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Endothelial glycocalyx damage as a systemic inflammatory microvascular endotheliopathy in COVID-19

Microvascular endotheliopathy in COVID-19

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Abstract

In atherosclerosis patients, vascular endothelial dysfunction is commonly observed alongside damage of the vascular endothelial glycocalyx, an extracellular matrix bound to and encapsulating the endothelial cells lining the blood vessel wall. Although atherosclerotic risk factors have been reported in severe patients with coronavirus disease 2019 (COVID-19), the exact mechanisms are unclear. The mortality associated with the COVID-19 outbreak is increased by comorbidities, including hypertension, diabetes, obesity, chronic obstructive pulmonary disease (COPD), and cardiovascular disease. Besides, older individuals and smokers have significantly worse outcomes. Interestingly, these comorbidities and risk factors are consistent with the pathophysiology that causes vascular endothelial glycocalyx damage. Moreover, vascular glycocalyx dysfunction causes microvascular leakage, which results in interstitial pulmonary abnormal shadows (multiple patchy shadows with a ground glass inter-pneumonic appearance). This is frequently followed by severe acute respiratory distress syndrome (ARDS), closely related to coagulo-fibrinolytic changes contributing to disseminated intravascular

coagulation (DIC) and Kawasaki disease shock syndrome, as well as inducing activation of the coagulation cascade, leading to thromboembolism and multiple organ failure. Notably, SARS-CoV-2, the causative virus of COVID-19, binds to ACE2, which is abundantly present not only in human epithelia of the lung and the small intestine, but also in vascular endothelial cells and arterial smooth muscle cells. Moreover, COVID-19 can induce severe septic shock, and sepsis can easily lead to systemic degradation of the vascular endothelial glycocalyx. In the current review, we propose new concepts and therapeutic goals for COVID-19-related vascular endothelial glycocalyx damage, based on previous vascular endothelial medicine research.

Keywords: Vascular endothelial dysfunction, systemic inflammatory response, cytokine storm, ARDS, Kawasaki disease shock syndrome

Introduction

It has been said that scientists could win the Nobel Prize in physiology or medicine if they could invent a cure for the common cold. Since the common cold is caused by various viruses, which can easily mutate their genes, it has been extremely difficult to develop any specific medicine or vaccine for influenza infection. For this reason, following infection, individuals are advised to wait for recovery by taking coping medications for the symptoms, such as fever, cough/sputum, diarrhea, and headache, as well as getting sufficient nutrition and rest. It is known that 15% of common colds are caused by conventional human coronavirus (HCoV) infections (e.g., HCoV-229E, HCoV-0C43, HCoV-HKU1, and HCoVNL63). Coronavirus disease 2019 (COVID-19) was initially thought to be a slightly stronger viral infection compared to the seasonal common cold or flu. However, it has become clear that the infectious power of severe acute respiratory coronavirus 2 (SARS-CoV-2), the virus causing COVID-19, is remarkable, and as a result has led to life-threatening complications in a significant proportion of patients.

SARS-CoV-2 spread rapidly throughout the world, largely due to asymptomatic viral transfer. According to the COVID-19 dashboard website by the Center for Systemics Science and Engineering at Johns Hopkin University, the number of global deaths due to COVID-19 was 291,487 as of May 12, 2020. A subset of infected patients go on to develop a more severe form of disease, which is characterized by expanding pulmonary lesions, sepsis, acute respiratory distress syndrome, and respiratory failure[1]. The fight against SARS-CoV-2 is decisively different from that against conventional viral infections. Since many infected people are asymptomatic, SSARS-CoV-2 is easily spread to others indiscriminately, which has led to the formation of huge clusters of severe COVID-19 patients, especially in situations where there are increased numbers of individuals with opportunistic infections, such as in hospitals and nursing homes for the elderly. There are numerous differences between COVID-19 and the common cold-induced by traditional coronaviruses or flu. For instance, thrombotic complications are emerging as a critical complication in patients with COVID-19[2]. In line with this, COVID-19 patients often present with special features, such as increasing D-dimer and fibrin degradation levels,

prolonged prothrombin time, and the development of disseminated intravascular coagulation (DIC)[3], which have all been associated with poor prognosis in severe COVID-19 patients[4]. Indeed, microvascular thrombosis can induce swollen hands, and toes like frost have been reported in COVID-19 patients.

It is known that patients with chronic obstructive pulmonary disease (COPD) are more likely to develop pneumonia as a result of COVID-19 infection. Furthermore, according to accumulating evidence, high-risk patients with critical COVID-19 are more frequently older (>65 years of age), male, obese, smokers, and have common comorbidities, such as hypertension (57%), obesity (42%), and diabetes (34%)[5]. Thus, some cardiologists speculated that a large number of acute coronary syndrome (ACS) patients might be at an increased risk of mortality from COVID-19, because these risk factors were thought to coincide with traditional coronary risk factors of atherosclerotic diseases. However, as COVID-19 outbreak spread, a significant number of patients without ACS were struck with severe disease in a short period of time, and DIC has been observed in 71.4% of non-survivors[4]. Most of the medical doctors were at a loss to what type of SARS-CoV-2-infected patients

should be considered at high risk of critical COVID-19. Recent evidence revealed that severe COVID-19 patients have cardiac arrhythmias, myocardial injury and heart failure, DIC, and pulmonary embolism[6]. Interestingly, these phenomena can be explained centrally with one concept: the vascular endothelial glycocalyx, an extracellular matrix bound to and encapsulating the endothelial cells lining the blood vessel wall.

In the current review, previously unrevealed key components in severe COVID-19 pathophysiology will be outlined, with the aim to accelerate related research for a diagnostic and therapeutic approach in the fight against COVID-19.

Specific features of SARS-CoV-2

SARS-CoV-2 binds to the transmembrane angiotensin-converting enzyme 2 (ACE2) protein to enter type II alveolar epithelial cells, macrophages, and other cell types[7]. The spike protein of SARS-CoV-2 is primed by transmembrane protease, serine-2 (TMPRSS2). The primary symptoms of COVID-19 are cough (67.8%), diarrhea (3.8%), and fever (total 88.7% during hospitalization)[3], which might provide possible routes of infection via the

respiratory tract and intestines, as the entry for SARS-CoV-2.

ACE2 is also present on vascular endothelial cells and arterial smooth muscle cells in all organs[8]. As a result, SARS-CoV-2 can directly adhere to vascular endothelial cells, and induce vascular endothelial dysfunction, followed by microvascular leakage, microvascular coagulation, excessive release of inflammatory cytokines, and disruption of cell-cell contact. The most notable features in COVID-19 infection are asymptomatic pneumonia detected by chest X-ray or computed tomography (CT). Furthermore, multiple ground glass patchy shadows are common radiological findings in SARS-CoV-2 infected patients with mild symptoms. The difficulty in perceiving signs of worsening COVID-19 may make it difficult to notice the rapid deterioration in the condition of patients, which may also increase the number of sudden deaths before hospitalization. It is known that blood oxygen saturation (SpO_2) is decreased in COVID-19 patients before symptoms such as shortness of breath and dyspnea; however, an increase in respiratory rate is observed just before the decrease in SpO_2. Therefore, if the respiratory rate is 20 times/min or more, patients should be carefully observed for worsening respiratory conditions.

SARS-CoV-2 has a long viral spreading time (median, 20.0 days; interquartile range, 17.0-24.0 days) in survivors[9]; indeed, the longest period of virus excretion was 37 days. Furthermore, in non-survivors SARS-CoV-2 could be detected up until their death[9]. This long viral excretion has contributed significantly to the rapid spread of the disease, and provides the rationale for further isolation of infected patients and optimal antiviral therapeutic strategies.

Life-threatening complications in COVID-19

In Zhongnan Hospital of Wuhan University in Wuhan, China, of 138 hospitalized patients with SARS-CoV-2-infected pneumonia, 36 patients (26.1%) were transferred to the intensive care unit (ICU) because of complications, including ARDS (61.1%), arrhythmia (44.4%), and shock (30.6%)[10]. Compared to the 102 patients not treated in the ICU, patients treated in the ICU were much older and were more likely to have comorbidities[10].

Indeed, it is now understood that the mortality associated with the COVID-19 outbreak is increased by the presence of comorbidities, including

hypertension, diabetes, COPD, and cardiovascular disease. Furthermore, elderly individuals (> 65 years of age) and smokers have been shown to have significantly worse outcomes. Interestingly, these comorbidities and risk factors are consistent with the pathophysiology that causes damage to vascular endothelial glycocalyx[11], a negative charged brush-like monolayer of endothelial cells[12].

The generation of a cytokine storm induces organ damage, followed by edema, air exchange dysfunction, ARDS, acute cardiac injury, and secondary infection, all of which may lead to death. The presence of a cytokine storm is an important factor that leads to the exacerbation of COVID-19 or even death[13]. Therefore, avoidance of a cytokine storm may be the key to the treatment of COVID-19 patients[14].

Coagulation disorders occur in patients infected with COVID-19, SARS-CoV-1, and MERS-CoV[2]. Regarding COVID-19, DIC has been observed in 71.4% of non-survivors[4]. In line with this, D-dimer levels of 2.0 μg/mL or more (4-fold increase) on admission can predict in-hospital mortality in patients with COVID-19, which indicates that D-dimer could be an early marker to improve the management and stratification of COVID-19 patients[15].

Furthermore, dysregulation of the coagulation cascade and the subsequent formation of intra-alveolar or systemic fibrin clots are prominent findings in coronavirus infections associated with severe respiratory disease. In addition, microvascular endothelial failure and peripheral thrombosis may induce frost-like swollen hands and toes. Therefore, severe COVID-19 patients should be given treatments for coagulation disorders in order to prevent multiple organ failure[2].

COVID-19 and vascular endothelial dysfunction

Underlying cardiovascular disease is associated with an increased risk of in-hospital death among patients hospitalized with COVID-19[16]. According to a previous report of COVID-19 in Wuhan, 48% of patients had comorbidities, including hypertension (39%), diabetes (19%), and coronary heart disease (8%)[9]. Furthermore, patients with preexisting coronary risk factors and cardiovascular disease had the highest mortality rates (10.5%) following infection with SARS-CoV-2[17]. Data have shown that SARS-CoV-2-infected patients ≥ 60 years old have more systemic symptoms and more severe pneumonia than patients aged ≤ 60 years[18]. In support of this, multivariable

regression analysis showed increased odds of in-hospital death with old age[9]. It appears that COVID-19 is more likely to deteriorate due to an increased in comorbidities in elderly, which may lead to immune dysfunction in elderly COVID-19 patients. In other words, microvascular leakage, which acts as a window for SARS-CoV-2 organ invasion, is caused by more advanced vascular endothelial glycocalyx damage in elderly patients[19]. In addition, the vascular endothelial glycocalyx is more easily damaged in elderly people than young, and common comorbidities are known to perturbate the vascular endothelial glycocalyx[20]. The vascular endothelial glycocalyx is systemically damaged under the conditions of old age and multiple comorbidities, which may be a potent mechanism for the development of lethal complications in COVID-19 patients.

Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and SARS-CoV-2 bind to ACE2, which is abundantly present not only on human epithelia of the lung and the small intestine, but also on vascular endothelial cells, arterial smooth muscle cells, cardiomyocytes, cardiofibroblasts, and coronary endothelial cells[21]. The exploitation of ACE2 by coronavirus may impair the renin-angiotensin-aldosterone system

(RAAS). Furthermore, ACE2 is highly expressed on failing human hearts and pericytes, which could lead to the development of microvascular dysfunction[22], and explain the greater propensity for ACS[23, 24]. Therefore, careful attention must be paid to the exacerbation of COVID-19 in patients with highly expressed ACE2. On the contrary, in severe COVID-19 patients, it is necessary to pay attention to cardiovascular diseases, such as microvascular endothelial dysfunction (Figure 1), the onset and exacerbation of heart failure, and the onset of ACS.

ACE2 is a potent cardioprotective and counterregulatory enzyme that degrades angiotensin II to angiotensin-(1-7), thereby attenuating its effects on vasoconstriction, sodium retention, and fibrosis[25]. MasR, an endogenous receptor of angiotensin-(1-7), has emerged as a physiological antagonist that counter-regulates RAAS activation via the ACE2/angiotensin-(1-7)/MasR axis. The angiotensin II/AT1 receptor is critically involved in disease progression leading to non-ischemic, ischemic, and diabetic cardiomyopathy, and to obesity-associated cardiac dysfunction. ACE2 shifts the balance to the cardioprotective ACE2/angiotensin-(1-7)/MasR axis through converting angiotensin II to

angiotensin-(1-7)[21]. In a recent paper that reported on 8,910 patients with COVID-19, no harmful association of ACE inhibitors or angiotensin-receptor blockers with in-hospital death was confirmed[16].

Vascular endothelial glycocalyx

Glycocalyx is defined as a thick mixture of protein lipids and post-translational sugar structures, which surround all living cells and act as a buffer between the cell and the extracellular matrix[26]. The monolayer of vascular endothelial cells constitutes the inner cellular lining of vasculatures such as arteries, veins, and capillaries. The luminal layer is in direct contact with blood as a vascular protective barrier between blood and organs. The vascular endothelial glycocalyx on the luminal surface of all endothelial cells plays an essential role to regulate coagulation, inflammation, trans-capillary flux, and microvascular permeability[27, 28]. The glycocalyx is a complex gel-like layer of sialic acid-containing glycoproteins, membrane-bound proteoglycans (e.g., syndecans and glypicans), and glycosaminoglycan side chains (e.g., heparin sulfate and chondroitin sulfate), and long chains of hyaluronan

(HA)[29, 30]. The vascular endothelial glycocalyx is stabilized by shear stress[31], which is pivotal for proper nitric oxide (NO) production[32, 33]. Glycosaminoglycans are constantly degraded through enzymes, and also synthesized and extruded through vesicles of the Golgi apparatus to maintain homeostatic balance[34]. As shown in Figure 2, homeostasis is broken down, and vascular endothelial glycocalyx shedding/degradation occurs in conditions of cellular stress, ischemia/reperfusion injury[35], the presence of endotoxins[36], inflammatory mediators[37], atrial natriuretic peptide, excessive reactive oxygen species[38], hyperglycemia[39, 40], high-salt intake[41], hypertension[42], familial hypercholesterolemia[43], and oxidized low-density lipoprotein (ox-LDL)[44]. Of note, rosuvastatin administration has been shown to partially restore damaged vascular endothelial cells in patients with heterozygous familial hypercholesterolemia[43]. Moreover, unfavorable lifestyle, including smoking and physical inactivity, also induces glycocalyx degradation. Indeed, it has been previously shown that a smoking cessation program using varenicline or nicotine replacement therapy for 3 months resulted in a decrease of carbon monoxide (CO), oxidative stress, arterial stiffness, and restored the endothelial glycocalyx[45]. Physical

inactivity induces systemic low shear stress in the body, and it has been demonstrated that AMP-activated protein kinase regulates glycocalyx impairment due to hyaluronan degradation and macrophage recruitment in response to low shear stress in a mice common carotid artery ligation model[46]. Moreover, the vascular endothelial glycocalyx is perturbed by various unfavorable disease conditions, including dehydration, acute infectious disease[47], trauma[48], sepsis[49], ARDS[50], preeclampsia[51], gestational diabetes mellitus[52], and chronic disease conditions, such as hypertension, diabetes[19, 53], chronic kidney disease[54], atherosclerosis[55-60], stroke[61, 62], dementia[63], microvascular angina[64], ACS[65], and heart failure[66]. In ApoE knockout mice, an inhibitor of hyaluronan synthesis, 4-metylumbelliferone (4-MU) has been shown to interfere with the protective function of the endothelial glycocalyx, thereby facilitating leukocyte adhesion, subsequent inflammation, and progression of atherosclerosis[56].

The vascular endothelial glycocalyx is crucial to endothelial function[67], as it is involved in microvascular reactivity, and modulates the interaction between the endothelium and blood constituents[68]. In addition, the vascular endothelial glycocalyx protects endothelial cells from shear

stress caused by blood flow, and serves as a vascular permeability barrier[69]. As shown in Figure 3, the intact vascular endothelial glycocalyx harbors various cytokines and chemokines, receptors, growth factors, gap junction proteins, and enzymes, including extracellular superoxide dismutase (ecSOD), endothelial nitric oxide synthase (eNOS), ACEs, lipoprotein lipase, xanthine oxidase, and antithrombin III, all of which play a central role in endothelial function and blood/microvascular/tissue interactions[68]. Vascular endothelial dysfunction and vascular failure occur in situations where the endothelial glycocalyx is impaired, which has roles in the development of various cardiovascular diseases[70, 71].

The vascular endothelial glycocalyx has the potential to not only function as a physical cytoprotective barrier for vascular endothelial cells, but also as a mechanism to regulate intracellular cell signaling. IQGAP1, an essential scaffolding protein that binds to vascular endothelial growth factor (VEGF) receptor-2[72], has roles in many different aspects of cell physiology and interacts with numerous proteins[73]. IQGAP1 modulates the actin cytoskeleton through Rac1 and Cdc42, while cell-cell adhesion through VE-cadherin and β-catenin regulates the mitogen-activated protein kinase

pathway and forms a complex with the hyaluronan receptor CD44 to regulate cell migration and proliferation[74]. In vascular endothelial cells, IQGAP1 induces angiogenesis through binding to VEGF receptor-2 and VE-cadherin containing adherens junctions in a ROS-dependent manner[75]. IQGAP is required for the establishment of cell-cell contact, and is presumably necessary to collaborate with the vascular endothelial glycocalyx.

Virus infectious disease and vascular endothelial glycocalyx

Among viral infectious diseases, research on the relationship between dengue fever and vascular endothelial glycocalyx is progressing. Dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) is characterized by vascular leakage and shock. The dengue virus nonstructural protein 1 (NS1) is the only membrane-associated protein that anchors its replication complex to the cellular membrane. Increased circulating levels of vascular endothelial glycocalyx layer components, such as hyaluronic acid, heparin sulfate, claudin-5, and syndecan-1, have been associated with disruption of the vascular endothelial glycocalyx, and the subsequent development of plasma leakage and severe dengue disease[76, 77]. Sialic acid has been

established as an important determinant of endothelial barrier function in both *in vitro* and *in vivo* studies[78, 79]. This evidence emphasizes the importance of evaluation and therapy targeted to vascular endothelial glycocalyx in severe conditions induced by viral infections, potentially including COVID-19.

Severe inflammation induces vascular endothelial glycocalyx dysfunction

The vascular endothelial glycocalyx maintains homeostasis of the vasculature, including the control of vascular permeability and microvascular tone, prevention of microvascular thrombosis, and regulation of leukocyte adhesion[80]. During sepsis, the glycocalyx is degraded via inflammatory factors, such as metalloproteinases, heparinase, and hyaluronidase[81].

Systemic damage to the delicate layer of the vascular glycocalyx results in increased protein and water transit to the extra-vascular space. In septic conditions, the vascular endothelial glycocalyx is perturbated and the layer becomes thinner, which induces microvascular excessive permeability and contributes to interstitial edema in various organs[81, 82].

The systemic breakdown of the glycocalyx occurs dramatically in fatal disease conditions, such as severe infectious diseases, sepsis, hemorrhagic shock, burn, traumatic brain injury[83], and traumatic endotheliopathy, a syndrome associated with high mortality[84]. Figure 4 shows a schematic image of severe COVID-19 comorbidity induced by vascular endothelial glycocalyx damage. Patients with underlying diseases have systemic endothelial glycocalyx disorders due to complicated mechanisms. Once these patients are infected with SARS-CoV-2, COVID-19-induced systemic vascular inflammatory endotheliopathy is more likely to develop serious complications such as ARDS, DIC, Kawasaki disease shock syndrome, microvascular thrombosis, and arrhythmias.

Arrhythmia and sudden death following vascular endothelial glycocalyx damage

In acute cytokine storm models utilized to examine systemic inflammatory response syndrome (SIRS), intravenous injection of proinflammatory cytokines, including interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF), induces vascular hyperpermeability. It has been suggested that the

inhibition of connexin43 (Cx43) hemichannels could counteract TNF-induced SIRS-associated vascular permeability and lethality in mice[85]. Cx43 is an important cardiac gap junction protein, and excessive opening of Cx43 hemichannels are observed in ischemic or inflammatory conditions[86, 87]. Inhibition of Cx43 protects against vascular leakage, hypothermia, and mortality in a TNF-induced SIRS mouse model. Furthermore, altered Cx43 expression produces an arrhythmia substrate in the heart, which contributes to the arrhythmias of sudden cardiac death[88]. Interestingly, vascular endothelial glycocalyx degradation disrupts endothelial Cx43 proteins, likely blocking the inter-endothelial molecular transport that maintains endothelial cell and vascular tissue homeostasis to resist disease[89]. Endothelial glycocalyx damage in sepsis induced by severe viral infections like COVID-19 may occur as a result of a change in Cx43 expression in the microvasculature and the heart. SAR-CoV-2 binds to ACE2, which is also abundantly expressed in cardiomyocytes, cardiofibroblasts, and coronary endothelial cells[21]; consequently, the virus has the potential to induce lethal arrhythmia in COVID-19 patients via damaging Cx43 in these cell types. Examination of the blood electrolyte levels is important to predict lethal

arrhythmias, because COVID-19 patients tend to have hyponatremia or abnormalities of other electrolytes. Furthermore, all COVID-19 patients should receive an electrocardiogram to check for iatrogenic QT prolongation or other arrhythmias. Because many antiviral drugs can cause cardiac arrhythmia or other cardiovascular disorders, the presence of cardiac toxicity must be closely monitored[90].

Kawasaki disease shock syndrome and vascular endothelial glycocalyx

Kawasaki disease is an acute febrile systemic vasculitis that predominantly occurs in children below 5 years of age. Systemic vasculitis is particularly observed in small- and medium-sized arteries. Because Kawasaki disease shows seasonal, temporal, and regional patterns, an infectious agent is thought to cause or trigger the disease presentation[91]. According to a previous serological test, the development of Kawasaki disease is involved in human coronavirus (HCoV)-229E infection[92]. Although its exact etiopathogenesis is unclear, it is thought to be a complex interplay of genetic factors, infections, and immunity[93]. Though self-limiting in many cases, Kawasaki disease can lead to severe complications, such as coronary artery aneurysms and thrombo-embolic occlusions; thus, early diagnosis and

urgent attention tis required to avoid these complications. The presence of coronary aneurysms was significantly and positively correlated with male sex, IVIG resistance, higher neutrophil/lymphocyte ratio, inotrope treatment, cardiac failure, abdominal pain, and neurological symptoms[94]. In both Kawasaki disease and COVID-19, some clinical symptoms such as fever, rash, and eye redness (conjunctival injection) are present in many infected children. The first case of Kawasaki disease with concurrent COVID-19 was reported in April 2020 [95], since then, between April 29 and May 3, 2020, 15 cases were reported by the New York City Health Department, and 64 cases statewide were reported from the New York State Department of Health. Furthermore, an uptick in Kawasaki disease or Kawasaki-like disease was established among children coincident with the COVID-19 outbreaks in the U.K., Italy, and Spain.

Kawasaki disease shock syndrome, a severe subtype of Kawasaki disease, is a RARE complication of Kawasaki disease that can lead to significant sequelae and poor outcome[96]. According to a previous report of 187 consecutive patients with Kawasaki disease, 13 (7%) met the definition for Kawasaki disease shock syndrome[96]. Furthermore, Kawasaki disease shock

syndrome has been shown to be characteristic of more severe inflammatory cytokine production, and a tendency to develop IVIG non-responsiveness and coronary abnormalities[97]. Experts have indicate that there may be a small increase in the numbers of children with severe COVID-19 and features consistent with toxic shock syndrome (abdominal pain and gastrointestinal symptoms), which appear to be similar to those of Kawasaki disease shock syndrome.

Surprisingly, circulating endothelial glycocalyx components (syndecan-1 and hyaluronan) were significantly elevated at the acute phase, and serum hyaluronan was determined as the biomarker that is the best predictor of future development of coronary artery lesions in Kawasaki disease[98]. Serum levels of soluble syndecan-1 (sCD138), one of the major core proteins expressed on the vascular endothelial glycocalyx, is considered to reflect vascular endothelial damage and inflammation in Kawasaki disease[99]. Considering the common pathophysiology between Kawasaki disease and COVID-19, it is expected that the knowledge on vascular endothelial glycocalyx-related Kawasaki disease can be applied to research on new therapeutic strategies and biomarkers for predicting deterioration in

patients with severe COVID-19.

Vascular endothelial glycocalyx dysfunction induces a severe phenotype in disease

COVID-19 can induce severe septic shock, and sepsis can easily magnitude systemic degradation of the vascular endothelial glycocalyx. Vascular endothelial glycocalyx dysfunction contributes to septic-induced vascular endothelial cell damage leading to altered microvascular permeability. Therefore, vascular endothelial glycocalyx may have a key regulatory role in maintaining the pulmonary vascular barrier and its homeostasis[100]. The recent findings on vascular endothelial glycocalyx are outlined below, in the context of COVID-19-related complications.

1) Septic shock

Degradation of vascular endothelial glycocalyx represents one of the earliest and most significant sites of injury during sepsis[101]. In mice models, the total volume of vascular endothelial glycocalyx has been shown to be drastically reduced in sepsis. Excessive ROS and proinflammatory cytokines, such as TNF-α and IL-1β, are considered the

main actors in endothelial glycocalyx degradation in sepsis[102]. Both mechanisms activate the sheddases heparinase and matrix metalloproteases (MMPs). Furthermore, a thinner and sparser endothelial glycocalyx is associated with vascular permeability and resulting edema, hypovolemia, vasodilation troubles, leukocyte attraction, platelet aggregation, and lung injury[81, 103]. The increase in pulmonary vascular permeability as a result of sepsis, manifests acute lung injury and ARDS[104].

2) Acute respiratory distress syndrome (ARDS)

The main features of ARDS are lung endothelial cell injury, severe inflammatory responses, neutrophil adhesion or infiltration, and interstitial edema. Vascular endothelial glycocalyx and inflammatory responses are crucial for the pathogenesis of ARDS[105]. Pulmonary edema associated with albumin leakage is closely related to degradation of the endothelial glycocalyx[100]. The endothelial glycocalyx not only acts as a physical barrier to prevent albumin exudation, but also as signaling molecules to participate in hemodynamics[100, 106-109].

3) Microvascular thrombosis/DIC

The vascular endothelial glycocalyx is an important regulator of microvascular permeability preventive thrombus formation[110]. Coagulation disorders occur in coronavirus infected patients with COVID-19, SARS-CoV-1, and MERS-CoV[2]. Moreover, DIC has been observed in 71.4% of non-survivors of COVID-19[4]. Recently the International Society on Thrombosis and Hemostasis (ISTH) DIC Scientific Standardization Committee has proposed a new category termed "sepsis-induced coagulopathy (SIC)" to facilitate earlier diagnosis of DIC, which is hoped to lead to more rapid interventions in these critically ill patients[111].

4) Multiple organ failure

Systemic ischemia occurs in various life-threatening clinical settings, including cardiac arrest, hemorrhagic shock during trauma, or ST-elevation myocardial infarction complicated by cardiogenic shock[112, 113]. During these situations, distortion of the glycocalyx structure and function contributes to the multiorgan dysfunction that follows global ischemia of various etiologies, such as renal, cardiac, pulmonary, and hepatic ischemia/reperfusion injuries[68, 114]. Damage of the vascular endothelial glycocalyx in these ischemia/reperfusion injuries is

largely mediated by ROS, specifically through the activation of endothelial NADPH oxidase 2 (NOX2) and xanthine oxidase that are bound to glycosaminoglycans anchored at the endothelial surface layer[115, 116].

Remaining question: Could vascular endothelial glycocalyx damage influence the sex difference in COVID-19?

It has been reported that there is a clear sex difference in severe COVID-19 and the rate of in-hospital mortality. The relationship between COVID-19 severity and male hormones, or the possibility that male smokers are included to a greater extent in these studies has been investigated, although there remain no definitive conclusions. We propose that sex differences in the vascular endothelial glycocalyx could represent a crucial factor for the sex difference of COVID-19 severity and mortality. In ACS patients, it has been reported that males shed more syndecan-1 than females[65]. Circulating levels of syndecan-4 have been associated with incident myocardial infarction, and the association is stronger in women than in men[117]. These data imply either an increase in the amount of glycocalyx, a denser glycocalyx, or higher protease activity in male

endothelial cells[118]. Therefore, the mechanisms underlying sex the differences in atherosclerosis progression and ischemic cardiovascular disease may be explained by the sex difference of the vascular endothelial glycocalyx.

Possible therapeutic targets on COVID-19 associated with the vascular endothelial glycocalyx

Most of the patients in China receive antiviral therapy such as ribavirin, lopinavir/ritonavir, and remdesivir[9, 119, 120]. Clinical trials using ivermectin, avigan, and remdesivir are ongoing worldwide to clarify their effectiveness on COVID-19. Furthermore, more recent clinical trials have tested the efficacy of inhibition of TMPRSS2 by camostat mesylate, the recombinant form of human soluble ACE2[121], monoclonal antibodies against IL-6 receptor, and interferon-α 2b for the treatment of patients with COVID-19. Convalescent plasma transfusion has also been reported to be beneficial in the treatment of critically ill patients with COVID-19[122, 123]. Anticoagulant therapy resulted in lower mortality in patients with sepsis-induced coagulopathy, as well as lower mortality in COVID-19

patients with increased levels of D-dimer. However, there were no overall benefits for patients following the administration of low molecular weight heparin for at least 7 days[124]. Thus, hypothesis-driven studies based on the knowledge of the molecular details of virus-cell interaction are still crucial for the identification of therapeutic targets to treat COVID-19[125]. Degradation of the vascular endothelial glycocalyx significantly increased endothelial cell uptake of nanoparticle vehicles designed for drug delivery compared to the intact glycocalyx[126]. Ultra-small gold nanospheres coated with polyethylene glycol were successfully delivered intravenously in the glycocalyx degradation mouse model[127]. These lines of evidence suggest that vascular endothelial glycocalyx dysfunction induced by SARS-CoV-2 may be targeted for enhanced drug delivery, offering a new therapeutic approach for COVID-19. In particular, the possibility of a therapeutic approach focusing on vascular endothelial glycocalyx is explored in this section as follows:

1) A disintegrin and metalloprotease 17 (ADAM17)

ADAM17 was initially described to specifically cleave the precursor of TNF-α (pro-TNF-α)[128]. ADAM17 activity is induced in sepsis, and leads

to shedding of components of leukocytes and endothelial cell tether machinery, facilitating systemic inflammation[129]. It is already known that ADAM17 can release the ectodomains of a diverse variety of membrane-anchored cytokines, cell adhesion molecules, receptors, ligands, and enzymes. Since ADAM17 leads to shedding of membrane-bound ACE2 and release of the soluble extracellular domain of ACE2[130], ADAM17 and other proteases to do ACE2 shedding are expected to be valid as treatments for patients with COVID-19[131]. Of relevance, ADAM17 is co-expressed with syndecan-1 and has been shown to mediate syndecan-1 shedding in lung epithelial cells, which may aggravate endothelial glycocalyx disorders[132, 133]. Thus, careful consideration should be given to an ADAM17-related therapy, which is expected to shed membrane-bound ACE2, for COVID-19 patients.

2) Glycocalyx administration

Vascular endothelial glycocalyx has cardiovascular protective effects. Since it has been shown to protect against myocardial edema in a rat model[134], investigators have expected that intravenous administration of glycocalyx may improve damage to the vascular endothelial glycocalyx[135].

Restoring the vascular endothelial glycocalyx by infusion of the combination of hyaluronan and chondroitin sulfate was confirmed in an animal model[136]. The effectiveness of administration of glycocalyx to restore the vascular endothelial glycocalyx was examined using hyaluronan and chondroitin sulfate[137]. However, a similar effect has not yet been confirmed in COVID-19 patients.

3) Inhibitors of glycocalyx sheddase

Heparanase inhibitor: A protein heparinase inhibitor, PG545 plays a deleterious role in the development of renal injury and kidney dysfunction, attesting heparinase inhibition as a therapeutic approach for acute kidney disease[138, 139].

MMP inhibitors: Matrix metalloprotease (MMP) inhibitors have both pro-adhesion effects, by reducing sheddase activity, and anti-adhesion effects by inhibiting glycocalyx shedding and subsequent exposure of adhesion molecules on the endothelial cell surface[140].

Sulodexide: A heparin sulfate-like compound resistant to degradation by heparase, sulodexide can accelerate endothelial glycocalyx regeneration *in vitro* and *in vivo*. Type 2 diabetes is associated with

glycocalyx perturbation and increased vascular permeability, which are partially restored following sulodexide administration in these patients[53].

4) Anti-inflammatory mediators

Numerous anti-inflammatory mediators, such as TNF-α or its receptor inhibitor (etanercept)[37], allopurinol[38], sphingosine-1 phosphate (S1P)[89], and hydrocortisone, have been shown to have protective roles on the vascular endothelial glycocalyx[141]. Since these substances are expected to have anti-inflammatory and anti-oxidative effects, which impair vascular endothelial glycocalyx, they affects not only vascular endothelial cells but also vascular endothelial glycocalyx composition.

5) Fresh frozen plasma and albumin[142, 143]

The simplest way to achieve protection of the endothelial glycocalyx is to maintain a sufficiently high concentration of plasma proteins[20]. Indeed. the early and empiric use of fresh frozen plasma in hemodynamically unstable patients with bleeding has led to a decrease in early hemorrhagic deaths[144, 145]. Endothelial dysfunction not only leads to coagulation abnormalities, but also to inflammation and the breakdown

of organ-specific endothelial and epithelial barrier integrity[146]. Fresh frozen plasma reduced lung inflammation and injury in a rodent model of hemorrhagic shock that was correlated with restitution of syndecan-1[147]. Together, these observations suggest that after hemorrhagic shock, TNF-α induces syndecan-1 shedding in an ADAM17-dependent manner, which is inhibited by fresh frozen plasma[146].

6) Stem cell therapy

Cell-based approaches primarily using mesenchymal stem cells, have demonstrated safety and possible efficacy in patients with ARDS[148]. Intravenous administration of clinical-grade human mesenchymal cells into patients with COPD-19 was also shown to improve functional outcomes[149].

7) Antioxidant

Shedding of the vascular endothelial glycocalyx is triggered by redox stress encountered during reperfusion, and therefore, should be alleviated by the radical scavenger NO. The cardioprotective effect of NO in post-ischemic reperfusion includes the prevention of coronary vascular leak and interstitial edema, as well as a tendency to forestall

both no-reflow and degradation of the endothelial glycocalyx[150]. In theory, antioxidants seem to be a therapeutic option for COVID-19; however, the results of various large-scale clinical trials to date suggest that this would be difficult to induce. The reason being that many antioxidants lose their effectiveness immediately after administration, and may affect the redox regulatory control necessary to maintain homeostasis.

8) Ivermectin

Ivermectin, an FDA-approved anti-parasitic previously shown to have broad-spectrum antiviral activity in vitro, inhibits the replication of SARS-CoV-2 in vitro[151]. The previous study revealed that ivermectin is a specific inhibitor of importin α/β-mediated nuclear importable to inhibit replication of HIV and dengue virus[152]. It has already reported that ivermectin can improve the prognosis of patients with COVID-19, and ivermectin is currently considered to be one of the drugs with the highest potential.

9) Antithrombin III[153]

Antithrombin III is a physiological inhibitor of serine proteases (e.g.,

thrombin, elastase), which inhibits coagulation abnormalities and reduces inflammatory responses[154]. The combination of antithrombin III and hydrocortisone has also been reported to be effective. However, randomized control trials of Antithrombin III are not sufficient, and further trials with prespecified inclusion criteria and good bias protection is needed[154].

10) Sevoflurane

Sevoflurane is a modulator of the inflammatory response triggered by ischemia-reperfusion lung injury[155, 156]. Sevoflurane protects the lung endothelial glycocalyx in an *in vivo* lung auto-transplant model in pigs, and reduces the expression of leukocyte on the vessels[157]. These data may explain the beneficial outcomes linked to clinical use of volatile anesthetics after ischemia-reperfusion.

11) Intravenous immunoglobulin (IVIG)

IVIG is the standard treatment for Kawasaki disease. IVIG should be started within 7 days from the onset of fever in high suspicious patients for Kawasaki disease with COVID-19[158], because coronary artery aneurysms could occur in up to 25% of children with Kawasaki disease without timely

treatment[159]. IVIG is used to neutralize bacterial super-antigens and other infectious agents, inhibit the production of proinflammatory cytokines, neutralize pathogenic autoantibodies, enhance regulatory T cells (as well as inhibit other T cells), inhibit differentiation of Th17 cells, and reduce excessive ROS[93]. However, IVIG is ineffective in approximately 15% of children with Kawasaki disease, and insufficient control of monocyte suppression and T-cell activation, especially in terms of the CD8-T cells, are associated with IVIG resistance[160]. Given the pathological treatment of Kawasaki disease, IVIG treatment should be considered as an effective therapeutic option for severe COVID-19.

12) Tranexamic acid

The serine protease inhibitor, tranexamic acid, may prevent degradation of the glycocalyx. The effect of tranexamic acid administration on stress-related vascular endothelial glycocalyx damage has been examined in human umbilical vein endothelial cells (HUVECs)[161], in which it was shown to prevent vascular endothelial glycocalyx degradation via inhibition of endothelial sheddase activation of ADMA17 and MMP-9 *in vitro*.

13)Antihyperglycemic agents

Empagliflozin has been reported to restore the integrity of the endothelial glycocalyx in cultured human abdominal aortic endothelial cells treated with heparinase III-mediated glycocalyx disruption[162]. Although Empagliflozin is known to reduce cardiovascular events, the mechanism is still unclear. Therefore, a clinical study with the treatment of COVID-19 associated with vascular endothelial glycocalyx should be performed.

A traditional anti-diabetic drug, metformin, has been demonstrated to have a protective role in cardiovascular disease. In db/db mice, 2 weeks of metformin administration has been shown to improve obesity and diabetes-induced glycocalyx damage and hydration of the heart and kidney[163].

Summary and future outlook

To summarize, the vascular endothelial glycocalyx could explain the features of critical patients with COVID-19.

1) The vascular endothelial glycocalyx is perturbed by SARS-CoV-2

infection-induced inflammation, as well as in patients with hypertension, obesity, diabetes, cardiovascular disease, and who are current smokers.

2) SARS-CoV-2 can more easily infect the endothelial glycocalyx-damaged microvasculature in elderly people compared to young people, and in males mode than females.

3) Damage to the vascular endothelial glycocalyx leads to a rapid worsening of ARDS, microvascular thrombosis/DIC, Kawasaki disease shock syndrome, and may lead to arrhythmia and sudden death. Circulating levels of glycocalyx (e.g., syndecan-1 and hyaluronan) may be effective biomarkers to detect worsening signs earlier.

The composition of the vascular endothelial glycocalyx affects all aspect of severe COVID-19, including high risks of SARS-CoV-2 infection in the damaged endothelial glycocalyx, perturbed endothelial glycocalyx-induced microvascular leakage, thrombosis formation, excessive inflammatory cytokine release, leukocyte activation, platelet adhesion to the endothelium, and excessive ROS production (Figure 2).

The COVID-19 pandemic has fundamentally changed our lives. Many cities are,

or have been locked down, with people forced to stay at home and avoid contact with others. All unnecessary activities are encouraged to stop, and even educational and labor opportunities have been impacted by this infectious disease. We believe that the world will have to change as opposed to be restored to its previous state. Greater understanding of the virus will allow us to devise ways in which we can collectively survive the next "new-normal" era. Although the endothelial glycocalyx is a classical physical barrier common to many living creatures, this field has been poorly studies thus far. Given the international nature of the virus, we believe that it is necessary to share the latest knowledge from new research areas to offer the novel concept regarding the impact of vascular endothelial glycocalyx on COVID-19 to other researchers.

Figure legends

Figure 1. Comorbidities related to worsening of COVID-19 and vascular endothelial glycocalyx damage. The vascular endothelial glycocalyx is impaired due to factors such as smoking, physical inactivity, hypertension,

diabetes, obesity, and cardiovascular diseases. Severe acute respiratory coronavirus 2 (SARS-CoV-2) can easily infected the increased endothelial glycocalyx-damaged microvasculature that is observed to a greater extent in elderly people compared to young people, and in males more than females. ARDS: Acute respiratory distress syndrome, DIC: Disseminated intravascular coagulation, CKD: Chronic kidney disease, ROS: Reactive oxygen species, RAAS: Renin-angiotensin-aldosterone system, COPD: Chronic obstructive pulmonary disease.

Figure 2. Damaged vascular endothelial glycocalyx. Vascular endothelial glycocalyx damage is associated with vascular endothelial dysfunction, which leads to reduced nitric oxide (NO) bioavailability, excessive reactive oxygen species (ROS) production, inflammatory cytokine release, platelet adherence, coagulation, and leukocyte adhesion.
SARS-CoV-2: Severe acute respiratory coronavirus 2, VEGF: Vascular endothelial growth factor, VEGFR: VEGF receptor, ACE2: Angiotensin-converting enzyme 2, sACE2: Soluble ACE2, PAI-1: Plasminogen activator inhibitor-1, TF: Tissue factor, vWF: von Willebrand factor,

ox-LDL: Oxidized low-density lipoprotein, MMPs: Matrix metalloproteases, tPA: Tissue plasminogen activator, PGI2: Prostacyclin, TM: Thrombomodulin.

Figure 3. Intact vascular endothelial glycocalyx. In a situation where vascular endothelial cells are sufficiently covered with healthy vascular endothelial glycocalyx, even if severe acute respiratory coronavirus 2 (SARS-CoV-2) enters the body, it may be neutralized by the effects of appropriate reactive oxygen species (ROS) and soluble angiotensin-converting enzyme 2 (sACE2); consequently, it may be possible to prevent entry of the virus into the vascular endothelium.
VEGF: Vascular endothelial growth factor, VEGFR: VEGF receptor, NO: Nitric oxide, eNOS: Endothelial NO synthase, TM: Thrombomodulin, tPA: Tissue plasminogen activator, PGI2: Prostacyclin.

Figure 4. Severe COVID-19 comorbidity induced by vascular endothelial glycocalyx damage. The vascular endothelial glycocalyx can be damaged by various factors, including smoking, physical inactivity, hypertension, diabetes, obesity, and cardiovascular diseases. Various lethal conditions

in COVID-19 (e.g., acute respiratory distress syndrome [ARDS], disseminated intravascular coagulation [DIC], Kawasaki disease, microvascular thrombosis, and arrhythmias) may be caused by a common mechanism, damage of the vascular endothelial glycocalyx. CKD: Chronic kidney disease, ROS: Reactive oxygen species, RAAS: Renin-angiotensin aldosterone system, COPD: Chronic obstructive pulmonary disease.

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Figure 1

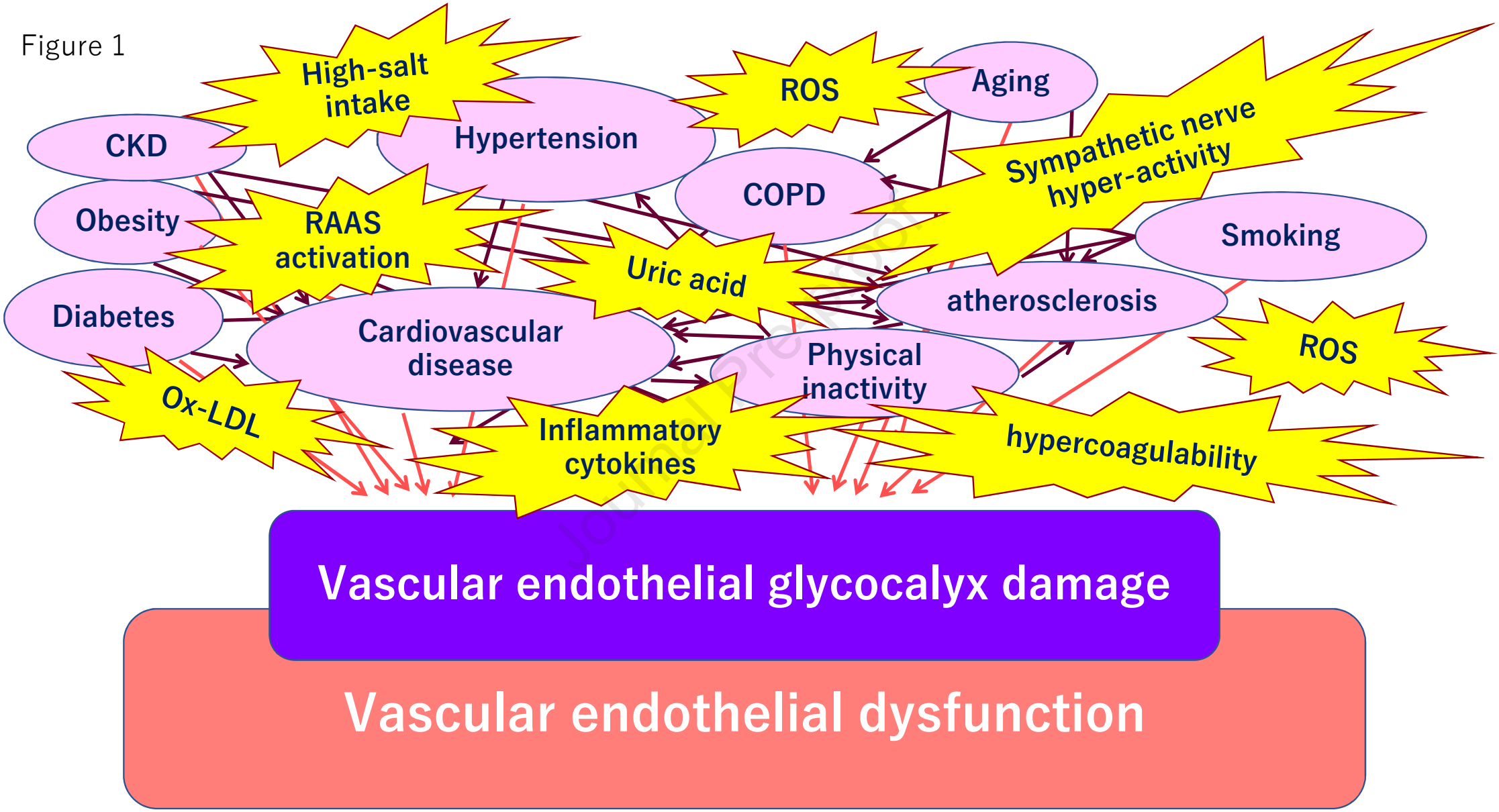

Figure 2

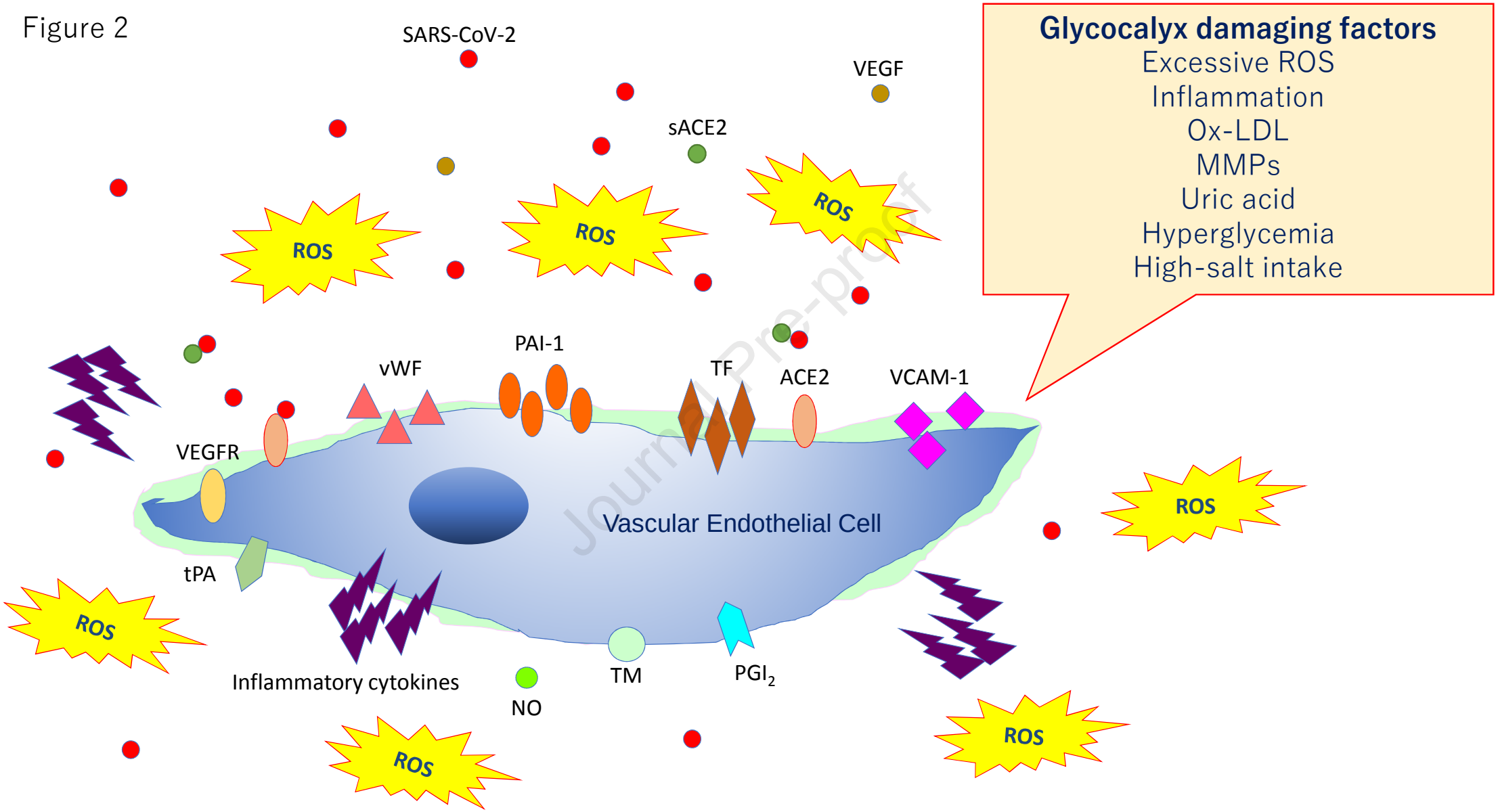

Figure 3

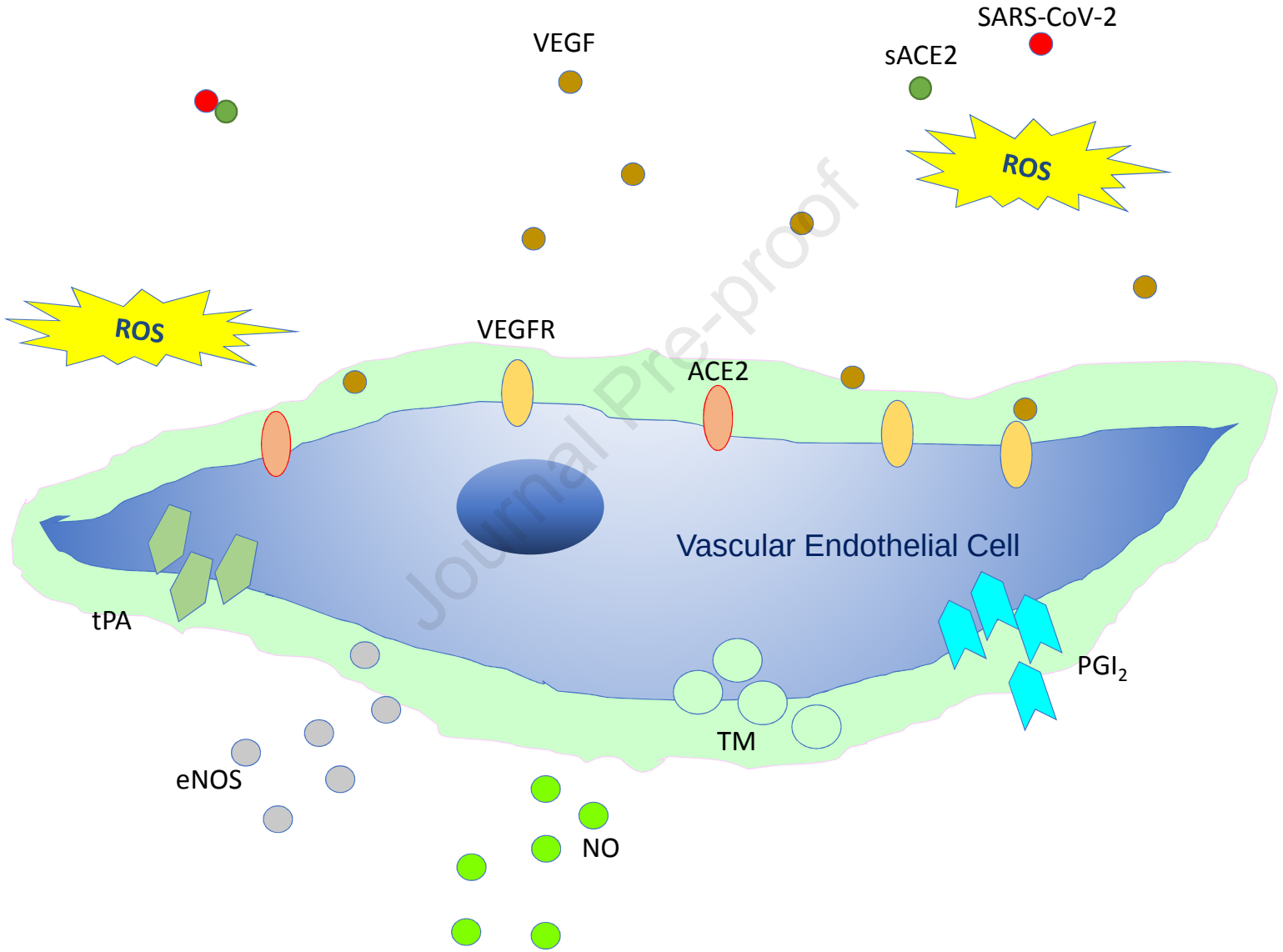

Figure 4