Mini Review

New coronavirus disease (COVID-19): Clinical symptoms other than pneumonia with emphasis on thrombosis

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Development of thrombosis in COVID-19 patients

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Abstract

The main manifestation of new coronavirus disease 2019 (COVID-19) is respiratory disease. The new coronavirus (SARS-CoV-2) attacks the lungs and may result in severe acute respiratory syndrome. In such cases, the infected person has difficulty breathing, which impairs oxygen uptake in the body. If pneumonia or another respiratory illness develops, and the host immune system is unable to combat SARS-CoV-2, the infected person may eventually die. Meanwhile, reports on unexpected symptoms in organs other than the nasal cavity, throat, and lungs are emerging. To elucidate the causes of such symptoms, we analyzed the expression status of angiotensin-converting enzyme 2 (ACE2), the host receptor used by SARS-CoV-2, in various tissues. This study confirmed that alveolar macrophages appear to be involved in pneumonitis and thrombus formation. In this report, we introduce some new symptoms.

Symptoms related to blood and blood vessels in patients with COVID-19.

In China, the United States, and Europe, a close relationship between SARS-CoV-2 infection and thrombotic events is gaining attention (1). Infection and SARS-CoV-2's invasion into vascular endothelial cells is assumed to be the main cause of increasing thrombotic events in patients. However, until now, the details of these diseases' pathogenic mechanisms have not been clarified. It has been reported that COVID-19-caused inflammation can lead to thrombus formation, which can cause serious damage, including "subclinical hypoxia." Based on research results obtained so far, pulmonary thrombosis causes asymptomatic hypoxia. Our research has disclosed that, in addition to type 2 alveolar epithelial cells, angiotensin-converting enzyme 2 (ACE2), which is the host receptor by which SARS-CoV-2 gains entry into the host cell, is also expressed in alveolar macrophages*1 (Figure 1a, Supplementary Data 1, Data 2). Furthermore, in COVID19 patients, alveolar macrophages and neutrophils reportedly invaded the arterial vessels of lung tissue (2). Apoptosis of alveolar vascular endothelial cells has been confirmed in patients infected with COVID-19 (2). Upon infection, neutrophils release neutrophil extracellular traps (NETs), composed of DNA, nuclear proteins, and proteases into the cytoplasm to process pathogens (3). Furthermore, neutrophils simultaneously induce thrombus formation by a coagulation reaction, activating possibly SARS-CoV-2-infected macrophages and platelets to prevent the progress of infection (3,4) (Figure 2). The microthrombi in the interalveolar septa of a lung from a patient who died from COVID-19 was found to differ from those in a lung from a patient infected with influenza virus (H1N1) (5). In Europe, young people infected with SARS-CoV-2 have frostbite-like symptoms, and this ripple of skin symptoms is spreading all over the world (6,7). Symptoms such as frostbite, that is, rash and blisters on the toes of the foot, and a unique symptom called "COVID toe,*2" have been observed in SARS-CoV-2 infected individuals (6,7). Numerous other reports have shown that blood clots can affect all organs, including the kidneys, blood vessels, intestines, liver, and brain. A Dutch study found that up to 38% of severely ill patients exhibited thrombosis-related symptoms. A report from Thailand showed that SARS-CoV-2-infected people had skin symptoms similar to those seen in cases of dengue fever. Skin symptoms, including erythema and petechiae, are typical symptoms of dengue fever (8). Due to the high incidence of dengue fever in Thailand, medical staff have been challenged differentiating between the symptoms of dengue fever and SARS-CoV-2 in outpatient clinics (8). In Europe and the United States, patients with Kawasaki disease-like symptoms have bene observed in pediatric medicine (9). Kawasaki disease is a childhood disease in which inflammation of blood vessels is observed throughout the entire body. Clinically, macrophage activation syndrome is associated with Kawasaki disease.

Symptoms in the brain and nerves

The most serious CNS-related symptom that may occur during SARS-CoV-2 infection is stroke. For instance, at the Mount Sinai Health System, five patients under 50 years presented with large-vessel stroke due to SARS-CoV-2 infection (10). The most likely cause of such strokes is a blood clot in the artery leading to the brain. Stroke has been observed even in young infected individuals. In patients with COVID-19, deep vein thrombosis, pulmonary embolism, and stroke

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may develop even if standard thromboprotective measures, such as low molecular weight heparin are taken (11). COVID-19 cases with D-dimer concentrations ≥ 2 μg/mL have been reported to have a poor prognosis (12). In addition, SARS-CoV-2 infection can cause mild neurological symptoms. The most prominent symptom of neurological disease is the loss of taste and smell; one study confirmed this symptom in 65% of individuals infected with SARS-CoV-2 (13). SARS-CoV-2 may directly affect the nervous system. In addition, comprehensive studies performed in Wuhan Province, China, and France have confirmed symptoms of neurological disease in SARS-CoV-2-infected patients.

In general, olfactory deficits, which occur after infection of the upper respiratory tract with viruses such as the common cold and influenza, are the most common causes of odor disorders in adults (2,13,14). About 40% of olfactory disorders are reportedly due to viral infections. Previous research results have revealed that coronaviruses, such as SARS-CoV-2, are neurotrophic and neuroinvasive. Our analysis indicates that ACE2 is not expressed in bronchial epithelial cells or nasopharyngeal epithelial cells (Supplementary Data 2). The olfactory dysfunction due to SARS-CoV-2 infection is probably caused by virus-derived neurotoxicity, which affects the olfactory system (15,16).

Cardiac symptoms

In the past few months, the association between COVID-19 prognosis and cardiovascular disease has been subject to study (17,18). In clinical practice, cardiac troponin, a myocardial escape enzyme, is measured to determine the presence of myocardial injury (19). Up to 37.5% of hospitalized patients with COVID-19 may present with elevated cardiac troponin levels and myocardial injury (20-22), which injury leads to severe heart failure and has a vast impact on life prognosis. Therefore, diagnosis of the presence or absence of myocardial injury is important. In addition to thrombus-related complications caused by blood vessel obstruction, COVID-19 increases the burden on the heart. Furthermore, it seems that the myocardium can become exhausted due to hypoxia or/and inflammation in infected persons with chronic lung disease. Our results indicate that ACE2 is strongly expressed in myocardial tissue (Figure 1b, Supplementary Data 1, Data 2). In addition, in some cases, it has been shown that SARS-CoV-2 directly infects and damages heart tissue.

Renal symptoms

Recent epidemiological studies have revealed that 20%–40% of SARS-CoV-2-infected patients develop acute renal failure (ARF) (23,24). In Japan, cases of ARF in SARS-CoV-2 infected patients have been confirmed. Although several dialysis machines are being used for chronic renal failure in Japan, it is difficult to provide sufficient dialysis machines for SARS-CoV-2-infected patients with ARF. Therefore, medical authorities are expected to mitigate this issue promptly. In Chinese and Italian studies conducted in the early stage of the outbreak, approximately 25%–27% of inpatients who died were found to have kidney damage (23,24). In patients with pneumonia, SARS-CoV-2 infection appears to be a frequent cause of kidney damage. However, the mechanism by which such kidney damage occurs remains unclear. The results of our analysis showed that ACE2 is strongly expressed in cells of the renal tubules (Figure 1c, Supplementary Data 1, Data 2), and SARS-CoV-2 can infect renal endothelial cells (2). Previous results suggest that thrombus formation in blood vessels, leading to systemic overactive inflammation, oxygen deficiency, direct infection, and attack of SARS-CoV-2 on the kidney may all be involved in ARF.

Immune system abnormalities

The symptoms of patients with COVID-19 may worsen due to a ‘cytokine storm*[3]’, which is abnormal activation of systemic inflammatory reactions. Even after the virus has been treated, the immune system begins to attack healthy tissues and organs. Cytokine storms may, therefore, exacerbate the already serious medical conditions caused by the virus. However, data on cytokine storm-related exacerbation of COVID-19 are limited. Previous studies have confirmed elevated blood levels of cytokines that enhance immune system activity in many deceased patients. Cytokine

inhibitors may be effective in fending off cytokine storms. Previous studies have suggested that early administration of anticoagulants may help prevent cytokine storms by inhibiting cytokine activation.

Conclusion

There are several unclear points regarding SARS-CoV-2 infection pathophysiology. However, many recent cases of COVID-19-related thrombosis have been reported from all over the world. In a recent study, analysis of blood test data from patients with COVID-19 identified fibrinogen/albumin ratio and platelet count as independent risk factors for disease aggravation (25). SARS-CoV-2 binds to various cells using the protein angiotensin-converting enzyme 2 (ACE2) as a receptor. ACE2 is also expressed on the surface of vascular endothelial cells. Vasculitis, caused by the involvement of alveolar macrophages, may develop. Vasculitis is triggered by ACE2 binding with the SARS-CoV-2 spike glycoprotein. Blood vessels spread to all organs throughout the body, and the target of SARS-CoV-2 can, therefore, may be practically all organs, not only the lungs. Prophylactic anticoagulant therapy is considered a rational treatment for severely affected COVID-19 patients. In addition, for men and smokers, who are generally known to be prone to vascular disorders, substantial medical treatment is necessary to avoid a detrimental course of COVID-19. People with high blood pressure, diabetes, obesity, and other chronic diseases characterized by vascular damage may also represent a high-risk group for developing more severe courses of COVID-19. The spread of SARS-CoV-2 infection has not stopped, and the risk of medical shortcomings are becoming apparent worldwide. Since the Great Depression, the world economy is expected to experience the largest decline, and governments are rushing to respond to the economy. The unprecedented situation calls for the earliest development of therapeutic agents and vaccines against COVID-19.

Footnote

Alveolar macrophage*[1]: Our analysis revealed that ACE2 was strongly expressed in alveolar macrophages, but not in classical monocytes, interstitial monocytes, or non-classical monocytes (Figure 1a, Supplementary Data 2). An alveolar macrophage (or dust cell) is a type of macrophage, a ‘professional’ phagocyte, found in the pulmonary alveoli, near the pneumocytes, but separated from the wall. Classical monocytes are MHC II^{negative}, CD64^{low}, CD11b^{high}, and Ly6C^{high}. Interstitial macrophages are MHC II^{high}, CD64^{high}, CD11b^{high}, CD11c^{high}, and Siglec F^{negative}. Monocyte-derived alveolar macrophages are CD64^{high}, CD11c^{high}, F4/80^{positive}, MerTK^{positive}, and Siglec F^{low}. Tissue-resident alveolar macrophages are CD64^{high}, CD11c^{high}, F4/80^{positive}, MerTK^{positive}, and Siglec F^{high}.

COVID toe*[2]: Please note that these skin symptoms do not mean that you are infected with the new coronavirus, SARS-CoV-2. It means that these skin symptoms may have been caused by the development of other diseases, such as true chicken pox and collagen disease.

Cytokine storm*[3]: A cytokine storm is an excessive and systemic immune response to external stimuli. Its pathogenesis is complex. The disease progresses rapidly, and mortality is high. During the COVID-19 epidemic, the severe deterioration observed in some patients has been speculated to be linked to cytokine storms.

Disclosure

The authors declare no potential conflicts of interest. The funders had no role in study design, data collection, or analysis; decision to publish; or preparation of the manuscript. The materials (manuscript and figures) presented here reflect original research, have not been published previously, and have not been submitted for publication elsewhere while under consideration.

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Author Contributions
T.H. and M.M. performed most of the experiments and coordinated the project; T.H. and M.M. conceived the study and wrote the manuscript. N.Y. carefully reviewed this manuscript and commented on the medical science. I.K. gave information on clinical medicine and oversaw the entire study.

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Materials and Methods are indicated in Supplementary Data 1 and Data 2

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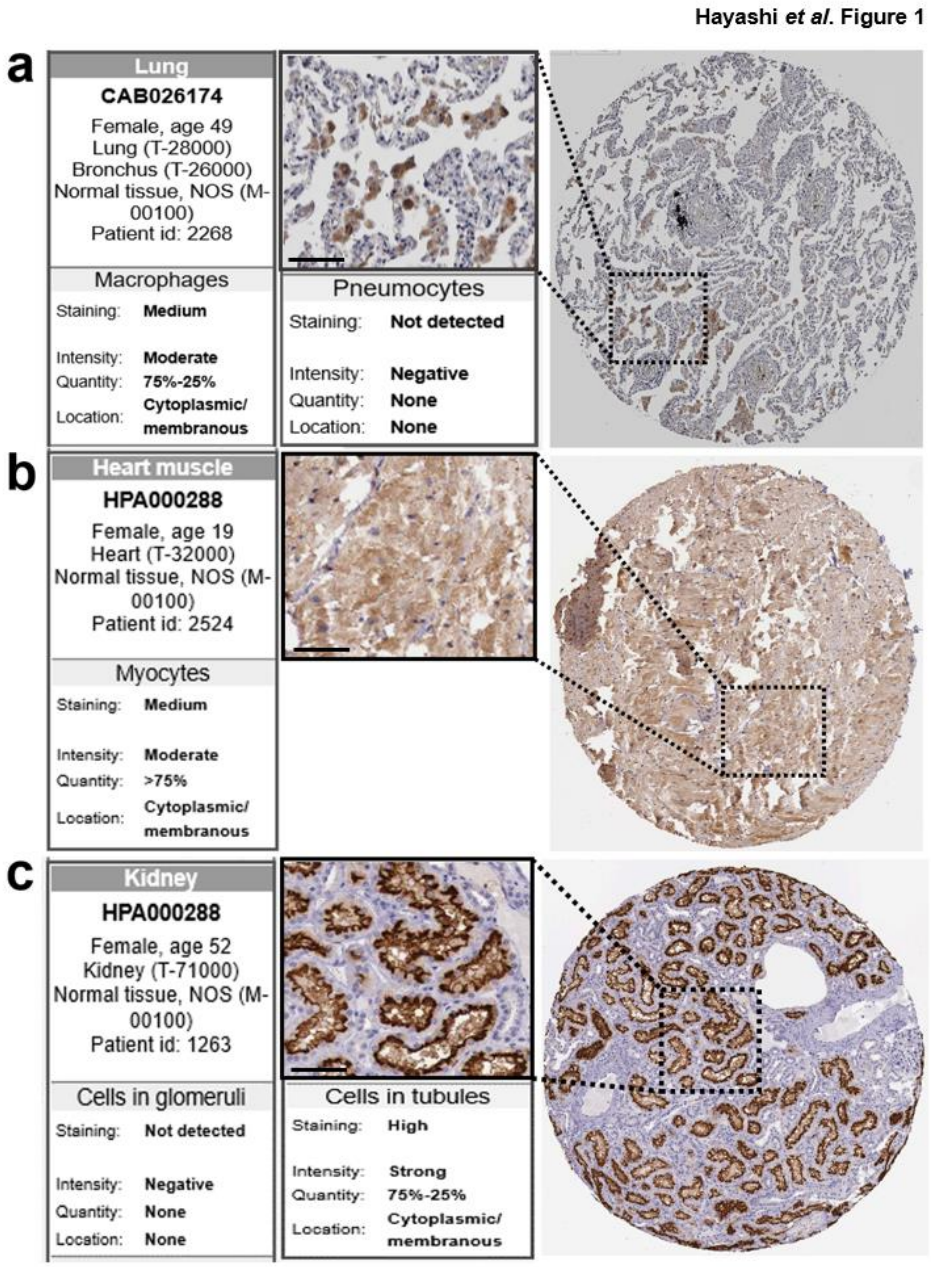

Figure 1. ACE2 expression in alveolar, myocardial, and renal tissues
To examine the expression status of ACE2, immunohistochemical staining was performed, using alveolar, myocardial, and kidney tissues. **A.** In alveolar tissue, ACE2 expression is found in alveolar macrophages, but not in type 1 and type 2 alveolar epithelial cells. **B.** In myocardial tissue, ACE2 is expressed abundantly in cardiomyocytes. **C.** In kidney tissue, ACE2 is strongly expressed in tubular cells, but not cells in cells of the glomeruli. Sections (5 μm) were stained with primary antibody. Figures were captured with a 40× objective lens. Scale bar = 100 μm. Details on the antibodies and tissues used for immunohistochemical staining are described in Supplementary Data 1 and Supplementary Data 2.

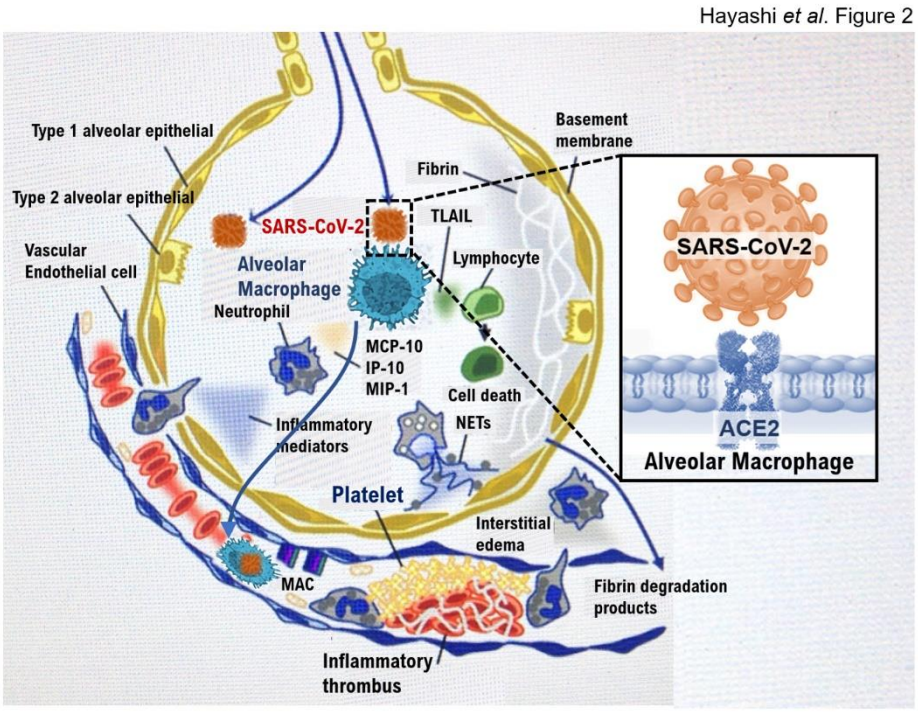

Figure 2. Intravascular thrombus formation in COVID-19

Within the alveoli, alveolar macrophages may be infected with SARS-CoV-2. Monocytes/alveolar macrophages, possibly SARS-CoV-2-infected alveolar macrophages, and neutrophils play important roles in thrombus formation during COVID-19. Cytokines, including MCP-10, IP-10, and MIP-1 released from activated alveolar macrophages induce activation of neutrophils. Apoptosis of alveolar vascular endothelial cells has been confirmed in patients infected with COVID-19 (2). In COVID-19 patients, alveolar macrophages and neutrophils were reported to invade the arterial vessels of lung tissue (2). When a neutrophil encounters a pathogen, it can respond in several ways: by phagocytosis, degranulation, or by releasing neutrophil extracellular traps (NETs). In recent studies, NETs have been described to play a central role in activating the inflammasome in macrophages or, possibly, SARS-CoV-2-infected alveolar macrophages. The cellular components of blood vessels, i.e. leukocytes, platelets, erythrocytes, macrophages, and vascular endothelial cells, form membrane attack complexes (MACs). MACs play significant roles in thrombus formation in combination with coagulation system activation (26). Thrombotic microangiopathy leads to peripheral organ failure and death of the host.

MCP-10; monocyte chemoattractant protein-1, IP-10; interferon gamma- induced protein 10, MIP-1; Macrophage inflammatory protein-1