations have been observed in COVID-19 patients, as compared with healthy subjects, and Ang II levels in patients have been associated with viral load and lung damage (34).

In addition to severe inflammation and hypoxemia in COVID-19 patients through vasoconstriction in small pulmonary vessels, Ang II induces the expression of the plasminogen activator-1 (PAI-1) inhibitor in endothelial cells through binding to the AT1R (35). Fibrin deposits in the alveoli of the patients lead to PAI-1 expression (36). In addition, excessive Ang II can be metabolized to angiotensin IV, which enhances the development of thrombosis, as hypercoagulation is observed in highly severe cases, suggesting that a decrease in ACE2 expression possibly contributes to an increase in thrombotic risk (36).

As ACE2 is known to facilitate viral entry into host cells, the question that has been raised is whether RAAS modulators (ACEIs and ARBs) can increase the risk of developing severe viral infection. This hypothesis is based on the results of studies on animal models that demonstrate increased levels of ACE2 expression following intravenous infusion of ACEI and ARB (37). To determine whether RAAS modulators increase the risk for severe disease, the activity of both ARBs and ACEIs was compared in patients with COVID-19 and concomitant hypertension; it was deduced that those receiving ARBs were less likely to develop severe COVID-19, while those being administered ACEIs did not exhibit similar outcomes to those receiving ARBs, as ACEIs inhibit the AGT hydrolysis step in the RAAS pathway, preventing the formation of Ang 1-7 that induces vasodilatory effects (38).

Based on the significant association of ACE2 expression with COVID-19 infection, several potential therapeutic approaches have been proposed. The majority of these strategies are based on results from animal models or *in vitro* studies, which however require additional and more in-depth research before therapies can be tested in humans and become available to the general public (39,40).

5. NO

Hypertension is believed to arise from remodeling of the vessel walls and from abnormal vascular volume, salt regulation and vascular tone (41). These abnormalities are in turn thought to be the result of an imbalance between NO and Ang II, as disruption of either pathway can lead to heart failure, renal failure, vascular and cerebrovascular disease (42). NO synthesis takes place in the endothelium through the actions of endothelial nitric oxide synthase (eNOS), which converts L-arginine to NO and citrulline (43). NO then initiates a cascade through the actions of cGMP, which activates protein kinase G (PKG) I and II, with PKG I being the kinase responsible for vasodilation and inhibition of platelet aggregation (44). The role of NO in the RAAS system mainly lies in antagonizing the effects of angiotensin II via downregulation of ACE and AT1R, thereby positively balancing cardiovascular activity overall (41). On the other hand, if there is inhibition of NO or if Ang II predominates over it, there is a disruption of homeostasis and increased production of aldosterone and superoxide anions (O_2^-), resulting in vasoconstriction and persistent hypertension (45,46). Furthermore, superoxide anions (O_2^-) cause oxidative stress and subsequently a reduction in NO with concomitant increase of ACE2, hence increasing the severity of COVID-19 infection (47).

NO and Ang II both interact with other vasoactive regulators. Ang II can induce the release of ET-1 from endothelial cells, which leads to more severe Ang II-induced vasoconstriction and hypertension (48,49). On the other hand, as aforementioned, the activation of ETb causes eNOS to synthesize NO, and this leads to a reduction in the ETAR calcium-induced vasoconstriction due to an ET-1 inhibiting function that is facilitated via a cGMP-dependent mechanism (50).

Upon SARS-CoV-2 infection, the virus enters the cell through the ACE2 receptor, which decreases its availability, leading to reduced conversion of Ang II to Ang 1-7 (51). Hence, this results in increased Ang II concentration and AT1R activity, inducing higher generation of reactive oxygen species (ROS), limiting the bioavailability of NO (52). This is proportional and correlates with the severity of the disease. Furthermore, reduced NO bioavailability can in part lead to endothelial dysfunction, by predisposing the vasculature towards prothrombotic and pro-inflammatory state expressing adhesion molecules and pro-inflammatory cytokines (53). These data further signify the vital role which NO plays in COVID-19 severity, along with being of immense importance in cardiovascular regulation (51).

6. AT1R and ETAR auto-antibodies

Autoantibodies (AAs) against AT1R and ETAR are functional agonists able to activate both receptors (54). They have similar binding abilities and functions to the natural ligands, including remodeling of the extracellular matrix, vasoconstriction and inflammation (55). AAs differ in their ability to dissociate; binding to the AT1R results in a 10-fold longer

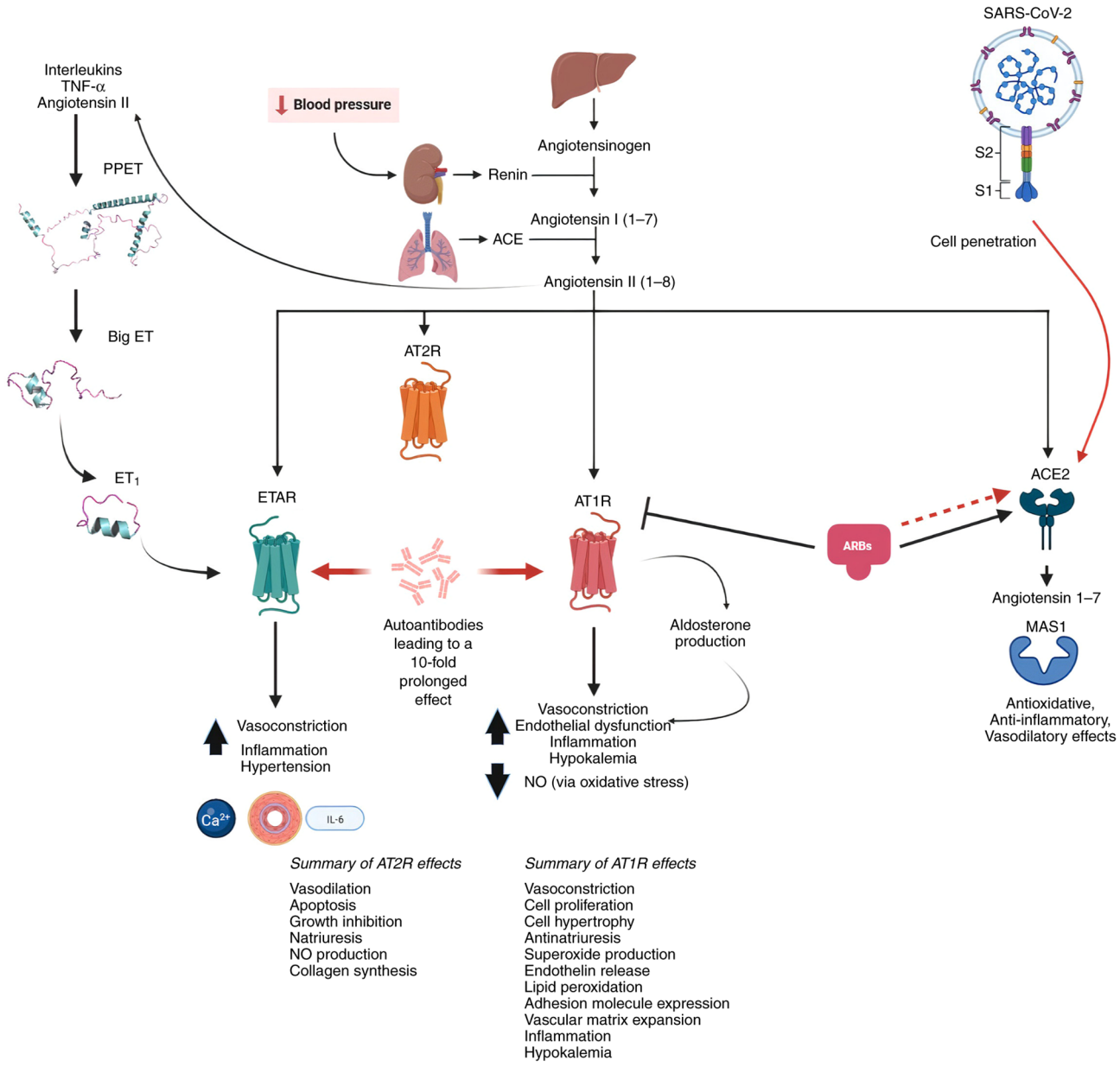

Figure 1. The renin-angiotensin system, endothelins and COVID-19. Red arrows outline known pathological mechanisms. Red dashed arrows outline hypothetical pathological mechanisms. PPET, preproendothelin; ET, endothelin; BigET, big endothelin; ACE, angiotensin converting enzyme; MAS1, mitochondrial assembly protein 1; AT1R, angiotensin II receptor type 1; AT2R, angiotensin II receptor type 2; ETAR, endothelin-1 receptor type A.

vasoconstriction as compared with Ang II (55,56), as shown in Fig. 1. Furthermore, the autoantibodies only bind to AT1R, which is responsible for inducing vasoconstriction inflammation and is actively involved in the production of ROS, reducing NO (57). Even though Ang II has the same binding affinity for both AT1R and AT2R, the latter has an opposing effect to the former, resulting in reduced vasoconstriction and inflammation and therefore to a positive regulation of AT1R activity (55). Similar to the AT1R AAs, the AAs against ETAR have the same effect, with the only difference in that they induce vasoconstriction and inflammation without utilizing ETBR (55). The presence of AAs has been observed in systemic sclerosis, where it has been associated with increased mortality and more severe disease, graft injury and loss in transplantation (58,59), as well as in more severe COVID-19 infections and end-stage cystic fibrosis (60,61). In general, the presence of these AAs is regarded as an independent risk factor, significantly related to adverse outcomes and death (62).

The mechanism behind AA generation is still not fully understood. A previous study speculated that they arise from damaged endothelium that is characterized by an accumulation of extracellular AT1Rs and ETARs (63). Additional investigations including more control groups will possibly shed more light into this field.

7. Conclusions

Taking all of the above systems and factors into consideration, it can be assumed that the abnormal interaction of RAAS, ET receptors, NO and AAs is a necessary prerequisite for severe manifestation of COVID-19 disease and increased mortality. Even in healthy individuals, disturbance of the aforementioned

systems and their respective interactions can lead to cardiovascular disease and chronic inflammation, and also contribute to the appearance of related pathologies.

So far, the presence of AT1R and ETAR AAs, even at low concentrations, has been associated with more severe forms of the disease, drastically reducing the time to mortality (62). Nonetheless, further investigations are warranted to confirm the precise mechanism of action and effect of AAs, and to facilitate the testing of more specialized therapies for COVID-19 in clinical trials.

The implication of ARBs in SARS-CoV-2 infection is still controversial (64). Initially they were developed to treat hypertension by blocking AT1R, inducing higher concentrations of ACE2 and converting Ang II into Ang 1-7, which results in MAS1 activation. The latter is regarded a major system protective pathway, which reduces inflammation organ fibrosis and downregulates several signaling pathways (65). ARBs also seem to be an optimal therapeutic strategy for patients with severe critical disorders (such as inflammatory lung disease, pneumonia, influenza, sepsis and Ebola), by maintaining insulin sensitivity, energy and lipid metabolism, protecting mitochondrial function and regulating the coagulation cascade (66-68). Despite the numerous clinical benefits conferred by ARBs to non-COVID patients, it is still not clear whether a similar effect would be achieved in COVID-19 patients, or whether higher ACE2 expression is responsible for a more severe disease. To this end, certain studies have hypothesized that COVID-19 patients with cardiovascular problems, diabetes and kidney disorders might have progressed to more severe disease and mortality following administration of ARBs (69,70). Although no harmful effects of ARBs has been observed on COVID-19 susceptibility, severity and mortality, the benefits are still uncertain and further research is warranted (71).

Another potential treatment and possible preventive measure against COVID-19 is the administration of NO. The latter has an antiviral action by inhibiting SARS-CoV-1 and SARS-CoV-2 replication (72). There are several different ways of supplementing NO, the most effective of which is thought to be the inhalation method, currently being investigated in clinical trials (including NCT04312243, NCT04338828 and NCT04305457) (72). Other methods of administration include supplementation of NO donor molecules such as citrulline, arginine and nitro glycerin, phosphodiesterase inhibitors such as Viagra and a high intake in nitrate-rich food such as beetroots, spices and leafy green vegetables (73,74). So far, inhalation of NO is the method that mostly targets the respiratory tract, while observed benefits include improved arterial oxygenation, reduced hypertension, as well as spread and density of pulmonary infiltrates (75). Furthermore, as NO is constantly produced by the epithelial cells of the nasopharynx and the paranasal sinuses, it activates the secretion of mucus and initiates ciliary movement which removes viral particles and foreign molecules from the respiratory tract (76-78). Another essential activity of NO is the capacity to prevent pulmonary infections by inducing antimicrobial/antiviral effects, a property that is facilitated via the modification and inactivation of nucleic acids and proteins that are vital for viral replication (79-81).

Last but not least, the presence of AAs could represent a potential diagnostic marker of the severity of COVID-19 disease. This could further direct the risk stratification of patients and guide more optimal treatment decisions. Preliminary data from ongoing clinical studies appear quite promising and hold much hope for a more effective diagnosis and treatment of this disease in the near future.

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Authors' contributions

TK, IT and KD conceptualized the study. TK and KD prepared the first draft of the manuscript. KD, IT, RC and NS wrote the manuscript. All authors were involved in the revision of the draft manuscript. KD and IT provided expertise in genomics. MA, DAS and VZ provided expertise in proteomics. RC, NS and VM provided expertise in clinical medicine. VM, RH, VZ and DAS supervised the study. KD and IT visualized data. TK, RH, MA, VZ, DAS and VM reviewed and edited the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

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Patient consent for publication

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Competing interests

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