Arteriosclerosis, Thrombosis, and Vascular Biology

BRIEF REVIEW

Overcoming Barriers

The Endothelium As a Linchpin of Coronavirus Disease 2019 Pathogenesis?

Dakota Gustafson, Sneha Raju, Ruilin Wu, Crizza Ching, Shawn Veitch, Kumaragurubaran Rathnakumar, Emilie Boudreau, Kathryn L. Howe, Jason E. Fish

OBJECTIVE: Coronavirus disease 2019 (COVID-19) is a global pandemic involving >5 500 000 cases worldwide as of May 26, 2020. The culprit is the severe acute respiratory syndrome coronavirus-2, which invades cells by binding to ACE2 (angiotensin-converting enzyme 2). While the majority of patients mount an appropriate antiviral response and recover at home, others progress to respiratory distress requiring hospital admission for supplemental oxygen. In severe cases, deterioration to acute respiratory distress syndrome necessitating mechanical ventilation, development of severe thrombotic events, or cardiac injury and dysfunction occurs. In this review, we highlight what is known to date about COVID-19 and cardiovascular risk, focusing in on the putative role of the endothelium in disease susceptibility and pathogenesis.

APPROACH AND RESULTS: Cytokine-driven vascular leak in the lung alveolar-endothelial interface facilitates acute lung injury in the setting of viral infection. Given that the virus affects multiple organs, including the heart, it likely gains access into systemic circulation by infecting or passing from the respiratory epithelium to the endothelium for viral dissemination. Indeed, cardiovascular complications of COVID-19 are highly prevalent and include acute cardiac injury, myocarditis, and a hypercoagulable state, all of which may be influenced by altered endothelial function. Notably, the disease course is worse in individuals with preexisting comorbidities that involve endothelial dysfunction and may be linked to elevated ACE2 expression, such as diabetes mellitus, hypertension, and cardiovascular disease.

CONCLUSIONS: Rapidly emerging data on COVID-19, together with results from studies on severe acute respiratory syndrome coronavirus-1, are providing insight into how endothelial dysfunction may contribute to the pandemic that is paralyzing the globe. This may, in turn, inform the design of biomarkers predictive of disease course, as well as therapeutics targeting pathogenic endothelial responses.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: biomarkers ■ cardiac injury ■ coronavirus ■ endothelium ■ pandemic ■ thrombosis

Emerging and re-emerging zoonotic diseases represent a persistent and unpredictable threat to global health. In the last 2 decades, the world has witnessed 2 major epidemics from genetically distinct and highly infectious betacoronaviruses; severe acute respiratory syndrome coronavirus (SARS-CoV-1, 2002) and middle east respiratory coronavirus (MERS-CoV, 2012).[1] Although causing respiratory, enteric, hepatic, and neurological diseases of varying severity, the cardiovascular complications associated with infection have been underappreciated until recently.

In early December 2019, China reported multiple cases of pneumonia of unknown etiology occurring in Wuhan, Hubei, China.[2] By early January, viral sequencing identified the causative agent as a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with a 75% to 80% sequence identity to SARS-CoV-1. Between the index case in early December and May 26, 2020, >5 500 000 cases and >300 000 deaths due to coronavirus disease 2019 (COVID-19) were reported worldwide. Similar to SARS-CoV-1 and MERS-CoV, the spectrum of clinical presentation can range from asymptomatic

Correspondence to: Kathryn L. Howe, MD, PhD, Toronto General Hospital Research Institute, Peter Munk Cardiac Centre, University Health Network, Division of Vascular Surgery, Department of Surgery, University of Toronto, 200 Elizabeth St, 6 EN-220 Toronto General Hospital, Toronto, ON, Canada, M5G 2C4, Email kathryn.howe@uhn.ca; or Jason E. Fish, PhD, Toronto General Hospital Research Institute, University Health Network, 101 College St, 3-309 Princess Margaret Cancer Research Tower, Toronto, ON, Canada, M5G 1L7, Email jason.fish@utoronto.ca

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Nonstandard Abbreviations and Acronyms

ACE2	angiotensin-converting enzyme 2
Ang II	angiotensin II
Angpt2	angiopoietin-2
ARDS	acute respiratory distress syndrome
COVID-19	coronavirus disease 2019
DIC	disseminated intravascular coagulation
ECs	endothelial cells
MERS-CoV	Middle east respiratory coronavirus
NO	nitric oxide
RAAS	renin-angiotensin-aldosterone system
SARS-CoV-1	severe acute respiratory syndrome coronavirus-1
vWF	von Willebrand Factor

Highlights

- Preexisting chronic endothelial dysfunction coupled with virus-mediated endothelial activation, permeability, and hypercoagulable state is likely a driving factor in Coronavirus disease 2019 (COVID-19) severity.
- Compensatory over-expression of ACE2 (angiotensin-converting enzyme 2) in the vasculature of patients with cardiovascular comorbidities may increase viral infection and dissemination.
- The endothelium may be considered as a linchpin in COVID-19 pathogenesis; blocking viral binding to ACE2 receptors on the endothelium and preventing capillary barrier disruption and the hypercoagulable state should be explored as therapies for COVID-19.

infection to severe disease. Early clinical studies revealed that initial symptoms of COVID-19 are consistent with viral pneumonia, most commonly fever (≈88%–89%), cough (≈57%–68%), and myalgia or fatigue (≈36%).[3] Although a majority of individuals seem to mount a controlled and appropriate viral response, others decompensate into respiratory distress requiring hospital admission for supplemental oxygen, or worse, develop acute respiratory distress syndrome (ARDS) necessitating mechanical ventilation and intensive care unit level care. Early meta-analyses of COVID-19 patient clinical characteristics revealed that ≈12% to 20% of all cases require intensive care, with the case fatality rate of admitted patients ranging from ≈4% to 26%.[4,5] While the cause of death is largely related to ARDS, there is a strong correlation between markers of cardiac injury and mortality, with up to 7% dying as a result of substantial myocardial damage and 33% dying of combined cardiopulmonary and cardiovascular failure.[6–9] Additionally, thrombotic events, both venous and arterial in nature, have been commonly observed in patients with COVID-19, further highlighting that cardiovascular complications are likely a strong predictor and contributor to the high mortality rate.[8,10,11]

Although rapidly evolving, our knowledge of COVID-19 pathophysiology remains incomplete. Consequently, our understanding of cardiovascular biology and the role of the endothelium is seen through the lens of lessons from SARS-CoV-1 and MERS-CoV, as well as emerging COVID-19 pathology reports.[11,12] While elements of this review may be speculative regarding the role of the endothelium in COVID-19, a case series showing that SARS-CoV-2 infects endothelial cells causing endotheliitis,[12] has begun to shed light on the unique pathogenesis of COVID-19 (Figure 1). Importantly, in this review, we endeavor to highlight research areas that will further our understanding and may lead to the development of therapies that address endothelial dysfunction.

VIRAL ENTRY TO ENDOTHELIAL CELLS AND THE ROLE OF ACE2

At the cellular level, the pulmonary endothelium forms a key part of the alveolar-capillary unit, providing an interface for efficient gas exchange between the alveolar space and red blood cells within the capillaries of the lung. Under homeostatic conditions, the endothelial barrier is maintained through interactions between adjacent endothelial cells (ECs) via intercellular junctions—tight junctions and adherens junctions—that are tethered to the actin cytoskeleton.[13] As a consequence of severe viral infection of the lower respiratory tract epithelium, there is attendant dysfunction and destruction of alveolar epithelia, with loss of junctional complexes between ECs leading to enhanced permeability of the capillary endothelium and edema in the lung. This is mediated in part by the impact of proinflammatory cytokines (referred to as cytokine storm) that are produced during the antiviral innate immune response.[14] Subsequent loss of surfactant production, together with alveolar collapse and fluid accumulation, leads to acute lung injury, pneumonia, and ARDS: which are common complications of viral infection. Clinical studies have revealed that ≈29% of hospitalized patients diagnosed with COVID-19 develop ARDS, with up to 32% of these patients requiring intensive care.[15,16] Concordant upregulation of EC inflammatory cascades promotes leukocyte recruitment, further driving EC activation, as well as activating coagulation pathways.[14] Indeed, the endothelium has been shown to be a central orchestrator of cytokine storm and tissue damage during lung viral infections.[17]

Strategically located between the circulation and other vascular layers, the endothelium is responsible for dictating niche-specific vascular homeostasis through the

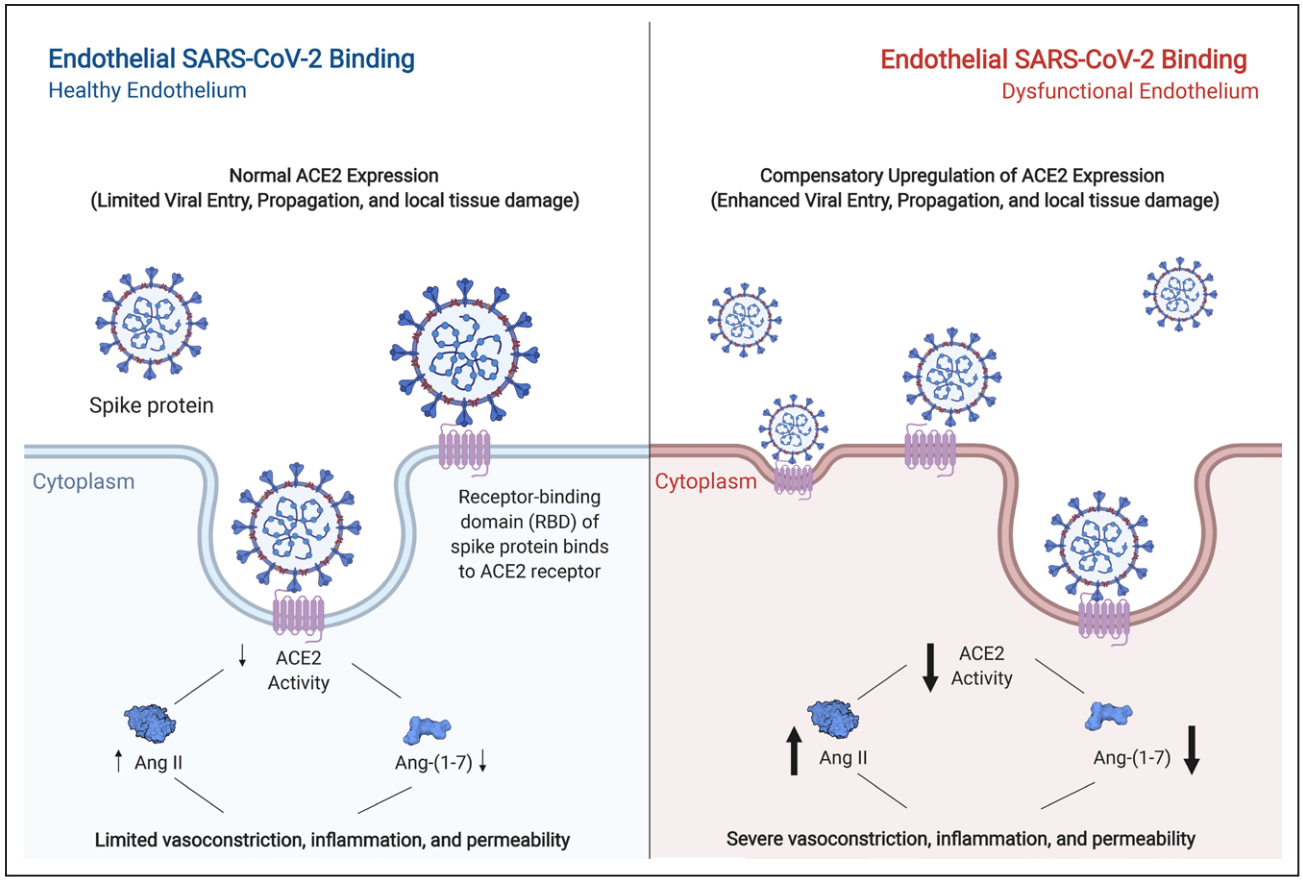

Figure 1. Proposed model of the pathological effect of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) on the endothelium.

Left, under conditions of endothelial quiescence, binding of SARS-CoV-2 is mediated in part by ACE2 (angiotensin-converting enzyme 2). Internalization of ACE2, and subsequent receptor interference, results in an upregulation of AngII (angiotensin II) and downregulation of Ang-(1-7) (angiotensin-[1-7]). Although contributing to vasoconstriction, inflammation, and permeability, limited expression of ACE2 within a relatively quiescent endothelium may result in limited viral entry and local and systemic dysfunction. Right, paradoxically, although a dysfunctional endothelium may have higher baseline levels of ACE2, enhanced viral entry may increase the degradation of ACE2 in the lysosome, enhancing inflammation and tissue damage. The resulting downregulation of ACE2 activity, upregulation of Ang-II, and downregulation of Ang-(1-7) thereby may have a starker induction of vasoconstriction, inflammation, and permeability as compensatory mechanisms are abrogated. Arrow width corresponds to intensity. This figure was created with the assistance of www.BioRender.com.

balanced release of autocrine and paracrine molecules. In conditions of dysfunction, this balance is disrupted, making the endothelium susceptible to vasoconstriction, platelet activation, thrombosis, and vascular inflammation, among other detrimental effects. Fundamental features of EC dysfunction include reduced local production of the vasodilator NO (nitric oxide), decreased anticoagulant factors (eg, heparan and dipeptidyl peptidase-4), increased secretion of vWF (von Willebrand Factor) and tissue factor, upregulation of leukocyte adhesion molecules (eg, intercellular adhesion molecule 1, E-Selectin, P-Selectin), and increased generation of reactive oxygen species; all of which lead to compromised vascular homeostasis and inflammation.[18–21]

Among pathways responsible for the development of endothelial dysfunction, the renin-angiotensin-aldosterone system (RAAS) is a pivotal signalling axis. RAAS is a major regulator of blood pressure, fluid and electrolyte balance, cardiovascular homeostasis, and facilitates a complex series of enzymatic reactions that culminate in the generation of Ang II (angiotensin II); a potent vasoconstrictor and proinflammatory effector molecule.[22,23] Although the initial conversion of Ang I to Ang II by angiotensin-converting enzyme 1 represents an important process in cardiovascular signalling, it is the negative regulator of this process, ACE2 (angiotensin-converting enzyme 2), which warrants in-depth examination in COVID-19. ACE2, a type I transmembrane zinc metallocarboxypeptidase, counterbalances angiotensin-converting enzyme 1 by converting Ang I and Ang II into Ang-(1-9) and Ang-(1-7), respectively, facilitating the attendant cardioprotective actions of NO release, vasodilation, and blunting of inflammation.[24] The ability of ACE2 to provide negative regulation of Ang II and the receptor (angiotensin II receptor type 1) is of particular biological significance in pathological conditions where RAAS may be dysfunctional. In line with these observations, ACE2 broadly influences the cardiovascular function of multiple

organs including the systemic vasculature, kidneys, liver, heart, and lungs through its broad expression in arterial and venous ECs, as well as vascular smooth muscle.[25]

Given its integral involvement in the regulation of cardiovascular function, it is unsurprising that ACE2 is dysregulated in numerous cardiovascular pathologies. Several clinical and experimental observations have suggested a beneficial role for ACE2 in cardiovascular function; yet, paradoxically, ACE2 expression is elevated at various stages of cardiovascular pathologies.[25] Among comorbidities prevalent in the general population, diabetics have increased levels of circulating ACE2 when micro- and/or macrovascular disease is present, suggesting that the upregulation of ACE2 may act as a compensatory mechanism in maintaining homeostatic vascular function as a means to counteract RAAS activation.[26,27] Increases in ACE2 have also been observed in cardiac tissue of patients with ischemic heart failure, idiopathic dilated cardiomyopathy, and pulmonary hypertension.[28,29]

In the setting of SARS-CoV-2 infection, enhanced levels of ACE2 may facilitate viral entry into multiple tissues (Figure 2).[30] Recent cryo-electron microscopy studies have demonstrated that similar to SARS-CoV-1, the major spike glycoprotein (S1) of SARS-CoV-2 is capable of direct binding to ACE2, albeit with a 10- to 20-fold greater affinity.[31–33] Although ACE2 has a seemingly prominent role in mediating viral entry as well as dictating cardiovascular phenotype, several other co-receptors/factors are likely involved in the process including transmembrane serine protease type II,[34] which is expressed on ECs[35,36] and plays a crucial role in viral entry by cleaving the viral spike protein into functional S1 and S2 subunits. While SARS-CoV-2 initially infects epithelial cells in the upper and lower respiratory tracts by binding to ACE2, viral dissemination to other organs requires the virus to cross the endothelium and enter the bloodstream. Indeed, SARS-CoV-2 RNA can be detected in the blood of patients that progress to severe disease and SARS-CoV-1 and SARS-CoV-2 have been shown to infect ECs, which are known to express ACE2 on their surface.[37–40] Importantly, previous preclinical studies with the H5N1 virus revealed that blocking viral replication in the endothelium limits systemic viral spread, disease symptoms, and mortality, emphasizing the importance of infection of the endothelium in viral dissemination.[41] Intriguingly, viral entry leads to internalization and degradation of ACE2, reducing its bioavailability, which may precariously reduce the much-needed activity of ACE2 in an already strained system.[30,34,42] Indeed, in animal models, loss of ACE2 activity has been linked to decreased cardiac contractility (reduced systolic function), coronary vasoconstriction, microcirculatory dysfunction, and reductions in myocardial blood flow,[25,28] while in the context of lung injury, loss of ACE2 results in worsened lung function, increased inflammatory cell infiltration, enhanced microvascular permeability, and decreased blood oxygenation.[43]

COVID-19 AND THE ENDOTHELIUM IN VASCULAR AND CARDIAC COMPLICATIONS

Although COVID-19 is primarily considered a respiratory infection, it has important systemic effects on the cardiovascular system. Similar to other viral infections (eg, influenza, SARS-CoV-1, and MERS-CoV), SARS-CoV-2 infection seems to more readily predispose individuals with comorbidities to cardiopulmonary failure and death; perhaps as a result of existing dysregulation of the RAAS.[44–46] In several large cohort studies, the clinical prevalence of these comorbidities among confirmed COVID-19 patients ranged between 8.3% and 10.5%; 7.3% for diabetes mellitus, 6.5% to 15.0% for hypertension, 2.5% for coronary artery diseases, and 1.4% for cerebrovascular disease.[47] Recent reports have described a high prevalence of at least one comorbidity among intensive care unit-admitted COVID-19 patients, ranging from ≈68% (Italian cohort) to ≈72% (Chinese cohort).[16,48] Hypertension and diabetes mellitus seem to be the most common, followed by other cardiovascular and pulmonary comorbidities.[49] This has raised the question as to whether these comorbidities share feature(s) that put individuals at higher risk.[50,51] As a result of these observations, collaborative efforts such as the COVID-19 host genetics initiative have been launched to better understand the role of host factors on COVID-19 susceptibility and severity.[52] In line with epidemiological observations, there are noted changes in RAAS with these conditions,[21] where the earliest stages of these disease conditions are often marked by structural and functional changes of the endothelium into a dysfunctional state (Figure 2).[53]

Similar to other viral outbreaks, worsening prognosis and increased adverse cardiovascular events associated with COVID-19 have been linked to the severity of inflammation and resulting cardiac dysfunction.[54] Observed abnormalities from laboratory tests, including elevation of cardiac-centric molecules such as troponins and BNP, as well as increases in endothelial-centric factors such as vWF and D-dimers have been reported in patients with COVID-19, reflective of cardiac damage and increased thrombosis risk, respectively.[3–5] Although the underlying mechanisms remain elusive, the importance of inflammation and their procoagulative effects highlight the need to focus on the role of the endothelium in virally induced cardiovascular pathology. While cardiac outcomes are prominent in older patients with COVID-19 with antecedent cardiovascular risk including hypertension, diabetes mellitus, and previous stroke, cardiac effects have also been observed in young and otherwise healthy patients with no known comorbidities.[55] A key clinical question is whether the virus (1) directly infects cells within the heart, including the vascular endothelium, to cause pathology; (2) impairs

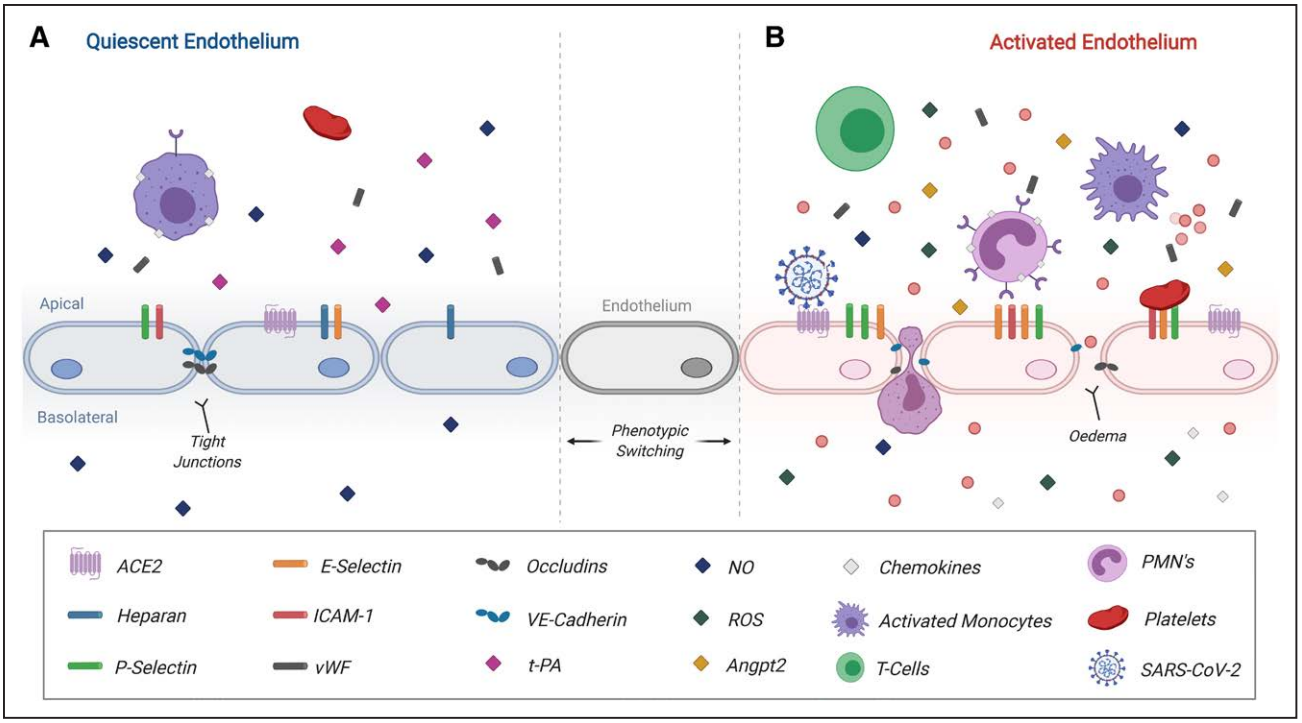

Figure 2. Proposed pathobiology of activated endothelium during severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2 infection).

A, A quiescent endothelium is shown on the left. Endothelial quiescence facilitates normal responses to angiogenic signalling, facilitates controlled immune surveillance, and provides homeostatic cues for coagulation. **B**, Chronic activation of the endothelium is marked by stark upregulation of P-selectin, E-Selectin, ICAM-1, and ACE2 (angiotensin-converting enzyme 2), along with their respective soluble forms. The subsequent loss of tight junction expression expedites edema and facilitates enhanced recruitment, attachment, and extravasation of immune cells across the vascular endothelium. Disruption of coagulation cues through the loss of heparan, DDP-4, and t-PA (tissue-type plasminogen activator) and secretion of vWF (von Willebrand Factor) exacerbates endothelial injury and induces coagulopathies. Further disruption of endothelial phenotype results in local reductions of secreted NO, increases in secreted reactive oxygen species (ROS), and enhanced Angpt2 secretion. SARS-CoV-2 reduces ACE2 availability further propagating endothelial dysfunction. While intended to assist in infection control, innate and adaptive immune responses can instead induce a storm of chemokines and cytokines, which further propagates underlying inflammation and dysfunction. This figure was created with the assistance of www.BioRender.com. ICAM-1 indicates intercellular adhesion molecule; NO, nitric oxide; PMN, polymorphonuclear leukocyte; and VE-Cadherin, vascular endothelial cadherin.

cardiac function indirectly through pulmonary insufficiency and increased cardiac load; and/or (3) damages the heart via the host response (ie, cytokine storm), inducing systemic inflammation and disseminated intravascular coagulation (DIC). Indeed, it may be that all of these scenarios occur simultaneously.

Although ACE2 expressed on the pulmonary epithelium facilitates viral entry, the antiviral response—including contributions by the pulmonary endothelium—may subsequently drive systemic cytokine storm, whereby ACE2 expressed on cells of the cardiovascular system may facilitate viral propagation and subsequent dissemination to the heart.[17] Notably, data from a small number of SARS-CoV-1 patients demonstrated that 35% of autopsied heart tissue had evidence of viral infection in the heart, with an average viral load of 4×10^6 copies/gram of tissue.[56] Importantly, there was also evidence of significantly decreased ACE2 expression in these hearts, implying that viral infection impairs ACE2-dependent functions.[56] Patients with COVID-19 and cardiac involvement had a significantly shorter duration of illness, suggestive of a more aggressive course.[57] It is possible that ACE2 expression in either the endothelium or myocardium of the heart may correlate with the severity of COVID-19 infection. This will require detailed pathological analysis of post-mortem tissues.[56] Unfortunately, there are scant pathology reports from patients with COVID-19 at this time. Of the available evidence, results of biopsies from multiple tissues in 3 patients who died from COVID-19 reported that blood vessels in the lung were congested, edematous with immune cell infiltration, and had hyaline thrombi in a small number of microvessels.[58] It is unclear whether the virus directly infects cells in the heart. Interestingly, single-cell sequencing datasets have revealed that 7.5% of myocardial cells express the mRNA that encodes for the ACE2 receptor.[59] Additionally, another recent single-cell atlas of abandoned donor hearts demonstrated high ACE2 mRNA expression in pericytes with the dominant EC-pericyte crosstalk between EC receptors and pericyte ligands suggesting that SARS-CoV-2 may affect the cardiac microvasculature.[60] Perhaps the most convincing experimental evidence to date of SARS-CoV-2 infection

of the vasculature was shown by Monteil et al,[39] who infected capillary organoids containing endothelial and pericyte cells with SARS-CoV-2 and showed that not only can viral RNA be recovered post-infection but also that progeny virus captured from supernatants are capable of infecting VeroE6 cells. Recent clinical evidence has also emerged with pathology reports demonstrating the presence of virions within ECs in conjunction with substantial endothelial cell inflammation and death.[12,61] Endotheliitis as a direct consequence of viral infection may therefore explain the systemic impairment of microcirculatory function in different vascular beds, and the unique cardiovascular sequelae in patients with COVID-19.[12,61]

Although many reports of cardiovascular complications in the adult population appeared in the early weeks of the COVID-19 pandemic, reports of acute vasculitis and hyperinflammatory shock, suggestive of atypical Kawasaki disease, have been emerging in the pediatric population.[62] Importantly, these pediatric cases reported elevated levels of C-reactive protein and D-dimers associated with COVID-19 exposure, despite initial negative tests for SARS-CoV-2, and alarmingly describe severe cardiovascular manifestations such as a fatal arrhythmia, and giant coronary aneurysms.[63] Although the etiology of Kawasaki disease is largely unknown, existing evidence points toward an infectious trigger, with studies describing associations between viral respiratory infections and the presentation of the disease.[64,65] Of relevance, during SARS-CoV-1, a case series of 3 patients described the findings of systemic vasculitis and infiltration of immune cells into vessel walls of numerous organs.[66] Although the mechanism may be the same, with microangiopathy of vessels, there is no direct evidence to date uncovering the role of SARS-CoV-2 in Kawasaki disease.

With the abundance of emerging evidence, it is likely that cardiac and vascular biomarkers could inform mechanisms of COVID-19 pathogenesis (Table 1). Several cardiac biomarkers have been found to be elevated in patients with COVID-19 and importantly, they portend substantially worse outcomes. Zhou et al[10] observed an early increase in cardiac structural proteins including troponin I in nonsurvivors compared with survivors. In a cohort study from Wuhan on 416 hospitalized patients, 19.7% had elevated troponin I with a higher mortality rate (51.2%) compared with those with normal troponin levels (4.5%), illustrating the significantly worse outcomes associated with higher troponin I.[6] In-hospital mortality for patients with elevated troponin T coupled with a history of cardiovascular disease was the highest at 69%.[8] A recent case report described progressively elevated levels of vWF antigen and activity in a patient during clinical deterioration and onset of COVID-19-induced ARDS, suggestive of EC activation or damage.[74] At this point, no endothelial-specific biomarkers have been routinely measured, apart from laboratory values that reflect generalized endothelial dysfunction such as D-dimers and DIC-related tests.[75] It is however notable that markers of systemic inflammation (eg, CRP, IL-6) are elevated in those with poor outcomes, as this may imply the presence of EC activation.[15,16,55] Together, the cardiac and generalized inflammatory biomarkers may provide useful indices across which endothelial-specific biomarkers could be compared. Assessment of endothelial-specific markers of dysfunction in animal models and clinical samples would provide insight into the contribution of EC dysfunction to disease course (Table 2). A starting point exists in the sepsis and infectious disease literature, for which there are several putative endothelial biomarkers, including Angpt2 (Angiopoietin-2), vWF, ADAMTS13 (a disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 13), thrombomodulin, soluble E-selectin/ICAM-1 (intercellular adhesion molecule 1), VCAM-1 (vascular cell adhesion molecule 1), and VEGF (vascular endothelial growth

Table 1. Emerging Biomarkers of Poor Cardiovascular Outcomes in COVID-19

Biomarker	Potential Mechanistic Insight	Predicted Clinical Outcomes
B-type natriuretic peptide (NT-proBNP/BNP)	Released into the circulation by cardiomyocytes in response to volume overload, increased cardiac stress, and hormone stimulation[67]	Myocardial stress and dysfunction
CK-MB	Released by cardiomyocytes following cardiac damage or myocardial injury[68]	Cardiac muscle damage
C-reactive protein	C-reactive protein is produced by the liver following IL-6 stimulation[69]	Inflammation
D-dimer	Fibrin degradation product that is produced during resolution of blood clot through fibrinolysis[70]	Inflammation and coagulation
Ferritin	Released by the liver as a protective mechanism depriving microbes of iron[10]	Associated with in-hospital mortality
IL-6	Proinflammatory cytokine that is elevated promptly following infection or tissue injury[71]	Inflammation, elevated immune response
Troponin I/T	Troponin proteins anchor within the actin filaments of the heart to facilitate muscle contraction. They are normally present at low levels in the circulation and are released into the blood upon cardiomyocyte injury[72]	Cardiomyocyte damage
vWF	vWF, predominantly released into the circulation by endothelial cells, may reflect the extent of endothelial activation or injury[73]	Inflammation and risk of thrombosis

CK-MB indicates creatine kinase-MB; and vWF, von Willebrand Factor.

Table 2. Unanswered Questions Regarding the Role of the Endothelium in COVID-19 Pathogenesis

Does SARS-CoV-2 directly infect cardiac endothelium and does this impact cardiac dysfunction and myocarditis?
Are measures of endothelial dysfunction (eg, NO pathway components, angiopoietins, vWF, circulating adhesion molecules) correlated with poor cardiac, pulmonary, and survival outcomes in COVID-19?
What is the role of the endothelium in the hypercoagulation observed in COVID-19 patients?
Can strategies to strengthen the endothelial barrier improve lung and cardiac function in COVID-19?
Is infection of the endothelium required for viral dissemination to additional organs (eg, the heart)? Can blocking endothelial ACE2 block viral dissemination?
Can animal models (with comorbidities) be developed that recapitulate effects on the cardiovascular system that are seen in humans?
Does SARS-CoV-2-mediated endothelial dysfunction dictate long-term cardiovascular risk?
What are the short- and long-term cardiovascular side-effects of current COVID-19 treatment strategies?
Do medications for preexisting cardiovascular diseases (eg, statins, β-blockers, ACE inhibitors, angiotensin II receptor blockers) impact COVID-19 disease progression?

ACE2 indicates angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; NO, nitric oxide; SARS-CoV-2, severe acute respiratory syndrome coronavirus-1; and VWF, von Willebrand Factor.

factor), some of which have been linked to mortality in ARDS (eg, Angpt2; Table 3).[85]

COVID-19 AND THE ENDOTHELIUM IN HEMOSTASIS AND THROMBOSIS

There is a shared etiology of generalized endothelial dysfunction in driving COVID-19 related cardiac and vascular complications. Specifically, hypercoagulable events—both venous and arterial—have been observed in patients with COVID-19, and an elevation of circulating D-dimers has been correlated with the severity of disease and poor outcomes.[86,87] While endothelial-specific biomarkers have not been broadly assessed in COVID-19, endothelial dysfunction in the setting of cytokine storm is likely a contributing factor to the hypercoagulable state and DIC reported in these patients (Table 3).[88] Indeed, plasma markers of endothelial dysfunction are associated with mortality and severity of coagulopathy in patients with sepsis-associated DIC.[89] Clinical observations have shown that mortality is higher for patients with COVID-19 with abnormal coagulation profiles, including elevated D-dimers and fibrin degradation products, with 71% of nonsurvivors meeting the criteria for DIC compared with 0.6% of survivors.[90] Importantly, elevated levels of vWF activity, D-dimers, and fibrin degradation products point toward a severe state of hypercoagulability rather than consumptive coagulopathy, as seen in classical DIC.[91] In support of this, analysis of critically ill patients from both Dutch and French cohorts has revealed a relatively high number of thrombotic events (adjusted cumulative incidence of up to 49% in the Dutch cohort), with the majority of events being pulmonary emboli.[11,92] Notably, deep venous thrombosis (DVT) was found in 58% of patients (n-value of 12) in a recent prospective autopsy study in which it was not suspected before death—perhaps suggesting the need for routine DVT screening in this patient group.[93] Although few cardiovascular imaging studies have been done to validate these findings, thoracic CT scans of patients with COVID-19 have described vascular thickening, vascular enlargement, or vascular congestion.[94] Large vessel arterial occlusions causing strokes have also been reported in young, otherwise healthy adults.[95] Importantly, Wang et al[96] describe a prothrombotic DIC with high rates of venous thromboembolism, as well as micro- and macrovascular arterial thromboses (eg, stroke, acute limb ischemia) in critically ill patients with COVID-19 necessitating off-label use of tissue-type plasminogen activator treatment. Recent case reports and working hypotheses have additionally highlighted the role of inflammatory dysregulation in the development both micro- and macrovascular thrombosis.[97–99] Although there is no direct evidence of a link between observed hypercoagulability and the endothelium, increased vWF alone points toward massive endothelial stimulation.[74] It is additionally unclear whether this dysregulation involves the ACE2 receptor or co-receptors, direct effects of virus entry on the endothelium, an unknown vulnerability of the patient (eg, thrombophilia), or whether it merely reflects the collateral damage of an overwhelming host response. Regardless of how the system becomes dysregulated, it seems clear that endothelial dysfunction—both preexisting and acute—resulting from multiple damaging hits likely plays a role in the severity of COVID-19.

MODULATING THE ENDOTHELIUM AS A POTENTIAL THERAPY FOR COVID-19

Despite a number of fast-tracked clinical trials testing existing antiviral and anti-inflammatory approaches for COVID-19, suitable preclinical models will be instrumental to understand cardiovascular complications and to develop novel treatments for COVID-19.[100] Nonhuman primates are permissive to SARS-CoV-1 and SARS-CoV-2 infection, and while they develop mild lung pathology, the studies are typically short-term and have not assessed cardiovascular involvement.[101,102] Interestingly, aged macaques develop an exaggerated innate immune response in response to SARS-CoV-1

Table 3. Circulating Endothelial-Derived Biomarkers That Predict the Development and/or Outcome of ARDS

Biomarker	Disease	Potential Mechanistic Insight	Predicted Clinical Outcomes
Angiopoietin-2 (Angpt2)	Acute lung injury (ALI)/ARDS	Angpt2 is a growth factor produced by endothelial cells that regulates vascular permeability, promotes cell death, and disrupts vascularization	Early increase of Angpt2 predicted the development of ARDS and identified patients at high risk for ALI[77]
		Hypoxia and inflammation induce Angpt2 expression, suggesting the presence of endothelial injury[76]	Angpt2 correlated with increased pulmonary oedema and mortality in patients with ARDS[78]
Endocan or endothelial cell-specific molecule 1 (ESM1)	ARDS	ESM1 is a dermatan sulfate proteoglycan that is mainly secreted by pulmonary and kidney vascular endothelial cells in response to inflammatory cytokines[79]	Elevated ESM1 can predict multiple organ dysfunction and mortality in ARDS patients[80]
Glycosamino-glycan (GAG)	ARDS	GAGs are a component of the endothelial glycocalyx and circulating fragments suggest endothelial glycocalyx degradation	GAGs, heparan sulfate, and hyaluronic acid, did not correlate with the severity of ALI/ARDS, however there was a correlation between plasma heparan sulfate concentration and length of stay in ICU[81]
		Heparin sulfate was increased following indirect lung injury (ie, sepsis and pancreatitis) and hyaluronic acid was increased following direct lung injury (ie, pneumonia)[81]	
Soluble thrombomodulin (sTM)	ARDS	TM is an integral membrane protein expressed by endothelial cells and sTM is released following EC injury in ARDS patients[82]	Higher levels of plasma sTM are associated with increased mortality in ARDS[82]
Soluble intercellular adhesion molecule-1 (sICAM-1)	ALI	sICAM-1 is widely secreted by multiple cell types, including vascular endothelial cells and respiratory epithelial cells at low levels	Patients with ALI have higher levels of sICAM-1 in plasma and edema fluid than patients with hydrostatic pulmonary edema. Also, higher sICAM-1 levels were associated with poor clinical outcomes[83]
		Proinflammatory cytokines significantly increase sICAM-1 expression permitting its use as a marker of endothelial activation/dysfunction[83]	
von Willebrand Factor (vWF)	ARDS	vWF is a glycoprotein produced predominantly by endothelial cells and to a lesser extent by platelets	Elevated plasma vWF levels in patients with ALI/ARDS were associated adverse outcomes, including death and organ failure[73,84]
		vWF is released from preformed stores into the circulation in the context of endothelial activation or injury[73]	

ARDS indicates acute respiratory distress syndrome; sICAM-1, soluble intercellular adhesion molecule-1; sTM, soluble thrombomodulin; and vWF, von Willebrand Factor.

infection, suggesting that comorbidities may be required to better model severe outcomes, and this may also be the case for cardiovascular pathology.[102] Small animals, particularly transgenic murine models that express human ACE2 can be infected with SARS-CoV-1 and SARS-CoV-2, and mouse-adapted strains of SARS-CoV-1 can cause lethal infection, but pathological effects on the cardiovascular system have not been observed.[38,103] Finally, while a hamster model is susceptible to SARS-CoV-1 and SARS-CoV-2 infection, they develop only mild cardiac disease and no mortality.[38] Interestingly, an immunocompromised hamster model, which may better model the lymphocytopenia observed in human patients with severe symptoms, showed evidence of multiorgan pathology, including viral replication and myocardial inflammation as well as significant mortality.[104] Considering existing evidence, the close association of underlying cardiovascular comorbidities with adverse outcomes of COVID-19—particularly cardiovascular outcomes—makes assessment of infection in rodent models of chronic comorbid disease a more likely source for valuable, clinically translatable information.

The implied necessity of SARS-CoV-2 to infect or transit the endothelium in an ACE2-dependent manner for dissemination outside the lungs to drive cardiovascular pathology represents a potential viral vulnerability. Proof of principle for the efficacy of ACE2 inhibition was provided with in vitro models of SARS-CoV-1, where small molecule inhibitors were able to inhibit SARS-CoV-1 entry by interfering with ACE2 binding and fusion.[105] Recombinant human soluble ACE2 was also recently shown to suppress SARS-CoV-2 infection of engineered human blood vessel organoids.[39] Additional strategies modulating the endothelium include preserving EC barrier function in the lung (and potentially other tissues) to preserve organ function, limiting systemic inflammatory responses, and impeding coagulation pathways, thereby limiting DIC. Importantly, therapies targeting cytokine storm have been largely unsuccessful in sepsis. This is in part due to the nuanced and biphasic role of inflammation in pathogenesis, making it difficult to determine the optimal timing of treatment.[106] This will likely also be the case with COVID-19, despite emerging favorable reports.[107–109] An alternative approach has been to utilize therapies that are intended to strengthen the endothelial barrier to withstand the detrimental effects of cytokine storm. Such approaches are effective in preclinical models of sepsis and influenza.[110,111] Thus, therapies that preserve endothelial barrier function may improve the clinical features of ARDS and may limit systemic cardiovascular damage. Given that maintenance of barrier function in ECs is driven by cell surface and adaptor proteins, extracellular matrix, and the actin cytoskeleton, the

opportunities for therapeutic design are plentiful. Candidate therapies include sphingosine-1-phosphate, Angiopoietin-1/Tie2, statins, interferon-β-1a, atrial natriuretic peptide, human recombinant soluble ACE2, and heparin, among others.[112–116]

LONG-TERM EFFECTS OF CORONAVIRUS INFECTION ON CARDIOVASCULAR DISEASE

Although significant resources and effort are being poured into addressing the immediate infection rate and reducing acute mortality, the long-term effects of COVID-19 on the cardiovascular system should be considered. Viral-mediated pneumonia is known to elicit long-term cardiovascular complications.[117] Hospitalization with pneumonia has been associated with a subsequent increase in the risk of cardiovascular disease, with up to a 4-fold increase in the first 30 days, and 1.5-fold increase years later.[118] Moreover, the magnitude of risk for cardiovascular disease events associated with pneumonia was similar or higher compared with the risk of cardiovascular disease associated with traditional risk factors, such as smoking, diabetes mellitus, and hypertension.[118] Experimental models of infection have pointed toward the induction of strong proinflammatory signaling leading to changes in cellular composition of atherosclerotic lesions making individuals more susceptible to coronary and cerebrovascular events.[119] Although studies of long-term follow-up in coronavirus patients indicate cardiovascular disease and altered metabolomics, the data are limited.[120] Nonetheless, the connections between systemic viral infection, endothelial ACE2 dysregulation, and postinfection cardiovascular outcomes warrant investigation.

CONCLUSIONS AND PERSPECTIVES FOR FUTURE RESEARCH

The endothelium plays a key role in the pathogenesis of infectious diseases, contributing to dysfunction of inflammation, vascular permeability, coagulation, and immune activation. Inferences from influenza, SARS-CoV-1, and MERS-CoV, although limited in their examinations of the endothelium, provide valuable pathophysiological insights into SARS-CoV-2. The abundance of ACE2 in the cardiovascular system and the inherent role of the endothelium as a gatekeeper to other organs highlights its potentially critical role as a mediator of disease severity, but many questions remain (Table 2). Emerging literature continues to suggest a key role for dysregulated endothelial function in SARS-CoV-2 infection. Detailed investigations are needed to unravel the complex interplay between SARS-CoV-2, the RAAS, and endothelial cell responses. Whether the expression of ACE2, as well as potential co-receptors and their modulation as a result of comorbidities, serve as a metric of disease susceptibility and severity warrants further investigation. Comprehensive disease-course assessments of endothelial activation and RAAS status may be relevant in guiding the appropriate implementation of treatment, as well as to uncover the potential long-term consequences of COVID-19.

ARTICLE INFORMATION

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Affiliations

From the Toronto General Hospital Research Institute, University Health Network, Canada (D.G., S.R., R.W., C.C., S.V., K.R., E.B., K.L.H., J.E.F.); Department of Laboratory Medicine and Pathobiology (D.G., R.W., S.V., J.E.F.) and Institute of Medical Science (S.R., C.C., K.L.H., J.E.F.), University of Toronto, Canada; and Division of Vascular Surgery (S.R., K.L.H.) and Peter Munk Cardiac Centre (K.L.H., J.E.F.), Toronto General Hospital, Canada.

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Disclosures

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