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# Interaction of the inflammatory response and megakaryocytes in COVID-19 infection

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## HIGHLIGHTS

- Coronavirus disease 2019 (COVID-19)—associated cytokine storm may result in aberrant megakaryocyte formation.
- Abnormal megakaryocyte behavior may cause severe COVID-19 clinical manifestations.
- Elevated megakaryocytes in COVID-19 may lead to thrombosis and abnormal coagulopathy.
- Patients with COVID-19 have large numbers of megakaryocytes in their cardiac and pulmonary systems.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly across the world and has resulted in more than 4.2 million global deaths as of July 30, 2021. For reasons that remain unknown, the coronavirus disease 2019 (COVID-19) clinically manifests itself in various levels of severity, with most patients positive for COVID-19 being asymptomatic or having only mild symptoms. However, clinical studies in severely ill patients have implicated that manifestations of this infection are due in part to abnormal megakaryocyte (MK) behavior. Additionally, COVID-19—associated cytokine storms have been found to induce aberrant MK formation, primarily through interleukin-6 and Janus kinase-signal transducer and activator of transcription signaling. Autopsy reports have revealed significantly higher rates of MKs in the pulmonary and cardiac systems, which may be responsible for the high rate of thrombotic complications and abnormal coagulopathies in patients with severe forms of COVID-19. This review examines MKs and their potential function in the clinical manifestations of SARS-CoV-2 infection.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which originated in the Hubei Province of Wuhan, China, in December 2019, is posing a public health threat to millions of individuals around the world because of its rapid transmittance rate and potentially severe clinical manifestations. As of July 30, 2021, a total of 197.31 million coronavirus disease 2019 (COVID-19) cases had been reported, with 4.2 million global deaths [1]. COVID-19 is an airborne disease that is transmitted through the inhalation of virus-containing droplets. The disease manifests itself in the infected individual after an incubation period ranging from 2 to 14 days [2]. Clinical manifestations of COVID-19 are variable, but include sore throat, fever, non-productive cough, difficulty breathing, and fatigue [3]. Disease

severity and complications vary significantly, with most individuals being asymptomatic or experiencing mild symptoms [2,3]. However, some individuals, particularly those with preexisting health conditions (diabetes, obesity, and cancer to name a few) and the elderly, are experiencing more severe complications such as pneumonia, multiple organ failure and dysfunction, hemodynamic shock, and death [3,4].

Clinical studies have recognized that among those patients with severe forms of COVID-19, the cytokine storm plays a significant role in symptomatology and outcomes. Cytokine storms are inflammatory responses characterized by the uncontrolled and excessive release of cytokines that lead to hyperinflammation [5,6]. In addition to contributing to the final prognosis of COVID-19 patients, these exacerbated responses have been determined to play a critical role in increasing the numbers and causing aberrant function of megakaryocytes (MKs) in patients infected with SARS-CoV-2 [7].

There is growing recognition of abnormal MK behavior in many patients with severe illness caused by COVID-19 [4]. Autopsies of patients with COVID-19 have revealed increased MK and platelet numbers with subsequent increased rates of thrombosis [4]. MKs are hematopoietic cells that reside within the bone marrow and are responsible for the production of platelets—the main mediators of thrombosis. The complex process of megakaryocytopoiesis commits hematopoietic stem cells (HSCs) to undergo a series of lineage commitment steps to produce MKs. These MKs then undergo unique maturation and differentiation processes including polyploidization and endomitosis to form platelets [5,8]. However, abnormalities in the production of platelets from the precursor MKs can have significant clinical manifestations in the form of thrombocytopenia or thrombocytosis. Thrombocytopenia, defined as platelet counts  $<150,000/\mu\text{L}$ , is associated with inadequate primary hemostasis and heightened risk of bleeding [9]. Conversely, thrombocytosis, defined as platelet counts  $>350,000/\mu\text{L}$ , contributes to pathologic thrombosis and abnormal coagulopathy, thereby inducing deep vein thrombosis (DVT) and pulmonary thromboembolism (PTE) [10]. This review summarizes insights into the potential role of immune responses and their impact on MK behavior in SARS-CoV-2 infection.

## SARS-COV-2 INFECTION AND CYTOKINE INVOLVEMENT

SARS-CoV-2 infection has highly variable clinical manifestations in different individuals, with the progression of the infection being

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largely dependent on the host immune system's ability to recognize and react effectively to the virus [11]. SARS-CoV-2 enters the body by binding to angiotensin-converting enzyme 2 (ACE2) and facilitating a cascade of immune responses [12]. After entry, viral infection elicits both innate and adaptive immune responses.

There are several cytokines have been determined to be upregulated in SARS-CoV-2 infection that also influence megakaryocytopoiesis (Table 1). One of the most prominent cytokines that is upregulated is interleukin-6 (IL-6), which is uniformly elevated in patients with severe COVID-19 infections [13]. IL-6 has been found to increase levels of thrombopoietin (TPO), the main megakaryocyte

growth factor responsible for the production of MKs and platelets. On binding to its receptor, c-Mpl, TPO stimulates the production of precursor MKs in addition to accelerating the proliferation of MK progenitor cells [14–16]. Inhibition of TPO has been associated with neutralizing thrombocytosis and preventing thromboembolism resulting from elevated levels of IL-6 [17]. Similarly, IL-9 has been determined to be upregulated in patients positive for COVID-19 [18]. Elevated levels of this cytokine also induce increased signaling of the JAK-STAT pathway, leading to higher concentrations of MKs [14,16]. Additional cytokines upregulated in the SARS-CoV-2 infection that are known to increase MK levels include granulocyte–macrophage

**Table 1** Upregulation of various proinflammatory cytokines and chemokines caused by COVID-19-associated cytokine storm<sup>a</sup>

Cytokine	Previously described levels in COVID-19	Impact on MKs	Impact on TPO	Impact on JAK-STAT pathway
G-CSF	↑ [13]			↑ and ↓ [16]
GM-CSF	↑ [18]	↑ [14]		↑ and ↓ [16]
IFN $\alpha$ 2	↑ [23]	↑ [14]	↓ [24]	↑ and ↓ [16]
IFN $\gamma$	↑ [13]	↑ [19]		↑ and ↓ [16]
IL-1 $\alpha$	↑ [23]	↑ [14]		
IL-1 $\beta$	↑ [13]	↑ [20]		
IL-1RA	↑ [25]			
IL-2	↑ [13]			↑ and ↓ [16]
IL-3		↑ [14]		↑ and ↓ [16]
IL-4	↑ [13]	↓ [26]		↑ and ↓ [16]
IL-5				↑ [16]
IL-6	↑ [13]	↑ [14]	↑ [14]	↑ and ↓ [16]
IL-7	↑ [13]			↑ and ↓ [16]
IL-8/CXCL8	↑ [13]			↑ [13]
IL-9	↑ [18]	↑ [14]		↑ [16]
IL-10	↑ [13]	↓ [27]		↑ and ↓ [16]
IL-12	↑ [13]			↑ and ↓ [16]
IL-13	↑ [23]			↑ and ↓ [16]
IL-15				↑ [16]
IL-17	↑ [23]			
IL-22				↑ and ↓ [16]
IL-27	↑ [23]			
IP-10/CXCL10	↑ [13]			
MCP-1/CCL2	↑ [13]			↑ [13]
MCP-3/CCL7	↑ [28]			
MIP-1 $\alpha$ /CCL3	↑ [13]			
MIP-1 $\beta$ /CCLC4	↑ [18]			
PDGF-AB/BB	↑ [18]			
TNF $\alpha$	↑ [13]			
VEGF-A	↑ [13]	↑ [14]		↑ [13]

G-CSF=granulocyte-colony stimulating factor; IP-10=interferon  $\alpha$ -inducible protein 10; MCP=monocyte chemoattractant protein; MIP=macrophage inflammatory protein; PDGF=platelet-derived growth factor; TPO=thrombopoietin; VEGF-A=vascular endothelial growth factor A.

<sup>a</sup>These cytokines have also been noted to increase and decrease MKs in addition to regulating TPO levels and the JAK-STAT pathway.

colony-stimulating factor (GM-CSF), interferon  $\gamma$  (IFN $\gamma$ ) and IL-1 $\beta$ , in addition to IL-6 and IL-9 [13,18–20]. Irrespective of the exact pathway, SARS-CoV-2 infection has been reported to elicit immune responses that are associated with a significant increase in MK numbers [4,21,22].

Cytokines use the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway, which is mediated by Janus kinases (JAKs) [29]. The JAK-STAT pathway is upregulated by pro-inflammatory cytokines involved in the COVID-19-associated cytokine storm that transduce extracellular signals from cytokines, lymphokines, and various growth factors [30]. Additionally, the JAK-STAT signaling pathway is involved in megakaryopoiesis and triggered when TPO binds to its receptor, c-Mpl [31,32]. Autopsy reports of SARS-CoV-2-infected patients have revealed increased levels of circulating IL-6, which consequently increase levels of TPO, both of which upregulate the JAK-STAT pathway. Taken together, these elevated levels of TPO and IL-6 may promote the JAK-STAT pathway to increase the production of MKs and platelets. As a result of the increased stimulation of the JAK-STAT signaling in COVID-19 patients, therapeutic strategies for the mitigation of COVID-19 clinical manifestations include the use of JAK inhibitors [29,30]. Although the use of JAK inhibitors to reduce cytokine activity is a plausible treatment option, several clinical studies have documented that some of these inhibitors may cause thrombosis and pulmonary emboli, both of which are common complications of SARS-CoV-2 infection [33]. However, it must be noted that several clinical trials using JAK inhibitors or the human monoclonal antibody against IL-6 as treatment options were discontinued because of the ineffectiveness of these treatments in improving survival rates in patients with COVID-19 [33].

## COVID-19—ASSOCIATED CYTOKINE STORM

Cytokine storms are suspected to be one of the main factors driving a severe clinical course in patients infected with SARS-CoV-2. Cytokine storms are systemic inflammatory responses that produce a cascade of pro-inflammatory cytokines [34]. Clinical findings of patients with COVID-19—associated cytokine storm have indicated high concentrations of pro-inflammatory cytokines and chemokines that attacked not only the virus, but also other tissues in the body [34]. As recognized for previous  $\beta$ -coronaviruses, such as SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 infection has increased levels of circulating interferon  $\gamma$ , IL-1 $\beta$ , IL-6, IL-12, C–X–C motif ligand 10 (CXCL10), and C–C motif chemokine ligand 2 (CCL2) [35,36]. The elevated levels of these cytokines and chemokines have been determined to activate T-helper type 1 (T<sub>h</sub>1) cells, which promote cell-mediated immune responses [36,37]. In particular, COVID-19 patients who had larger amounts of CXCL10, CCL2, and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) had higher rates of intensive care unit admission in comparison to those with lower concentrations of these cytokines and chemokines. The elevated levels of circulating pro-inflammatory cytokines have been found to correlate with the subsequent increase in MKs [36]. However, unlike SARS-CoV infection, COVID-19 exhibits increased secretion of IL-4 and IL-10, which are cytokines that function primarily to suppress inflammation [36,38].

Apart from the similarity between SARS-CoV-2 infection and previous  $\beta$ -coronaviruses with respect to the activation of various

cytokines and chemokines, recent research has revealed homology in the effects of cytokine storms on hematopoiesis. Coronaviruses have the ability to disrupt hematopoiesis by infecting bone marrow cells [39,40]. This direct infection of the bone marrow cells by SARS-CoV-2 infection induces the release of a large concentration of inflammatory cytokines triggering secondary hemophagocytic lymph histiocytosis (sHLH) [39]. A retrospective study of patients with COVID-19 ( $n = 150$ ) found that SARS-CoV-2 infection overactivated T cells, thereby increasing the production of GM-CSF. This overstimulation results in inflammatory macrophages, such as CD14 +CD16+, generating more IL-6 and other inflammatory factors that promote the cytokine storm [41], creating a vicious cycle. Additionally, an integrated immune analysis of COVID-19 patients ( $n = 50$ ) identified an impairment in the interferon (IFN) type I response in patients with SARS-CoV-2 with severe clinical manifestations [42]. This impairment results in a deficiency of type I IFN in the blood [42,43]. When paired with the enhanced responses of IL-6 and TNF- $\alpha$ , the type I IFN response exacerbates the inflammatory response in patients with COVID-19 [42,43]. This upregulation of cytokines may induce the overproduction of MKs, a characteristic of many severe prognoses of individuals infected with SARS-CoV-2.

Although several pro-inflammatory cytokines are involved in SARS-CoV-2 infection, IL-6 has been found to have the largest impact on COVID-19—associated cytokine storms and the final prognosis of patients. IL-6 is a crucial component of acute inflammation and is produced by many cells including lymphocytes, macrophages, and stromal cells [44]. This interleukin has been determined to be circulating in large quantities in patients with COVID-19 and can be used to predict the severity of the infection [36,45]. Increased IL-6 serum levels indicate higher disease severity and increased rates of mortality [36]. One study consisting of 29 patients positive for COVID-19 reported that IL-6 levels were higher in the critically ill than in patients who had milder clinical manifestations [45]. Likewise, studies conducted on patients with COVID-19 by Chen et al. [46] and Gao et al. [47] obtained similar results [46,47]. One of these studies ( $n = 21$ ) identified increased levels of IL-6 along with IL-10 and TNF $\alpha$  [46]. Because of the significance of this pro-inflammatory cytokine's role in COVID-19 in not only inducing cytokine storms, but also instigating elevated levels of MKs, other studies have encouraged the use of IL-6 inhibitors to decrease the prevalence of cytokine storms, which may also lower MK levels. Tocilizumab is one such anti-IL-6 monoclonal antibody. This is a humanized antibody that is clinically used to inhibit IL-6 accumulation during cytokine storms and has therefore been used in treating highly severe COVID-19 cases [34,36,48]. However, it is important to note that tocilizumab has been found to be ineffective in reducing mortality rates in critically ill patients with COVID-19 [49].

## SARS-CoV-2—Induced thrombocytopenia

Thrombocytopenia is a common consequence of many infectious diseases, including SARS-CoV-2 infection. Incidence rates of thrombocytopenia in patients with COVID-19 range from 5% to 41.7% [50,51]. A meta-analysis of patients with COVID-19 ( $n = 1779$ ) revealed that thrombocytopenia enhanced the risk of severe prognosis by threefold, and a separate retrospective study of patients with COVID-19 ( $n = 383$ ) detected a threefold correlation between thrombocytopenia at hospital admission and in-hospital mortality [51–53]. Thrombocytopenia and its relationship to mortality in patients with COVID-19 has been reported to correlate with older age [54].

IL-6 upregulates the process of megakaryocytopoiesis in bone marrow by stimulating TPO, thereby increasing the number of blood platelets in circulation [55]. Collectively, the increased levels of IL-6 in patients infected with SARS-CoV-2 should theoretically allow for increased megakaryocytopoiesis and increased levels of blood platelets. Several studies have determined that elevated blood platelet counts worsen the prognosis of patients with COVID-19. On the other hand, decreased blood platelet counts, in the form of thrombocytopenia, have also been found to be a key clinical manifestation in some severe cases of COVID-19. Despite the interplay of increased IL-6 and TNF $\alpha$  levels as a result of the SARS-CoV-2-associated cytokine storm, platelet levels continue to be low in critically ill patients. This phenomenon may be the result of an exhaustion of lymphocytes caused by the SARS-CoV-2-associated cytokine storm. Severely ill patients with COVID-19 have been found to have decreased levels of lymphocytes in comparison to patients with milder symptoms [34]. IL-6 induces lymphocyte necrosis, and increased levels of this pro-inflammatory cytokine cause cytotoxic exhaustion of the lymphocytes [34]. Collectively, it may be possible that the platelet-to-lymphocyte ratio (PLR) is the connection between the low platelet and lymphocyte counts. A recent study determined that larger PLR changes in patients with COVID-19 induce a more severe cytokine storm, thereby increasing the severity of the infection [56]. An additional rationale for this phenomenon may be explained by the association between thrombocytosis and the subsequent onset of thrombocytopenia. SARS-CoV-2-induced thrombocytosis can result in the production of excessive emboli, which consume large numbers of platelets and MKs. This consumption can then lead to such depletion of platelets and MKs that thrombocytopenia develops.

Although the exact mechanism of thrombocytopenia in SARS-CoV-2 infection has not been identified, currently several possible mechanisms have been proposed to cause the associated hematological changes. According to one mechanism proposed, COVID-19 directly infects the hematopoietic and bone marrow cells, thereby inhibiting hematopoiesis and thus causing a decrease in the formation of primary platelets [39,51]. The aberrant hematopoiesis and direct attack on the HSCs prevent the differentiation and maturation of MKs, thereby preventing the production of platelets in bone marrow [39,51]. With respect to the impacts of COVID-19 on HSCs, one study found that exposing HSCs and HPCs *ex vivo* to the SARS-CoV-2 spike protein inhibits their proliferative abilities [57]. This proposed mechanism is reliant on the known pathophysiology of previous coronaviruses that have nearly 82% homology with the SARS-CoV-2 infection [58]. These precursor coronavirus receptors on bone marrow cells and platelets can induce apoptosis and obstruct proliferation, thereby altering hematopoiesis. Based on the similarity between the novel coronavirus and previous coronaviruses, SARS-CoV-2 infection may be inducing thrombocytopenia through a similar mechanism [39]. Alternatively, SARS-CoV-2 infection may also increase levels of autoantibodies and immune complexes in individuals positive for COVID-19, thereby inducing an immune-mediated thrombocytopenia [39,51,59]. In this immune-mediated mechanism, the antibodies and immune complexes attack the HSCs and hematopoietic progenitor cells, thereby suppressing hematopoiesis [51,59]. This response may also induce damage to the circulatory system through the production of autoantibodies and immune complexes. Autoantibodies and immune complexes activate platelets, which hastens their consumption into micro- and macrothrombi and results in their shortened survival [51]. Additionally, disseminated

intravascular coagulopathy (DIC) results in thrombotic obstruction of blood vessels caused by systemic activation of the coagulation cascade [60]. This consequence is believed to have a possible role in the increased consumption of platelets in patients with COVID-19

## SARS-COV-2-INDUCED THROMBOSIS

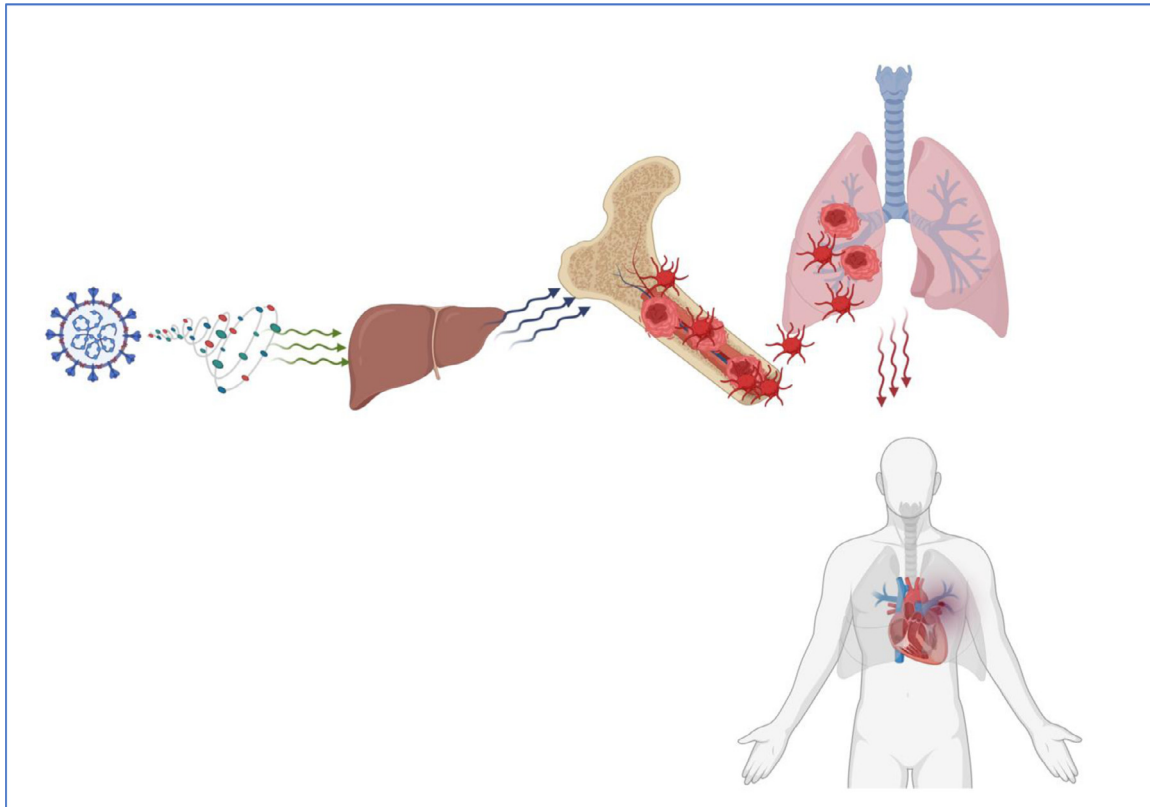
The thrombosis-associated clinical manifestations of SARS-CoV-2 infections are varied. Indeed, as discussed earlier, some patients exhibit thrombocytopenia while others exhibit significant increases in thrombosis (Figure 1). Recent studies have reported that coagulopathy occurs in nearly 50% of patients with severe cases COVID-19 [61]. The abnormal coagulopathy in critically ill patients triggers various thrombotic complications that have also been identified to instigate adverse effects on the final prognosis of patients positive for COVID-19. In autopsies of patients with COVID-19, both microvascular thrombosis and macrovascular thrombosis were identified, and in addition, many patients were found to have thrombi in the lung parenchyma [62]. Abnormal coagulopathy and thrombosis are positively correlated with the severity of infection, thrombocytosis, and elevated levels of MKs [61,62].

Thrombosis has been reported to exist in many critically ill patients with COVID-19, with many of the deceased patients meeting the criteria for DIC [54]. DIC contributes to the formation of platelet-fibrin thrombi in the microvasculature, thereby inducing multi-organ failure [54]. Histopathological reports of a COVID-19 patient's biopsy indicated the presence of intravascular coagulopathy within the small arterial vessels, suggesting that abnormal coagulopathy results in diffuse alveolar injury [63].

Two different phenotypes of thrombosis have been identified—one characterized by thromboembolism and the other by microthrombosis [64]. The microthrombotic angiopathy has been recognized to be the predominant form of thrombosis present in SARS-CoV-2 infection. Its presence in the lungs along with other organs induces widespread coagulation, increasing the likelihood of thrombotic events. Autopsy reports of lungs collected from patients with COVID-19 stated that thrombosis and microangiopathy of the lungs resulted in severe damage to the endothelial cells and cell membranes [64,65]. The damage sustained by the endothelial cells and cell membranes may be a result of the exacerbated release of cytokines, such as IL-6. Such hyperintense inflammatory responses would elicit the formation of MKs through the production of TPO and upregulation of JAK-STAT signaling, thereby inducing thrombosis.

Hence, the increased formation of MKs could induce the formation of a larger population of platelets. Despite this increased turnover of platelets, Wool and Miller [66] found that a significant portion of these platelets are reticulated or immature platelets, which are known to have increased functionality [66]. Clotting events and thrombosis significantly reduce platelet counts, and MKs respond to these low platelet numbers by producing reticulated or immature platelets [67]. Additionally, activated platelets produced from lung MKs have been noted to induce neoangiogenesis in patients affected by SARS-CoV-2 [67]. In patients with pulmonary veno-occlusive disease (PVOD), altered intraluminal flow from lung injury prompts vascular remodeling [68]. During this process, neoangiogenesis is increased excessively [68]. However, research regarding the role of neoangiogenesis in SARS-CoV-2 infection is warranted.





**Figure 1** COVID-19 viral infection stimulates the production of cytokines, including IL-6. This leads to synthesis of TPO by the liver, thereby stimulating an increase in MK production in the bone marrow. These MKs then travel throughout the bloodstream, causing the high levels of lung MKs in patients with COVID-19. Created with BioRender.com.

Autopsy reports have revealed notably larger numbers of MKs in the lungs and hearts of patients positive for COVID-19 that have been associated with hypercoagulability [21]. A separate case study of individuals who died as a result of COVID-19 in Italy found that nearly 50% died from cardiac comorbidities such as atrial fibrillation and ischemic heart disease that required anticoagulants for treatment [10,69]. The abnormal coagulopathy and enlarged rates of platelet-rich thrombi in the cardiovascular and pulmonary systems have been concurrent with the increased number of MKs in these organ systems [21,62]. Because the existence of MKs in the heart is a rare occurrence, it has been suggested that MKs may have a significant role in the formation of the platelet-rich microthrombi and thrombosis in patients with COVID-19 [4]. Specifically, the increased levels of IL-6 and JAK-STAT signaling that characterized many severe cases of the SARS-CoV-2 infection may be enacting a primary role in the development of large numbers of MKs in the pulmonary and cardiac vasculature. A retrospective study stated that the platelet count of patients with COVID-19 that was predictive of thrombosis was  $>450 \times 10^9/L^{70}$ . On this basis, the discrepancy between the large number of MKs present and the low concentrations of platelets may be attributed to the coagulation of platelets.

In addition to increased numbers and aberrant activities of MKs within cardiopulmonary systems, MKs have also been discovered occluding cortical capillaries within the brain of patients with COVID-19 [71]. These capillary occlusions may hinder the passage of blood to the brain, particularly to the cerebral cortex, leading to

the impaired awareness and confusion reported by many recovered patients with COVID-19 [71]. Such findings may be indicative of possible ischemic alterations to the brain parenchyma, thus leading to further neurologic impairments in patients infected with SARS-CoV-2 [71]

PTE and DVT have both been recognized in severe cases of SARS-CoV-2 infection because of the severe hypercoagulable state in these patients. PTE is the blockage of the pulmonary arteries by blood clots that may form because of DVT in the deep veins of the leg [10]. Blockage of the pulmonary arteries can cause severe damage to the lungs and significantly decrease oxygen levels [10,70]. Studies have suggested incidence rates of PTE and DVT to be as high as 40%, which may sustain a substantial role in progressive lung failure and acute respiratory distress syndrome (ARDS) in patients with COVID-19 [61]. The pro-inflammatory cytokines are the primary factors driving the development of abnormal formation of clots as hypercoagulability has been proven to be an outcome of inflammation and cytokine storms [61,72]. Moreover, pro-inflammatory cytokines have also been observed to play a role in the downregulation of anticoagulant pathways, suggesting that the cytokine storm has a major role in causing thrombotic complications. In particular, elevated serum levels of IL-6, IL-17A, and TNF $\alpha$  cytokines that have been observed in patients with COVID-19 are believed to play a critical role in thromboinflammation [61]. IL-17A has been reported to induce the development of thrombosis, particularly in the presence of TNF $\alpha$  [73]. Additionally, IL-17A has the capability to modulate arterial thrombus

formation, suggesting that elevated levels of this cytokine promote thrombotic states in patients infected with SARS-CoV-2 [73,74].

## MKS AND THE PULMONARY MANIFESTATIONS OF SARS-COV-2 INFECTIONS

The pathophysiology of SARS-CoV-2 infection has been reported to resemble that of complement-mediated thrombotic microangiopathy (TMA) [75]. TMA is a condition that manifests itself through thrombocytopenia, organ dysfunction, and microangiopathic hemolytic anemia [75]. Studies have determined that cells expressing ACE2 at high levels become target cells for SARS-CoV-2 infection [75]. ACE2 is highly expressed in podocytes and epithelial cells of the kidneys, with even higher levels in patients with pre-existing cardiac injuries [75]. This suggests that the thrombocytopenia characteristic of TMA is similar to that of COVID-19 in the lungs with respect to the pathway by which organ damage is impelled [75]. Because the lung tissue, particularly lung alveolar epithelial cells, express ACE2, injury induced by SARS-CoV-2 infection is similar to that of ACE2 expressed in the kidneys resulting in TMA injury [75]. The ACE2 molecules in lung tissue act as a receptor to which the SARS-CoV-2 infection can bind and therefore enter the host's immune system [63,75].

As mentioned previously, patients with COVID-19 have elevated levels of proinflammatory cytokines and chemokines that are required for the recruitment of various adaptive immune cells [76]. This increase in chemokines and cytokines has been determined to cause a collection of cells and fluid to build up in the lungs, leading to respiratory failure [76,77]. The combination of immune-active molecules results in a sustained inflammatory response in which the immune system begins attacking its own cells. Specifically, CXCL10 and CXCL8 have been found to be upregulated in ARDS in both mouse models and patients [36]. The overproduction of pro-inflammatory cytokines causes not only ARDS, but also extensive tissue damage and multiple organ failure [18]. Moreover, the dysregulation of the innate immune response to SARS-CoV-2 infection is believed to increase the severity of the clinical presentation of the virus [77]. The transition to the adaptive immune response of SARS-CoV-2 infection has been proven to be critical in determining the final prognosis of patients positive for COVID-19 [76]. Whether a protective or exaggerated inflammatory response occurs can determine if ARDS or other severe consequences develop [76,77]. A protective response is led by cytotoxic CD8 cells attacking infected cells in the body. On the contrary, exacerbated inflammatory responses result from uncontrolled release of immune cells, as in cytokine storms [76].

Although typically thought of only in their role of thrombus development, platelets and MKs also express T-cell co-stimulatory molecules to activate CD8+ T cells [78]. In addition, studies have determined that MKs live outside of the bone marrow, including within the lung [78]. Previous research revealed that lung MKs have a more inflammatory gene expression pattern compared with bone marrow MKs [78]. In fact, only lung MKs constitutively express immune molecules to present antigen to CD4+ T cells [78]. These location-specific differences in MK phenotype are driven in part by immune differentiating molecules secreted by lung epithelial cells. Lung MKs therefore are specialized immune cells that process and present antigen to T

cells. For example, MK immunophenotype was variable, and lung-specific MKs (MK-L) were under the influence of specific pathogen challenge and lung-associated immune molecules, such as IL-33. MK-L internalized and processed both antigenic proteins and bacterial pathogens [79]. Furthermore, MK-L induced CD4+ T-cell activation in a major histocompatibility complex (MHC) II-dependent manner both in vitro and in vivo [79]. These data indicated that MK-L had key immune regulatory roles dictated in part by the tissue environment [79]. Investigators have also found an important role for platelets specifically in a lung inflammatory model. Investigators used a murine model of lung inflammation, in which platelets were depleted by either thrombopoietin antisense oligonucleotides (TPO ASO) or anti-CD42b treatment, and observed a reduction in the accumulation of inflammatory immune cells, including monocytes and macrophages, in the lung [80].

A subpopulation of human bone marrow MKs with immune characteristics have been determined to contain the CD148 and CD48 surface markers [81]. These CD148+CD48+ MKs can elicit rapid immune responses while also serving as markers of acute inflammation [81]. Specifically, CD48+ MKs exhibit higher levels of expression of CD88, Fpr1, Tlr2, and Tlr4 in comparison to CD48- MKs in single-cell quantitative polymerase chain reaction analysis [81]. As such, this high level of expression enables these MKs to function as immune surveillance cells during acute inflammation, potentially leading to neutrophil mobilization and migration [81].

As a result of the lung damage caused by SARS-CoV-2 infection, it is also possible that the damaged lung tissue and endothelial cells are causing an increased amount of platelet consumption by activating platelets in the lungs for the formation of microthrombi. Autopsy reports of patients with COVID-19 have revealed significantly high levels of platelet-rich thrombi in the pulmonary and cardiac systems. As a result, we speculate that the SARS-CoV-2 viral infection impedes hematopoiesis and adversely affects the functioning of the MKs.

## CONCLUSIONS

Currently, there is a lack of data on the most effective therapeutic strategies to mitigate severe illness and mortality in SARS-CoV-2 infection. Because of the rapid spread of the virus globally, along with the emergence of highly transmissible mutant strains, such as the Delta variant, there is an immediate need for an effective treatment. Autopsies and postmortem reports identified significantly elevated levels of MKs in severe cases of COVID-19. Although not yet tested, it is interesting to speculate that the COVID-19-associated cytokine storm increases IL-6 and JAK-STAT signaling, which in turn increases MK levels [14,16,39]. Additionally, thrombosis and abnormal coagulopathy in the presence of increased levels of MKs in the bone marrow, pulmonary, and cardiac systems indicate the possible role of MKs in worsening the final prognosis for patients with COVID-19. Therefore, a potential adjuvant therapeutic intervention could include limiting MK production in patients with COVID-19 [80,82].

### Conflict of interest disclosure

The authors declare no competing financial interests.

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