Immunoinflammatory, Thrombohaemostatic, and Cardiovascular Mechanisms in COVID-19

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Abstract

The global coronavirus disease 2019 (COVID-19) pandemic has deranged the recent history of humankind, afflicting more than 27 million individuals to date. While the majority of COVID-19 patients recuperate, a considerable number of patients develop severe complications. Bilateral pneumonia constitutes the hallmark of severe COVID-19 disease but an involvement of other organ systems, namely the cardiovascular system, kidneys, liver, and central nervous system, occurs in at least half of the fatal COVID-19 cases. Besides respiratory failure requiring ventilation, patients with severe COVID-19 often display manifestations of systemic inflammation and thrombosis as well as diffuse microvascular injury observed postmortem. In this review, we survey the mechanisms that may explain how viral entry and activation of endothelial cells by severe acute respiratory syndrome coronavirus 2 can give rise to a series of events including systemic inflammation, thrombosis, and microvascular dysfunction. This pathophysiological scenario may be particularly harmful in patients with overt cardiovascular disease and may drive the fatal aspects of COVID-19. We further shed light on the role of the renin–angiotensin aldosterone system and its inhibitors in the context of COVID-19 and discuss the potential impact of antiviral and anti-inflammatory treatment options. Acknowledging the comorbidities and potential organ injuries throughout the course of severe COVID-19 is crucial in the clinical management of patients affecting treatment approaches and recovery rate.

Keywords
- COVID-19
- SARS-CoV-2
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- ACE2

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Introduction

The novel coronavirus disease 2019 (COVID-19) has rapidly progressed to a global pandemic infecting over 23 million people in 188 countries by the middle of August 2020.[1] The basis underlying COVID-19 is infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which originates from the Coronaviridae family of viruses that are usually associated with respiratory infections.[2,3] Although the respiratory manifestations of COVID-19 are well documented,[4,5] recent studies have also observed cardiovascular complications in patients.[6,7] Viral infection is associated with increased inflammatory biomarkers including interleukin-6 (IL-6) and D-dimer,[8] which may influence severe cardiovascular clinical features such as thrombosis and cardiac injury as observed in limited cohorts of COVID-19 patients.[9–11]

It is well established that outbreaks of acute respiratory infections such as influenza may trigger an increase in coronary deaths due to myocardial infarction or stroke.[12,13] Previously, similar viral epidemics including severe acute respiratory syndrome (SARS) reported common cardiovascular complications such as acute myocardial infarction and increased susceptibility to thrombosis.[14,15] In the case of SARS-CoV-2, however, the risk of ischemic stroke was 7.5-fold higher than that of influenza patients.[16] Furthermore, emerging evidence from the current COVID-19 pandemic suggests that individuals with preexisting cardiovascular risk factors including heart failure, hypertension, and diabetes may be more susceptible to severe infection.[4,17–19]

Although the interactions between COVID-19 and cardiovascular inflammation require further investigation, this review will focus on the potential mechanisms by which SARS-CoV-2 infects its host with a particular focus on vascular endothelial cell dysfunction. Specifically, we seek to describe the immunoinflammatory mechanisms that may disproportionately affect COVID-19 patients with underlying cardiovascular pathologies leading to their hypercoagulable states and cardiac injury. Finally, we discuss promising therapeutic options targeting the hyperinflammation associated with severe SARS-CoV-2 infection.

Mechanisms of Cellular Entry and Infection

Viruses cause infections in hosts by entering the cells to exploit the cellular machinery of the host to further replicate and spread from cell to cell. It has been established that the SARS-CoV-2 uses the protein angiotensin-converting enzyme-2 (ACE2) efficiently, even more so than the original SARS-CoV, to invade the host cells.[20–22] ACE2 is an extensively present cell surface enzyme. Li and colleagues recently analyzed the expression of ACE2 across 31 human tissues using datasets provided from Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA). They found the highest expression of the receptor in the small intestines, testes, kidneys, heart, thyroid, and adipose tissue, whereas the lowest expression was observed in the blood, spleen, bone marrow, blood vessels, and muscle.[23] Moderate expression levels were reported in the lungs, colon, liver, bladder, and adrenal gland. Nevertheless, these findings do not specify cell-specific expression of the receptor and remain to be further validated in protein levels. A study by Chen et al examined the cellular expression of ACE2 in the human heart via single nuclear transcriptome analysis and found that ACE2 expression was low in cardiomyocytes, whereas it was high and specific to pericytes.[24] Moreover, another study by Nicin and colleagues using single nuclei RNA sequencing likewise reported ACE2 expression particularly in pericytes.[25] They also reported the expression of the receptor in cardiomyocytes as well as mural cells and lower levels of expression were also observed in fibroblasts, endothelial cells, and leukocytes. Furthermore, cardiomyocyte expression of ACE2 was found to be significantly increased in patients with heart disease. The extensive presence of this receptor may be an explanation to the wide spectrum of symptoms and complications of COVID-19, such as respiratory and gastrointestinal distress, loss of taste and smell, and multiorgan dysfunction including cardiac and liver injury as well as renal failure. ACE2 is a central regulator in the renin–angiotensin aldosterone system (RAAS), a hormone system crucial for the maintenance of blood pressure as well as the fluid and electrolyte homeostasis in the body (►**Table 1**).[26] Imbalances in RAAS can lead to hypertension, and the components of this system are known to further augment cardiovascular risk factors such as inflammation, thrombosis, insulin resistance, and obesity (►**Table 1**).[27] Therefore, the doorway receptor of SARS-CoV-2, ACE2, plays a pivotal role in cardiovascular health and disease among other factors.

RAAS is activated in response to renin released by kidneys in the events of low blood supply and low sodium load. Circulating renin then cleaves its substrate angiotensinogen produced by the liver, which produces the peptide hormone angiotensin I. Predominantly occurring in the lungs, angiotensin I is further cleaved by ACE to produce angiotensin II (►**Table 1**).[28] Angiotensin II constricts blood vessels and increases blood pressure to replenish the blood supply to the kidneys in addition to stimulating aldosterone synthesis in the adrenal cortex for renal sodium reabsorption.[29] Consequently, RAAS activation leads to increased blood pressure and pharmacological blockade of the RAAS via ACE inhibitors (ACEis) and angiotensin II receptor blockers (ARBs) are used widely to treat hypertension in patients suffering from cardiovascular disease (CVD).[27]

While the RAAS is fulfilling its aim in assisting the kidneys via the effects of angiotensin II, its impact on the vasculature can introduce adverse cardiac outcomes such as left ventricular hypertrophy due to hypertension.[30] This impact may be further detrimental especially in the case of present underlying risks for CVDs including atherosclerosis. Angiotensin II and its receptor angiotensin II receptor type I (AT_1) promote inflammation at the vascular wall by several mechanisms including increased oxidative stress via reactive oxygen species, NF-kB-mediated adhesion molecule expression, and cytokine and chemokine release (►**Table 1**).[31] These events vastly contribute to endothelial dysfunction and arterial leukocyte recruitment, which are major drivers of atherosclerotic plaque development.[28] Moreover, RAAS has been shown to enhance insulin resistance. In the clinic, it could be demonstrated that type II diabetes in humans may be dependent on actions of angiotensin II as

Table 1 The role of RAAS in cardiovascular comorbidities associated with severe COVID-19 infection

Pathology	Relevant role of RAAS	References
Hypertension	• RAAS is activated in response to renin released by kidneys with low blood supply and it increases blood pressure via its vasoconstrictive hormone angiotensin II	26–28
	• Zhong et al showed that angiotensin II infusion in ACE2-deficient mice leads to hypertension as well as diastolic dysfunction	55
	• In contrast to angiotensin II produced by ACE, angiotensin (1–7) produced by ACE2 acts as a vasodilator and reduces blood pressure	26–28
Insulin resistance	• RAAS is shown to enhance insulin resistance and thus type II diabetes in humans via angiotensin II	32,33
	• RAAS inhibition by losartan, an angiotensin receptor blocker, in patients showed improved insulin resistance as well as glucose homeostasis	148
	• Angiotensin (1–7)/MasR axis is shown to promote glucose uptake by rat skeletal muscle in vivo and thereby improves insulin sensitivity	43
Obesity	• RAAS is activated in adipose tissue during obesity and promotes adipocyte growth and inflammation	36,39
	• Components of the RAAS were shown to be increased in obese patients	34,35,38
Endothelial dysfunction	• AT_1 receptor in activated RAAS drives endothelial oxidative stress and adhesion molecule expression via the NF-kB pathway, thus impairs endothelial function	28,31
	• Angiotensin (1–7)/MasR axis promotes nitric oxide release	44
Inflammation	• Angiotensin II–AT_1 axis promotes inflammation at the vascular wall via increased oxidative stress and NF-kB-mediated adhesion molecule expression along with cytokine and chemokine release	31,149
	• Angiotensin II supports endothelium–immune cell adhesion by stimulating endothelial vascular cell adhesion molecule-2 via NF-kB	149
	• ACE2/angiotensin (1–7) axis exerts anti-inflammatory and antifibrotic effects by inhibiting the MAPK/NF-kB pathway	45,46

Abbreviations: ACE2, angiotensin-converting enzyme-2; AT_1, angiotensin II receptor type I; RAAS, renin–angiotensin aldosterone system.

several studies have shown improved insulin resistance in patients treated with ACEis as well as ARBs (►**Table 1**).[32,33]

Furthermore, additional components of RAAS, such as aldosterone, renin, and angiotensinogen, were shown to be elevated in the circulation of obese patients revealing a significant link between RAAS and obesity (►**Table 1**).[34–38] RAAS is also upregulated locally in adipose tissue during obesity, which links angiotensin II to increased adipocyte growth and inflammation within the tissue.[39,40] In conclusion, activation of RAAS and thus its predominant effector hormone, angiotensin II, introduces several deleterious consequences which are critical mechanisms driving the pathophysiology of CVDs and its comorbidities.[41] The key switch antagonizing angiotensin II-driven effects of RAAS is the action of ACE2. Although structurally homologous to ACE, the physiological function of ACE2 is actually to counterbalance the functions of ACE and to establish a vital equilibrium in RAAS.[42] By hydrolyzing angiotensin II, ACE2 produces angiotensin (1–7) and ultimately diminishes angiotensin II levels and function. Moreover, angiotensin (1–7) reduces blood pressure by acting as a vasodilator in contrast to angiotensin II. Angiotensin (1–7) and its receptor MAS1 oncogene (Mas) offer further cardioprotective effects such as reduced insulin resistance, antithrombotic effects through nitric oxide release, and decreased inflammation by NF-kB pathway blockade (►**Table 1**).[43–46] Therefore, ACE2 is a crucial regulator of RAAS, overcoming its hostile side effects and thereby supporting cardiac health.[47]

In spite of its extensively protective roles as mentioned above, ACE2 provides an invasion pathway to SARS-CoV-2 via its extracellular domain that is recognized and targeted by the virus to gain intracellular access.[48] The virus expresses a class I fusion protein, known as the Spike (S) protein, on its envelope establishing its characteristic "crown-like" exterior hence its name "corona."[49] The S protein facilitates the engagement of the virus to the host cell via its subunit S1, which possesses the binding region to the extracellular domain of ACE2.[50] Viral attachment is followed by fusion and internalization of the virus into the target cell via the S protein subunit S2.[51] A crucial event enabling the S2 subunit-driven fusion is the priming of the S protein, which is executed by the host transmembrane protease serine 2 (TMPRSS2). Notably, TMPRSS2 is expressed in endothelial cells giving rise to their susceptibility as a target cell. Confirming its role in viral entry, an inhibitor of this serine protease involved in S protein priming can block cellular SARS-CoV-2 entry.[20,52] Internalization of the virus entails endocytosis of the virus presumably along with its bound receptor ACE2. As a result, the virus entry eliminates ACE2 from the cell surface and subsequently attenuates the receptor activity and its protective roles through the angiotensin (1–7)–Mas pathway leading to unbalanced RAAS.[47,53] This is supported by the findings that SARS-CoV-infected mice displayed reduced ACE2 levels in their lungs, which was likewise observed upon the recombinant SARS S protein treatment.[54] Moreover, Zhong and colleagues showed that angiotensin II infusion in ACE2-deficient mice led to hypertension, pathological hypertrophy, myocardial fibrosis, and diastolic dysfunction. However, this phenotype was alleviated in wild-type mice with recombinant human ACE2 (►**Table 1**).[55] Therefore, in addition to the known pulmonary consequences of COVID-19-related inactivation of

ACE2 receptors such as the acute respiratory distress syndrome (ARDS), ACE2 inactivation has great potential to also impair cardiovascular health in several ways.[56,57] Low ACE2 expression, due to various reasons such as older age, diabetes, or hypertension, in patients may increase the severity of SARS-CoV-2 infection.[58] This notion is also in line with the epidemiological statistics revealing that significant numbers of patients facing serious and even fatal manifestations of the COVID-19 consist of elderly and CVD patients.[59]

SARS-CoV-2 and Endothelial Dysfunction

As mentioned previously, SARS-CoV-2 can promote endothelial dysfunction by shifting the balance in RAAS to the angiotensin II/AT_1 axis, which elevates oxidative stress and inflammation. Endothelial dysfunction is characterized by a decrease in nitric oxide levels as a consequence of impaired endothelial nitric oxide synthase function. Nitric oxide is a vasodilator and its deficiency leads to hypertension by constricting the blood vessels, and it can further elicit thrombosis and vascular inflammation.[60–62] In addition to the RAAS-mediated effects, emerging evidence revealed that SARS-CoV-2 can also directly cause endothelial dysfunction by infecting endothelial cells. Varga and colleagues showed accumulation of viral bodies in endothelial cells of several organs, including the kidneys and small intestines, from COVID-19 patients, which was accompanied by increased endothelial cell inflammation and apoptosis.[63] The authors also reported "lymphocytic endotheliitis in lung, heart, kidney, and liver."[63] Moreover, SARS-CoV-2 induces systemic inflammation in the host leading to significantly increased levels of proinflammatory cytokines in the circulation, such as IL-6 and tumor necrosis factor-α (TNF-α).[17] As the vascular endothelium forms a protective layer between the organs and the circulatory system, endothelial cells are constantly exposed to various circulating molecules. Therefore, in the event of SARS-CoV-2-induced cytokine release, endothelial cells are primarily influenced by the potent effects of these inflammatory cytokines. Increased adhesion molecule expression and chemoattractant release are critical processes mediated by activated endothelial cells in response to inflammatory stimuli. These events further augment inflammation of the vascular wall by promoting leukocyte recruitment. In conclusion, SARS-CoV-2 can impair endothelial function by several mechanisms including direct-viral-infection-induced endotheliitis and endothelial injury leading to shifts in the angiotensin II/AT_1 axis and host inflammatory response.

Furthermore, Chen et al point out that pericytes express high levels of ACE2, which was especially increased in patients with basic heart failure leading to their evaluation of pericytes as the "cardiac target cell of SARS-CoV-2."[24] Additionally, the authors speculated that pericyte injury may lead to endothelial dysfunction at the capillary level and might compromise the microcirculation. Further complicating the issue, recent reports on detection of SARS-CoV-2 in the central nervous system (CNS) of COVID-19 patients support the notion that severe illness may be due to CNS involvement and neurological manifestations.[64] In the case of CNS involvement, it is clear that the blood–brain barrier and its endothelium represent a unique setting compared with other endothelial cells in the body due to its specific expression of enzymes and transport molecules.[65] Evidence from earlier SARS and MERS outbreaks suggest that SARS-CoV-2 likely invades the CNS through ACE2 as it does with other tissues; however, additional molecules including CD147 may also play a role in viral entry.[66]

Endothelial dysfunction is a common theme for numerous conditions known to be especially disadvantageous for COVID-19 patients including CVDs and their comorbidities.[67] SARS-CoV-2-driven systemic endothelial cell injury raises the threat of multiple organ failure, and patients who are already suffering from impaired endothelial function due to underlying conditions, like CVDs, are at much higher risk for severe complications of COVID-19. Accordingly, treatment strategies aimed at restoring endothelial function in COVID-19 patients, such as tackling nitric oxide deficiency, should be implemented strictly. For example, phosphodiesterase type 5 (PDE-5) inhibitors are used in the treatment of erectile dysfunction with the aim of restoring NO-mediated erectile smooth muscle relaxation, and the PDE-5 inhibitors, sildenafil and tadalafil, were shown to improve endothelial function by increasing flow-mediated vasodilation in patients with chronic heart failure and type 2 diabetes.[68,69] In addition to its endothelial-protective effects, nitric oxide is proven to protect against the original SARS-CoV. Akerström et al showed that nitric oxide interferes with S protein and ACE2-mediated viral fusion mechanism while also inhibiting viral replication in the early stages.[70,71] Consumption of nitric oxide boosting foods, such as beetroot, may be beneficial to improve the endothelial function and to limit thrombus formation as well as viral infection.[72] In addition, experimental modalities for directly and specifically protecting endothelial cells against damage-induced apoptosis, e.g., microRNA mimics, could be considered.[73]

Inflammation

While lung epithelial and vascular endothelial cell infection is the direct consequence of SARS-CoV-2, viral infection can also elicit severe systemic inflammation that may underlie the cardiovascular complications seen in COVID-19 patients. The severity of SARS-CoV-2 infection has been associated with immune cell dysregulation together with inflammatory cytokine storms (►**Fig. 1**).[17,18]

Pathological analysis of lungs from patients with COVID-19 compared with patients with influenza revealed similar total lymphocytic infiltration; however, CD4+ T cell subsets were increased in COVID-19 patients while CD8+ T cell subsets were decreased.[74] More specifically, CD4+ T cells resembled proinflammatory CC-chemokine receptor 6 (CCR6+) T helper 17 (Th17) cells while CD8+ T cells harbored higher percentages of cytotoxic granules.[75] A genome-wide association study from the Italian and Spanish epicenters observed an association between SARS-CoV-2 infection with polymorphisms at chromosome 3p21, which encodes a cluster genes for the ABO blood group as well as for chemokine receptors including chemokine receptor 9 (CCR9) and C-X-C motif receptor 6 (CXCR6).[76]

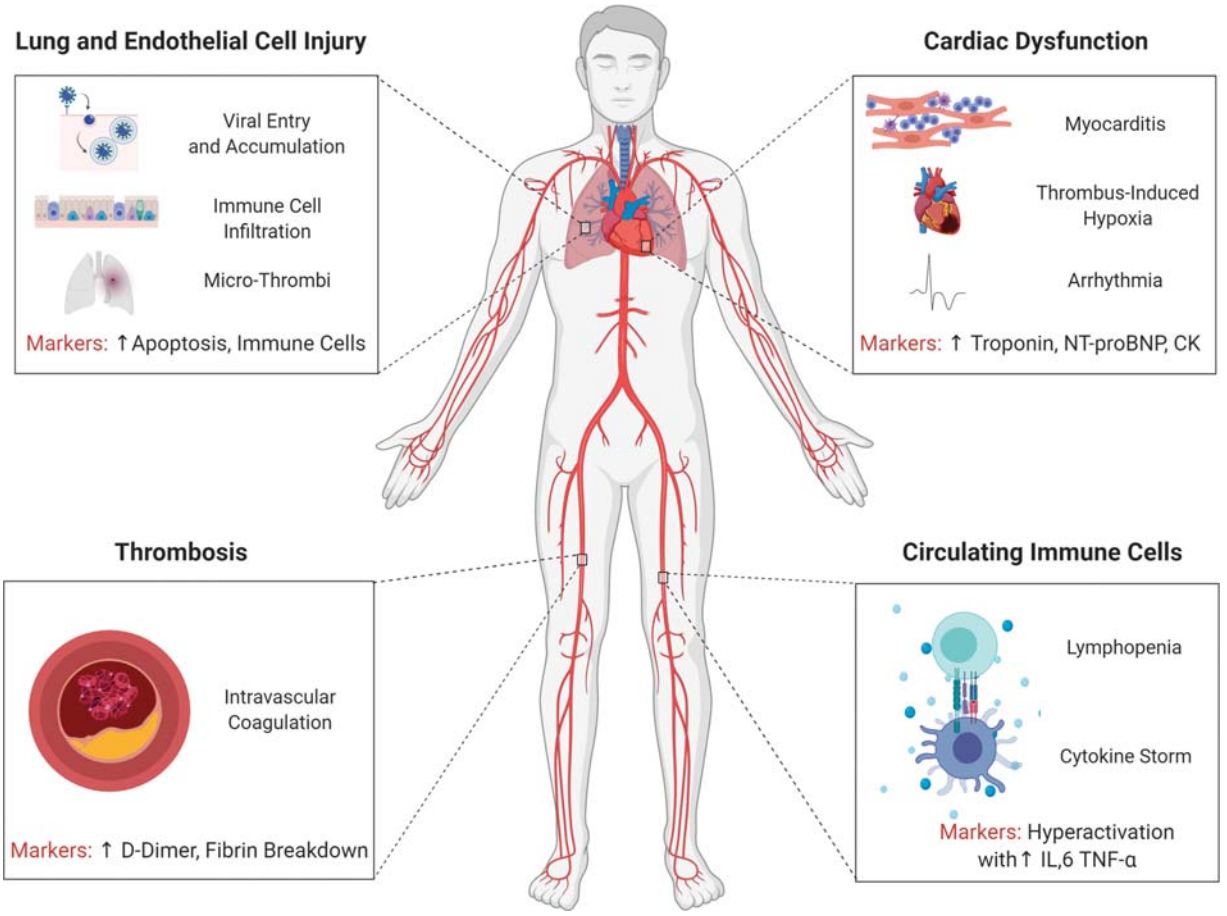

Fig. 1 Summary of systemic effects of SARS-CoV-2 infection on endothelial cells, immune cells, coagulation system, and cardiac inflammation. Viral infection first mediates endothelial dysfunction with observed changes in the RAAS system as well as inflammation, oxidative stress, upregulation of adhesion molecules for leukocyte recruitment, and intravascular coagulation leading toward microthrombi in the lungs. Inflammatory cytokine storms involving expression of macrophage IL-6 and TNF-α leading to hyperactivation and increased apoptosis of lymphocytes characterize systemic inflammation in severe COVID-19 patients. Ultimately, inflammation may be tied to both elevated levels of thrombosis and cardiac injury as observed in markers such as the D-dimer and troponin. Created with Biorender. CK, creatine kinase; IL-6, interleukin-6; NT-proBNP, NT-proB-type natriuretic peptide; RAAS, renin–angiotensin aldosterone system; TNF-α: tumor necrosis factor α.

Interestingly, both chemokines control T cell migration, which may link them to both Th17-mediated lung and atherosclerotic inflammations.[77–79]

Outside of the lungs, significant lymphopenia in the blood is associated with severe infection.[80,81] Flow cytometric analysis revealed that T cells from COVID-19 patients were hyperactivated with increased expression of human leukocyte antigen-DR isotope (HLA-DR) and CD38.[75] Furthermore, hyperactivated T cells from COVID-19 patients were shown to upregulate CD25 and IL-2 expression while T regulatory-associated forehead box P3 (Foxp3) expression was downregulated, which may lead to unregulated T cell proliferation in response to SARS-CoV-2 infection.[82] Clinical characteristics of COVID-19 patients reported cytokine storms with increased concentrations of several inflammatory cytokines including IL-2, IL-6, and TNF-α[17]. Overactivation of proinflammatory Th17 and high cytotoxicity of CD8+ cells may help explain the severe lung injury presented in some COVID-19 patients. Although this cytokine storm may be in part attributed to T cells, several reports have noted a low level of interferon responses in COVID-19 patients suggesting SARS-CoV-2 has more distinct transcriptional response compared with other respiratory viruses.[83,84]

Macrophages, on the other hand, represent another likely source of the cytokine storm. The systemic cytokine profile observed in COVID-19 patients has been compared with macrophage activation syndrome (MAS), which is typically characterized by uncontrolled activation and expansion of both macrophages and T cells.[85,86] In addition to resident lung macrophages, proinflammatory monocyte-derived macrophages appeared to be abundant in the bronchoalveolar fluid of COVID-19 patients. Interestingly, RNA-sequencing (RNA-seq) analysis of those macrophages revealed an upregulation of inflammatory cytokines including IL-1B and IL-6 as well as chemokine receptors such as CCL2 and CCL3 in severe COVID infections, which may suggest recruitment of inflammatory monocytic cells together with neutrophils.[87] The hyperactivation of macrophages with its subsequent cytokine profile may account for the severe lymphopenia observed in COVID-19 patients as one study revealed increased expression of the death receptor FAS on T cells that could mediate activation-induced

Table 2 Potential therapeutics for treating the hyperinflammation observed in severe COVID-19 patients

Potential treatments	Targets and action	References
Anticoagulants	• Low-molecular-weight and unfractionated heparin as first line of treatment to prevent thrombotic events through activation of antithrombin III	112,113
	• Heparin may have additional antiviral and anti-inflammatory properties that prevent viral entry into cells by displacing surface proteoglycans including the S protein of SARS-CoV-2 as well as prevention of vascular-occluding neutrophil extracellular traps	89
	• Danaparoid, typically prescribed to patients with thrombocytopenia and venous thromboembolism, may be a secondary option which inhibits factor Xa and thrombin	112,113
	• Concentrated danaparoid dosage nebulized into the lungs may direct its effect toward the lung, but no published reports exist for COVID-19 usage	112
RAAS inhibitors	• RAAS has been shown to drive inflammation through the angiotensin II–AT_1 axis. Inhibitors of RAAS such as ACEis and ARBs interfere with the ACE2-driven angiotensin II production and angiotensin II binding to its receptor, respectively. Therefore, RAAS inhibitors may decrease RAAS-driven inflammation	31,127
Cytokine-blocking therapies	• Monoclonal antibody treatments targeting cytokines produced during the hyper-inflammatory state in COVID-19 patients have been previously shown to reduce risk in several diseases including atherosclerosis	102,103
	• The COVACTA trial which utilized tocilizumab to target the IL-6 receptor reported that patient status and mortality were not improved after 4 weeks of treatment	104,140
	• Ongoing clinical trials are testing the effectiveness of IL-1 inhibition through the use of high-dose anakinra and canakinumab	103,141,142
Corticosteroids	• Systemic glucocorticoid treatment has been shown to reduce viral shedding in previous SARS and MERS outbreaks on top of their known anti-inflammatory and immunosuppressive effects • The RECOVERY trial demonstrated a 6 mg daily dosage of dexamethasone reduced the 28-day mortality rate of patients receiving oxygen	143,144

Abbreviations: ACE2, angiotensin-converting enzyme-2; ACEi, ACE inhibitor; ARB, angiotensin II receptor blockers; AT_1, angiotensin II receptor type I; IL, interleukin; MERS, Middle East respiratory syndrome; RAAS, renin–angiotensin aldosterone system; SARS, severe acute respiratory syndrome.

cell death.[88] Recently, severe COVID-19 has been characterized by a highly pronounced formation and aggregation of neutrophil extracellular traps (NETs) inside microvessels, leading to rapid occlusion, disturbed microcirculation, and organ damage. Neutrophil granulocytes are strongly activated and adopt a low-density phenotype prone to spontaneously form NETs, and accordingly markers of NET turnover are increased in COVID-19 and linked to disease severity. This process could potentially be targeted by heparin (►**Table 2**).[89]

However, several studies have also reported increased T cell exhaustion in severe infections as noted by increased expression of programmed cell death protein 1 (PD-1) on T cells from COVID-19 patients, which might be a consequence of T cell hyperactivation that leads to lymphopenia.[81,90] Postmortem autopsies revealed that SARS-CoV-2 infection resulted in increased apoptosis of T cells in lymph nodes and spleen, which may be mediated by direct infection though lymphocytic ACE2 expression, which is still questionable.[75,88] In recovering COVID-19 patients, single cell (sc) RNA-seq and T cell receptor sequencing (TCR-seq) revealed high levels of expression for inflammatory genes, but decreased T cell expansion compared with healthy controls further suggesting that T cell exhaustion plays an important role in SARS-CoV-2 infection.[91]

Importantly, inflammation from immune cells like T cells and macrophages plays a key role in CVDs such as atherosclerosis. Due to this inflammation, COVID-19 patients have a higher risk for cardiovascular manifestations including myocardial infarction and stroke.[16,92] A link between acute infections and adverse cardiovascular events has been established, but the cytokine storm observed in severe COVID-19 patients may heighten the risk.[93] Using hyperlipidemic mice models, previous research has established proatherogenic roles for inflammatory cytokines within the cytokine storm such as IL-6 and TNF-α.[94,95] Both cytokines are actively produced by innate and adaptive immune cells, possibly in response to initial complement cascades or innate immune cell inflammasome activation and subsequent IL-1β production[96–98] leading to microvascular injury and thrombotic microangiopathy in some patients with COVID-19 (►**Fig. 1**). Inflammasome activation has been previously linked to pyroptosis of macrophages and endothelial cells leading to massive thrombosis, which may be fundamental to understanding the unusual thrombosis risks associated with COVID-19.[99,100]

IL-1β activates endothelial cells during vascular inflammation to upregulate adhesion molecules allowing leukocytes to infiltrate and expand atherosclerotic lesions. Within the plaque, IL-1β induces collagenase, metalloproteinase, and cytokine expression leading to plaques that are more vulnerable to rupture.[101] Plaque rupture leads to the activation of platelets and thrombosis formation, which may occlude the vessel lumen leading to potential cardiovascular complications. In humans, the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) demonstrated the ability of IL-1β

inhibition to reduce adverse cardiac events.[102,103] Although most studies have not reported an increase in IL-1β levels, IL-1 receptor blockade was associated with clinical improvement in COVID-19 patients without invasive ventilation.[104] Similarly, IL-6 antagonism using tocilizumab has proven to be an effective treatment for severe infection.[105] Considering several studies have reported hypercoagulable states in COVID-19 patients, further studies investigating a link between SARS-CoV-2 infection, vascular inflammation, and atherothrombosis are needed.

Thrombosis

In severe COVID-19 cases, patients develop a type of ARDS, which is characterized by alveolar damage and fibrosis that may be due to the infiltration of immune cells and cytokines as mentioned previously. Fibrin deposition may be a consequence of the hyperactivation of macrophages and T cells during MAS, which leads to increased endothelial cell damage and diffuse lung injury.[106] Supporting the inflammatory hypothesis for increased thrombosis, serum proteomic analysis revealed that elevated IL-6 was a critical marker for upregulation of coagulation markers including Factor 5, 7, and 10 in the most severe COVID-19 patients.[107] Thromboelastometry measures may be beneficial to distinguish the difference in hypercoagulability of mild and severe cases as prolonged clot formation time and ThromboDynamic Index were reported in critically ill patients needing invasive ventilation.[108] Ultimately, severe SARS-CoV-2 infection presents with pulmonary intravascular coagulation that appears to be similar to disseminated intravascular coagulation.[106] Several studies observe consistent hematological parameters such as increased D-Dimer with moderate thrombocytopenia that support an increase in thrombus formation as well as the breakdown of fibrin products (►**Fig. 1**).[4,9,109] Further complicating the issue, a small study comparing the clot lysis between control samples and COVID-19 samples described impaired lysis pointing to fibrinolytic resistance on top of the hypercoagulability during severe SARS-CoV-2 infection.[110]

When compared with influenza patients, COVID-19 patients had nine times as many alveolar capillary microthrombi leading to significant capillary occlusion.[74] A series of autopsies found an interesting link between the increase in thrombosis and ACE2 expression. Thrombotic microangiopathy was not observed in tissues not expressing ACE2 such as vasculature of the kidneys; however, multiple thrombotic events were discovered in ACE2-expressing lung and brain parenchymal capillaries.[111] Therapeutic anticoagulant treatment has been associated with decreased mortality in COVID-19 patients highlighting thrombosis as a critical turning point in SARS-CoV-2 infection.[112] Specifically, low-molecular-weight and unfractionated heparin has been proposed as the first line of treatment, which may possess both anti-inflammatory and antiviral properties via disrupting viral interaction with ACE2 (►**Table 2**).[113,114] More targeted anticoagulant therapies, including inhaled danaparoid, may allow a directed approach to tailor treatment toward the thrombus-induced inflammation in the lungs.[113]

While microthrombi contribute to the development of respiratory dysfunction, they may also lead to multiorgan damage including cardiovascular complications such as heart failure. Lung injury due to increased thrombosis may induce pulmonary hypertension, which leads to observable increases in cardiac troponin, creatine kinase (CK), and N-terminal pro-B type natriuretic peptide levels in critically ill COVID-19 patients.[6,11,18,115] Outcomes from an in-hospital study reported 32% of COVID-19 patients had heart failure. However, the numbers were skewed toward nonsurvivors when comparing nonsurvivors (52% heart failure) to survivors (12% heart failure), suggesting heart failure may correlate with disease severity rather than infection itself.[8] Similarly, in a cohort of 799 COVID-19 patients, heart failure was the second most common cause of death after ARDS.[116] The combination of right ventricular heart failure together with lung fibrosis might contribute to decreased lung perfusion leading to a hypoxic state observed in severe cases.[117] Interestingly, one postmortem study observed increased pulmonary angiogenesis in COVID-19 patients, suggesting new vessel growth was necessary for accurate lung perfusion.[74] Furthermore, arterial or venous thrombosis accounted for 16.4% of COVID-19 hospital readmissions in a cohort of 1,368 patients likely influencing ischemic conditions in severe cases.[118] Preexisting cardiovascular comorbidities including hypertension and diabetes were associated with COVID-19 case severity, which likely exacerbate heart failure and other cardiac injuries.[4,17,19]

Cardiac Injury

Although heart failure represents one side of the cardiac injury involved in SARS-CoV-2 infection, several studies have also reported cardiac arrhythmia and myocarditis in COVID-19 patients. A retrospective study identified the heart was the earliest damaged tissue after the lungs following SARS-CoV-2 infection.[119] While most studies have not investigated specific arrhythmias, one report observed arrhythmic complication in 16.7% of COVID-19 patients, making it the most common complication after ARDS (►**Fig. 1**).[18] Furthermore, arrhythmias may manifest in more severe cases as one report found arrhythmias more often in patients admitted to an intensive care unit.[18] Thrombus-induced hypoxia or inflammation may in part explain the high prevalence of arrhythmias in COVID-19 patients especially those with preexisting cardiovascular risk factors.[120] However, elevated cardiac troponin and CK levels may indicate an underlying myocardial inflammation considering irregular ventricular arrhythmias can be associated with myocarditis.[121,122]

To date, there is limited clinical evidence of myocarditis with only a few case reports in COVID-19 patients all with varying degrees of myocardial inflammation (►**Fig. 1**).[6,7,123–125] However, the mechanism behind this cardiac injury in COVID-19 remains unclear particularly as a primary or secondary effect of SARS-CoV-2 infection. Interestingly, the receptor for viral entry, ACE2, is expressed in pericytes of the cardiovascular system, and its expression appears to be upregulated in failing hearts.[24] Additionally, single-cell RNA sequencing revealed that cardiomyocytes also express ACE2, which was

upregulated in patients receiving ACEis for pre-existing cardiovascular conditions.[25] However, COVID-19 patients present acute cardiac injury symptoms on average 15 days after the onset of symptoms suggesting direct infection may not be the likely cause of myocardial inflammation.[8,126] A secondary immune-mediated effect of SARS-CoV-2 might be the more likely explanation considering the timeline of symptoms. Particularly, inflammatory hyperactivation, as observed by the cytokine storm, and subsequent increase in inflammatory biomarkers have been associated with myocardial damage and cardiac injury.[11,127] To distinguish the cardiac injury mechanisms at play in SARS-CoV-2 infection, further investigation requires a systematic elevation of larger cohorts of severe COVID-19 cases as well as experimental work using both in vitro and in vivo models.

Outlook for Targeting SARS-CoV-2 Inflammation

Hyperinflammation appears to be a common theme in the immunomodulatory, thrombotic, and cardiovascular complications associated with SARS-CoV-2 infection. Therefore, a variety of anti-inflammatory treatments have been purposed for severe COVID-19, including RAAS inhibitors, cytokine-blocking therapies, and corticosteroids. However, preliminary evidence for each therapy demonstrates both advantages and disadvantages depending on their target.

Inhibitors of RAAS, such as ACEis and ARBs, are widely used to treat hypertension. In the context of atherosclerosis, these inhibitors were also shown to be effective in suppressing inflammation as well as oxidative stress (►**Table 2**).[128] The use of RAAS blockers in COVID-19 patients, however, has caused a great dilemma among health care workers, due to their probable impact on ACE2-SARS-CoV-2 dynamics.[129–131] Keidar and colleagues showed that mineralocorticoid receptor blockade via spironolactone, aimed at hindering the activity of aldosterone, increased ACE2 expression and activity in monocyte-derived macrophages collected from patients with congestive heart failure.[132] Another study showed that an ARB named telmisartan reduced ACE2 levels in the aorta of spontaneous hypertensive rats.[133] Moreover, Ferrario and colleagues revealed that the treatment of Lewis rats with angiotensin II receptor antagonist losartan increased cardiac messenger RNA (mRNA) levels and activity of ACE2.[134] In view of that, RAAS inhibitor-related ACE2 upregulation has been hypothesized by several scientists to increase the risk and incidence of SARS-CoV-2 infection as there would be theoretically more doorways available for the virus entry.[135] Moreover, there is no scientific evidence to support the theoretical concern that RAAS[136] blockers may increase the threat or severity of the SARS-CoV-2 infection. Meanwhile, Milne and colleagues tested mRNA expression levels of ACE2 in human lung tissues upon ACEi and ARB treatments and disclosed a decrease in ACE2 levels via ACEi treatment whereas ARB treatment did not cause any differences.[137]

Of note, several studies investigating the association between the risk of SARS-CoV-2 infection and the use of RAAS inhibitors disclosed that RAAS inhibitors do not impose an increased risk of viral infection. Mancia and colleagues reported no association between the use of ACEi and ARB and COVID-19 in a case–control study in Lombardy, Italy with a total of 6,272 cases and 30,759 matched controls.[138] Another study in New York City, United States evaluating the connection between the likelihood of testing positive for COVID-19 as well as the severity of the disease and the use of RAAS inhibitors among other treatments, such as β-blockers and calcium-channel blockers, disclosed no association between any of these treatments and the risk of infection as well as the disease severity.[139] Finally, another study by Mehra and colleagues with a database from 169 hospitals in Asia, Europe, and North America reported that underlying CVDs are indeed associated with an increased risk of death among the hospitalized COVID-19 patients, whereas ACEi and ARB treatment was not associated with in-hospital death.[59]

It seems, regardless of its amount, the presence of ACE2 is sufficient to support virus entry and a decrease of the receptor activity empowers the severity of the illness due to the abolished protective roles of ACE2. In this case, targeting increased activity of ACE2 may be of benefit rather than a disadvantage to restrict the impact of the COVID-19, both for the pulmonary and cardiovascular systems.[140] Besides, COVID-19 has been shown to cause a strong inflammatory response in patients, leading to a so-called cytokine storm, which also strongly contributes to an ARDS.[17] This rise in inflammation further feeds chronic inflammatory diseases, such as atherosclerosis, and therefore worsens the patients' prognosis. The immunomodulatory benefit of the ACE2–angiotensin (1–7)–Mas axis contrasting the proinflammatory role of RAAS is especially advantageous with regard to the management of the inflammation and therefore the manifestations of chronic inflammatory diseases.

The massive immune response observed during SARS-CoV-2 infection has prompted a search for therapeutics primarily targeting the inflammatory cytokine storm. Unlike broad immunosuppression, cytokine-blocking therapies such as those targeting IL-6 and IL-1β likely should not dampen the host's response to the virus. While initial reports of IL-6R antagonists were promising, the results from phase III of the COVACTA trial recently announced that tocilizumab did not meet its primary endpoint of improved clinical status of COVID-19 patients (►**Table 2**).[104,141] Nevertheless, clinical trials are still pursuing the clinical relevance of other cytokine-blocking therapies including IL-1β inhibition using anakinra and canakinumab (►**Table 2**).[142,143]

A recent breakthrough heralded in a press release only at the time of submission may provide further evidence of a more global role of excessive inflammation and the importance of its control in COVID-19. The randomized controlled RECOVERY trial enrolled 2,100 patients who received a low dose of the corticosteroid dexamethasone for 10 days, and compared them against 4,300 patients who received standard care. The results revealed a striking effect of dexamethasone among critically ill patients on ventilators and those receiving oxygen therapy, reducing their mortality by up to 30% (►**Table 2**).[144] Dexamethasone is a type of glucocorticoid, which are known to exert potent anti-inflammatory

effects and are therefore used in the treatment of several autoimmune and inflammatory diseases such as asthma and ulcerative colitis.[145] Therefore, glucocorticoids may be very useful in the treatment of heightened immune response to COVID-19, including the cytokine storms. Moreover, dexamethasone treatment might potentially offer further benefits to patients besides immunosuppression. Despite the association of glucocorticoids with venous thromboembolism,[146] a study by van Giezen et al investigating hemostatic effects of dexamethasone on rats showed a twofold decrease in arterial thrombosis and reduced platelet aggregation with low-dose treatments (up to 1 mg/kg).[147] It is important to note, however, that higher doses of dexamethasone (from 1 mg/kg onwards) yielded a decrease in fibrinolytic activity and counteracted the arterial thrombosis. Further research is needed to explore such potential benefits of dexamethasone as well as its dose-dependent effects.

Without a doubt, COVID-19 presents an immense challenge for the health care system due to its wide-ranging impact on the health of diverse groups of patients. Although severe COVID-19 cases typically present with similar thrombotic and inflammatory characteristics, data describing the most representative biomarkers are still evolving. Therefore, future treatments for thromboinflammation may need to be tailored to better fit the patients' individual needs.[148] The safety of the drugs intended to treat COVID-19 patients should be carefully considered, especially for those with underlying health problems, such as CVDs. Larger studies investigating these drugs in the context of CVDs are needed to identify groups of patients who are at higher risk for suffering from serious and even lethal consequences of these treatments.

Conclusion

In this review, we aimed to highlight the immunoinflammatory mechanisms and subsequent thrombohemostatic and cardiovascular effects of COVID-19 especially in patients with underlying cardiovascular risk factors. Considering the novel nature of the virus, our knowledge is still growing with regard to the systemic and local effects of SARS-CoV-2 infection. With this in mind, many questions remain unanswered about the primary and secondary causes of the cardiovascular manifestations of COVID-19 patients. In the upcoming months, systematic analyses of larger patient cohorts, in particular at a genome-wide genetic level, are needed to dissect and explain differential predisposition in different blood groups and ethnicities. Together with experimental work, researchers may be able to shed more light on the identification of the underlying mechanisms of inflammation, thrombosis, and cardiac injury in COVID-19 patients.[79] In the meantime, careful evaluation of new therapeutics for SARS-CoV-2 should highlight their effects on the cardiovascular system as many studies have observed cardiovascular complications ranging from ischemic stroke to myocarditis in severe cases especially with hypertensive and diabetic patients. Considering we have seen several similar coronaviruses in the past, careful and thorough research in SARS-CoV-2 will likely improve our understanding of future coronaviruses.

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Conflict of Interest
None declared.

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