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Hypothesis: Alpha-1-antitrypsin is a promising treatment option for COVID-19

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ABSTRACT

No definitive treatment for COVID-19 exists although promising results have been reported with remdesivir and glucocorticoids. Short of a truly effective preventive or curative vaccine against SARS-CoV-2, it is becoming increasingly clear that multiple pathophysiologic processes seen with COVID-19 as well as SARS-CoV-2 itself should be targeted. Because alpha-1-antitrypsin (AAT) embraces a panoply of biologic activities that may antagonize several pathophysiologic mechanisms induced by SARS-CoV-2, we hypothesize that this naturally occurring molecule is a promising agent to ameliorate COVID-19. We posit at least seven different mechanisms by which AAT may alleviate COVID-19. First, AAT is a serine protease inhibitor (SERPIN) shown to inhibit TMPRSS-2, the host serine protease that cleaves the spike protein of SARS-CoV-2, a necessary preparatory step for the virus to bind its cell surface receptor ACE2 to gain intracellular entry. Second, AAT has anti-viral activity against other RNA viruses HIV and influenza as well as induces autophagy, a known host effector mechanism against MERS-CoV, a related coronavirus that causes the Middle East Respiratory Syndrome. Third, AAT has potent anti-inflammatory properties, in part through inhibiting both nuclear factor-kappa B (NFκB) activation and ADAM17 (also known as tumor necrosis factor-alpha converting enzyme), and thus may dampen the hyper-inflammatory response of COVID-19. Fourth, AAT inhibits neutrophil elastase, a serine protease that helps recruit potentially injurious neutrophils and implicated in acute lung injury. AAT inhibition of ADAM17 also prevents shedding of ACE2 and hence may preserve ACE2 inhibition of bradykinin, reducing the ability of bradykinin to cause a capillary leak in COVID-19. Fifth, AAT inhibits thrombin, and venous thromboembolism and *in situ* microthrombi and macrothrombi are increasingly implicated in COVID-19. Sixth, AAT inhibition of elastase can antagonize the formation of neutrophil extracellular traps (NETs), a complex extracellular structure comprised of neutrophil-derived DNA, histones, and proteases, and implicated in the immunothrombosis of COVID-19; indeed, AAT has been shown to change the shape and adherence of non-COVID-19-related NETs. Seventh, AAT inhibition of endothelial cell apoptosis may limit the endothelial injury linked to severe COVID-19-associated acute lung injury, multi-organ dysfunction, and pre-eclampsia-like syndrome seen in gravid women. Furthermore, because both NETs formation and the presence of anti-phospholipid antibodies are increased in both COVID-19 and non-COVID pre-eclampsia, it suggests a similar vascular pathogenesis in both disorders. As a final point, AAT has an excellent safety profile when administered to patients with AAT deficiency and is dosed intravenously once weekly but also comes in an inhaled preparation. Thus, AAT is an appealing drug candidate to treat COVID-19 and should be studied.

Abbreviations: AAT, alpha-1-antitrypsin; ACE2, angiotensin converting enzyme 2 (receptor for SARS-CoV-2); SERPIN, serine protease inhibitor.

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Introduction

"I have devised seven separate explanations, each of which would cover the facts as far as we know them. But which of these is correct can only be determined by the fresh information which we shall no doubt find waiting for us."

Sherlock Holmes – The Adventure of the Copper Beeches

The number of COVID-19 cases worldwide is approaching 30 million as of September 2020. One of the most serious manifestations of COVID-19 is acute respiratory distress syndrome, especially in the elderly and those with cardiopulmonary disorders [1]. Curiously, the onset of respiratory compromise often occurs >7 days following known or suspected exposure or 5–7 days after symptom onset [2]. This delayed onset of respiratory compromise has been attributed to various pathophysiologic processes including diffuse alveolar damage, *in situ* microthrombi formation, venous thromboembolism, immunothrombosis, cardiac dysfunction, and hyper-inflammatory cytokine responses [3–7].

There is currently no definitive treatment for COVID-19 [8]. No efficacy was seen with combined lopinavir and ritonavir [9]. Despite initial optimism with hydroxychloroquine, a recent observational study found that it had no significant impact on the composite end point of endotracheal intubation or death in hospitalized COVID-19 patients [10]. Remdesivir initially showed a trend in reducing the time to clinical improvement [11]. A more recent, double-blind, placebo-controlled study showed that remdesivir significantly reduced the recovery time from COVID-19 by approximately four days and there was a trend toward improved mortality [12].

Glucocorticoid was initially not recommended by some during the early period of the COVID-19 pandemic [13,14]. A plausible rationale – which may still be true – is that a potent, initial pro-inflammatory response is necessary for viral clearance. However, in the more delayed severe cases, where an overzealous inflammatory response ("cytokine storm") may result in lung tissue damage, there is increasing evidence that glucocorticoids are therapeutic. Thus, timing of administration and severity of disease are likely important factors in whether glucocorticoids are effective or not [15]. The large RECOVERY trial showed that compared to placebo, daily intravenous or oral dexamethasone 6 mg – beginning ≥ 7 days into the symptomatic phase for up to 10 days of treatment – reduced death rate by one-third in ventilated patients and by 20% in patients who required supplemental oxygen only [16]. This benefit of delayed glucocorticoid administration coincides with the belated onset of respiratory insufficiency and lends credence to the notion that a delayed hyper-inflammatory response is implicated in the oxygenation failure. In contrast, the use of dexamethasone in milder COVID-19 cases showed a trend toward increased mortality in the RECOVERY trial [16]. In a *meta*-analysis of 7 randomized clinical trials of systemic glucocorticoid use in critically ill COVID-19 patients, glucocorticoid was associated with a lower 28-day all-cause mortality [17]. Hydrocortisone for 7 days was also linked to reduced number of days requiring ICU-based respiratory or cardiovascular support for those with severe COVID-19 [18]. Other, more targeted anti-inflammatory drugs are also being investigated as treatments for COVID-19, including inhibitors/antagonists to Janus kinase, interleukin-1 (IL-1), IL-6, IL-6 receptor, and tumor necrosis factor-alpha (TNFα) in the hope of further limiting the hyper-inflammatory response and resultant multi-organ damage [7]. Despite initial optimism with the use of neutralizing agents against IL-6 signaling [19], the recent multicenter, randomized, double-blind, placebo-controlled COVACTA trial of hospitalized patients with severe COVID-19 pneumonia found that tocilizumab (anti-IL-6 receptor antibody) had no significant efficacy as analyzed by clinical status, mechanical ventilation use, or mortality [20]. Until definitive anti-viral treatments are developed against SARS-CoV-2 and an effective prophylactic vaccine comes to fruition, the scientific community should continue to investigate existing drugs – with acceptable side effect profiles – that may target SARS-CoV-2 and the pathophysiologic mechanisms of COVID-19.

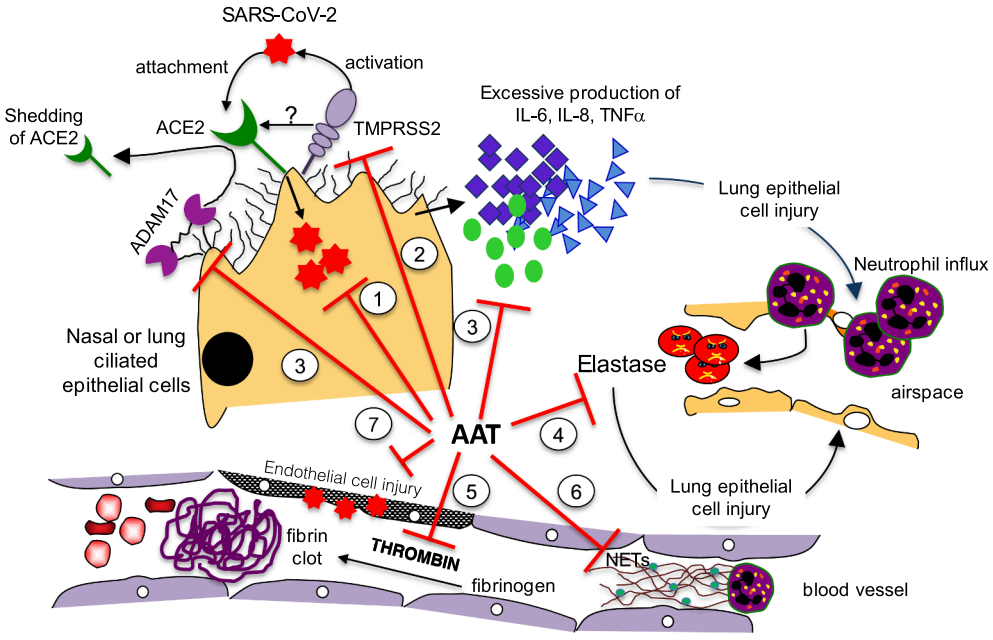

Fig. 1. Hypothesized mechanisms by alpha-1-antitrypsin (AAT) may be therapeutically efficacious against COVID-19. We hypothesize that AAT is a promising therapeutic against COVID-19 *via* at least seven mechanisms (see accompanying text for full description). In brief, we posit that AAT will: *(1)* augment host immunity against SARS-CoV-2 by enhancing autophagy, *(2)* inhibit TMPRSS-2 activity, mitigating a key and necessary step prior to SARS-CoV-2 entry into cells, *(3)* antagonize inflammation, *(4)* inhibit neutrophil elastase and ameliorate acute lung injury, *(5)* inhibit thrombin, retarding microthrombi formation, *(6)* inhibit neutrophil extracellular traps (NETs) adherence, limiting immunothrombosis seen with COVID-19, and *(7)* protect against endothelial cell apoptosis, curbing COVID-19-associated endothelial injury. Whereas TMPRSS-2 may also process ACE2 to facilitate binding and entry of SARS-CoV, it is not known whether such activity also enhances SARS-CoV-2 binding to ACE2; this uncertainly is denoted by the question mark. ACE2 = receptor for SARS-CoV-2; TMPRSS-2 = serine protease necessary to "activate" SARS-CoV-2; T-shaped "arrows" = inhibition; red heptagon = SARS-CoV-2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Hypothesis

alpha-1-antitrypsin is a promising treatment option for COVID-19.

Alpha-1-antitrypsin (AAT) is a serine protease inhibitor (SERPIN) and the third most abundant protein in circulation. AAT plasma level can increase 3- to 5-fold in states of systemic inflammation and / or infection, perhaps indicative of a homeostatic role of AAT but which may be deficient or overwhelmed in severe cases of COVID-19 [21–22]. While the best described function of AAT is that it irreversibly inhibits the serine protease elastase [22], it has a panoply of biological activities that may be independent of its SERPIN activity. Thus, because AAT possesses several biological functions that may antagonize both SARS-CoV-2 infection and the array of pathophysiologic processes that have been ascribed to COVID-19, we hypothesize that AAT is a promising candidate for the successful treatment of COVID-19.

In the U.S., it is estimated that there are ~300,000 individuals with frank AAT deficiency and the vast majority are undiagnosed. Even in the absence of AAT shortage, the AAT response to a systemic infection may be inadequate in some individuals. An estimated 9% of individuals in the U.S. (>20 million) are carriers of a single AAT gene mutation (with over 400 different mutations identified) with most having no ill effects but an uncertain number have inadequate AAT response to infections or inflammation [21]. Vianello and Braccioni [23] showed that in Italy there was geographic co-localization between those with AAT deficiency and the number of COVID-19 cases. Furthermore, since oxidation of methionine 351 and/or 358 residues of normal AAT may lead to loss of its SERPIN activity, the increased oxidative stress seen with COVID-19 may render even normal or elevated levels of AAT ineffective [24–25].

Support for the hypothesis

alpha-1-antitrypsin embraces a panoply of functions that can antagonize COVID-19

Our central hypothesis is that AAT, a naturally occuring molecule which has been utilized at pharmacologic doses for decades, is a promising agent against COVID-19. We describe below seven potential mechanisms by which AAT could antagonize both SARS-CoV-2 and some of the known pathogenic processes of COVID-19. The seven mechanisms discussed coincide with the numbers shown in Fig. 1.

AAT protects against microbes including RNA viruses

AAT augments host immunity against a wide variety of pathogens including influenza [26], HIV [27–31], *Pseudomonas aeruginosa* [32], and *Mycobacterium intracellulare* [33]. AAT antagonizes HIV by several mechanisms including interacting with gp41 to block HIV entry into $CD4^+$ lymphocytes, inhibiting HIV replication through alteration of IκBα ubiquitination and inhibition of nuclear factor-kappa B (NFκB) activation (a transcription factor that induces HIV replication), and inducing prostaglandin synthase-2, which inhibits HIV replication [27–31]. A mechanism by which AAT reduces the burden of cell-associated *M. intracellulare* is through sequential inhibition of NFκB, reduction in the expression of A20 (a deubiquitinating enzyme that inhibits autophagosome maturation by inhibiting TRAF6 from ubiquitinating a key autophagic protein Beclin-1), and induction of autophagy [33]. Autophagy has been implicated in controlling MERS-CoV, a related coronavirus that causes the Middle East Respiratory Syndrome (MERS) [34]. Given the similarities between the highly pathogenic coronaviruses, we posit that AAT augmentation of autophagy is likely important in the host immune response to SARS-CoV-2.

AAT inhibition of TMPRSS-2 impedes SARS-CoV-2 entry into cells

The coronaviruses that may cause fatal disease – SARS-CoV, MERS-CoV, and SARS-CoV-2 – all utilize the host cell serine protease TMPRSS-2 to process the viral spike protein so that it may bind to the cell surface receptor ACE2 (or DPP4 in the case of MERS-CoV) on host cells to gain intracellular entry. TMPRSS-2 may also process ACE2 to facilitate entry of SARS-CoV [35] but whether this applies to SARS-CoV-2 is not known. The SERPIN camostat inhibits TMPRSS-2 and entry of SARS-CoV and SARS-CoV-2 into cells [36–38]. Camostat was also shown to inhibit influenza replication and cytokine production in airway epithelial cells, likely due to inhibition of the host serine protease hepsin [39].

Because AAT is a potent SERPIN, it also has the potential to inhibit viral entry into cells. Indeed, in HEK293T cells engineered to overexpress TMPRSS-2, physiologic concentrations of AAT potently inhibited TMPRSS-2 activity using the fluorogenic substrate Boc-Gln-Ala-Arg-7-amino-4-methylcoumarin [40]. Wettstein *et al* [41] further demonstrated that AAT has inhibitory properties against SARS-CoV-2 infection of cells. From a pooled 20 L volume of bronchoalveolar lavage fluid, they analyzed different fractions of a peptide/protein library in their ability to inhibit SARS-CoV-2 entry of epithelial cells. After MALDI-TOF-MS (matrix-assisted laser-desorption ionization time-of-flight mass spectrometry) analysis of the fraction that best inhibited viral infection, AAT was identified as the principal inhibitor [41]. These findings were confirmed by the ability of exogenous AAT in physiological concentrations to inhibit SARS-CoV-2 infection of human airway epithelial cells as well as TMPRSS-2-expressing Vero E6 cells. The specificity of AAT to inhibit SARS-CoV-2 was demonstrated by the inability of AAT to inhibit pseudoparticles carrying the G-protein of the vesicular stomatitis virus, a negative sense RNA virus [41]. Oguntuyo and colleagues [42] reported that sera from SARS-CoV-2 naïve individuals inhibited cellular entry of SARS-CoV-2 and identified AAT as the molecule responsible. de Loyola and co-workers [43] also showed that AAT inhibits disintegrin/metalloproteinase 17 (ADAM17), a protease that can cause shedding of ACE2 (which would decrease viral entry) [35] but may also process membrane-bound ACE2 and enhance SARS-CoV entry [44] although this latter finding is controversial [35]. To the best of our knowledge, whether ADAM17 processing of ACE2 to enhance SARS-CoV-2 cellular entry is not known.

AAT has potent anti-inflammatory activities

While much has been written of the injurious role the delayed hyperinflammatory response may play in COVID-19 and evinced by the numerous clinical trials being undertaken to counter inflammation, this concept is not completely established because a recent trial of neutralizing antibodies to the IL-6 receptor showed no significant efficacy [20]. Furthermore, genome-wide association studies have not implicated any targetable inflammatory pathways as linked to COVID-19 risk [45]. But negative studies in which only one pro-inflammatory cytokine was targeted does not rule out the potential injurious role an array of cytokines may play as evinced by the benefit of delayed administration of glucocorticoid in severe COVID-19.

AAT also has potent anti-inflammatory properties [46–48]. A mechanism by which AAT attenuates inflammation is by inhibiting NFκB activation, a prototypical pro-inflammatory transcription factor, through binding of IκBα and/or altering IκBα ubiquitination [31,47,49]. AAT also binds extracellular IL-8, preventing the chemokine from binding to its receptor CXCR1 and activating Akt signaling pathway [46]. Because Akt signaling inhibits the early stages of autophagy, perhaps this binding of AAT to IL-8 attenuates Akt activation, thereby inducing autophagy [50]. In addition, since neutrophilia is associated with worse outcome in COVID-19 [51], the ability of AAT to sequester IL-8, a chemokine for neutrophils, may limit both neutrophil influx and acute lung injury. COVID-19 is also associated with increased oxidative stress [25] and AAT has been shown to inhibit neutrophil superoxide production [52].

Another anti-inflammatory mechanism of AAT is inhibition of ADAM17 [43]. Also known as TNFα-converting enzyme, ADAM17 is a cell surface metalloprotease that is activated by the spike protein of coronaviruses and cleaves membrane-bound TNFα to soluble TNFα. But

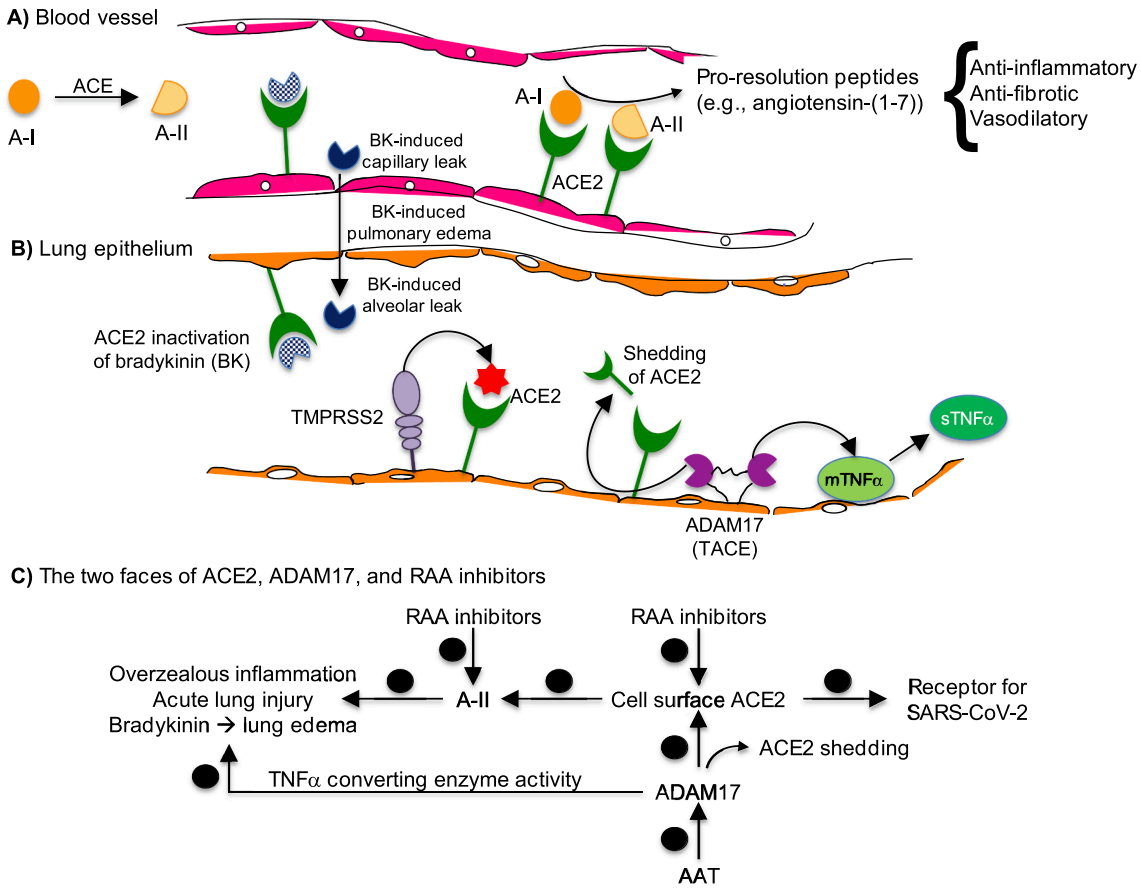

Fig. 2. The two faces of ACE2, ADAM17, and RAA inhibitors. (A) In blood vessels, angiotensin converting enzyme (ACE) converts angiotensin I (A-I) to angiotensin II (A-II). ACE2 then metabolizes A-I and A-II into angiotensin-(1–7) and angiotensin-(1–9), with the latter metabolites also known as pro-resolution peptides because, unlike A-II, they have anti-inflammatory, anti-fibrotic, and vasodilatory properties. (B) In the nasal and lung epithelium, ACE2 is the receptor for SARS-CoV-2 after the viral spike protein is processed by the serine protease TMPRSS-2. However, ACE2 is also anti-inflammatory and protects against various forms of acute lung injury through metabolism of pro-inflammatory A-II to anti-inflammatory angiotensin-(1–7) and angiotensin-(1–9), inhibition of bradykinin production, and preservation of cell viability but the precise ligand in the airways that ACE2 catalyzes is not known. ADAM17 is pro-inflammatory in that it converts membrane TNFα to soluble TNFα as well as causes shedding of ACE2, reducing the latter's anti-inflammatory effects. Thus, (C) the two faces of ACE2 are that it is the receptor for SARS-CoV-2 and yet is anti-inflammatory and protects against lung injury. ADAM17 causes ACE2 shedding, reducing cell surface expression of the SARS-CoV-2 receptor but also induces a pro-inflammatory state. Inhibition of the renin-angiotensin-aldosterone (RAA) axis is also known to induce ACE2 expression but inhibits inflammation by reducing A-II expression. Thus, both RAA inhibition and AAT would increase ACE2 expression but both would inhibit A-II expression and inflammation. A-I = angiotensin I; **A-II** = angiotensin II; BK = bradykinin; RAA = renin-angiotensin-aldosterone; mTNFα = membrane-bound tumor necrosis factor-alpha; sTNFα = soluble TNFα; TACE = TNFα converting enzyme; Negative sign = inhibit or reduce; Positive sign = augment.

as previously mentioned, ADAM17 also causes shedding of ACE2 (Fig. 1) [35]. In the blood compartment or within tissues, shedding of ACE2 by ADAM17 would increase inflammation since ACE2 normally converts angiotensin I and pro-inflammatory angiotensin II [53] to "resolution peptides" – angiotensin-(1–7) and angiotensin-(1–9) – that are anti-inflammatory, anti-fibrotic, and vasodilatory (Fig. 2A) [54]. While these biochemical functions of ACE2 may also occur in the airway lumen, the specific ligand(s) other than SARS-CoV or SARS-CoV-2 that bind ACE2 in the airways is not known (Fig. 2B). Neverthless, normal ACE2 expression has been shown to protect the lungs from injury – by reducing both bradykinin production and neutrophil infiltration [55] as well as catalyzing angiotensin II to the anti-inflammatory angiotensin-(1–7) and angiotensin-(1–9); both these two metabolic products also protect lung epithelial cells from death [56]. Consequently, ADAM17-induced shedding of ACE2 may cause excessive inflammation, resulting in lung injury whereas AAT would dampen these host-deleterious responses [35]. To summarize these complex interactions, ACE2, while being a receptor for SARS-CoV-2, also has anti-inflammatory properties (Fig. 2C). ADAM17, by inducing ACE2 shedding, would decrease the cell surface receptor for SARS-CoV-2 but also negate ACE2 inhibition of overzealous inflammation, acute lung injury, and lung edema (Fig. 2C). AAT inhibition of ADAM17 would reduce inflammation by decreasing both soluble TNFα formation and ACE2 shedding.

Pharmacologic inhibition of the renin-angiotensin-aldosterone (RAA) axis (*e.g.*, ACE inhibitors) appear to have a neutral effect on the course of COVID-19. This neutrality may be due to off-setting effects in that RAA inhibition induces ACE2 cell surface expression (potentially increasing viral entry) but decreases angiotensin-II (which would decrease overzealous inflammation) [57]. As shown in Fig. 2C, both RAA inhibition and AAT would theoretically increase ACE2 expression and both would decrease angiotensin-II expression and inflammation.

McElvaney and colleagues [58] reported in 40 hospitalized COVID-19 patients (20 stable and 20 requiring intensive care) and 15 patients with critically ill non-COVID-19 community-acquired pneumonia that there is a blunted AAT acute-phase response in the critically-ill COVID-19 patients but not in non-COVID-19 patients requiring intensive care. In addition, they found that an increased IL-6:AAT ratio predicted prolonged ICU stay and mortality, while relative reduction in IL-6:AAT ratio was associated with clinical resolution [58]. Thus, their findings support our hypothesis that an inadequate AAT response may be responsible for the hyper-inflammatory response associated with COVID-19 and predict a worse outcome.

AAT inhibition of neutrophil elastase may limit acute lung injury

Neutrophil elastase at sites of acute inflammation is known to mediate acute lung injury [59]. Elastase contributes to excessive inflammation by inducing the release of IL-8 from neutrophil vesicles and facilitating conversion of pro-IL-1β to IL-1β [60]. AAT is a potent and irreversible inhibitor of elastase. In a rat model of lipopolysaccharide / ventilator-induced lung injury, pre-treatment with AAT resulted in improved outcome, as evinced by increased PaO_2/FiO_2 ratio and decreased wet/dry lung weight ratio as well as decreased protein levels, pro-inflammatory cytokines, and cell count in the bronchoalveolar lavage fluid [61]. Recently, Mehraban and colleagues [62] showed that broken down elastic fibers as a result of elastase plus another insult such as lipopolysacchardide was pro-inflammatory. However, there have been mixed results with the use of neutrophil elastase inhibitors in various forms of non-COVID-19-related acute lung injury [63]. This may be due, in part, to the fact that neutrophil elastase may not only incite excessive inflammation but is also required for optimal intracellular killing of gram-negative bacteria [64].

Acute lung injury is also manifested by non-cardiogenic pulmonary edema due to leakage of exudate through the alveolar-capillary membrane (Fig. 2). Bradykinin may play a role in this leakage [55,65]. Since ACE2 inactivates bradykinin by cleaving a single amino acid from its carboxyl terminus, ACE2 can protect against this mechanism of acute lung injury. Given that ADAM17 causes shedding of ACE2 [35], ADAM17 would theoretically exacerbate the capillary leakage by attenuating ACE2 inhibition of bradykinin. But since AAT inhibits ADAM17, AAT would mitigate the pulmonary edema caused by the virus-induced bradykinin pathway (Fig. 2C) [55]. While AAT inhibition of ADAM17 would inhibit ADAM17 shedding of ACE2 and in theory enhance viral binding, AAT was found to inhibit SARS-CoV-2 viral entry into cells through inhibition of TMPRSS-2 [41–42].

AAT inhibition of thrombin may retard thrombus formation

Both venous thromboembolism as well as *in situ* micro- and macrothrombi are increasingly recognized with COVID-19 and likely contribute to the hypoxemia seen with the acute lung injury [6,66–67]. AAT has been shown to antagonize thrombin, a serine protease [68]. Because most of the enzymes in the coagulation cascade are also serine proteases, AAT has the potential to inhibit other pro-coagulant proteins in addition to thrombin. Thus, while AAT itself is unlikely to be thrombolytic in those with established thrombi, it may help retard thrombus formation [42].

AAT alteration of neutrophil extracellular traps may limit COVID-19 immunothrombosis

Increased absolute neutrophil number, percentage of neutrophils, and neutrophil:lymphocyte ratio in the blood of COVID-19 patients are predictive of progression to severe disease [51]. While this association may simply be a non-specific reflection of increased severity, there is increasing evidence that aberrant formation of neutrophil extracellular traps (NETs) – essentially comprised of neutrophil-derived decondensed chromatin (cell-free DNA) and proteins such as elastase, cathepsin G, and histones to trap and kill extracellular pathogens – play a pathogenic role in the immunothrombosis, mucous secretions, and cytokine production seen with COVID-19 based on autopsy results, *ex vivo* NETs formation studies, and blood biomarkers for NETs (cell-free DNA, myeloperoxidase DNA, and citrullinated histone H3) [5,69–73]. Since elastase plays a key role in NETs formation by degrading specific histones and promoting chromatin decondensation [74], AAT has the potential to inhibit NETs formation and reduce the excessive inflammation and immunothrombosis seen with COVID-19. Indeed, Frenzel and coworkers [75] showed that while AAT did not decrease the formation of phorbol myristate acetate-induced NETs, it changed the shape and adherence of the NETs in *ex vivo* experiments using blood neutrophils.

AAT inhibition of apoptosis may limit endothelial injury by SARS-CoV-2

SARS-CoV-2 infects endothelial cells and COVID-19 lungs demonstrate endothelial cell injury, microthrombi and angiogenesis [66]. This endothelial injury seen with COVID-19 may be part of a spectrum of pulmonary pathology observed that includes acute lung injury, microthrombi formation, and extra-pulmonary multi-organ dysfunction associated with the most severe cases.

Another disorder increasingly recognized in gravid patients with severe COVID-19 is pre-eclampsia or pre-eclampsia-like syndrome [76–77]. One obvious common denominator for both pre-eclampsia and severe COVID-19 is endothelial injury / pathology as a hallmark of pre-eclampsia is disrupted placentation which leads to endothelial dysfunction and end-organ damage [78]. An indirect support for a similar underlying pathophysiology of pre-eclampsia and severe COVID-19 is that both disorders may manifest with non-cardiogenic pulmonary edema, venous thromboembolism, and/or multi-organ dysfunction. Two other specific pathogenic elements that are increased in both COVID-19 and non-COVID-19 pre-eclampsia is further evidence that the two disorders are linked pathogenically. One is that NETs – as previously mentioned with mounting evidence of a pathologic role in COVID-19 – have also been implicated in the pathogenesis of non-COVID-19-related severe pre-eclampsia [79]. Another is that the presence of anti-phospholipid antibodies (aPLA) is a major risk factor for pre-eclampsia [80] and one study found that 52% of COVID-19 patients had elevated aPLA levels [81]. This very high prevalence of pro-thrombotic aPLA in COVID-19 is most likely a consequence of its induction by SARS-CoV-2 rather than being a pre-existing state.

AAT inhibits endothelial cell apoptosis and thus may antagonize the endothelial injury seen with COVID-19 [82–83]. More specifically, murine models revealed that in endothelial cells, AAT inhibits caspase-3, an executioner caspase in the classical apoptotic pathway [83]. In addition, AAT treatment of endothelial cells decreased oxidative stress, inflammation, and cell wall deterioration [83]. Interestingly, low levels of plasma AAT have been associated with severe non-COVID-19-related pre-eclampsia [84]. Furthermore, AAT suppresses oxidative stress in both murine and molecular models of non-COVID-19 pre-eclampsia [85–86]. Thus, AAT may also be a promising agent against the pre-eclampsia-like syndrome seen in pregnant women with severe COVID-19 and should be studied. While large prospective studies have not evaluated AAT therapy in pregnancy, there are case reports of safe use in pregnancy with normal neonatal outcomes [87]. Before administering AAT – as part of a study or as augmentation therapy in those with AAT deficiency – screening for IgA deficiency should be done since those with IgA deficiency are more likely to develop hypersensitivity reactions due to the potential presence of antibodies directed against IgA.

Summary and fulfilment of the hypothesis

In summary, given its anti-viral, SERPIN (anti-TMPRSS-2 and anti-elastase), anti-inflammatory, anti-thrombin, anti-NETs, and anti-apoptotic activities, AAT is a promising therapeutic for COVID-19. It is also important to note that AAT is routinely prescribed to those with AAT deficiency, has an excellent safety profile, and normal plasma AAT levels may be achieved with once weekly intravenous administration. Moreover, an inhaled AAT formulation is available although its efficacy remains to be fully determined. It is important to also be cognizant that even if a truly effective prophylactic vaccine is developed against SARS-CoV-2, there will continue to be barriers, including the challenge of administering one or more doses of the vaccine to each person in the world, vaccine efficacy likely will not be 100%, vaccine refusal by a significant number of individuals, and the looming specter of mutations of SARS-CoV-2, rendering the vaccine less effective, *vis-à-vis* what is seen with influenza. If mounting evidence shows that AAT does have

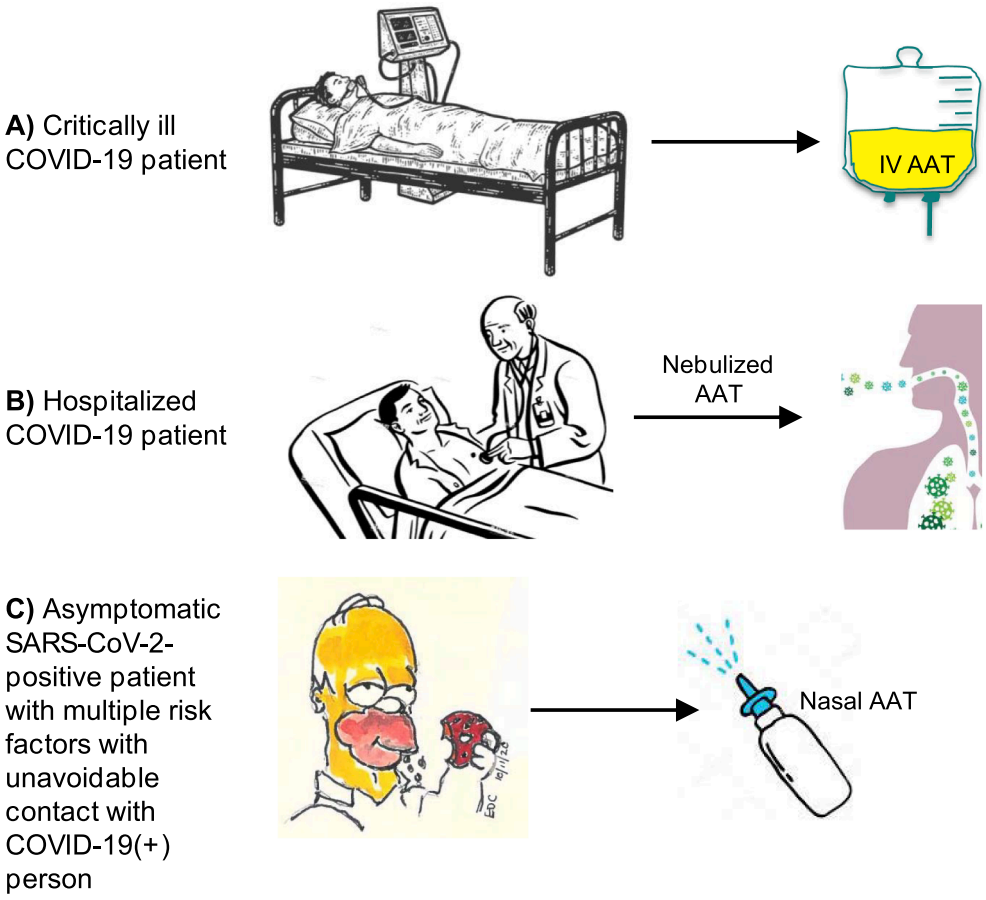

Fig. 3. Potential use of AAT depending on the severity. If more evidence acrue that AAT has great potential against SARS-CoV-2 infection and COVID-19 complications, AAT should be studied in patients with SARS-CoV-2 infection by randomized, placebo-controlled trials with AAT administered by different means depending on the severity of the SARS-CoV-2 infection. As shown, (A) AAT administered intravenously (IV) in critically-ill COVID-19 subjects, (B) AAT administered by nebulization in non-critically-ill patients with COVID-19 pneumonia, or (C) AAT administered prophylactically by a nasal spray (yet-to-be-developed) in asymptomatic persons with multiple risk factors for severe COVID-19 with unavoidable close contacts with COVID-19 positive individuals. AAT = alpha-1-antitrypsin.

significant activity against SARS-CoV-2 infection, it can be studied by randomized, placebo-controlled trials in which is AAT administered by different means depending on the severity of the SARS-CoV-2 infection (Fig. 3). For example, AAT administered intravenously (IV) along with an anti-oxidant to protect AAT from oxidation in critically-ill COVID-19 subjects (Fig. 3A), by nebulization in non-critically-ill patients with COVID-19 pneumonia (Fig. 3B), or prophylactically by a nasal spray (yet-to-be-developed) in asymptomatic persons with multiple risk factors for severe COVID-19 with unavoidable close contact with COVID-19 positive individuals (Fig. 3C).

Declaration of Competing Interest

Dr. Robert A. Sandhaus is the Medical Director of AlphaNet.

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This work is dedicated to the patients who have suffered from or lost their lives to severe COVID-19 as well as to all the health-care workers who have risk their lives in taking care of them.

References

[1] Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054–62.
[2] Johnson RM, Vinetz JM. Dexamethasone in the management of covid-19: Preliminary trial results are mostly good news, but timing is everything. BMJ 2020. E-pub.
[3] Guan WJ, Ni ZY, Hu Y, et al. China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020 [Epub ahead of print].
[4] Long B, Brady WJ, Koyfman A, Gottlieb M. Cardiovascular complications in COVID-19. Am J Emerg Med 2020;38:1504–7.
[5] Middleton EA, He X-Y, Denorme F, et al. Neutrophil Extracellular Traps (NETs) Contribute to Immunothrombosis in COVID-19 Acute Respiratory Distress Syndrome. Blood 2020; Online ahead of print.
[6] Wang J, Hajizadeh N, Moore EE, et al. Tissue Plasminogen Activator (tPA) Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Case Series. J Thromb Haemost 2020 [Epub ahead of print].
[7] Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. Int J Antimicrob Agents 2020; [Epub ahead of print].
[8] NIAID-RML. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. 2020. Available from: https://www.covid19treatmentguidelines.nih.gov/.
[9] Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med 2020 [Epub ahead of print].
[10] Geleris J, Sun Y, Platt J, et al. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. N Engl J Med 2020;382:2411–8.
[11] Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2020:E-pub.
[12] Beigel JH, Tomashek KM, Dodd LE, et al. ACTT-1 Study Group Members. Remdesivir for the Treatment of Covid-19 - Preliminary Report. N Engl J Med 2020; Online ahead of print.
[13] Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. Lancet 2020;395:473–5.
[14] Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA. Intern Med 2020 [Epub ahead of print].
[15] McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines Including Interleukin-6 in COVID-19 Induced Pneumonia and Macrophage Activation Syndrome-Like Disease. Autoimmun Rev 2020; Online ahead of print.
[16] Horby PW, Landry MJ. RECOVERY Collaborative Group. Effect of dexamethasone in hospitalized patients with COVID-19 - Preliminary Report. medRxiv preprint 2020.
[17] Sterne JAC, Murthy S, Diaz JV, et al. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group. Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. JAMA Cardiol 2020; Online ahead of print.
[18] Angus DC, Derde L, Al-Beidh F, et al. Writing Committee for the REMAP-CAP Investigators. Effect of Hydrocortisone on Mortality and Organ Support in Patients With Severe COVID-19: The REMAP-CAP COVID-19 Corticosteroid Domain Randomized Clinical Trial. JAMA 2020; Online ahead of print.

[19] Somers EC, Eschenauer GA, Troost JP, et al. Tocilizumab for treatment of mechanically ventilated patients with COVID-19. Clin Infect Dis 2020.
[20] Lipworth BJ, Chan R, Kuo CR. Tocilizumab for severe COVID-19 pneumonia. Lancet Rheumatol 2020; published online ahead of print.
[21] de Serres FJ, Blanco I, Fernández-Bustillo E. Genetic epidemiology of alpha-1 antitrypsin deficiency in North America and Australia/New Zealand: Australia, Canada, New Zealand and the United States of America. Clin Genet 2003;64: 382–97.
[22] Hazari YM, Bashir A, Habib M, et al. Alpha-1-antitrypsin deficiency: Genetic variations, clinical manifestations and therapeutic interventions. Mutat Res 2017; 773:14–25.
[23] Vianello A, Braccioni F. Geographical overlap between alpha-1 antitrypsin deficiency and COVID-19 infection in Italy: Casual or causal? Arch Bronconeumol 2020:E-pub.
[24] Taggart C, Cervantes-Laurean D, Kim G, et al. Oxidation of either methionine 351 or methionine 358 in alpha 1-antitrypsin causes loss of anti-neutrophil elastase activity. J Biol Chem 2000;275:27258–65.
[25] Wang JZ, Zhang RY, Bai J. An anti-oxidative therapy for ameliorating cardiac injuries of critically ill COVID-19-infected patients. Int J Cardiol 2020 [Epub ahead of print].
[26] Harbig A, Mernberger M, Bittel L, et al. Transcriptome profiling and protease inhibition experiments identify proteases that activate H3N2 influenza A and influenza B viruses in murine airway. J Biol Chem 2020; Online ahead of print.
[27] Zhou X, Liu Z, Zhang J, Adelsberger JW, Yang J, Burton GF. Alpha-1-antitrypsin interacts with gp41 to block HIV-1 entry into CD4+ T lymphocytes. BMC Microbiol 2016;16:172.
[28] Bryan CL, Beard KS, Pott GB, et al. HIV infection is associated with reduced serum alpha-1-antitrypsin concentrations. Clin Invest Med 2010;33:E384–9.
[29] Zhou X, Shapiro L, Fellingham G, Willardson BM, Burton GF. HIV replication in CD4+ T lymphocytes in the presence and absence of follicular dendritic cells: inhibition of replication mediated by α-1-antitrypsin through altered IκBα ubiquitination. J Immunol 2011;186:3148–55.
[30] Whitney JB, Asmal M, Geiben-Lynn R. Serpin induced antiviral activity of prostaglandin synthetase-2 against HIV-1 replication. PLoS ONE 2011;6:e18589.
[31] Shapiro L, Pott GB, Ralston AH. Alpha-1-antitrypsin inhibits human immunodeficiency virus type 1. FASEB J 2001;15:115–22.
[32] Pott GB, Beard KS, Bryan CL, Merrick DT, Shapiro L. Alpha-1 antitrypsin reduces severity of pseudomonas pneumonia in mice and inhibits epithelial barrier disruption and pseudomonas invasion of respiratory epithelial cells. Front Public Health 2013;1:19.
[33] Bai X, Bai A, Honda JR, et al. Alpha-1-antitrypsin enhances primary human macrophage immunity against non-tuberculous mycobacteria. Front Immunol 2019;10:1417.
[34] Gassen NC, Niemeyer D, Muth D, et al. SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection. Nat Commun 2019;10:5770.
[35] Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pöhlmann S. TMPRSS2 and ADAM17 Cleave ACE2 Differentially and Only Proteolysis by TMPRSS2 Augments Entry Driven by the Severe Acute Respiratory Syndrome Coronavirus Spike Protein. J Virol 2014;88:1293–307.
[36] Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020; E-pub: pii: S0092-8674(0020)30229-30224.
[37] Kawase M, Shirato K, van der Hoek L, Taguchi F, Matsuyama S. Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry. J Virol 2012;86:6537–45.
[38] Matsuyama S, Nao N, Shirato K, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci U S A 2020;117:7001–3.
[39] Yamaya M, Shimotai Y, Hatachi Y, et al. The serine protease inhibitor camostat inhibits influenza virus replication and cytokine production in primary cultures of human tracheal epithelial cells. Pulm Pharmacol Ther 2015;33:66–74.
[40] Azouz NP, Klingler AM, Rothenberg ME. Alpha 1 antitrypsin is an inhibitor of the SARS-CoV2-priming protease TMPRSS2. bioRxiv 2020; Pre-print.
[41] Wettstein L, Conzelmann C, Müller JA, et al. Alpha-1 antitrypsin inhibits SARS-CoV-2 infection. bioRxiv 2020:pre-print.
[42] Oguntuyo KY, Stevens CS, Siddiquey MN, et al. In plain sight: the role of alpha-1-antitrypsin in COVID-19 pathogenesis and therapeutics. bioRxiv 2020; Preprint.
[43] de Loyola MB, Dos Reis TTA, de Oliveira GXLM, da Fonseca PJ, Argañaraz GA, Argañaraz ER. Alpha-1-antitrypsin: A possible host protective factor against Covid-19. Rev Med Virol 2020;26:e2157.
[44] Haga S, Nagata N, Okamura T, et al. TACE antagonists blocking ACE2 shedding caused by the spike protein of SARS-CoV are candidate antiviral compounds. Antiviral Res 2010;85:551–5.
[45] Ellinghaus D, Degenhardt F, Bujanda L, et al. Severe Covid-19 GWAS Group. Genomewide Association Study of Severe Covid-19 with Respiratory Failure. N Engl J Med 2020; Online ahead of print.
[46] Bergin DA, Reeves EP, Meleady P, et al. α-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8. J Clin Invest 2010; 120:4236–50.
[47] Chan ED, Pott GB, Silkoff PE, Ralston AH, Bryan CL, Shapiro L. Alpha-1-antitrypsin inhibits nitric oxide production. J Leuk Biol 2012;92:1251–60.
[48] Pott GB, Chan ED, Dinarello CA, Shapiro L. Alpha-1-antitrypsin is an endogenous inhibitor of pro-inflammatory cytokine production in whole blood. J Leuk Biol 2009;85:886–95.
[49] Ehlers MR. Immune-modulating effects of alpha-1 antitrypsin. Biol Chem 2014; 395:1187–93.
[50] Bai D, Ueno L, Vogt PK. Akt-mediated regulation of NFkB and the essentialness of NFkB for the oncogenicity of PI3K and Akt. Int J Cancer 2009;125:2863–70.
[51] Lu Y, Sun K, Guo S, et al. Early Warning Indicators of Severe COVID-19: A Single-Center Study of Cases From Shanghai, China. Front Med; 7: 432.
[52] Bucurenci N, Blake DR, Chidwick K, Winyard PG. Inhibition of neutrophil superoxide production by human plasma alpha 1-antitrypsin. FEBS Lett 1992;300: 21–4.
[53] Dandona P, Dhindsa S, Ghanim H, Chaudhuri A. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. J Human Hypertension 2007;21:20–7.
[54] Iwai M, Horiuchi M. Devil and agel in the renin-angiotensin system: ACE-angiotensin II-AT1 receptor axis vs. ACE2-angiotensin-(1–7)-Mas receptor axis. Hypertens Res 2009;32:533–6.
[55] Sodhi CP, Wohlford-Lenane C, Yamaguchi Y, et al. Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg(9) bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration. Am J Physiol Lung Cell Mol Physiol 2018;314: L17–31.
[56] Samavati L, Uhal BD. ACE2, much more than just a receptor for SARS-CoV-2. Front Cell Infect Microbiol 2020;10:317.
[57] Driggin E, Madhavan MV, Bikdeli B, et al. Cardiovascular Considerations for Patients, Health Care Workers, and Health Systems During the COVID-19 Pandemic. J Am Coll Cardiol 2020;75:2352–71.
[58] McElvaney OJ, McEvoy NL, McElvaney OF, et al. Characterization of the Inflammatory Response to Severe COVID-19 Illness. Am J Respir Crit Care Med 2020. In Press.
[59] Ishii T, Doi K, Okamoto K, et al. Neutrophil Elastase Contributes to Acute Lung Injury Induced by Bilateral Nephrectomy. Am J Pathol 2010;177:1665–73.
[60] Alfaidi M, Wilson H, Daigneault M, et al. Neutrophil Elastase Promotes Interleukin-1β Secretion from Human Coronary Endothelium. J Biol Chem 2015;290: 24067–78.
[61] Wang X, Gong J, Zhu J, Jin Z, Gao W. Alpha 1-antitrypsin for treating ventilator-associated lung injury in acute respiratory distress syndrome rats. Exp Lung Res 2019;45:209–19.
[62] Mehraban S, Gu G, Ma S, Liu X, Turino G, Cantor J. The proinflammatory activity of structurally altered elastic fibers. Am J Respir Cell Mol Biol 2020; Online ahead of print.
[63] Polverino E, Rosales-Mayor E, Dale GE, Dembowsky K, Torres A. The Role of Neutrophil Elastase Inhibitors in Lung Diseases. Chest 2017;152:249–62.
[64] Belaaouaj A, McCarthy R, Baumann M, et al. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. Nat Med 1998; 4:615–8.
[65] Garvin MR, Alvarez C, Miller JI, et al. A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm. Elife 2020;9:e59177.
[66] Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med 2020; Online ahead of print.
[67] Levi M, Hunt BJ. Thrombosis and coagulopathy in COVID-19: An illustrated review. Res Pract Thromb Haemost 2020;4:744–51.
[68] Gans H, Tan BH. α1-antitrypsin, an inhibitor for thrombin and plasmin. Clin Chim Acta 1967;17:111–7.
[69] Rao AN, Kazzaz NM, Knight JS. Do neutrophil extracellular traps contribute to the heightened risk of thrombosis in inflammatory diseases? World J Cardiol 2015;7: 829–42.
[70] Zuo Y, Yalavarthi S, Shi H, et al. Neutrophil extracellular traps in COVID-19. JCI Insight 2020;5:e138999.
[71] Zuo Y, Yalavarthi S, Shi H, et al. Neutrophil extracellular traps (NETs) as markers of disease severity in COVID-19. medRxiv 2020; 2020: Pre-print.
[72] Zuo Y, Zuo M, Yalavarthi S, et al. Neutrophil extracellular traps and thrombosis in COVID-19. medRxiv 2020; 2020: Pre-print.
[73] Barnes BJ, Adrover JM, Baxter-Stoltzfus A, et al. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med 2020;217:e20200652.
[74] Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol 2010;191:677–91.
[75] Frenzel E, Korenbaum E, Hegermann J, et al. Does augmentation with alpha1-antitrypsin affect neutrophil extracellular traps formation? Int J Biol Sci 2012;8: 1023–5.
[76] Kayem G, Lecarpentier E, Deruelle P, et al. A snapshot of the Covid-19 pandemic among pregnant women in France. J Gynecol Obstet Hum Reprod 2020; Online ahead of print.
[77] Mendoza M, Garcia-Ruiz I, Maiz N, et al. Pre-eclampsia-like syndrome induced by severe COVID-19: a prospective observational study. BJOG 2020; Online ahead of print.
[78] Burton GJ, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. BMJ 2019;366:l2381.
[79] Hu Y, Li H, Yan R, et al. Increased Neutrophil Activation and Plasma DNA Levels in Patients with Pre-Eclampsia. Thromb Haemost 2018;118:2064–73.
[80] Steegers EAP, von Dadelszen P. D'uvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010;376:631–44.
[81] Zuo Y, Estes SK, Gandhi AA, et al. Prothrombotic antiphospholipid antibodies in COVID-19. medRxiv 2020; pre-print.

[82] Petrache I, Fijalkowska I, Zhen L, et al. A novel antiapoptotic role for alpha1-antitrypsin in the prevention of pulmonary emphysema. Am J Respir Crit Care Med 2006;173:1222–8.
[83] Petrache I, Fijalkowska I, Medler TR, et al. Alpha-1 antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis. Am J Pathol 2006;169:1155–66.
[84] Twina G, Sheiner E, Shahaf G, et al. Lower circulation levels and activity of α-1 antitrypsin in pregnant women with severe preeclampsia. J Matern Fetal Neonatal Med 2012;25:2667–70.
[85] Feng YL, Wang N, Xu J, et al. Alpha-1-antitrypsin functions as a protective factor in preeclampsia through activating Smad2 and inhibitor of DNA binding 4. Oncotarget 2017;8:113002–12.
[86] Feng YL, Yin YX, Ding J, et al. Alpha-1-antitrypsin suppresses oxidative stress in preeclampsia by inhibiting the p38MAPK signaling pathway: An in vivo and in vitro study. PLoS ONE 2017;12:e0173711.
[87] Gaeckle NT, Stephenson L, Reilkoff RA. Alpha-1 Antitrypsin Deficiency and Pregnancy. COPD 2020;17:326–32.