3 Endothelial Dysfunction as a Primary Consequence of SARS-CoV-2 Infection

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Abstract

A number of different viral species are known to have effects on the endothelium. These include dengue, Ebola, Marburg, Lassa fever, yellow fever and influenza viruses, cytomegalovirus and coronaviruses. There are currently seven human endemic coronaviruses, all of which cause respiratory diseases and bind to receptors found within the endothelium. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes the coronavirus disease 2019 (COVID-19) is highly infectious. Like its predecessor, SARS-CoV, it binds to angiotensin-converting enzyme-2 (ACE-2), which is expressed in many cell types, particularly in the lung, including endothelial cells. The initiation of a cytokine storm by the virus along with infection of endothelial cells leads to apoptosis and structural and functional changes that attenuate vascular integrity in many organs including the lungs, heart, liver and kidney. Endothelial damage also enhances the coagulation pathway leading to thrombus formation in major vessels and capillaries. Infection with SARS-CoV-2 has an adverse outcome for individuals with particular comorbid diseases, e.g. hypertension, obesity, type 2 diabetes and cardiovascular disease. It is possible that this is due to the presence of pre-existing endothelial dysfunction and systemic inflammation in subjects with these diseases. Therapies for COVID-19 that target the endothelium, the inflammatory response and the coagulation pathway are currently under trial.

Keywords

Endothelial dysfunction · Inflammation · COVID-19 · SARS-CoV-2

3.1 Introduction

Coronaviruses are a group of positive-sense single-stranded RNA viruses that were first identified over 50 years ago and known to infect both birds and mammals [1]. In humans, coronaviruses cause the common cold [2], severe acute respiratory syndrome (SARS),

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Middle East respiratory syndrome (MERS), gastroenteritis and hepatic and neurological disorders [3, 4]. More recently, a new strain of coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified as the causative agent in the current pandemic of coronavirus disease 2019 (COVID-19) [5]. This is an atypical pneumonia in which patients present with shortness of breath and diarrhoea, as well as cold and flulike symptoms such as fatigue, fever, headaches, sore throat, cough, muscle aches, nausea, loss of taste or smell and runny nose [6, 7]. As of July 23, 2020, according to statistics from the World Health Organization (WHO), just over 15 million people had been infected with SARS-CoV-2 globally, of which the Americas accounted for 53% of confirmed cases, while 4% of confirmed cases were in Africa [8].

The SARS-CoV-2 is a spherical enveloped virus consisting of four structural proteins, spike (S), envelope (E), membrane (M) and nucleocapsid (N), eight accessory proteins (3a, 3b, 6, 7a, 7b, 8a, 8b and 9b) and 16 nonstructural proteins (nsp1–16) [9]. Stemming from the family of *Coronaviridae*, SARS-CoV-2 shares up to 80% sequence similarity to its predecessor, SARS-CoV [10]. Although SARS-CoV-2 is more infectious than SARS-CoV, it has a lower case fatality rate of 3.7%, compared to 10% for SARS-CoV [11]. The other five coronaviruses known to be found in humans are MERS-CoV, HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1.

The endothelium consists of a layer of squamous cells that lines the internal surface of blood and lymph vessels. Acting as the interface between circulating blood and the wall of blood vessels, it has several functions and is regarded as an organ. Some of these functions include maintaining vascular integrity, inducing angiogenesis, controlling coagulation and reducing inflammation [12]. The endothelium equally plays a protective role, shielding the organs from damage, as it controls the movement of substances across the vessels into and out of the tissues. Damage to the endothelium or prolonged activation of endothelial cells via exposure to high cytokine levels, particularly of interleukin-6 (IL-6) and tumour necrosis factor (TNF), can cause endothelial dysfunction leading to activation of the coagulation pathways, enhanced inflammation and loss of vascular integrity. Recently, a study conducted by Varga et al. [13] showed that SARS-CoV-2 is able to infect endothelial cells resulting in endotheliitis in several organs including the lung, heart, kidney, liver and small intestine.

It is now known that SARS-CoV-2 and a number of other viruses can cause endothelial dysfunction by both direct and indirect methods, and this will be the focus of the current chapter.

3.2 Viruses and the Endothelium

Endothelial dysfunction is a common consequence of a number of different viral infections. One of the most intensely researched forms of virally induced endothelial damage is that associated with human immunodeficiency virus (HIV). It is thought to be a major cause of the increased prevalence of cardiovascular diseases (CVDs) observed in subjects infected with the virus [14]. This process is not thought to involve HIV entry into endothelial cells but may be due to the HIV accessory proteins, Nef and Tat. These viral proteins, particularly Nef, are thought to cause endothelial dysfunction via activation of the NF-*k*B pathway and by reactive oxygen species (ROS) generation within the endothelium [15]. Activation of endothelial cells results in the increased expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelial leukocyte adhesion molecule-1 (E-selectin) [16] and the propagation of atherosclerosis. The soluble versions of these molecules are used as biomarkers of endothelial dysfunction and have been shown to be present at high levels in the serum of subjects infected with HIV [17]. The causative association of HIV with endothelial dysfunction is further highlighted by the obser-

vation that antiretroviral therapy causes a reduction in the serum levels of markers of endothelial dysfunction [18, 19].

A number of other viruses have been associated with endothelial dysfunction and atherosclerosis [20]. The most prominent of these is cytomegalovirus (CMV), also known as human herpesvirus 5. This virus has been detected in multiple cell types within the vasculature, including the endothelium, causing vascular inflammation, endothelial dysfunction and the progression of atherosclerotic plaque formation [21]. Influenza viruses have also been implicated in atherosclerosis. The virus infects endothelial cells leading to apoptosis, and it also initiates a cytokine storm that activates the molecular pathways involved in plaque formation [22]. Furthermore, increased vascular permeability in the lungs is caused by influenza-induced endothelial dysfunction leading to pulmonary oedema.

Viral haemorrhagic fevers (VHFs) are a group of severe diseases caused by four families of RNA viruses, i.e. filoviruses, flaviviruses, arenaviruses, and bunyaviruses. These diseases include Ebola virus disease, Marburg disease, Lassa fever, yellow fever and dengue fever. A characteristic feature of many of these diseases is endothelial dysfunction and associated vascular damage and leakage. Haemorrhage is present but varies in severity across these viral types [23]. The mechanisms underlying the endothelial damaged caused by these viruses include infection of the endothelium and of monocytes, macrophages and dendritic cells leading to the production of inflammatory cytokines that induce endothelial dysfunction [24, 25]. A further mechanism specific to the flaviviruses involves production of the viral peptide nonstructural protein 1 (NS1) [26]. This secreted peptide blocks the function of endothelial glycocalyx leading to reduced cell adhesion and increased permeability of the endothelium [26, 27]. It is interesting to note that the Zika virus is also a flavivirus, and studies of human foetal tissue have shown that Zika viral peptides can be found in brain endothelial cells [28].

It is therefore clear that a number of different viral species can negatively affect endothelial function, and recent studies have confirmed that SARS-CoV-2, and possibly other coronaviruses, are endothelial-tropic.

3.3 Infection of Endothelial Cells by SARS-CoV-2

Electron microscopies of lung and kidney tissue obtained from subjects who died from COVID-19 have demonstrated the presence of SARS-CoV-2 in endothelial cells from both tissue types [29]. The virus gains entry into cells by binding to angiotensin-converting enzyme-2 (ACE-2) via the S1 region of the viral S protein. The S protein is then cleaved by the host cell transmembrane serine protease 2 (TMPRSS2) at the boundary of the S1 and S2 subunits, with the latter then mediating fusion of the viral and host cell membranes [30]. The ACE-2 protein is expressed in several organs such as the kidney, intestine, heart and lungs, as well as on the surface of lung alveolar epithelial cells, enterocytes of the small intestine, smooth muscle cells and endothelial cells [31]. The ACE-2 protein is also used by SARS-CoV and HCoV-NL63 as a receptor for host cell entry [32]. The host cell protein used for viral entry by MERS-CoV is dipeptidyl peptidase-4 (DPP-4) [33], while aminopeptidase N (APN) is the receptor of HCoV-229E [34]. The receptor interactions of the coronavirus family are complex, and each virus can recognize multiple host cell proteins [35]. It is interesting to note that in humans, coronavirus receptors are often peptidases, but the biological significance of this is not fully understood. All the proteins used by endemic human coronaviruses to access host cells are widely expressed across various tissues, but, most importantly, each of these viral receptors is expressed in endothelial cells. This suggests that all of these viruses are capable of causing some degree of endothelial dysfunction, and it is interesting to note the similar symptomology of subjects infected with the different coronavirus species [36].

3.4 Signs of Endothelial Dysfunction in COVID-19 Cases

Histological studies have shown direct evidence of structural changes to, and SARS-CoV-2 infection of, endothelial cells [13, 29]. Clinical symptoms of infection with the virus also suggest endothelial involvement in COVID-19. Endothelial dysfunction can also be detected using blood-based biomarkers, but only a few studies have investigated these in subjects infected with SARS-CoV-2 [37, 38]. Such biomarkers include soluble forms of endothelial leukocyte adhesion molecule-1 (E-selectin), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and vascular adhesion protein-1 (VAP-1) and von Willebrand factor [37, 38]. The four adhesion molecules function in forming and stabilizing cell-to-cell interactions and also promote leukocyte transmigration through the endothelium [39], while von Willebrand factor plays an important role in haemostasis [40]. All these molecules are expressed by endothelial cells, and the levels of the adhesion molecules rise in the presence of inflammation [41]. Each of these biomarkers, with the exception of VAP-1, has been used to demonstrate the presence of endothelial dysfunction in subjects with HIV infection [39]. The plasma levels of soluble VCAM-1 and ICAM-1, as well as the numbers of circulating endothelial cells, have also been shown to be elevated in subjects with dengue virus infection [42, 43], a known cause of endothelial dysfunction.

Currently, only a small number of studies have analysed the levels of blood-based biomarkers of endothelial dysfunction in subjects with COVID-19. In an investigation involving 39 COVID-19 patients (9 with severe disease and 30 with mild disease) and 32 uninfected control participants, serum levels of fractalkine, ICAM-1, VCAM-1 and VAP-1 were significantly higher in cases than controls, with the levels of each biomarker increasing with disease severity and decreasing with disease recovery [37] (Fig. 3.1). Fractalkine (CX3CL1) is a chemokine and adhesion molecule that is also expressed on endothelial cells and has similar functions to ICAM-1 and VCAM-1 [44].

Two studies have investigated the level of circulating endothelial cells (CECs) in blood from COVID-19 patients [45, 46]. These cells are released from damaged endothelium and have been used as markers of vascular trauma. Their levels have been shown to be increased in a variety of disease conditions including CVD, inflammation and infection [47]. Blood levels of CECs have also been shown to be increased in the presence of untreated HIV infection [48]. In the first study, CECs were measured in 66 COVID-19 cases and 30 uninfected subjects [46] (Fig. 3.2), while in the second study, CECs were measured in blood taken from 30 COVID-19 patients and 6 healthy control subjects [45]. In both investigations, the CECs were found at significantly higher levels in the COVID-19 cases upon admission compared with uninfected subjects. Interestingly, in the first study, COVID-19 patients that had been treated with only anticoagulation therapy (n = 10) had lower CEC numbers than in untreated cases (n = 47), and COVID-19 patients treated with both an anticoagulation therapy and ACE inhibitors or angiotensin receptor blockers had the lowest CEC numbers [46].

Measuring these biomarkers of endothelial dysfunction in subjects with COVID-19 may aid in the assessment of disease severity and in monitoring the effectiveness of any treatment being administered, particularly in patients with pre-existing conditions associated with endothelial dysfunction.

The SARS-CoV-2 virus is able to infect endothelial cells, and studies show that markers of endothelial dysfunction are increased in subjects with COVID-19. It is important to understand how the virus is able to modulate endothelial function and how this may affect disease outcomes. A number of studies have investigated these topics and will be discussed in the following section of this chapter.

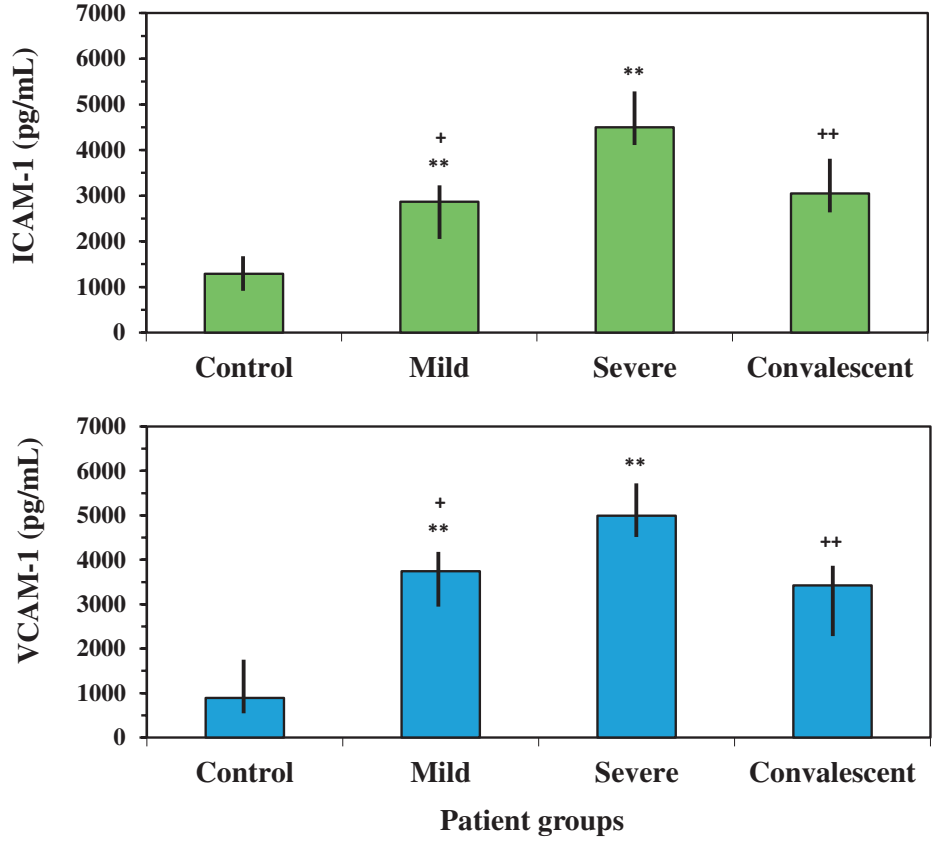

Fig. 3.1 Serum VCAM-1 and ICAM-1 levels in 32 non-infected control subjects and 30 mild and 9 severe COVID-19 cases. Data were also obtained from the severe cases of COVID-19 after convalescence. The data are given as median with interquartile range; $^{**}p < 0.01$ vs. controls, $^{+}p < 0.05$ and $^{++}p < 0.01$ vs severe. (Data taken from [37])

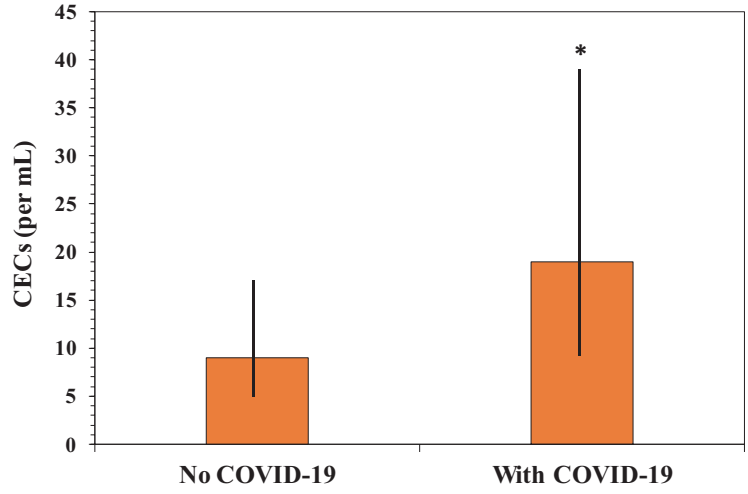

Fig. 3.2 Blood levels of circulating endothelial cells (CECs) in 30 non-infected and 66 COVID-19 cases. The data are given as median with interquartile range;*p = 0.008 vs no COVID-19. (Data taken from [46])

3.5 Causes and Results of Endothelial Dysfunction in COVID-19 Cases

The endothelial dysfunction observed in subjects with SARS-CoV-2 infection may be due to two main causes. Firstly, infection of endothelial cells by the virus may directly cause dysfunction. Microscopic examination of SARS-CoV-2-infected endothelial cells from COVID-19 cases has shown disrupted membrane structures in these cells and apoptosis [13, 29]. An equally important source of endothelial dysfunction may be induced by the inflammatory response that is characteristic of SARS-CoV-2 infection. A cross-sectional study conducted in China by Qin and colleagues involving 452 COVID-19-infected patients, 22–95 years of age, of whom 63.3% were severely infected, revealed plasma levels of TNF-α, IL-2R, IL-6, IL-8 and IL-10 to be elevated above the normal reference range and to be significantly higher in severely infected patients compared with less severe cases [6]. This observation of a so-called cytokine storm in COVID-19 patients has been confirmed in multiple reports. Thus, in a longitudinal study conducted by Liu et al. involving 40 COVID-19 patients, 34–62 years of age, the severely infected patients, who comprised approximately a third of the cohort, had higher serum levels of IL-2, IL-6, IL-10 and IFN-γ compared with the mild cases [49].

The inflammatory response observed in COVID-19 is complex and involves a major input from endothelial cells. The virus firstly interacts with type 2 alveolar epithelial cells (APCs) in the alveolar space of the lungs and activates the innate immune system, leading to the production of high levels of cytokines at the site of infection [50]. Endothelial cells, which lie in close proximity to the APCs, then become targets of the SARS-CoV-2 virus. As mentioned previously, the virus enters these cells via the ACE-2 receptor leading to apoptosis. In addition, the high levels of cytokines, particularly IL-6 and TNF, stimulate endothelial cells themselves to secrete more cytokines [IL-6, IL-8 and monocyte chemoattractant protein (MCP)-1] further enhancing the inflammatory milieu. This cytokine storm causes loosening of endothelial cell-to-cell interactions further increasing vascular permeability. The apoptosis of endothelial cells in combination with the loss of cell adhesion leads to vascular leakage resulting in oedema and in severe cases, respiratory failure [51].

The high cytokine levels also cause activation of the coagulation pathways by stimulating the endothelial cells to secrete von Willebrand factor, P-selectin and fibrinogen. These allow platelet binding to the endothelium, and the former also secrete vascular endothelial growth factor (VEGF) which stimulates the endothelial cells to produce tissue factor, a strong activator of coagulation. In addition, exposure of the thrombogenic collagen fibres of the basement membrane following endothelial cell detachment and apoptosis also leads to activation of the coagulation cascade [51]. A study of 77 cases of severe SARS-CoV-2 infections, matched with 145 non-COVID-19 controls (all cases and controls had acute respiratory distress syndrome), demonstrated that coagulation parameters in the cases were greater than those in the controls, and the cases had a sixfold higher risk of pulmonary embolism compared to the controls [52]. This activation of the coagulation cascade leads to thrombus formation in pulmonary arteries and capillaries as observed in autopsy tissue sections from COVID-19 cases [29].

Damage sustained to the pulmonary endothelium reduces the barrier function of this tissue, allowing SARS-CoV-2 to be transported to other organs. Studies have shown that endothelial cell damage is observed in multiple organs, including the kidneys, heart, liver and small intestine [13].

These studies suggest that SARS-CoV-2, by both direct and indirect mechanisms, can cause endothelial dysfunction which in turn leads to major vascular problems in multiple organs. Endothelial dysfunction is also a feature of many of the comorbid diseases that increase the risk of severe COVID-19, and this will be discussed below.

3.6 Comorbid Diseases, Endothelial Dysfunction and Severity of COVID-19

Reports from the United States and the United Kingdom have shown that individuals admitted to hospitals with COVID-19 have a high prevalence of comorbid diseases such as heart disease, hypertension, type 2 diabetes and obesity [53, 54]. Additionally, in a study comparing data from China and Italy, age and gender were associated with COVID-19 mortality, with individuals above 60 years of age and males having an increased risk [55]. In a retrospective study conducted in South Africa involving 22,308 COVID-19 patients, mortality was associated with diabetes, hypertension, male sex, increasing age and chronic kidney disease [56]. This study also showed that HIV and TB infection were associated with a 2.14- and a 2.70-fold increased risk of COVID-19-associated mortality, respectively, while diabetic subjects with poor glycaemic control (HbA1c $\geq$ 9.0%) had a 12.07-fold increased risk of death [56]. These studies, from both high- and low-to-middle-income countries, showed evidence of an association of both non-communicable and infectious diseases with higher mortality in subjects with COVID-19.

The reason for the higher risk of severe COVID-19 and mortality in subjects with comorbid diseases is not fully understood. However, it has been hypothesized that this may be due to the high level of endothelial dysfunction and systemic inflammation in subjects with cardiometabolic diseases [57, 58]. Many subjects with obesity, hypertension, type 2 diabetes or CVD have underlying endothelial dysfunction and systemic inflammation. Thus, the building blocks for vascular leakage and the cytokine storm are already in place, and infection with SARS-CoV-2 will build upon these pre-existing pathologies increasing the risk for severe COVID-19 (Fig. 3.3). Subjects with HIV infection also have underlying endothelial dysfunction and systemic inflammation [39], and this may partially explain the higher mortality rate observed for subjects coinfected with HIV and SARS-CoV-2 [56]. However, the immune suppression that is characteristic of HIV may also explain this finding.

The other endemic human coronavirus species, i.e. MERS-CoV, SARS-CoV, HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1, have also been associated with more severe disease in subjects with CVDs [36]. Alongside the similar symptoms seen on infection with these viruses, this observation again suggests that all seven coronaviruses have similar effects on the endothelium and the inflammatory response.

Inflammation and endothelial damage and dysfunction are aetiological factors in atherosclerotic plaque formation [59]. It is therefore possible that coronavirus infection may enhance atherosclerosis [60] and that even after the infection has passed, plaque formation may progress, especially in those with pre-existing risk factors such as obesity, diabetes or dyslipidaemia. Long-term follow-up studies of survivors of SARS-CoV-2 infection are necessary to investigate if the incidence of atherosclerotic diseases such as coronary artery disease or subclinical atherosclerosis, as assessed using carotid intima-media thickness, is different from that in subjects who were never infected. A 12-year follow-up study comparing survivors of SARS-CoV-1 infection to those with no infection has been performed [61]. The number of study subjects was small (n = 25 per group), but it was shown that incident cardiovascular abnormalities were more common (44% vs 0%) in the infected group, although no details were given of these abnormalities.

Comorbid diseases obviously increase the risk of severe COVID-19 and of mortality and must therefore be monitored during infection and controlled as optimally as possible. Therapies for the treatment of COVID-19 itself are being intensively investigated, and the possible use of agents that improve endothelium function must be considered.

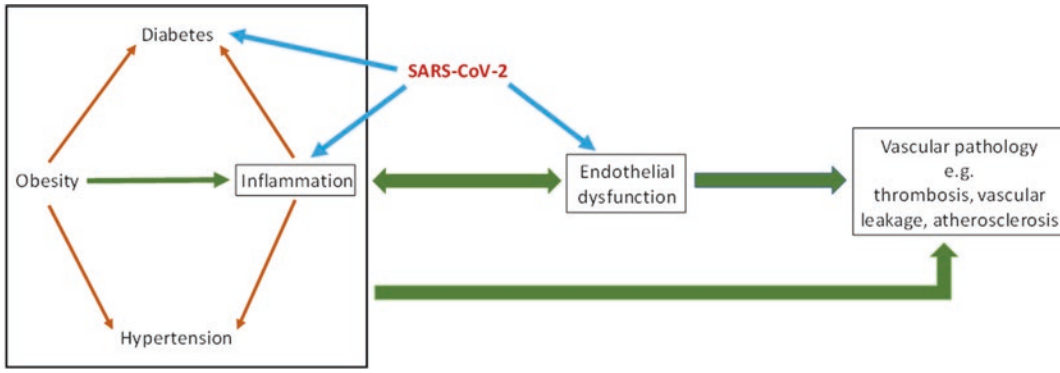

Fig. 3.3 The relationships between comorbid diseases, inflammation, vascular function and SARS-CoV-2. Obesity is an inflammatory state due to the secretion of cytokines such as IL-6 and TNF from adipose tissue. These cytokines cause insulin resistance and thus increase the risk of other comorbid diseases such as type 2 diabetes and hypertension. Obesity can also lead to diabetes and hypertension by mechanisms other than inflammation. Inflammation causes endothelial dysfunction as do obesity, hypertension and diabetes, and endothelial dysfunction can also enhance inflammation by the production of inflammatory cytokines from activated endothelial cells. Endothelial dysfunction is involved in vascular pathology including increased vascular permeability, enhanced coagulation leading to thrombosis and atherosclerotic disease. Comorbid diseases and inflammation can also affect the vasculature independently of their effects on endothelial dysfunction; for example, diabetes can have profound effects on major blood vessels and capillaries via chronic hyperglycaemia. The SARS-CoV-2 virus causes both inflammation and endothelial dysfunction enhancing the effects of the comorbid diseases. It should be noted that the virus has been associated with new-onset diabetes and worsening of glycaemic control in pre-diagnosed diabetics, and it has been suggested that the virus may also cause elevated blood pressure. Both of these effects are thought to be mediated by the ACE-2 protein

3.7 Targeting the Endothelium in Treatments for COVID-19

The endothelium is a major target of the cytokine response to SARS-CoV-2 infection, leading to dysfunction and weakening of the vascular barrier. Thus, attenuating this cytokine storm may protect the endothelium and reduce vascular leakage, and a number of anti-inflammatory agents are currently being assessed as therapeutic interventions for COVID-19. Dexamethasone, a strong anti-inflammatory agent, has already shown positive responses in severe COVID-19 cases [62]. It has been suggested that IL-6 should be the main focus of anti-cytokine therapy, because this molecule is one of the main drivers of the inflammatory response to SARS-CoV-2 infection and has major effects on endothelial function [49]. However, the use of anti-inflammatory therapies must be considered with caution as its usage is hindered by several factors such as correct timing of the treatment, secondary infections and cytokine measurement. It is thought that the use of IL-6 antagonists such as tocilizumab, which is a humanized monoclonal antibody targeting the IL-6 receptor, might only be beneficial in severely infected patients with elevated serum levels of IL-6 [63]. Moreover, COVID-19-infected patients do not share the same inflammatory profile. Significant fluctuation in serum levels of IL-6 in severely infected COVID-19 patients has been reported [49]. Despite these misgivings, small clinical trials without the use of a control group have shown both positive and negative effects of tocilizumab therapy in severe COVID-19 cases [64, 65]. These studies need to be replicated in larger populations set within more stringent clinical trial frameworks. Anti-TNF agents have also been suggested as therapy options for COVID-19 [66]. This cytokine is also a prominent role player in the cytokine storm with endothelial effects [51]. A small study of the anti-TNF monoclonal antibody infliximab in seven treated and non-treated COVID-19 cases did show clinical improvements with the therapy, but again,

these results need to be confirmed in larger clinical trials.

Statins are known to have both anti-inflammatory and pro-endothelial effects, and it has been hypothesized that they may be an effective therapy for COVID-19. These drugs have been used to treat other viral infections such as influenza and Ebola, with some success [67, 68]. In fact, in the studies on Ebola, statins were used in conjunction with angiotensin receptor blockers (ARBs), as both agents have been reported to counteract endothelial dysfunction. The use of ARBs to treat COVID-19 is contentious as these drugs are known to upregulate expression of ACE2, the SARS-CoV-2 receptor [69].

Disruption of endothelial function by coronavirus leads to activation of the coagulation pathway [51]. Therapies directed at downregulation of this pathway have therefore been suggested for treatment of COVID-19, and it has been recommended that anticoagulants be used prophylactically in severe COVID-19 cases to reduce the risk of thrombosis [70].

There are a number of therapeutic agents which act directly or indirectly on the endothelium to attenuate inflammation, endothelial dysfunction and thrombotic events and hence improve vascular function. Many of these therapies have not yet completed testing in properly controlled clinical trials, although a number of such studies are currently in progress. The use of these drugs in combination must also be considered in future trials.

3.8 Conclusions

The endothelium has gained attention in recent years as a target for many different viral infections, and this has been strongly highlighted with the current SARS-CoV-2 pandemic. Its multiple functions have ensured that any pathological changes induced in this tissue will have profound effects on health. The targeting of therapies toward the endothelium is therefore essential, and the development of such agents has been augmented by studies showing that pre-existing, commonly used drugs do have positive effects on endothelial function. The outcome of current clinical trials on new therapies that modulate endothelial activity in the context of COVID-19 is eagerly awaited.

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