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Review

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COVID-19 and acute limb ischemia: latest hypotheses of pathophysiology and molecular mechanisms

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Abstract: Coronavirus disease 2019 (COVID-19) is a multi-system disease that can lead to various severe complications. Acute limb ischemia (ALI) has been increasingly recognized as a COVID-19-associated complication that often predicts a poor prognosis. However, the pathophysiology and molecular mechanisms underlying COVID-19-associated ALI remain poorly understood. Hypercoagulability and thrombosis are considered important mechanisms, but we also emphasize the roles of vasospasm, hypoxia, and acidosis in the pathogenesis of the disease. The angiotensin-converting enzyme 2 (ACE2) pathway, inflammation, and platelet activation may be important molecular mechanisms underlying these pathological changes induced by COVID-19. Furthermore, we discuss the hypotheses of risk factors for COVID-19-associated ALI from genetic, age, and gender perspectives based on our analysis of molecular mechanisms. Additionally, we summarize therapeutic approaches such as use of the interleukin-6 (IL-6) blocker tocilizumab, calcium channel blockers, and angiotensin-converting enzyme inhibitors, providing insights for the future treatment of coronavirus-associated limb ischemic diseases.

Key words: Acute limb ischemia (ALI); Coronavirus disease 2019 (COVID-19) infection complication; Hypercoagulability; Thrombosis; Vasospasm; Hypoxia-inducible factor 1α (HIF-1α); Angiotensin-converting enzyme 2 (ACE2); Type I interferon (IFN-I); Tocilizumab

1 Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China at the end of 2019 (Zhu et al., 2020). The outbreak caused a large number of deaths and challenged global healthcare systems. Although most deaths from COVID-19 result from acute respiratory distress syndrome (ARDS) and multiple organ failure, thromboembolic complications such as myocardial infarction, pulmonary embolism, and ischemic stroke have also led to morbidity and mortality (Chen et al., 2020). Acute limb ischemia (ALI), the sudden disruption of blood flow to the arms or legs, is one of the manifestations of COVID-19-associated ischemic lesions. Although ALI is commonly caused by cardiovascular emboli, thrombotic occlusion, atherosclerosis, and vascular injury, an unexpected increase in the number of ALI patients was found during the COVID-19 pandemic (Bellosta et al., 2020). These patients often experienced local ischemia of the limbs with symptoms of cyanosis, ulceration, blisters, and even necrosis (Fig. 1). The appearance of ALI predicts a poor disease prognosis (Goldman et al., 2020; Bellosta

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et al., 2021). However, most studies have been based on case reports, and a few retrospective studies had problems such as a small number of cases or inconsistent inclusion criteria (Putko et al., 2021; Jain et al., 2022). In addition, the molecular mechanisms of COVID-19 infection and its effects on the circulatory system are not well understood, resulting in limited knowledge of the pathophysiology and molecular mechanisms of COVID-19-associated ALI. The purpose of this article is to analyze the common characteristics of previous cases and discuss relevant hypotheses for COVID-19-associated ALI by reviewing the literature from the past three years. We also explore the risk factors and new treatment strategies for this disease from a mechanistic perspective, providing ideas and therapeutic strategies for future research on ALI associated with COVID-19 and other coronavirus diseases.

2 Pathophysiology

There are many pathophysiological hypotheses regarding COVID-19-associated ALI (Fig. 2). Most studies suggest that hypercoagulability and thrombosis are key processes, as changes in coagulation markers have been observed in many cases, and the presence of thrombi has been confirmed through imaging studies (Bellosta et al., 2020; Omar et al., 2022). Additionally, vasoconstrictive ischemia, such as vasospasm, is also considered an important functional change in patients where thrombi are not detected, as well as in mild cases with localized skin manifestations. Moreover, since ALI occurs mainly in patients with severe COVID-19 infection, systemic hypoxia and acidosis may also lead to ALI.

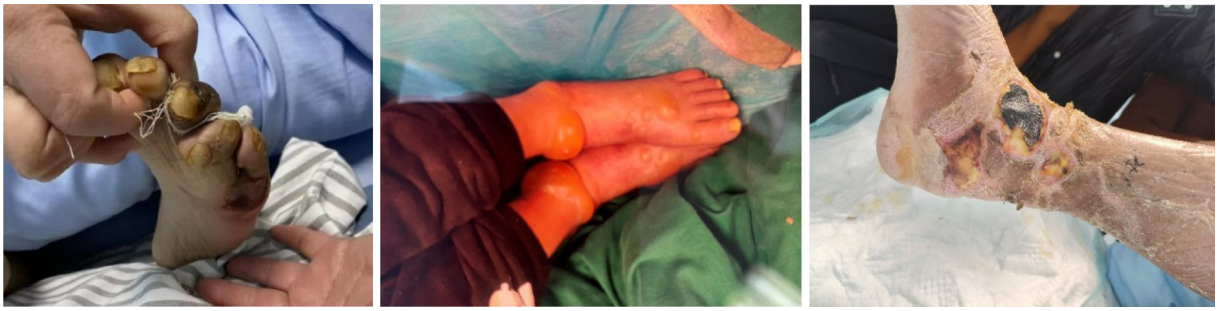

Fig. 1 Clinical presentations of coronavirus disease 2019 (COVID-19)-associated acute limb ischemia (ALI). All patients presented with severe limb ischemic symptoms within one month after COVID-19 infection, but the specific manifestations varied, including cyanosis, ulceration, blisters, and necrosis. Severe cases may result in death.

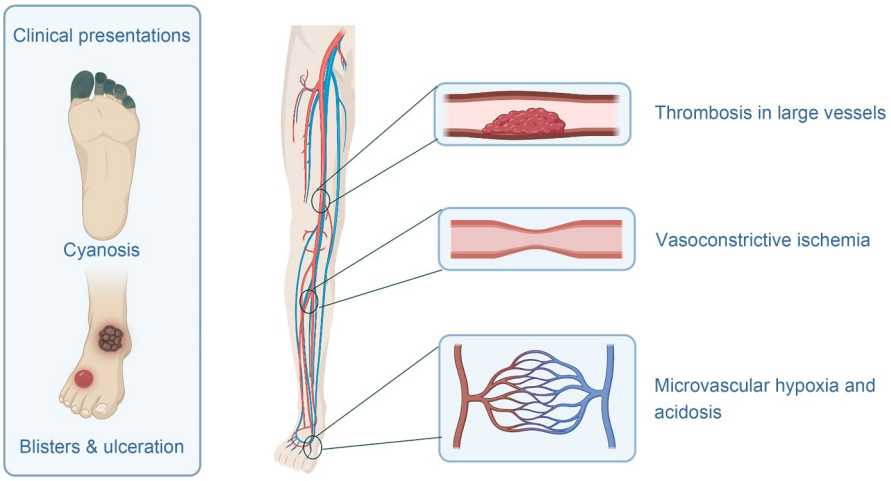

Fig. 2 Pathophysiological hypotheses of coronavirus disease 2019 (COVID-19)-associated acute limb ischemia (ALI). Hypercoagulability and thrombosis are widely recognized as important pathophysiological mechanisms, but recent evidence and hypotheses suggest that vasoconstrictive ischemia, such as vasospasm, as well as microvascular hypoxia and acidosis, also plays important roles. However, these mechanisms have different effects on different vascular segments and have different impacts on patients with variable degrees of severity. Note that although COVID-19-associated ALI occurs mostly in the lower extremities, the upper extremities can also have similar presentations.

2.1 Hypercoagulability and thrombosis

In previous studies of SARS-CoV-1 and coronavirus of Middle East respiratory syndrome (MERS-CoV), hypercoagulable state and thrombosis were important complications. In some cases of SARS-CoV-1 postmortem autopsies, fibrin thrombi were found in the pulmonary vasculature, suggesting a prothrombotic effect of the SARS-CoV-1 virus (Nicholls et al., 2003; Ng et al., 2005). Laboratory parameters of both SARS-CoV-1 and MERS-CoV patients demonstrate dysregulation of the coagulation cascade indicating a prothrombotic state (Lee et al., 2003; Ng et al., 2005; Algahtani et al., 2016). Giannis et al. (2020) suggested that this phenomenon can be attributed to an attempt to prevent diffuse alveolar haemorrhage, but it leads to the formation of harmful blood clots instead. In addition to SARS-CoV-1 and MERS-CoV, similar laboratory findings and clinical manifestations have been observed in SARS-CoV-2 infection. Klok et al. (2020) reported an incidence of thrombotic complications of 31% in intensive care unit (ICU) patients with COVID-19, with pulmonary thromboembolism being the most common type. Tang et al. (2020) found 71.4% of disseminated intravascular coagulopathy (DIC) in COVID-19 non-survivors. Individuals exhibited markedly elevated levels of D-dimer and fibrin degradation products (FDPs), as well as prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT). A hypercoagulable state and thrombosis are considered important pathological mechanisms of ALI in COVID-19 patients. In a systematic review, Putko et al. (2021) found evidence to support a hypercoagulable state as being the main cause of ALI in 10 of 12 studies. An elevated D-dimer level is a common characteristic observed in most case studies (Zhang Y et al., 2020; Zhou et al., 2020; Ozbey and Algan, 2022; Alitter et al., 2023). D-dimer is a metabolite of fibrin and indicates fibrin deposition and subsequent degradation. However, D-dimer is a relatively non-specific indicator, as it can also increase in many other inflammatory conditions.

2.1.1 Debates on DIC

There are numerous explanations for the hypercoagulable state observed in patients with COVID-19. One possible explanation is that the severe inflammatory response induced by COVID-19 leads to ARDS and subsequent DIC. However, it is still debated whether ALI caused by COVID-19 is a result of DIC. In a case study by Khattab et al. (2021) found that the elevation of D-dimer was not accompanied by hypofibrinogenemia or thrombocytopenia, which is inconsistent with the possibility of DIC. According to Iba et al. (2020), the prolonged PT and aPTT, as well as decreased antithrombin activity, are relatively infrequent in COVID-19 patients. The hypercoagulable state caused by COVID-19 may have its unique pathophysiological mechanism (Abou-Ismail et al., 2020).

2.1.2 Inflammatory thrombosis

It is widely accepted that the mechanisms underlying hypercoagulability and thrombosis involve vascular injury and inflammatory thrombosis. Conway et al. (2022) summarized the pathophysiological mechanisms of COVID-19-associated coagulopathy (CAC), which involve three main steps: vascular endothelial cell dysfunction, hyperinflammatory immune response, and hypercoagulability. Under normal conditions, endothelial cells can express antithrombotic molecules to prevent both platelet activation (prostacyclin and ectonucleotidases) and coagulation (tissue factor (TF) pathway inhibitor). Vascular endothelial cell dysfunction is often observed in viral infections. However, under a COVID-19 condition, although early hypotheses suggest that SARS-CoV-2 can cause injury by directly infecting endothelial cells, recent studies have shown that angiotensin-converting enzyme 2 (ACE2) expression on the surface of endothelial cells is relatively low (Zhao et al., 2020). Ahmetaj-Shala et al. (2020) found that primary human endothelial cells are not susceptible to infection by SARS-CoV-2 in vitro, indicating that COVID-19 mainly damages blood vessels through indirect pathways. The specific cellular and molecular mechanisms discussed below involve mainly the release of inflammatory cytokines, the activation of the complement system, and the activation of various inflammatory cells, ultimately leading to endothelial cell activation. This impairs the original antithrombotic function of the endothelial cells. Elevated levels of von Willebrand factor (VWF) and factor VIII (FVIII) could be observed in plasma (Escher et al., 2020). In addition, the activity of TF-positive extracellular vesicles (EVs), released by TF-expressing cells such as endothelial cells, was also increased in the plasma of COVID-19 patients (Mackman et al., 2021). Tissue damage can induce more activation of the complement system and

neutrophils, thereby causing positive feedback loops. One reported mechanism by which COVID-19 can promote clotting is through increased production of neutrophil extracellular traps (NETs). Activated neutrophils release NETs, which are intricate networks consisting of DNA, histones, microbicidal proteins, oxidant enzymes, coagulation factors, and complement factors. Increasing NETs can promote clotting by increasing the expression of functional TF and capturing platelets (Middleton et al., 2020). Platelets are also activated in this process (Althaus et al., 2021). Moreover, there have been reports of COVID-19 causing the production of anti-phospholipid antibodies in certain cases, leading to thrombosis (Stelzer et al., 2021). Finally, COVID-19 can regulate coagulation and fibrinolytic proteins at the genetic level (Mast et al., 2021). Bouck et al. (2021) found that COVID-19 patients had increased thrombin generation potential and increased endogenous plasmin potential compared with sepsis patients. COVID-19 can upregulate genes encoding procoagulant proteins and downregulate genes encoding anticoagulants (Conway et al., 2022).

2.2 Vasoconstrictive ischemia

Vasospasm is widely recognized as an important mechanism underlying ischemic lesions, particularly in cardiovascular and cerebrovascular events (Maseri et al., 1978; Arning et al., 1998). While vasospasm of limb vessels can occur via the contraction of smooth muscle in muscular arteries, few reports have focused on this phenomenon in large vessels of the extremities (Al Yacoub et al., 2022). Taking the lower extremity as an example, occlusion of one of the three vessels supplying the calf and foot (anterior and posterior tibial arteries and peroneal artery) does not typically result in severe outcomes. However, spasms in larger vessels such as the popliteal artery can lead to ALI (Winckiewicz et al., 2007). Some case reports have observed vasospasm in the femoropopliteal or peroneal artery followed by ALI (Bory et al., 1979; Winckiewicz et al., 2007). Kaneyama et al. (2014) reported that spontaneous vasospasm can present with both “acute” and “chronic” limb ischemia. Kuchynkova et al. (2017) reported a case of prolonged vasospasm leading to ALI in all four limbs. Virus-associated vasospasm has been observed following infection by human immunodeficiency virus (Orrapin et al., 2015) and varicella zoster virus (Limb and Binning, 2009; Keser et al., 2018), but some cases are due to interactions between virus infection and drug metabolism such as ergotamine (Demir et al., 2010; Orrapin et al., 2015; Mohamedi et al., 2021). For COVID-19 patients, vasospasm is believed to play an important role in ischemic lesions. A high Lindegaard’s ratio (middle cerebral artery/ipsilateral extracranial internal carotid artery mean velocity) indicates severe vasospasm (Park et al., 2022). Monov et al. (2022) retrospectively observed a higher Lindegaard’s ratio in COVID-19-positive subarachnoid haemorrhage patients between January 2020 and December 2021, indicating worse brain perfusion due to vasospasm (Monov et al., 2022). Aikawa et al. (2022) proposed that vasospastic angina could be a cause of post-acute COVID-19 syndrome. Vasospasm is considered to be an important causal mechanism of ALI (Teixeira et al., 2022; Alitter et al., 2023). Alitter et al. (2023) emphasized the importance of vasospasm in their case report and questioned the role of microthrombi with no other organ involvement and negative workup. For smaller vessels, COVID-19 is associated with atypical Raynaud’s phenomenon or pernio (chilblain)-like lesions, such as the notorious “COVID toe” (Alonso et al., 2020; Cappel et al., 2021). Although not life-threatening like ALI, COVID-19-associated skin ischemia is often a precursor or combined symptom of ALI. Ischemic skin lesions (chilblains and erythema-edema) have been reported with a high incidence in retrospective studies in France and Spain (de Masson et al., 2020; Galván Casas et al., 2020). Cappel et al. (2021) suggested that the pathogenesis likely involves vasospasm and a type I interferon (IFN-I) immune response. However, Kolivras et al. (2020) proposed that there is confusion between chilblain-like lesions and ALI, with chilblain-like lesions often occurring in young patients with mild COVID-19 symptoms, and ALI often occurring in severely ill patients with a hypercoagulable state. Cappel et al. (2021) also reported misuse of the term in this field, but they proposed that systemic mechanisms may explain the two types of manifestations. Based on the results of the above studies, we believe that vasospasm is one of the important pathophysiological mechanisms underlying the presentation of limb ischemia caused by COVID-19 infection, affecting both large and small vessels. However, vasospasm may play a more important role in small vessels. COVID-19-induced vasospasm is caused mainly by (1) the ACE2 and angiotensin

pathways, (2) the IFN-I pathway, and (3) the use of vasopressors.

2.2.1 ACE2 and angiotensin pathways

Angiotensin (Ang) is an important molecule for vasoconstriction in the body, primarily through the pathway of the renin–angiotensin–aldosterone system (RAAS). ACE2 is an important target for the SARS-CoV-2 virus when attacking cells. Virus invasion can cause ACE2 downregulation, leading to Ang II accumulation and exacerbating the occurrence of vascular spasm. The specific mechanisms will be detailed in the mechanism section. Wu et al. (2020) reported elevated plasma Ang II levels in 82 non-hypertensive COVID-19 patients, demonstrating a positive correlation between plasma Ang II levels and COVID-19 severity. However, Rieder et al. (2021) did not find a significant change in Ang II levels in mild COVID-19 patients (Rieder et al., 2021). This indicates that Ang II level alterations can be observed only in critically ill patients, who are likely to develop ALI. Additionally, Villard et al. (2020) found that aldosterone and C-reactive protein levels were significantly elevated in critically ill COVID-19 patients, with a linear relationship between aldosterone levels above 102.5 pmol/L and C-reactive protein. Aldosterone levels and C-reactive protein have great clinical significance in patients with coronary vasospasm (Inoue et al., 2009). In summary, we hypothesize that the ACE2 and Ang pathways may be a specific mechanism for COVID-19-induced vasospasm. However, in addition to direct effects, ACE2 may indirectly induce microvascular spasms through inflammation. Further animal experiments are required to confirm these mechanisms in COVID-19-associated ALI.

2.2.2 IFN-I pathway

Kolivras et al. (2020) reported that COVID-19-associated chilblain-like lesions are pathologically characterized by lymphocytic vasculitis, with a prominent perivascular lymphocytic infiltrate. This pathological feature is consistent with that of chilblains (Herman et al., 1981). Recent studies on familial chilblain lupus have found that IFN-Is are continuously activated and cause an autoimmune response (Zimmermann et al., 2019). In chilblains, cold stimulation can cause local inflammation through increased oxidative stress and the activation of IFN-I (Zimmermann et al., 2019). IFN-I has been found to inhibit the endothelial nitric oxide (NO) synthase pathway in this process (Buie and Oates, 2014), resulting in local vasoconstriction and vasospasm, leading to the occurrence of chilblains (Shahi et al., 2015). Considering the common pathological manifestations of COVID-19-associated chilblain-like lesions and chilblains, IFN-I might play an important role in their pathophysiological mechanisms (Kolivras et al., 2020; Cappel et al., 2021). Furthermore, these skin manifestations usually occur in warm seasons and are frequently associated with COVID-19 contacts, indicating that the abnormal IFN-I response caused by COVID-19 is highly likely to be involved (Galván Casas et al., 2020). Interestingly, this lymphocytic vasculitis was not found in the skin pathology of critically ill patients with ALI (Cappel et al., 2021), suggesting that it exists only in mild skin ischemic manifestations involving small blood vessels.

2.2.3 Vasopressor use in COVID-19 patients

Although low blood pressure is not a direct pathological change caused by COVID-19 infection, vasopressors are often used to treat critically ill patients. High-dose, short-term use of vasopressors such as norepinephrine, epinephrine, and dopamine may lead to ALI as an adverse reaction. Therefore, vasopressors have been discussed in numerous articles as one of the causes of vasospastic ischemia. In a systematic review by Putko et al. (2021) reported that 10.6% of COVID-19-associated ALI patients were treated with vasopressors. However, further research is needed to evaluate whether the use of vasopressors is associated with COVID-19-associated ALI and whether it should be continued. Lari et al. (2020) observed that the limb ischemia they found was confined to the distal phalanx, and they suggested that it may be secondary to vasopressor infusions. However, Schultz and Wolf (2020) reported two cases of digital ischemia in patients who received vasopressors, and they believed that unilateral ischemia would not be caused by vasopressors. Currently, there is still controversy over whether vasopressors are involved in ALI. Many articles have discussed the role of vasopressors (Kartikasari et al., 2021; Nowroozpoor et al., 2021). The results showed that the drug concentration of vasopressors was very low when limb ischemia occurred (Nowroozpoor et al., 2021). Xie et al. (2023) suggested that ischemic events in critically ill patients may be caused by increasing

vasopressor support, but their further research found no significant association between vasopressor use and post-revascularization amputation.

2.3 Hypoxia and acidosis

Hypoxia is rarely reported as a pathological mechanism of ALI. However, for severe respiratory diseases such as COVID-19, hypoxia and acidosis are the most common pathological changes in the body. A systematic review by Putko et al. (2021) showed that 17.0% of COVID-19-associated ALI patients required ventilators, indicating that hypoxia and acidosis may be the underlying mechanisms. Hypoxia-inducible factor 1α (HIF-1α) is an important sensor of oxygen tension in inflammatory environments in the body (Dang et al., 2011). It regulates the balance between regulatory differentiation of regulatory T (Treg) cells and T helper 17 (Th17) cells. On the one hand, it can enhance Th17 development by increasing interleukin-17 (IL-17), and on the other hand, it can attenuate Treg development by binding Fokhead box protein P3 (Foxp3) and targeting it for proteasomal degradation (Dang et al., 2011). Treg plays an important anti-inflammatory role in the body, and its inhibition will lead to immune function hyperactivity. In addition, HIF-1α is considered an important cofactor in COVID-19 infection, further indicating the important role of hypoxia in activating the body's immune response (Jahani et al., 2020). Note that, regardless of whether severe ALI related to thrombosis or mild skin ischemia related to vasospasm is involved, the common result is the relative hypoxia of local tissues. Thus, a change in HIF-1α may be a common mechanism of these two symptoms. In addition, hypoxia and acidosis are important factors leading to microcirculatory disorders. In severe COVID-19 patients, septic shock is very common, especially when there is secondary bacterial or fungal infection, which is also common in ALI. When septic shock occurs, the sympathetic nervous system is overexcited, causing an increase in catecholamine release, which in turn causes constriction of arterioles and precapillary sphincters, leading to local tissue ischemia. The RAAS is also activated in this process. At the same time, platelets produce more thromboxane A_2, which can further contract blood vessels and cause thrombus formation. The large amount of lactate and inflammatory substances produced by tissue hypoxia can further damage the vascular endothelium, causing thrombosis and increased vascular permeability. Various factors promote each other and lead to a vicious cycle.

3 Molecular mechanisms

The pathological and physiological mechanisms underlying ALI are usually caused by systemic reactions and complex mechanisms. Note that COVID-19 infection has been proven to have many complex effects with numerous clinical manifestations and sequelae. In the following section, we will illustrate the body's response after COVID-19 infection involving several important molecular mechanisms and the role of these mechanisms in ALI.

3.1 ACE2 mechanism

Infection of COVID-19 is facilitated by the binding of the spike protein on the virus and ACE2 on host cells (Zhang HB et al., 2020) (Fig. 3). ACE2 is a homolog of ACE1, which is a transmembrane glycoprotein composed of 805 amino acids. The extracellular catalytic domain can cleave an amino acid from Ang I to form Ang1–9 and remove an amino acid from Ang II to form Ang1–7 (Vickers et al., 2002). For the SARS-CoV-2 virus to enter cells, it must first bind to ACE2 and then the transmembrane protease serine 2 (TMPRSS2) facilitates viral ingress. During this process, surface ACE2 expression is down-regulated (Walls et al., 2020), and the spike protein of the virus can also trigger endocytosis of ACE2 (Gheblawi et al., 2020). Ultimately, the weakening of ACE2 function directly leads to an increase in Ang II and a reduction in Ang1–7. An "Ang II storm" has been described in SARS-CoV-2 infection (Ramos et al., 2021). Ang II can bind to the Ang II type 1 receptor (AT1R), causing a series of pro-inflammatory, pro-fibrotic, and procoagulant cascades. In addition, AT1R activation can cause vasoconstriction by decreasing NO release. Conversely, the AT2R activated by Ang1–7 increases NO release and has antithrombotic and anti-inflammatory effects (Forrester et al., 2018). This mechanism can also explain certain vaccine-related thrombotic events. The free spike protein may down-regulate ACE2 expression by binding to ACE2 (Deshotels et al., 2014; Angeli et al., 2021b), and cause thrombus formation by binding with ACE2 on platelets (Zhang S et al., 2020).

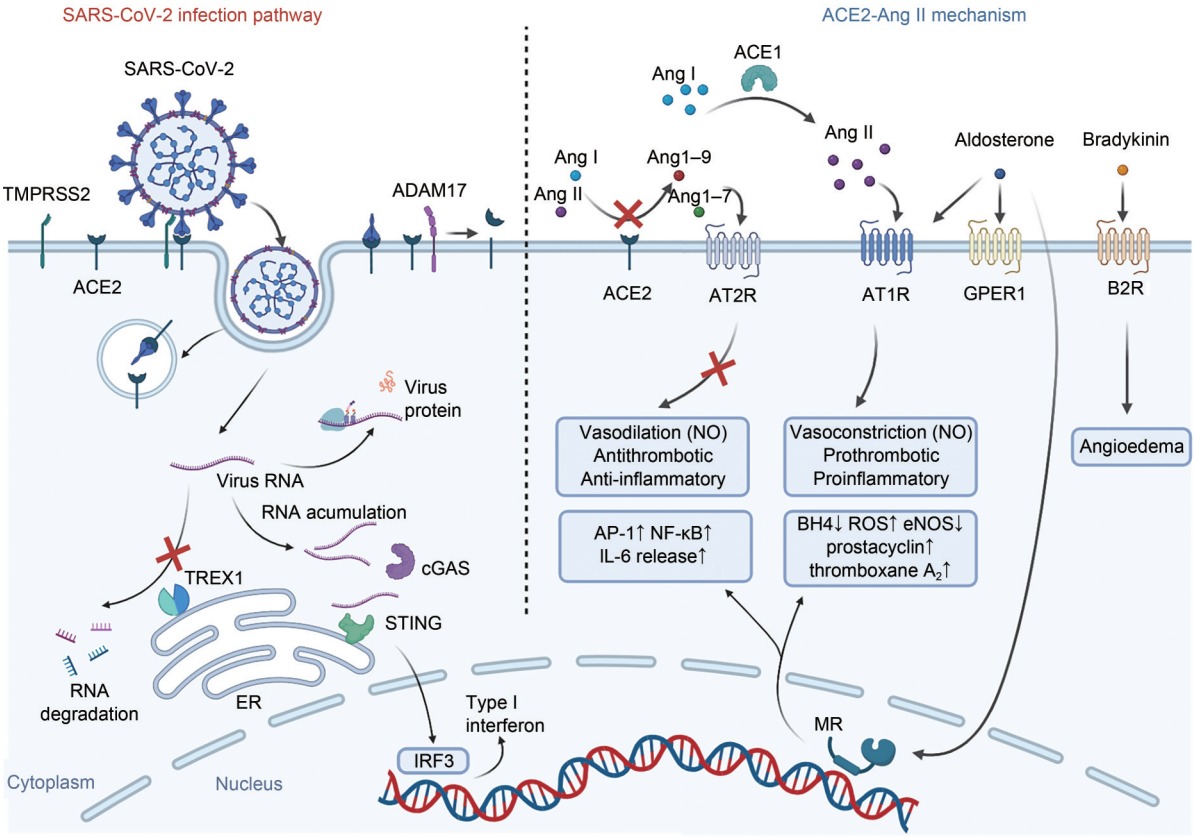

Fig. 3 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and angiotensin-converting enzyme 2 (ACE2)-angiotensin (Ang) II mechanism. SARS-CoV-2 entry into cells requires the involvement of both ACE2 and transmembrane protease serine 2 (TMPRSS2). The entry results in the internalization of ACE2 and reduces ACE2 number on the cell surface. Besides, the free spike protein can bind to ACE2 and inhibit its function. Activation of A disintegrin and metalloprotease 17 (ADAM17) can also lead to ACE2 cleavage. Therefore, SARS-CoV-2 can cause a significant reduction in ACE2. ACE2 can modify Ang I and Ang II to produce Ang1–9 and Ang1–7, respectively, which activate Ang II type 2 receptor (AT2R) and have vasodilatory and anti-inflammatory effects. Downregulation of ACE2 leads to the formation of a large amount of Ang II through ACE1, which activates mainly Ang II type 1 receptor (AT1R) and has vasoconstrictive, pro-inflammatory, and pro-coagulant effects. In addition, aldosterone secretion increases. This can regulate endothelial function through the nuclear receptor mineralocorticoid receptor (MR), membrane receptor AT1R, G protein-coupled estrogen receptor-1 (GPER1), and the expression of inflammatory factors, nitric oxide (NO) synthesis, cyclooxygenase (COX)-related prostaglandin synthesis, and thromboxane A_2. Furthermore, an increase in bradykinin can cause angioedema. Additionally, SARS-CoV-2 RNA in the cytoplasm can be degraded by three prime repair exonuclease 1 (TREX1) on the endoplasmic reticulum. Inactivation of TREX1 for various reasons can lead to RNA accumulation and recognition by intracellular receptor cyclic guanosine monophosphate-adenosine monophosphate (GMP-AMP) synthase (cGAS), stimulator of interferon gene (STING), and activation of transcription factors to increase the expression of type I interferon. ER: endoplasmic reticulum; IRF3: interferon regulatory factor 3; B2R: type 2 bradykinin receptor; AP-1: APETALA-1; IL-6: interleukin-6; NF-κB: nuclear factor-κB; BH4: tetrahydrobiopterin; ROS: reactive oxygen species; eNoS: endothelial NO synthase.

3.1.1 Aldosterone alteration

Activation of AT1R can also induce downstream aldosterone synthesis. This can then regulate the physiological functions of the body through genomic pathways involving mineralocorticoid receptor (MR) or non-genomic pathways mediated by MR or other receptors such as G protein-coupled estrogen receptor-1 (GPER1) or angiotensin receptor type 1 (Hermidorff et al., 2017). Besides, endothelial dysfunction caused by aldosterone primarily includes vascular tone dysfunction, vascular inflammation, aldosterone-related atherosclerosis, and vascular remodeling (Chen et al., 2019), with the first two mechanisms having a strong correlation with the occurrence of ALI. Aldosterone can affect the production of vascular NO through mechanisms such as causing a deficiency of cofactor tetrahydrobiopterin (BH4) and generating reactive oxygen

species (ROS) (Nagata et al., 2006). It can also directly lower the activity of endothelial NO synthase (eNOS) by dephosphorylating its phospho-eNOS (Ser1177) (Nagata et al., 2006). In addition, aldosterone can increase cyclooxygenase-2 (COX-2)-derived prostacyclin and thromboxane A_2 (Xavier et al., 2008). Blanco-Rivero et al. (2005) demonstrated through rat experiments that aldosterone can weaken the vasodilatation effect of acetylcholine by producing prostacyclin. Aldosterone can also directly or indirectly lead to vascular inflammation. Apart from causing an increase in ROS, aldosterone has been found in animal models to increase the expression of inflammation-related factors such as transcription factors activator protein (AP)-1 and nuclear factor-κB (NF-κB) (Fiebeler et al., 2001). Aldosterone can induce oxidative stress damage in tissue cells and the release of a series of inflammatory factors, including IL-6, through the MR/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/NF-κB pathway (Chou et al., 2018). In addition, aldosterone can interact with inflammatory cells and promote their proliferation by acting on the receptors on the surface of dendritic cells and T cells together with Ang II (Chang and Wei, 2015). Cappel et al. (2021) found that the large amount of IL-6 and IFN-I produced by inflammatory cells can cause the manifestations of COVID-19-associated limb ischemia through the Janus kinase-signal transducer and activator of transcription 1 (JAK-STAT1) and JAK-STAT3 pathways, respectively. Furthermore, they suggested that, due to their different responses to inflammatory factors, young people are more affected by IFN, while old people are mainly affected by IL-6. This results in IFN-related chilblains in young people and IL-6-related thrombosis and retiform purpura in the elderly (Fig. 4).

3.1.2 Kallikrein-kinin system

Apart from aldosterone, the kallikrein-kinin system is also regulated by ACE2 (Sodhi et al., 2018). Bradykinin is a vasoactive peptide that can relax vascular smooth muscle cells and increase vascular permeability (Marceau et al., 2018). Although ACE2 does not directly act on bradykinin, it can inactivate des-Arg9-bradykinin (DABK, the active metabolite of bradykinin) (Sodhi et al., 2018). Therefore, COVID-19 infection can cause kallikrein-kinin system activation, leading to localized angioedema (van de Veerdonk et al., 2020). Although the effects of kinins and the RAAS system on vascular constriction and dilation are opposite, considering the potent constrictive effects of angiotensin and aldosterone on blood vessels and that kinins are primarily regulated by ACE1 rather than ACE2, the overall effect on vascular tension is still constriction. The angioedema caused by kinins may be related to local inflammation, fluid accumulation, and thrombus formation in blood vessels.

These studies have shown that ACE2 and downstream RAAS system dysfunction can affect vascular function in multiple ways, leading to ALI through vascular constriction, thrombus formation, and other mechanisms. Since ACE2 is a specific target of coronavirus, these mechanisms are unique to COVID-19 infection and may play important roles in COVID-19-associated ALI.

3.2 Inflammatory mechanisms

COVID-19 infection can cause severe immune reactions in the body, which are typically seen as cytokine storms. This phenomenon is the result of a complex molecular mechanism that involves multiple inflammatory cells (Fig. 5). Systemic inflammation can lead to a hypercoagulable state and local inflammatory

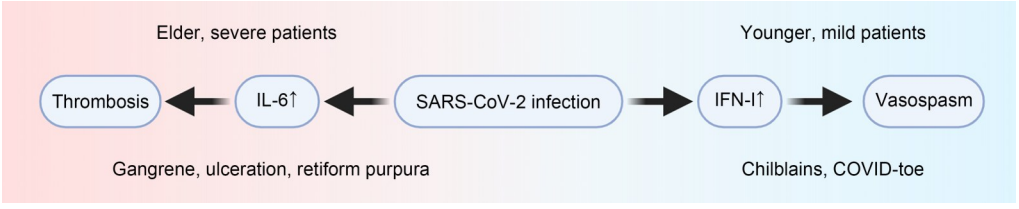

Fig. 4 Hypothesis of interleukin-6 (IL-6) and type I interferon (IFN-I) balance in coronavirus disease 2019 (COVID-19)-associated acute limb ischemia (ALI). Studies have found that COVID-19-associated ALI can present as severe limb thrombosis or mild chilblains-like symptoms, with the former occurring mainly in elderly patients with severe COVID-19 infection and the latter occurring mainly in younger patients with milder symptoms. Studies suggest that this is related to the balance of IL-6 and IFN-I in patients. Under different conditions, different inflammatory factors dominate the body, with IL-6 mainly causing thrombosis and IFN-I potentially causing strong vasospasm (Cappel et al., 2021).

thrombosis, which can result in ALI. Notably, these inflammatory changes usually first occur in lung tissue and then cause systemic changes throughout the body.

3.2.1 Inflammatory cells

Regarding white blood cell counts, many studies have reported an increase in neutrophil count and a decrease in lymphocyte count (Deng et al., 2020; Fan, 2020; Li et al., 2020). Liao et al. (2020) analyzed bronchoalveolar lavage fluid (BALF) and found that severely affected COVID-19 patients had a higher proportion of macrophages and neutrophils, along with increased levels of inflammatory cytokines IL-6, IL-1β, and IL-8, while T cells were reduced. This may have resulted from the action of different chemokines. For example, chemokine (C-C motif) receptor 1 (CCR1) and chemokine (C-X-C motif) receptor 2 (CXCR2) recruit monocytic cells and neutrophils in severely affected COVID-19 patients and increase CXCR3 and CXCR6 production to attract T cells in moderately affected COVID-19 patients. These results indicate that macrophages play a crucial role in the inflammation of COVID-19 infection, especially in severe patients who are at high risk of a hypercoagulable state and ALI. The activation of macrophages is followed by excessive and sustained release of IL-6 and tumor necrosis factor-α (TNF-α), which can cause a series of systemic COVID-19 inflammation symptoms (Giamarellos-Bourboulis et al., 2020). This presentation is similar to that of the macrophage-activation syndrome (MAS), but MAS usually requires ferritin levels above 4420 ng/mL, which is not very common in patients with COVID-19 (Kyriazopoulou et al., 2017; Abou-Ismail et al., 2020). Meanwhile, activated macrophages can also cause an upregulation of TF expression, activating platelets and promoting thrombosis through protease-activated

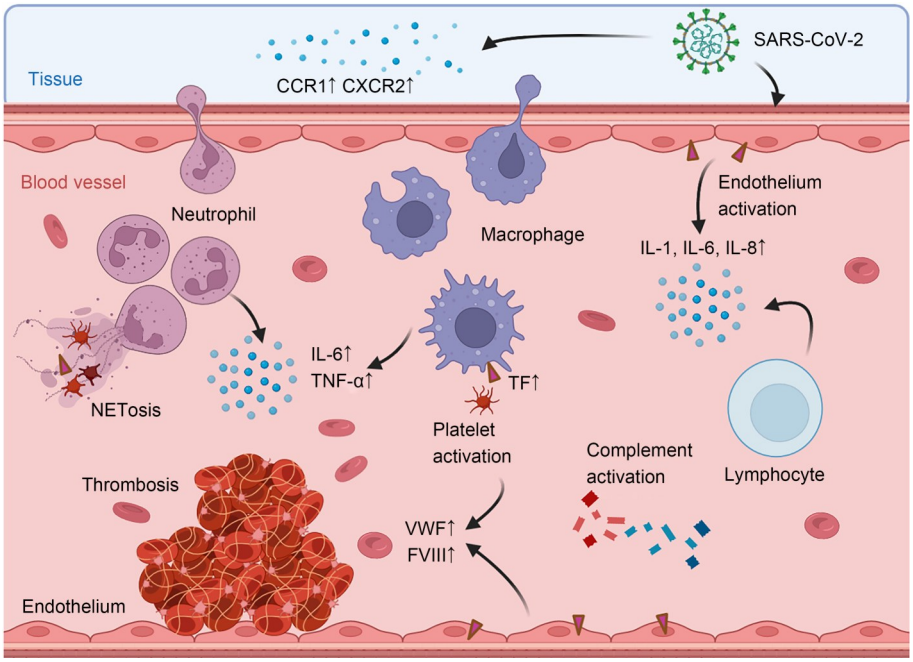

Fig. 5 Inflammation and hypercoagulable state in coronavirus disease 2019 (COVID-19)-associated acute limb ischemia (ALI). The initial effect of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is the release of a large amount of inflammatory cytokines from tissue cells and the activation of vascular endothelial cells. Among patients with ALI, the levels of chemokine receptors chemokine (C-C motif) receptor 1 (CCR1) and chemokine (C-X-C motif) receptor 2 (CXCR2) are significantly increased, causing massive accumulation of neutrophils and macrophages. Neutrophils can release a large amount of inflammatory cytokines and form neutrophil extracellular traps (NETs) through neutrophil extracellular trap release (NETosis), which can capture functional tissue factor (TF) and platelets. Macrophages play an important role by continuously releasing large amounts of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and by activating platelets through TF production. SARS-CoV-2 can directly activate endothelial cells and lymphocytes to produce a large amount of cytokines, while the levels of von Willebrand factor (VWF) and factor VIII (FVIII) in blood vessels are significantly increased. The complement system is also significantly activated in this process. The ultimate result is inflammation and thrombosis in blood vessels. Note that although this mechanism occurs mainly in pulmonary tissue, the inflammatory response can occur throughout the body via blood vessels, especially in patients with ALI.

receptor (PAR) signaling pathways (Foley and Conway, 2016).

3.2.2 Cytokines

The COVID-19 cytokine spectrum includes IL-6, TNF-α, IL-1β, IL-7, IL-2, IL-10, IFN-γ, and others, and these cytokines are released not only by macrophages but also by mesenchymal cells and fibroblasts (Foley and Conway, 2016; Abou-Ismail et al., 2020; Kang and Kishimoto, 2021). Among these cytokines, IL-6 plays a particularly important role and has some association with ALI. The IL-6 receptor has three signaling pathways: classic signaling, trans-signaling, and trans-presentation (Kang and Kishimoto, 2021) (Fig. 6). In classic signaling, IL-6 binds to membrane-bound IL-6 receptor (mIL-6R) and glycoprotein 130 (gp130) to activate the JAK-STAT3 pathway. In trans-signaling, IL-6 binds to soluble IL-6 receptor (sIL-6R) in blood and tissue fluids and then binds to cells that express gp130 but not mIL-6R, such as endothelial cells. In trans-presentation, mIL-6R on dendritic cells binds to IL-6 and presents it to T cells with gp130, resulting the priming of Th17 cells (Kang and Kishimoto, 2021). In the presence of high concentrations of IL-6, the main pathway activated is trans-signaling, causing endothelial damage and activation (Kang and Kishimoto, 2021). JAK kinases bind to gp130 and are phosphorylated after the IL-6/sIL-6R complex activates gp130. JAK activates STAT3 phosphorylation and induces homodimerization. STAT3 homodimer can induce the transcription of functional genes. The activation of endothelial cells by the IL-6/sIL-6R complex leads to the release of IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1), which in turn recruit immune cells and plasminogen activator inhibitor-1 (PAI-1), thereby promoting the activation of the

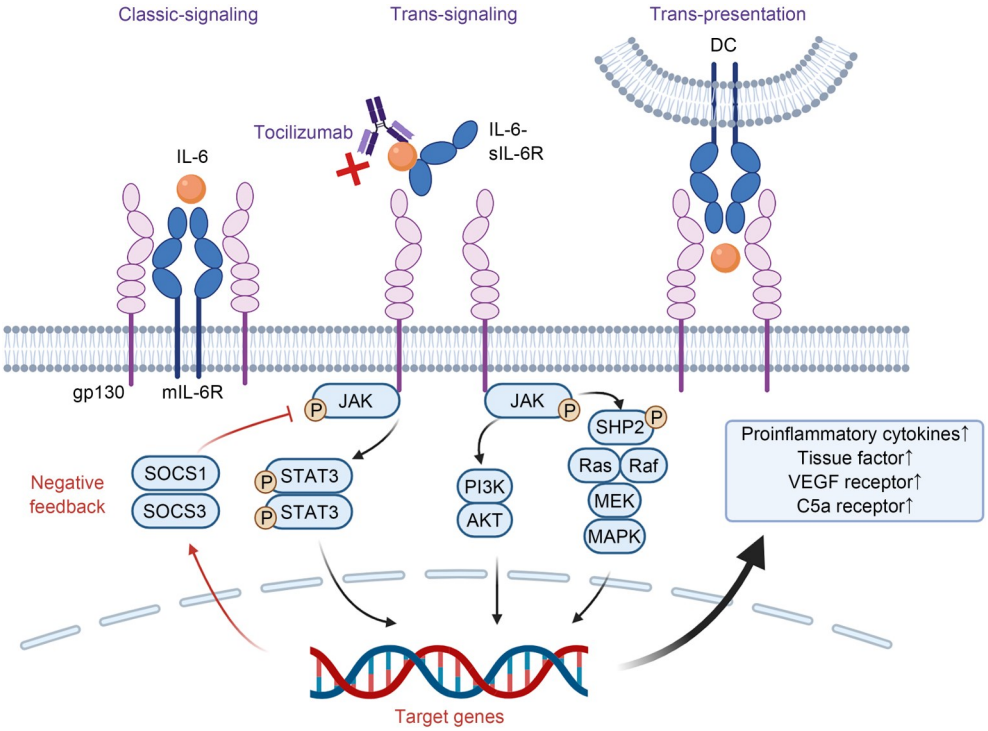

Fig. 6 Interleukin-6 (IL-6) signaling in cells. IL-6 has three signaling modes: classic signaling, trans-signaling, and trans-presentation. Within vascular endothelial cells, trans-signaling is the predominant mode of IL-6 signaling. Binding of the IL-6/soluble IL-6 receptor (sIL-6R) complex to the glycoprotein 130 (gp130) receptor triggers the activation of several signaling pathways including Janus kinase-signal transducer and activator of transcription 3 (JAK-STAT3), JAK-src homology 2 domain-containing tyrosine phosphatase 2 (SHP2)-mitogen-activated protein kinase kinase (MEK)-mitogen-activated protein kinase (MAPK), and JAK-phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT). These pathways ultimately result in changes in gene transcription levels, leading to the release of more inflammatory cytokines and upregulation of tissue factor (TF), vascular endothelial growth factor (VEGF) receptor, and C5a receptor expression. In addition, cells can negatively regulate JAK signaling by expressing suppressor of cytokine signaling (SOCS) proteins such as SOCS1 and SOCS3; mIL-6R: membrane-bound IL-6 receptor; DC: dendritic cell.

coagulation cascade (Kang et al., 2020). IL-6 also has been shown to increase the expression of complement component 5a (C5a) receptor on vascular endothelial cells and cause VE-cadherin disassembly, leading to vascular leakage. Furthermore, IL-6 upregulates TF on monocytes, which can trigger the coagulation cascade (Neumann et al., 1997). In vascular endothelial cells, IL-6 mainly uses trans-signaling. Binding of the IL-6/sIL-6R complex to gp130 activates the JAK-STAT3, JAK-src homology 2 domain-containing tyrosine phosphatase 2 (SHP2)-mitogen-activated protein kinase kinase (MEK)-mitogen-activated protein kinase (MAPK), and JAK-PI3K-AKT signaling pathways to induce changes in gene transcription levels, resulting in the release of more inflammatory cytokines and upregulation of TF, vascular endothelial growth factor (VEGF) receptor, and C5a receptor expression. Additionally, cells can negatively regulate JAK signaling by expressing suppressor of cytokine signaling 1 (SOCS1) and SOCS3.

3.2.3 Neutrophil extracellular traps

Neutrophilia and an elevated neutrophil-lymphocyte ratio (NLR) are common features of COVID-19 (Liu et al., 2020; Wang et al., 2020). Neutrophils play a crucial role in ALI, primarily through the release of NETs. NETs are formed when neutrophils undergo programmed cell death, referred to as neutrophil extracellular trap release (NETosis). We have briefly described the definition of NETs and how they facilitate thrombosis. NETs are often elevated in the blood of patients with COVID-19 and are frequently accompanied by neutrophilia (Zuo et al., 2020). High levels of NETs are associated with a poor prognosis (Zhu et al., 2018). Vascular NETosis initiates and contributes to thrombotic events in arteries (Döring et al., 2017), veins (Brill et al., 2012), and the microvasculature (Tanaka et al., 2014). The mechanisms involve electrostatic interactions that activate the contact pathway of coagulation (Gould et al., 2014) and bidirectional interplay between NETs and platelets (Gould et al., 2014). NETs can also present TF to activate the intrinsic coagulation pathway (Kambas et al., 2014). Additionally, serine proteases in NETs dismantle anticoagulant mechanisms, such as TF pathway inhibitor and antithrombin (Massberg et al., 2010).

3.2.4 Complement system

The complement system also plays an essential role in the inflammatory response, promoting thrombus formation. There are three pathways through which COVID-19 activates the complement system: the classic pathway via the formation of complement-fixing immunoglobulin G (IgG) and IgM antibody immune complexes (Holter et al., 2020), the alternative pathway by competing with the negative regulator factor H (Yu et al., 2020), and the lectin pathway through viral nucleocapsid protein-mediated enhancement of Mannan-binding lectin serine protease 2 (MASP2) cleavage of complement component C4 (Ali et al., 2021). Ultimately, the cleavage of complement component C5 leads to the release of prothrombotic factors from platelets. Furthermore, C5 cleavage can result in TF activation and increased P-selectin expression on endothelial cells, leading to the aggregation of inflammatory cells and thrombus formation.

3.3 Mechanisms of platelet activation

For decades, platelets and the immune cells were thought to act independently. However, in recent years, with the increasing understanding of the mechanism of immunothrombosis, it has been discovered that platelets play an important role in the immune system, especially in viral infections. Platelets can directly and indirectly interact with viruses through various mechanisms. On the one hand, viruses can bind to platelet surface integrins, surface lectins, and Toll-like receptors (TLRs) (Peerschke et al., 2010; Assinger et al., 2014), and particularly ACE2 which has been found to bind to SARS-CoV-2 (Zhang S et al., 2020), resulting in direct platelet activation and destruction. On the other hand, the FcγRII receptor on the platelet surface can recognize immune complexes formed by viruses and immunoglobulins, resulting in Fc receptor-mediated platelet activation, aggregation, and platelet clearance (Anderson et al., 1995). This mechanism requires the engagement of IgG and aIIbb3, and adenosine diphosphate (ADP) and thromboxane A_2 feedback mechanisms also participate (Arman et al., 2014). Furthermore, the antibodies produced by the body against viruses may cross-react with platelet glycoprotein IIb/IIIa (GPIIb/IIIa) (Goeijenbier et al., 2012), causing reactions similar to idiopathic thrombocytopenic purpura (ITP). In the case of COVID-19, although a decrease in platelet count is relatively rare, heightened platelet activation is often detectable (Conway et al., 2022; Iba and Levy, 2022). SARS-CoV-2 activates platelets mainly through an inflammation-mediated pathway and a SARS-CoV-2-specific pathway (Iba and Levy, 2022). The

inflammation-mediated pathway is due to a cytokine storm that causes platelets to express TF and release procoagulant microvesicles (Bautista-Vargas et al., 2020). The SARS-CoV-2-specific pathway is due to SARS-CoV-2 directly causing platelets to release the contents of α and dense granules, including VWF, platelet factor 4 (PF4), adenosine diphosphate, and serotonin, resulting in platelet aggregation (Zhang S et al., 2020). Manne et al. (2020) also reported distinct changes in the gene-expression profile of platelets in COVID-19 patients, such as upregulation of platelet surface P-selectin and increased MAPK pathway activation. Interestingly, ACE2 expression was not detected in platelets in their study. Platelets also play an immunomodulatory role by producing chemokines such as chemokine (CXC motif) ligand 4 (CXCL4) and chemokine ligand 5 (CCL5) to recruit leukocytes (Klinger et al., 1995; Assinger et al., 2014). The direct interaction with leukocytes can also mediate immune responses during viral infections (Yeaman, 2014). The mutual modulation between platelets and virus infection suggests that platelets are not only passive participant in the host response but also an active defense mechanism. Activation of the coagulation cascade might provide a host defense mechanism to limit pathogen dissemination (Antoniak and Mackman, 2014), but it may also lead to adverse consequences such as ALI.

3.4 Post-vaccination thrombosis mechanism

The previous section briefly mentioned post-vaccination thrombosis. Recent news and research have mainly reported a few adverse clotting events associated with the AstraZeneca and Janssen vaccines (Tiede et al., 2021; Tobaiqy et al., 2021). Among these events, vaccine-induced immune thrombotic thrombocytopenia (VITT) stands out as the primary pathophysiology, manifesting as severe thrombotic events such as cerebral venous sinus thrombosis, visceral vein thrombosis, pulmonary embolism, and arterial thrombosis (Liu et al., 2021). Baker et al. (2021) revealed, from a structural perspective, that the adenovirus vector chimpanzee adenovirus Y25 (ChAdOx1) used in the AstraZeneca vaccine can interact with PF4 to form a stable complex. This interaction has been shown to induce the production of PF4 antibodies, thereby promoting the occurrence of VITT (Dotan and Shoenfeld, 2021).

4 Risk factors

Most studies to date have focused on the prognosis of ALI and outcomes under different treatment regimens (Putko et al., 2021; Jain et al., 2022; Predenciuc et al., 2023). However, research on risk factors and predictive factors related to ALI is scarce. Some studies recognized that COVID-19-associated ALI patients are elderly individuals with severe COVID-19 infection, but several case studies have reported that young patients with no medical history can also exhibit severe limb ischemia that may ultimately lead to death (Mazzotta and Troccoli, 2020; Perini et al., 2020; Teixeira et al., 2022). In addition, many studies suggest that ischemic changes in skin tissue do not belong to the concept of ALI, and their main impact is on younger patients (Kolivras et al., 2020; Cappel et al., 2021). We have discussed the correlation of these two manifestations in terms of their pathophysiology and molecular mechanisms. In this section, we will discuss these manifestations from three perspectives: genetic background, age, and gender (Table 1).

Table 1 Hypotheses of risk factors of coronavirus disease 2019 (COVID-19) associated acute limb ischemia (ALI)

Risk factor	Hypotheses
Genetics	TREX1 inactivation
	ACE2 polymorphism
	Recombination of chromosome structure and epigenetic change induced by SARS-CoV-2
Age>65 years	Lack of ACE2 expression
	IFN-I and IL-6 levels
	Comorbidity impact
Male	*ACE2* gene location
	TLR7 gene and IFN-I
	Estrogen protection

TREX1: three-prime repair exonuclease 1; *ACE2*: angiotensin-converting enzyme 2; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; IFN-I: type I interferon; IL-6: interleukin-6; *TLR7*: Toll-like receptor 7.

4.1 Genetic background

Genetics is currently the hottest research topic. In COVID-19-related genome-wide association study (GWAS), many important gene mutation sites have been discovered that predispose patients to susceptibility and thrombosis (The Severe Covid-19 GWAS Group, 2020). Jabalameli et al. (2022) found an important gene

region, 3p21.31, associated with ischemic skin manifestations. They emphasized that three-prime repair exonuclease 1 (*TREX1*) gene inactivation can increase the accumulation of viral RNA fragments in the cytosol, promote the recognition of viral pattern recognition receptors such as cyclic GMP-AMP synthase (cGAS) and stimulator of interferon gene (STING), and induce the expression of IFN-I (Fig. 3). IFN-I has been described earlier as playing an important role in small-vessel limb ischemic changes. In a study of ACE2 polymorphism, Möhlendick et al. (2021) also proposed that the ACE2 rs2285666 G-allele is an independent risk factor for COVID-19 fatality. A recent study by Wang et al. (2023) found that SARS-CoV-2 can recombine the host's chromosome structure and change its epigenetic features. This study further proves the close relationship between COVID-19 infection and genetics. Further research is needed to confirm other genetic factors that may influence susceptibility to COVID-19-associated ALI.

4.2 Age

The age of patients has been widely discussed, including its effect on inflammatory factors, ACE2 expression, and vascular reactivity. The United States Centers for Disease Control and Prevention (CDC) has released information on underlying medical conditions associated with a higher risk for severe COVID-19, with age being the biggest risk factor (Centers for Disease Control and Prevention, 2023). Age>65 years is considered to be of higher risk (Centers for Disease Control and Prevention, 2023). In their systematic review of COVID-19-associated ALI, Putko et al. (2021) reported that the average patient age was 67.6 years. The occurrence of this phenomenon is thought to be related to a lack of ACE2 in older patients (Angeli et al., 2021a). Xie et al. (2006) found that ACE2 expression in the lungs of rats was markedly reduced among an older group. Yoon et al. (2016) found that the ACE2-Mas receptor axis was reduced and the ACE-Ang II-AT1R axis was increased in the aortas of an aging group of mice. Furthermore, the oxidative stress level of blood vessels increased, and a marker of endothelial dysfunction, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (Nox2), also increased (Yoon et al., 2016). Therefore, aging and COVID-19 infection have a similar effect on the RAAS, and elderly patients have a lower level of ACE2 and are more susceptible to developing ALI. From the perspective of inflammatory factors, dendritic cells secrete less IFN-I in aged people (Agrawal, 2013). However, as age increases, testosterone and estradiol decrease, which can cause more IL-6 production (Maggio et al., 2006; Kim et al., 2012). IL-6 plays a dominant role in COVID-19 elderly patients and increases the risk of COVID-19-associated ALI. In addition, age itself is an important risk factor for cardiovascular events, and the combination of hypertension, diabetes, and atherosclerosis can directly cause ALI.

4.3 Gender

Regarding gender factors, in their systematic review, Putko et al. (2021) reported that 79.6% of patients with COVID-19-associated ALI were male. This result is similar to the severe COVID-19 risk factors reported by the CDC. This phenomenon can also be explained by the ACE2 mechanism. The *ACE2* gene is located on the X chromosome, which can escape inactivation in XX cells and has higher expression in females (Tukiainen et al., 2017). Additionally, the Toll-like receptor 7 (*TLR7*) gene, which encodes TLR7 and induces the production of IFN-a, is also located on the X chromosome. Similar to the ACE2 mechanism, it can cause increased IFN-I response in females (Souyris et al., 2018). As previously mentioned, the balance between IFN and IL-6 determines the specific manifestations of ALI. Individuals with a stronger IFN response will have mild ischemic changes in small blood vessels, while those with a stronger IL-6 response will have thrombosis and severe limb ischemia. Furthermore, due to the protective effect of IFN following viral infection, COVID-19 nucleic acid tests can even be negative when mild ischemic changes occur (Hubiche et al., 2021). In addition, estrogen in females can protect the endothelium from damage and prevent thrombosis formation (Xing et al., 2009).

5 Clinical management

The treatment of COVID-19-associated ALI can be broadly categorized into pharmacotherapy (such as anticoagulation and antibiotics), intervention (such as vascular recanalization), and surgery (including surgical thrombo-embolectomy, vascular reconstruction, and debridement). However, most treatments have only

moderate efficacy. For example, Zhang Y et al. (2020) reported no clinical benefit in seven cases treated with therapeutic low-molecular-weight heparin, and re-thrombosis can develop even in patients who receive intense anticoagulant therapy (Gomez-Arbelaez et al., 2020). In contrast, the systematic review by Putko et al. (2021) showed that combined medical and surgical therapy achieved a higher resolution rate and lower mortality. Nevertheless, another systematic review found that the limb salvage rate for COVID-19-associated ALI was approximately 57%, with a mortality rate of 33% (Jain et al., 2022).

Some novel therapeutic strategies have been developed for COVID-19-associated ALI, which are based on the pathophysiological mechanisms described above. One such strategy is the use of the IL-6 blocker tocilizumab. IL-6 is one of the most important cytokines involved in the cytokine storm caused by COVID-19, and its role in immune activation, vascular injury, and thrombosis formation has been described above. Tocilizumab is an antagonist for IL-6R and has been used in clinical trials for COVID-19 patients. Although it can produce anti-inflammatory effects and reduce the need for mechanical ventilation, as well as shorten ICU stay, it has not significantly improved COVID-19 mortality rates (Rosas et al., 2021; Salama et al., 2021). Papamichalis et al. (2020) reported the successful use of tissue plasminogen activator (tPA) and tocilizumab to save patients with COVID-19-associated ALI, while Schultz and Wolf (2020) reported two cases treated with tocilizumab, one of whom survived. These studies highlight the involvement of immune-mediated thrombosis in COVID-19-associated ALI.

For vessel spasm, calcium channel blockers (CCBs) may be helpful in treating COVID-19-associated ALI when thrombosis is not detected on imaging, as vasoconstriction may be a more important mechanism in this case (Alitter et al., 2023). In addition, angiotensin-converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB) are often used in patients with hypertension to target the RAAS. However, some studies have suggested that the use of ACEI/ARB may increase the expression of ACE2 receptors, which may increase the risk of COVID-19 infection. A meta-analysis by Zhang X et al. (2020) found no significant impact of ACEI/ARB exposure on the incidence or mortality rate of COVID-19. Nevertheless, further research is needed to determine the effectiveness of these drugs in patients with COVID-19-associated ALI.

6 Conclusions

Ischemic lesions caused by COVID-19 are significant complications, among which ALI is a rare but poor prognostic factor. While previous studies have focused mainly on the importance of hypercoagulable states and thrombosis in pathophysiology, we also emphasize the effects of vascular constriction and hypoxia. COVID-19 is a multisystem disease, and there are complex cellular and molecular mechanisms that can cause ALI. In this article, we summarize the possible mechanisms by which COVID-19 may cause ALI, including ACE2-related molecular mechanisms, cytokine storm, and the latest platelet immune mechanism hypothesis. Currently, there is no highly effective treatment for COVID-19-associated ALI. However, by gaining a deeper understanding of its pathophysiology and molecular mechanisms, risk factors have been identified and novel treatment strategies proposed for COVID-19-associated ALI. Although the COVID-19 pandemic has largely ended, it has provided valuable experience in understanding ALI and preparing for future coronavirus infection events.

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Author contributions

Hui LU, Olga ALENIKOVA, Sahar Ahmed ABDALBARY, and Zhenfeng LIU designed the study. Chengjun YAO, Yanzhao DONG, Haiying ZHOU, Xiaodi ZOU, Ahmad ALHASKAWI, Sohaib Hasan Abdullah EZZI, Zewei WANG, Jingtian LAI, Vishnu Goutham KOTA, and Mohamed Hasan Abdulla Hasan ABDULLA performed the literature collection and analyzed the results. Chengjun YAO, Yanzhao DONG, Haiying ZHOU, Xiaodi ZOU, and Ahmad ALHASKAWI drafted the manuscript. Chengjun YAO, Sahar Ahmed ABDALBARY, Zewei WANG, Jingtian LAI, Vishnu Goutham KOTA, and Mohamed Hasan Abdulla Hasan ABDULLA critically revised the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Chengjun YAO, Yanzhao DONG, Haiying ZHOU, Xiaodi ZOU, Ahmad ALHASKAWI, Sohaib Hasan Abdullah EZZI, Zewei WANG, Jingtian LAI, Vishnu Goutham KOTA, Mohamed Hasan Abdulla Hasan ABDULLA, Zhenfeng LIU, Sahar Ahmed ABDALBARY, Olga ALENIKOVA, and Hui LU declare that they have no conflicts of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation

(The First Affiliated Hospital of Zhejiang University School of Medicine, China) and with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from all patients included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

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