Vascular Endothelial Damage: The Role of Syndecan-1 and Hyaluronan as Severity Indicators in COVID-19

Azwar Anas¹, Arie Utariani², Bambang Pujo Semedi³*

Anesthesiology and Intensive Care Department Faculty of Medicine Universitas Airlangga Surabaya 60286 Indonesia¹³

Corresponding Author: ³*

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ABSTRACT

SARS-CoV-2 was first isolated in bronchoalveolar lavage (BAL) fluid from three patients with COVID-19 at Jinyintan Hospital in Wuhan, Hubei Province, China. The cases are increasing quickly with global mortality rate of 2.12%. The main cause of COVID-19 death is hypoxic respiratory failure due to acute respiratory distress syndrome (ARDS). Endothelial cell damage has a central role in ARDS pathogenesis and multi-organ failure in COVID-19. The endothelium, under homeostasis condition, is surrounded by mural cells (pericytes), which maintain vascular integrity and barrier function. These cells prevent inflammation by limiting the interaction of endothelial cells with immune cells and platelets and inhibit coagulation by expressing coagulation inhibitors and blood-clotting enzymes and producing glycocalyx. Vascular endothelial glycocalyx has a crucial role in endothelial function and is degraded systemically in elderly conditions and various comorbidities, which can be a potential mechanism for the development of lethal complications from COVID-19. Glycocalyx degradation due to endotheliopathy in SARS-CoV-2 infection causes increased levels of its fragments such as syndecan-1 and hyaluronan in the blood. Data from previous studies showed that the levels of these two biomarkers increased significantly in septic patient and several viral infections such as Kawasaki and dengue. These biomarkers are also markers of organ damage. Therefore, it can be indicated that hyaluronan and syndecan-1 are significant prognostic markers for morbidity and survival in COVID-19 patient.

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1. INTRODUCTION

SARS-CoV-2 was first isolated in bronchoalveolar lavage (BAL) fluids from three patients suffering from COVID-19 at the Jinyintan Hospital of Wuhan, Hubei Province, China [37]. Coronavirus is an unsegmented, positive-stranded RNA virus and has four main structural proteins, namely spike protein (S), membrane (M), envelope (E) and nucleocapsid (Lu, n.d.) SARS-CoV-2 has a diameter of 60-100 nm and a round/oval shape. SARS-CoV-2 can be inactivated by UV light or high temperature 56°C for 30 minutes and is sensitive to diethyl ether, 75% ethanol, chlorine, peracetic acid, and chloroform [23]. As of early August 2021, according to WHO, the number of confirmed cases of COVID 19 was 199,466,523 and the
number of deaths was 4,244,541. The global mortality rate is 2.12%. The first COVID-19 cases were reported in Indonesia on March 2, 2020 in a total of two cases. Data as of early August 2021, there were 3,532,567 cases of COVID-19 with the number of death was 100,636 cases and a mortality rate due to COVID-19 reaching 2.85%. Currently, patients with COVID-19 are the main source of infection [17]. Transmission in COVID-19 cases is through droplets that come out when someone coughing or sneezing [8]. Several cases have also been reported related to SARS-CoV-2 infection in neonates where vertical transmission from pregnant women to fetuses has not been proven to occur. If it can happen, studies show that the chances of vertical transmission are low [7]. The main replication of SARS-CoV-2 occurs in the mucosal epithelium of the upper respiratory tract (nasal cavity and pharynx) and undergoes multiplication in the lower respiratory tract and gastrointestinal mucosa [4]. Human angiotensin converting enzyme 2 (ACE2) is known to be a SARS-CoV-2 receptor found in the nasal mucosa, bronchi, lungs, heart, esophagus, kidney, stomach, bladder and ileum. The incubation period for SARS-CoV-2 averages 1 to 14 days, most requiring 3-7 days [7]. However, another study showed that incubation can occur for up to 24 days [15].

2. DISCUSSION

2.1 Inflammation, Immunity, and COVID-19

The pathogenesis of SARS-CoV-2 is still not widely known, but it is suspected that it is not much different from that of SARS-CoV which is more widely known. The S protein in the coronavirus plays an important role in the viral invasion process to the host cell where it will bind to the Angiotensin converting enzyme 2 (ACE2) receptor and a fusion process occurs with the membrane [12]. Next, inside the cell, the viral RNA genome will be released into the cell cytoplasm and translated into two polyproteins and a structural protein. Next, the viral genome will begin to replicate. Glycoproteins in the newly formed viral envelope enter the endoplasmic reticulum or Golgi membrane of the cell. The formation of a nucleocapsid which is composed of the RNA genome and nucleocapsid proteins. In the final stage, vesicles containing viral particles will fuse with the plasma membrane to release new viruses [12]. Virus and host factors have important role in SARS-CoV infection. The cytopathic effect on the virus and the ability to fight the immune system determines the severity of the infection. Dysregulation of the immune system further contributes to tissue damage caused by SARS-CoV-2 infection. Inadequate immune response leads to viral replication and tissue damage. On the other hand, excessive immune response can also cause tissue damage. The immune response caused by SARS-CoV 2 is also not known with certainty. However, it can be studied based on the mechanism that occurs in SARS-CoV and MERS-CoV infection. When the virus enters the cell, the viral antigen will be presented to the antigen presentation cells (APC). Viral antigen presentation binds to major histocompatibility complex (MHC) class I and some to MHC class II. Antigen presentation then stimulates the body's humoral and cellular immune responses mediated by virus-specific T and B cells. In the humoral immune response, IgM and IgG are formed against SARS-CoV. IgM against SAR-CoV is lost by the end of the 12th week and IgG can persist long term. The results of a study of patients who had recovered from SARS showed that after 4 years, CD4+ and CD8+ memory T cells were specific for SARS-CoV, but their numbers decreased gradually in the absence of antigen [25].
Viruses have mechanisms to evade the host immune response. SARS-CoV can produce double-membrane vesicles that do not have pattern recognition receptors (PRRs) and replicate within these vesicles so that they cannot be recognized by the host. The IFN-I pathway is also inhibited by SARS-CoV and MERS-CoV. Antigen presentation can also be inhibited in infections caused by the presence of MERS-CoV [15].

2.2 COVID-19 and Vascular Endothelial Dysfunction
The main cause of death in COVID-19 patients is hypoxic respiratory failure due to acute respiratory distress syndrome (ARDS) [35]. Currently, pulmonary endothelial cells have been viewed as therapeutic targets in COVID-19, but recent studies have shown that these cells contribute to the initiation and propagation of ARDS by impairing the integrity of the vascular barrier, triggering pro-coagulation conditions, inducing vascular inflammation (endothelitis), and mediates inflammatory cell infiltration [34]. Therefore, a better understanding of the role of vascular disorders is very important [31]. The endothelium, under homeostasis condition, is surrounded by mural cells (pericytes), which maintain vascular integrity and barrier function. These cells prevent inflammation by limiting the interaction of endothelial cells with immune cells and platelets and inhibiting coagulation by expressing coagulation inhibitors and blood clotting inhibitory enzymes and producing glycocalyx (a protective layer composed of glycoproteins and glycolipids) with anticoagulation activity [34]. After the initial phase of viral infection, 30% of hospitalized patients with COVID-19 develop clinical deterioration with progressive lung damage, as a result of an exaggerated inflammatory response. Pulmonary complications, mechanically, result from breakdown of the vascular barrier, resulting in tissue edema (leading to accumulation of fluid in the lungs), endothelium, activation of the coagulation pathway with potential for disseminated intravascular coagulation (DIC) and unregulated inflammatory cell infiltration. As in ARDS due to other causes, endothelial cell damage has a central role in the pathogenesis of ARDS and multi-organ failure in COVID-19 [35].

Vascular leakage and pulmonary edema in COVID-19 patients are caused by multiple mechanisms. First, the virus can directly affect endothelial cells because SARS-CoV-2-infected endothelial cells are found in various organs in deceased patients [34]. These endothelial cells undergo extensive endothelitis characterized by endothelial dysfunction, lysis, and death. Second, to enter cells, SARS-CoV-2 binds to the ACE2 receptor, leading to impaired activity of ACE2 (an enzyme with the opposite function of the vasopressor angiotensin). Decreased ACE2 activity indirectly activates the kallikrein- bradykinin pathway, which increases vascular permeability. Third, activated neutrophils, recruited to pulmonary endothelial cells, produce histotoxic mediators such as reactive oxygen species (ROS). Fourth, immune cells, inflammatory cytokines and vasoactive molecules cause an increase in endothelial cell contractility and widening of the inter-endothelial junction which causes a widening of the inter-endothelial distance.
Furthermore, the cytokines IL-1β and TNF activate glucoronidase which degrades the glycocalyx and causes upregulation of hyaluronic acid synthase 2, which results in the deposition of hyaluronic acid in the extracellular matrix and triggers fluid retention. Simultaneously, these mechanisms lead to increased vascular permeability and leakage [31]. Cardiovascular disease is also associated with increased mortality in hospitalized COVID-19 patients [20]. According to a previous report in Wuhan, 48% of patients had comorbid diseases, including hypertension (39%), diabetes (19%), and coronary heart disease (8%). Furthermore, patients with coronary risk factors and cardiovascular disease had the highest mortality rate (10.5%) from SARS-CoV-2 infection [13]. Data have shown that COVID-19 patients aged over 60 years have more systemic symptoms and pneumonia with more severe degrees than patients aged less than 60 years [5].

Multivariable regression analysis showed an increase in in-hospital mortality in elderly patients. This suggests that COVID-19 tends to worsen more easily in elderly patients with comorbidities, leading to immune dysfunction in elderly COVID-19 patients. In other words, microvascular leakage, which acts as a window for SARS-CoV-2 invasion of organs, is caused by more severe damage to the endothelial glycocalyx in the elderly. The vascular endothelial glycocalyx is more easily damaged in the elderly than in the young, and is known to be a comorbidity that can facilitate glycocalyx damage. Vascular endothelial glycocalyx is degraded systemically in elderly conditions and various comorbidities, which can be a potential mechanism for the development of lethal complications from COVID-19 [36].

**Figure 2.** Comorbidities associated with worsening of COVID-19 and damage to the vascular endothelial glycocalyx. Damage to the vascular endothelial glycocalyx is caused by various factors such as smoking, physical inactivity, hypertension, diabetes, obesity, and cardiovascular disease. SARS-CoV-2 can easily infect the microvascular endothelial glycocalyx that has been compromised, which is more common in the elderly than in the young, and more in men than women. 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 [36].

### 2.3 Vascular Endothelial Glycocalyx

The vascular endothelium is covered by a protective layer on its luminal surface called the glycocalyx. Glycocalyx is a gel-like layer composed of glycoproteins containing sialic acid, membrane proteoglycans (syndecan-1 and glypican), side chain glycosaminoglycans (such as heparin sulfate and chondroitin sulfate), and long chain hyaluronan (HA). Glycocalyx functions to maintain endothelial permeability, regulate leukocyte migration, and inhibit intravascular coagulation [18]. Hyaluronan (a glycosaminoglycan) and syndecan-1 (a proteoglycan) play an important role in maintaining the integrity of the glycocalyx. Vascular endothelial glycocalyx is stabilized by shear stress, which has a central role in nitrous oxide production [9].
Glycosaminoglycans are constantly degraded by enzymes, and are also synthesized and excreted through the vesicles of the Golgi apparatus to maintain a balanced homeostasis [26]. On the other hand, homeostasis is impaired and degradation of vascular endothelial glyocalyx occurs under conditions of cellular stress, ischemia/reperfusion injury, presence of endotoxin, inflammatory mediators, atrial natriuretic peptide, and reactive oxygen species in excessive levels, hyperglycemia [24], consumption excessive salt [27], hypertension, familial hypercholesterolemia, and oxidized low-density lipoprotein (ox-LDL). In addition, unhealthy lifestyles such as smoking and physical inactivity also trigger glyocalyx degradation. It is known that homeostasis is impaired, and vascular endothelial glyocalyx degradation occurs under conditions of cellular stress, ischemia/reperfusion injury, the presence of endotoxins, inflammatory mediators, atrial natriuretic peptide, and reactive oxygen species in excessive levels, hyperglycemia, excessive salt intake, hypertension, familial hypercholesterolemia, and oxidized low-density lipoprotein (ox-LDL). In addition, unhealthy lifestyles such as smoking and physical inactivity also trigger glyocalyx degradation.

Vascular endothelial glyocalyx has a crucial role in endothelial function, because it plays a role in microvascular reactivity and regulates interactions between the endothelium and blood and its components [1]. In addition, the vascular endothelial glyocalyx protects endothelial cells from shear stress caused by blood flow, and acts as a vascular permeability barrier [36]. Intact vascular endothelial glyocalyx contains a variety of cytokines and chemokines, receptors, growth factors, gap junction proteins, and enzymes including extracellular superoxide dismutase (eNOS), ACEs, lipoprotein lipase, xanthine oxidase, and antithrombin III, all of which play a central role in supporting endothelial function and blood/microvascular/tissue interactions [1]. A conformational change in the glyocalyx, which can be induced by blood flow, triggers the release of nitric oxide, thereby contributing to the regulation of vasomotor tone and peripheral oxygen distribution. Based on this interaction, the most important role of the endothelial glyocalyx is vascular protection through inhibition of coagulation and leukocyte adhesion [2]. Vascular endothelial dysfunction and vascular failure occur in situations where the endothelial glyocalyx is compromised, which contributes to various cardiovascular diseases [33].

Figure 3. Complications in COVID-19 due to vascular endothelial damage [36].

2.4 Syndecan-1 and Hyaluronan as Biomarkers of Vascular and Organ Endothelial Glyocalyx Damage
Glyocalyx fragments, such as syndecan-1 and/or hyaluronan, have been studied, and their validity is currently being tested. Glyocalyx degradation, characterized by increased concentrations of glyocalyx fragments such as plasma syndecan-1 and hyaluronan, has attracted the attention of critical illness experts. It is thought that this glyocalyx fragment is a diagnostic and prognostic indicator in various pathological conditions. The glyocalyx also has a function as a barrier for albumin filtration. Therefore, glyocalyx fragments can be biomarker in kidney disease. Although the estimates of the role of this glyocalyx...
component have been made in several previous studies, it remains important to measure its prognostic value in future studies so that it can be used in the management of critically ill septic patients. This information can complement routine biomarkers such as procalcitonin (PCT) and C-reactive protein (CRP) [10]. In the cohort study by [2], hyaluronan and syndecan-1 levels were significantly increased in septic patients compared to healthy individuals. The levels of both are even higher in severe sepsis and septic shock than in other septic patients. Hyaluronan and syndecan-1 were also significantly different in survivors and non-survivors (p<0.001). In this study, it was stated that the cut off values for predicting mortality were 441 ng/ml and 898 ng/ml for hyaluronan and syndecan-1, respectively. Both markers were significantly correlated with APACHE II scores and SOFA scores [2]. Systemic damage to the smooth lining of the vascular glycocalyx causes increased amounts of protein and water to move into the extra-vascular space. In septic conditions, the vascular endothelial glycocalyx is disrupted and its layer becomes thinner, which causes an increase in vascular permeability and causes interstitial edema in various organs [32].

Research on vasculopathy in viral infections, eg the association between dengue fever and damage to the vascular endothelial glycocalyx, has been 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 is a marker of the replication complex in cellular membranes. Elevated levels of components of the vascular endothelial glycocalyx layer, such as hyaluronan, heparin sulfate, claudin-5, and syndecan-1, have been associated with disruption to the vascular endothelial glycocalyx, and subsequent plasma leakage and severe dengue disease [29]. This evidence demonstrates the importance of evaluation and therapy of the vascular endothelial glycocalyx in severe conditions caused by viral infections, including COVID-19 [36]. On the other hand, patients with underlying disease tend to have systemic endothelial glycocalyx disruption due to complex mechanisms. When these patients are infected with SARS-CoV-2, endotheliopathy due to COVID-19-induced systemic vascular inflammation is very likely to develop into serious complications such as ARDS, DIC, Kawasaki shock syndrome, microvascular thrombosis, and arrhythmias [36]. In the case of Kawasaki Disease Shock Syndrome, there is an excessive production of inflammatory cytokines, and a tendency to be non-responsive to IVIG and coronary abnormalities. Experts indicate that there may be a slight increase in the number of children with severe COVID-19 symptoms with characteristics similar to Kawasaki Disease Shock Syndrome. Surprisingly, the circulating endothelial glycocalyx components (syndecan-1 and hyaluronan) were significantly elevated in the acute phase, and serum hyaluronan was established as a biomarker that was the best predictor of coronary artery lesion formation in Kawasaki disease [21]. Serum levels of syndecan-1 (sCD138), one of the major core proteins that make up the vascular endothelial glycocalyx, are thought to reflect inflammation and damage to the vascular endothelium in Kawasaki disease. Taking into account the similar pathophysiology between Kawasaki disease and COVID-19, it is envisaged that knowledge of the pathophysiology of vascular endothelial glycocalyx disruption in Kawasaki disease can be applied to research new therapeutic strategies and biomarkers to predict worsening of COVID-19 patients with severe symptoms [36].

The ACE2 receptor as a SARS-CoV-2 receptor is also present on vascular endothelial cells and arterial smooth muscle cells in all organs. Consequently, SARS-CoV-2 can directly adhere to vascular endothelial cells, and cause vascular endothelial dysfunction, which is followed by microvascular leakage, microvascular coagulation, excessive release of inflammatory cytokines, and impaired cell-to-cell connections. Recent autopsy studies on COVID-19 patients have shown that SARS-CoV-2 causes endothelitis in various organs, including the lungs, intestines, liver, kidneys, and stomach [34]. The National Health Service in the UK reported the incidence of systemic inflammatory disease similar to Kawasaki disease in COVID-19 patients. Specifically, this phenomenon is characterized by inflammation of
the walls of blood vessels, including arteries, veins, and capillaries, throughout the body. This indicates that COVID-19 is a systemic disease associated with thrombosis, endothelial dysfunction, and inflammation. However, it remains unclear whether this virus directly triggers the coagulation cascade or other mechanisms are involved. This virus triggers systemic inflammation and can cause lesions that affect blood vessels. However, viral infections can directly cause vasculitis or inflammation of blood vessels [22]. In experimental studies, it was explained that together with other pathophysiological conditions, disruption of the vascular endothelium in vital organs often causes organ damage [3]. Therefore, elevated levels of syndecan-1 and hyaluronan may be associated with specific organ damage. In the research of [11] revealed that plasma levels of syndecan-1 can be a predictor of the risk of acute kidney failure, hospital death, and mortality within 6 months. If elevated levels of syndecan-1 reflect endothelial dysfunction, a relationship may be found between plasma levels of syndecan-1 and the onset of acute renal failure [11].

Besides being the main component of the endothelial glycocalyx [6], HA (Hyaluronic Acid) is also an important component of the extracellular matrix of the lung. The lungs have the largest surface blood vessels in the body so they contain a very large amount of HA. It has been widely studied previously that based on the chain length, HA molecules have different biological activities: high molecular weight (HMW) HA which helps maintain lung tissue properties, homeostasis, and tissue repair; while low molecular weight HA (LMW) can be a marker of lung tissue damage and plays an important role in the activation of the acute inflammatory response [30]. In the context of ARDS, LMW-HA fragments have been reported to trigger inflammation by eliciting a “danger signal” that triggers leukocyte infiltration through activation and maturation of dendritic cells and the release of proinflammatory cytokines such as IL-1β, Tumor Necrosis Factor (TNF)α, IL-6, and IL-12 by several cell types [28]. According to studies, the accumulation of LMW-HA molecules in the small airways not only stimulates macrophages to release chemokines, cytokines, and growth factors, but also causes fluid retention in the extracellular space, thereby contributing to interstitial and alveolar edema [14]. HA with a molecular weight of <10 kDa is also associated with unbalanced tissue remodeling, depending on the severity of tissue damage, and contributes to extracellular matrix deposition and an increased risk of pulmonary fibrosis [30]. In the research of [16] the investigators correlated serum HA concentrations and BALF with lung injury score (Lung Injury Score (LIS)) and systemic severity as measured by SOFA score. The authors reported a positive correlation between serum HA levels and BALF at day 0 and LIS values, particularly through the association between HA levels and the degree of hypoxemia and PEEP. By looking for the relationship between HA levels and SOFA scores as an index of systemic severity, illustrating that, HA levels in BALF only positively correlated with the respiratory component of SOFA scores. Serum HA levels were elevated in patients with worsening respiratory, coagulation, hepatic, cardiovascular and renal failure, based on evaluation of SOFA scores. Main findings of Esposito's study et al are serum HA and BALF levels, derived from structural changes affecting the lung, associated with the severity of lung disease and the severity of organ dysfunction [16].

3. CONCLUSION
Vascular endothelial damage has an important role in the pathogenesis of COVID-19. Glycocalyx degradation due to endotheliopathy in SARS-CoV-2 infection causes increased levels of syndecan-1 and hyaluronan in the blood. These biomarkers are also markers of organ damage. Therefore, it can be indicated that hyaluronan and syndecan-1 are significant prognostic markers for morbidity and survival in COVID-19 patients.

4. REFERENCES


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