Arteriosclerosis, Thrombosis, and Vascular Biology

BRIEF REVIEW

Coagulation Abnormalities and Thrombosis in Patients Infected With SARS-CoV-2 and Other Pandemic Viruses

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ABSTRACT: The world is amid a pandemic caused by severe acute respiratory syndrome-coronavirus 2. Severe acute respiratory syndrome-coronavirus causes serious respiratory tract infections that can lead to viral pneumonia, acute respiratory distress syndrome, and death. Some patients with coronavirus disease 2019 (COVID-19) have an activated coagulation system characterized by elevated plasma levels of D-dimer—a biomarker of fibrin degradation. Importantly, high levels of D-dimer on hospital admission are associated with increased risk of mortality. Venous thromboembolism is more common than arterial thromboembolism in hospitalized COVID-19 patients. Pulmonary thrombosis and microvascular thrombosis are observed in autopsy studies, and this may contribute to the severe hypoxia observed in COVID-19 patients. It is likely that multiple systems contribute to thrombosis in COVID-19 patients, such as activation of coagulation, platelet activation, hypofibrinolysis, endothelial cell dysfunction, inflammation, neutrophil extracellular traps, and complement. Targeting these different pathways may reduce thrombosis and improve lung function in COVID-19 patients.

Key Words: coronavirus ■ fibrin ■ orthomyxoviridae ■ pandemics ■ thrombosis

PANDEMIC RESPIRATORY VIRUSES

In the last century, several new viruses have emerged, including different strains of influenza virus A virus (IAV), severe acute respiratory syndrome-coronavirus (SARS-CoV), Middle East respiratory syndrome-coronavirus (MERS-CoV), and most recently, SARS-CoV-2, that have caused epidemics and pandemics. IAV transmission from zoonotic reservoirs into humans has caused the last 4 influenza pandemics: 1918 H1N1 Spanish flu, 1957 H2N2 Asian flu, 1968 H3N2 Hong Kong flu, and 2009 H1N1.[1–3] The SARS-CoV epidemic occurred between 2002 and 2004 and infected ≈8000 people with at least 774 deaths worldwide.[4–6] MERS-CoV appeared in 2012 and infected ≈2500 people with over ≈850 deaths, and cases still occur.[7–9] In December 2019, SARS-CoV-2 emerged in China and quickly spread throughout the world. As of June 17, 2020, there have been over 8.2 million diagnosed cases of coronavirus disease 2019 (COVID-19) with >445 000 deaths worldwide (Johns Hopkins Coronavirus Resource Center, https://coronavirus.jhu.edu/map.html).

Influenza viruses and coronaviruses are enveloped viruses with a single-stranded RNA genome (either a positive- or negative-sense RNA). Influenza viruses enter cells via endocytosis that requires binding and proteolytic cleavage of hemagglutinin on epithelial cells.[10,11] SARS-CoV, MERS-CoV, and SARS-CoV-2 all belong to the coronavirus family. They are called coronaviruses because they have large spike proteins on the capsid surface that create a crown-like shape. Entry of coronaviruses into host cells involves binding of the spike proteins with host receptors, followed by proteolytic cleavage of the spike protein to expose the S2 fusion domain with subsequent membrane fusion.[12,13] MERS-CoV uses DPP4 (dipeptidyl peptidase 4) as

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For Sources of Funding and Disclosures, see page 2040.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

ACE2	angiotensin-converting enzyme 2
ARDS	acute respiratory distress syndrome
AT	antithrombin
COVID-19	coronavirus disease 2019
CRP	C-reactive protein
DIC	disseminated intravascular coagulation
DPP4	dipeptidyl peptidase 4
FVIII	factor VIII
FXI	factor XI
FXII	factor XII
IAV	influenza A virus
ICAM-1	intercellular adhesion molecule 1
IL	interleukin
IMPROVE	Intermediate or Prophylactic Dose Anticoagulation for Venous or Arterial Thromboembolism in Severe COVID-19
MERS-CoV	Middle East respiratory syndrome-coronavirus
MPO	myeloperoxidase
NET	neutrophil extracellular trap
PAI-1	plasminogen activator inhibitor 1
PE	pulmonary embolism
PT	prothrombin time
SARS-CoV	severe acute respiratory syndrome-coronavirus
TF	tissue factor
TLR3	toll-like receptor 3
TNFα	tumor necrosis factor-alpha
VCAM-1	vascular cell adhesion molecule 1
VTE	venous thromboembolism
VWF	von Willebrand factor

a cellular receptor, whereas SARS-CoV and SARS-CoV-2 use ACE2 (angiotensin-converting enzyme 2) as entry receptors.[14–18] Importantly, SARS-CoV-2 has a stronger binding to ACE2 compared with SARS-CoV.[19] ACE2 is predominantly expressed in epithelial cells of subsegmental bronchial branches.[20] Interestingly, one study found low levels of ACE2 in alveolar epithelial cells and endothelial cells in uninfected control lungs but an increased expression of ACE2 in both cell types in the lungs of COVID-19 patients.[21] In a physiological setting, ACE2 cleaves and inactivates angiotensin I and angiotensin II and, therefore, plays a critical role in regulating the renin-angiotensin system.[22] Differences in tissue expression of these receptors and activating proteases may contribute to unique aspects of the pathophysiology of each virus.

Highlights

- Coronavirus disease 2019 (COVID-19) patients have an increased risk of arterial and venous thrombosis.
- Elevated levels of D-dimer are associated with increased thrombosis and mortality.
- Multiple pathways likely contribute to thrombosis in COVID-19 patients.

ACUTE RESPIRATORY DISTRESS SYNDROME ASSOCIATED WITH PANDEMIC RESPIRATORY VIRUSES

Super pandemic viruses, such as IAV H1N1, SARS-CoV, MERS-CoV, and SARS-CoV-2, cause serious respiratory tract infections that can lead to viral pneumonia and acute respiratory distress syndrome (ARDS).[23–25] ARDS is a type of respiratory failure characterized by widespread local and systemic inflammation.[26] Both viral infection of cells and the host response to infection damage the epithelial-endothelial cell barrier that separates the alveoli from capillaries. This injury compromises the lung's ability to exchange oxygen and carbon dioxide.[26] Lung stillness, fluid-filled alveoli, and a rise in carbon dioxide levels lead to hypoxemia and respiratory distress.[27] The primary treatment for ARDS is mechanical ventilation and supportive treatment in an intensive care unit.[28] IAV patients with classic ARDS that requires mechanical ventilation have decreased lung compliance with elevated plateau pressures.[29] One study reported that 46 (23%) of 199 patients hospitalized with SARS developed ARDS, and these patients had a mortality rate of 37% at 28 days.[30] Similarly, 20% of hospitalized COVID-19 patients in New York required mechanical ventilation.[31] Surprisingly, some COVID-19 patients with ARDS have well-preserved lung mechanics despite severe hypoxia.[32,33] This has led to the suggestion that microvascular thrombosis rather than decreased lung compliance contributes to the impaired oxygenation in COVID-19 patients.

CYTOKINE STORM IS ASSOCIATED WITH PANDEMIC RESPIRATORY VIRUSES

Infection with pandemic respiratory viruses can lead to an overproduction of numerous cytokines that is termed the cytokine storm.[34–36] This hyperinflammatory response contributes to disease severity and death. TNFα (tumor necrosis factor-alpha), IL (interleukin)-1β, and IL-6 orchestrate the inflammatory response.[34,37] Both IAV and SARS-CoV infection are associated with a cytokine storm.[38,39] Davey et al[40] reported the results of 2 international cohort studies that measured the association of 25 plasma biomarkers with disease progression in 837

IAV(H1N1)pdm09 patients. Seven biomarkers, including IL-6, were associated with disease progression in outpatients and inpatients, whereas 5 biomarkers, including TNFα and IL-8, were associated with disease progression among hospitalized patients. Critically ill patients with IAV(H1N1)pdm09 also exhibited higher levels of IL-6 compared with patients with bacterial pneumonia.[41] A recent study suggested that monocytes and macrophages play a key role in the hyperinflammatory response in COVID-19 patients.[42] Indeed, severe SARS-CoV-2 infection is associated with increased circulating levels of various inflammatory mediators, including IL-6 and CRP (C-reactive protein).[43–47] We observed increased levels of CRP in severe IAV H1N1 patients.[48] Zhou et al[47] observed a serial increase in IL-6 in nonsurviving patients but not in surviving patients. Accordingly, a pilot study analyzed the effect of the IL-6 receptor antagonist tocilizumab on survival of 129 hospitalized COVID-19 patients with moderate or severe viral pneumonia. Tocilizumab significantly reduced the number of life-support interventions and deaths compared with the control group (https://www.clinicaltrialsarena.com/news/french-early-trial-tocilizumab-covid-19/). This preliminary study led the Food and Drug Administration to approve a phase 3 trial of tocilizumab for the treatment of severe COVID-19 patients (https://www.clinicaltrials.gov; unique identifier: NCT04361552), with additional tocilizumab clinical trials underway. However, it remains unclear whether IL-6 targeting alone will be adequate to improve outcomes caused by a plethora of cytokines. In addition, it is unclear whether tocilizumab will mitigate the thrombotic propensity in COVID-19 patients, although the expected reduction of IL-6–dependent CRP expression has been observed.[49,50]

THROMBOSIS ASSOCIATED WITH PANDEMIC RESPIRATORY VIRUSES

Critically ill patients exhibit a rate of venous thromboembolism (VTE; deep vein thrombosis or pulmonary embolism [PE]), of VTE 5% to 10% despite thromboprophylaxis.[51] VTE, pulmonary microvascular thrombosis, and arterial thrombosis have been associated with IAV and pandemic coronavirus infections. One study of hospitalized H1N1 patients observed 7 (5.9%) thrombotic vascular events (4 venous and 3 arterial) in 119 patients.[52] Another study observed a higher rate of VTE (44%) in hospitalized H1N1 patients (n=36) with severe ARDS compared with 29% in non-H1N1 patients with ARDS.[24] Thrombotic complications have also been observed in SARS-CoV patients.[53] A study from a single hospital in Singapore reported that one-third of SARS-CoV patients experienced VTE despite the use of low-molecular-weight heparin at doses to achieve anti-Xa levels of 0.5 to 1.0 IU/mL[54]; however, no additional details of the VTE events were provided. Arterial ischemic stroke was observed in a small number of SARS-CoV patients.[54] Surprisingly, perhaps, there are no reports of thrombosis in MERS-CoV patients.

Recently, several studies have reported VTE rates ranging from 0.9% to 6.5% for noncritically ill hospitalized COVID-19 patients and 8% to 69% in COVID-19 patients in the intensive care unit (Table 1).[55–64] Rates of PE were between 16.7% and 35% in severely ill COVID-19 patients, and rates of deep vein thrombosis were between 0% and 46.1% for nonseverely ill COVID-19 patients (Table 1). Rates of arterial thrombotic events were between 2.8% and 3.8%.[57,62] There are several factors that could explain the wide variation in thrombosis rates in the different studies that include differences in clinical practice, such as if venous ultrasound is performed as a screening strategy or if thromboprophylaxis is routinely used, reporting of symptomatic versus asymptomatic VTE, and also differences in patient populations. Notably, however, several groups have reported that VTE may occur despite standard thromboprophylaxis (Table 1), which is like what was observed in SARS-CoV infection. Although initial reports suggested that COVID-19 patients had higher rates of thrombosis compared with patients with other types of pneumonia, a recent study found that the rate of VTE in COVID-19 patients was 2% compared with 3.6% in patients with non–COVID-19 community-associated pneumonia.[65] Furthermore, it is important to note that a recent study reported a VTE rate of 4.8% and a rate of overall bleeding of 4.8% in COVID-19 patients.[62] Another study of 353 COVID-19 patients in Boston found that the cumulative incidence of thrombotic events was 10.2% and major or fatal bleeding of 20.8% in hospitalized COVID-19 patients (J. Zwicker, unpublished data, 2020). At present, the optimal antithrombotic prophylactic strategy for patients with COVID-19 is unclear. A new clinical trial (IMPROVE [Intermediate or Prophylactic Dose Anticoagulation for Venous or Arterial Thromboembolism in Severe COVID-19]; https://www.clinicaltrials.gov; unique identifier: NCT04367831) will hopefully shed light on this question.

Several studies have reported pathological findings from autopsies of patients infected with pandemic coronaviruses. Pulmonary thromboemboli within the main pulmonary artery or segmental pulmonary arteries, thrombi in small vessels, and fibrin within pulmonary vessels were observed in SARS-CoV patients.[66–68] A recent series of studies have described the findings of autopsies of COVID-19 patients.[33,69–71] Fibrin-rich thrombi were found in small vessels and capillaries in the lung, as well as foci of hemorrhages.[33,70] Interestingly, CD61+ megakaryocytes were observed within alveolar capillaries.[70] Some fibrin and platelets within small vessels were also associated with neutrophils. Intra-alveolar fibrin deposition was observed in a subset

Table 1. Incidence of Thrombosis in COVID-19

Country	No. of Patients	ICU	Non-ICU	AC	VTE, %	PE, %	DVT, %	ATE, %	IS, %	Reference
The Netherlands	184	+		Y	37	35	0.5	3.8	2.7	87
China	81	+		N	…	…	25	…	…	88
France	26*	+		Y	69	23	69	…	…	59
The Netherlands	74	+		Y	25	…	…	…	…	61
The United States	144	+		Y	7.6	…	…	…	…	62
France	107	+		Y	…	20.6	…	…	…	58
France	150	+		Y	…	16.7	…	…	…	60
China	45	+		N	6.7	…	…	…	…	65
The United States	400	+	+	Y	4.8	…	…	2.8	…	62
The Netherlands	124		+	Y	6.5	…	…	…	…	61
The United States	166		+	Y	3.1	…	…	…	…	62
China	143		+	N	…	…	46.1	…	…	64
Italy	388		+	Y	…	…	0	…	…	63
Spain	156†		+	Y	…	…	14.7‡	…	…	56
China	211		+	N	0.9	…	…	…	…	65

AC indicates anticoagulant; ATE, arterial thromboembolism; COVID-19, coronavirus disease 2019; DVT, deep vein thrombosis; ICU, intensive care unit; IS, ischemic stroke; N, no; PE, pulmonary embolism; VTE venous thromboembolism; and Y, yes.

*Complete duplex ultrasound standard of care.

†D-dimer >1000 ng/mL.

‡Asymptomatic.

of severe COVID-19 patients consistent with a loss of vascular integrity.[69] One autopsy study found that 7 of 12 (58%) COVID-19 patients had a deep vein thrombosis that was not suspected antemortem, and PE was the direct cause of death in 4 of these patients.[72] A recent study performed autopsies on 7 COVID-19 patients and compared the findings to 7 H1N1 patients.[21] There was widespread thrombosis and microangiopathy in the lungs of COVID-19 patients, and capillary microthrombi were 9× more prevalent than in H1N1, which suggested a different pathological process.[21]

PANDEMIC RESPIRATORY VIRUSES ACTIVATE THE COAGULATION SYSTEM

The innate immune response is activated in response to invading pathogens to counteract the infection. This is generally accompanied by activation of coagulation that, in part, serves to localize the infection.[73,74] However, excessive and widespread activation of coagulation can lead to disseminated intravascular coagulation (DIC), defined as fulminant activation of coagulation, consumption of coagulation factors, and bleeding.[75,76] Classic DIC caused by bacterial sepsis is associated with prolonged activated partial thromboplastin time, prothrombin time (PT), thrombocytopenia, elevated D-dimer, and microangiopathic thrombosis in multiple organs.[75,76] D-dimer is a product of plasmin-mediated degradation of cross-linked fibrin.

Elevated plasma D-dimer is associated with a higher risk of disease progression in hospitalized IAV(H1N1) pdm09-infected patients.[40] Two other studies of patients with probable IAV H1N1 infection found that D-dimer predicted disease progression.[77,78] Elevated plasma levels of D-dimer have also been reported in SARS-CoV–infected patients.[79] D-dimer has attracted attention as a prognostic marker in COVID-19 patients.[43,44,46,47,60,80–82] As expected, COVID-19 patients with VTE had higher D-dimer levels than non-VTE patients.[55] A series of articles from China reported higher D-dimer levels in severely affected patients compared with those with a nonsevere disease and higher D-dimer levels in nonsurvivors compared with survivors (Table 2).[43–47,80] Similarly, studies from France and Italy found high D-dimer levels in COVID-19 patients in the intensive care unit (Table 2).[60,81] Two studies found that a higher D-dimer level on admission was associated with increased mortality.[47,64] One study used 2.0 µg/mL as a cutoff for D-dimer and found a mortality rate of 0.37% (1 of 267 COVID-19 patients, <2.0 µg/mL) versus 17.9% (12 of 67 COVID-19 patients, ≥2.0 µg/mL).[64] In contrast, a study from France observed a less impressive separation of mortality rates based on the same D-dimer cutoff (10.4% [8 of 77 COVID-19 patients], <2.0 µg/mL versus 18.3% [17 of 93 COVID-19 patients], ≥2.0 µg/mL) and suggested that the Chinese study had selection bias.[83]

Thrombocytopenia was observed in 45% to 55% of SARS-CoV patients, but overt DIC was rarely observed.[53,79,84] Thrombocytopenia was also found to be evident in a subset of MERS-CoV patients.[85–88] Similarly, thrombocytopenia and an elevated PT was observed in 2 fatal cases of MERS-CoV patients, consistent with a diagnosis of DIC.[86] Many COVID-19 patients have mild

Table 2. D-Dimer Levels in Patients With COVID-19

All Patients	Nonsevere	Severe	Nonsurvivors	*P* Value	Reference
0.5 (0.3–1.3), n=41	0.5 (0.3–0.8), n=28	2.4 (0.6–14.4), n=13		0.042	43
0.5 (0.4–1.8), n=21	0.3 (0.3–0.4), n=10	2.6 (0.6–18.7), n=11		0.029	44
0.6 (0.4–1.5), n=183	0.6 (0.3–1.3), n=162	2.1 (0.8–5.3), n=21		<0.001	80
0.8 (0.4–3.2), n=191	0.6 (0.3–1.0), n=137		5.2 (1.5–21.1), n=54	<0.001	47
1.1 (1.0–1.2), n=214	0.6 (0.3–1.3), n=161		4.6 (1.3–21.6), n=113	0.029	46
		2.2 (1.1–20.0), n=150			60
		5.5 (2.5–6.5), n=16			81
		4.8 (1.2–16.9), n=24			82

Less than 0.5 mg/L median (IQR). COVID-19 indicates coronavirus disease 2019; and IQR, interquartile range.

thrombocytopenia (100—150×10^9/L) at most and do not exhibit an increase in PT or decrease in AT (antithrombin) levels.[33,43–46,60,80–82,89] These results suggested the absence of a consumptive coagulopathy in most patients. However, several studies found that nonsurviving patients had slightly prolonged PT and a further decrease in platelet count.[46,47,80] Interestingly, patients with severe SARS-CoV-2 infection also have elevated levels of fibrinogen ranging from 1.3 to 2.0× above the normal range (2–4 g/L; Table 3).[60,80–82] We observed increased levels of fibrinogen in severe IAV H1N1 patients.[48] Ranucci et al[81] showed an association between IL-6 and fibrinogen levels. In addition, FVIII (factor VIII) and VWF (von Willebrand Factor) levels were increased in COVID-19 patients by 2- to 2.3-fold and 3- to 4.1-fold above the normal range, respectively.[60,82]

Taken together, these results indicate that most COVID-19 patients have an activated coagulation system that is associated with increased levels of D-dimer; however, it is unlike classic DIC since there is little change in PT and the thrombocytopenia is generally mild. Elevated levels of FVIII and fibrinogen likely contribute to the prothrombotic state in COVID-19 patients. Elevated FVIII and VWF may reflect activated/infected endothelium, whereas elevated fibrinogen likely reflects enhanced production by hepatocytes as part of the host's acute phase responses driven by IL-6. In the later stages of disease, nonsurviving COVID-19 patients may develop classic DIC with prolongation of the PT, moderate-to-severe thrombocytopenia (platelet count, <50×10^9/L), and decreased fibrinogen (<1.0 g/L).

MOUSE MODELS OF PANDEMIC VIRUS INFECTION

Several mouse models have been developed to study the pathological changes in the lung associated with infection with IAV H1N1, SARS-CoV, and MERS-CoV. One study reported that over 3500 genes were differentially regulated in the lungs of mice following SARS-CoV infection.[90] Importantly, mice infected with 1918 and 2009 IAV H1N1 strains exhibited similar transcriptional signatures, which suggested a common mechanism of lung injury.[90] Infection with IAV H1N1, SARS-CoV, and MERS-CoV is associated with lung hemorrhages.[90–92] Infection of mice with different coronavirus mouse hepatitis virus strains also caused severe pneumonia and lung hemorrhage.[93] However, thrombosis has also been observed in the lungs of mice expressing human DPP4 infected with MERS-CoV.[94]

POSSIBLE MECHANISMS DRIVING THROMBOSIS IN PANDEMIC VIRUS INFECTION

At this time, we can only speculate about the mechanisms of thrombosis in COVID-19 patients based on the available plasma biomarkers and clinical presentation. Several recent comments/reviews have described the coagulation abnormalities and thrombosis occurring in COVID-19 patients.[95–99] There is clear evidence for activation of different cell types, such as lung epithelial cells, macrophages, neutrophils, endothelial cells, and platelets, as well as different systems, such as coagulation, inflammation, and complement, in the lungs of COVID-19 patients (Figure). We will briefly summarize some of these pathways and refer to reviews that cover some of the pathways in more detail.

TF Pathway

Aberrant TF (tissue factor) expression is associated with most forms of thrombosis.[100] Importantly, TF is a key mediator of activation of coagulation in different forms of ARDS.[101–104] Viral infection of a variety of cell

Table 3. Fibrinogen Levels in Severe COVID-19 Patients

Healthy	Severe	Reference
2–4	5.1 (3.7–5.7), n=21	80
2–4	7.0 (6.1–7.7), n=150	60
2–4	7.9 (5.8–9.3), n=16	81
2.6 (1.6–3.5)	6.8 (2.3–13.4), n=24	82

Normal range, 2–4 g/L. COVID-19 indicates coronavirus disease 2019.

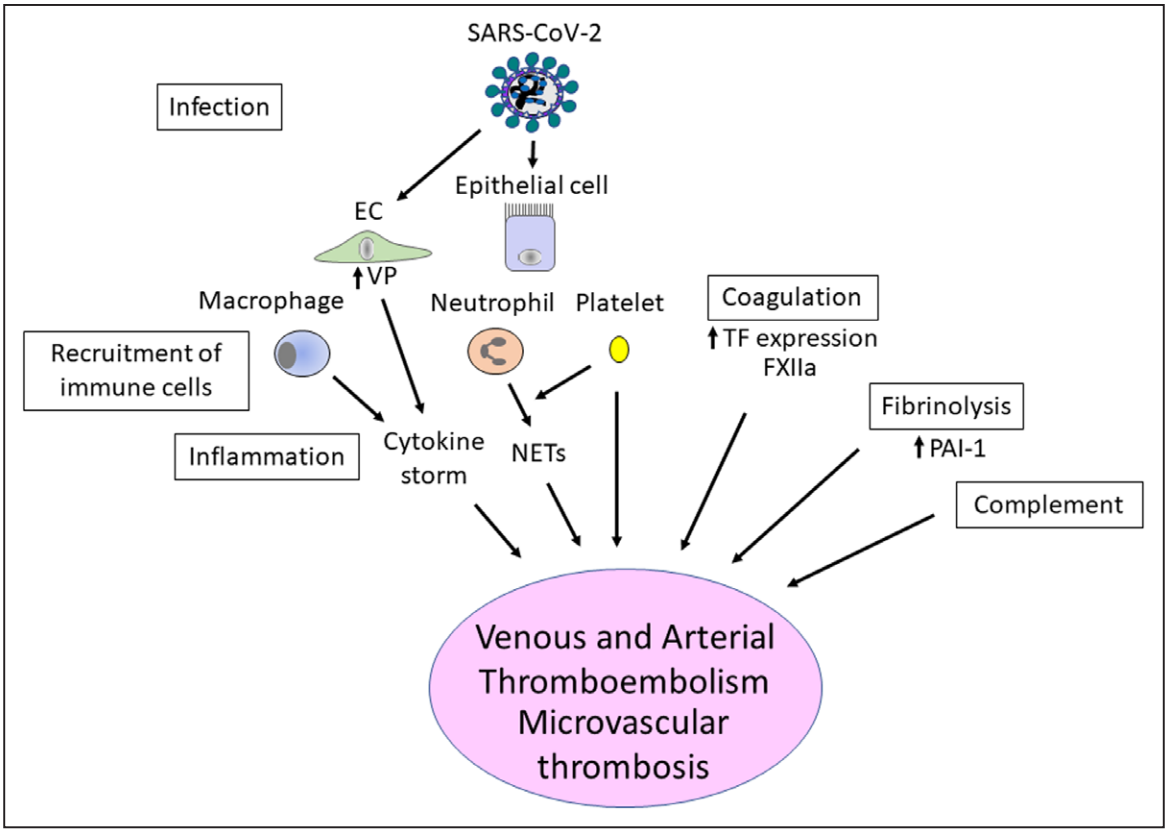

Figure. Potential pathways that drive thrombosis in coronavirus disease 2019 (COVID-19) patients.
Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infects lung epithelial cells and endothelial cells (ECs), which leads to the recruitment of a variety of immune cells, such as macrophages and neutrophils. Activated macrophages and ECs contribute to the cytokine storm. EC activation also increases vascular permeability (VP). Neutrophils release neutrophil extracellular traps (NETs). Activated platelets likely contribute to thrombosis and NET formation. TF (tissue factor) expression is likely to be increased in activated epithelial cells, macrophages, and ECs and will activate the coagulation system. Similarly, FXIIa (factor XIIa) can increase coagulation. SARS-CoV-2 infection also activates the fibrinolytic system and may increase PAI-1 (plasminogen activator inhibitor 1), which would reduce fibrin degradation. Finally, the complement system is activated in COVID-19 patients, and cellular damage would increase the activation of the coagulation system.

types, including lung epithelial cells, endothelial cells, and monocytes induces TF expression.[74,91,105] In addition, TF expression in endothelial cells is induced by activation of TLR3 (toll-like receptor 3)—a pattern-recognition receptor that detects single-stranded RNA.[106,107] Interestingly, TLR3 was shown to protect mice from SARS-CoV infection.[108] Cytokines produced during the cytokine storm (TNFα, IL-1β, and IL-6) induce TF expression in endothelial cells, and IL-6 induces TF expression in mononuclear cells.[109–111] Angiotensin II can also induce TF expression in vascular smooth muscle cells and endothelial cells.[112,113] Herpes simplex virus infection of endothelial cells increases TF expression.[114] Similarly, one would expect that SARS-CoV-2 infection of the endothelium would increase TF expression and microvascular thrombosis. Therefore, there are a variety of mechanisms for increasing TF expression in different cell types in the lung during viral infections. We found that plasma levels of extracellular vesicle TF activity were increased in severe influenza virus patients and were associated with mortality.[48] Increased TF is also observed after infection of mice with IAV H1N1, SARS-CoV, and MERS-CoV[90,91] (T. Sheahan, unpublished data). We found that IAV H1N1 infection of mice increases TF expression in lung epithelial cells and activates coagulation.[91] Furthermore, both a genetic reduction of TF in epithelial cells and administration of anticoagulants to wild-type mice was associated with increased alveolar hemorrhage.[91,115] This suggests that TF-dependent activation of coagulation is part of the host innate immune response to viral infection that helps protect against intrapulmonary hemorrhage. However, a complication of this response is thrombosis. Therefore, it seems likely that TF plays a central role in thrombosis in COVID-19 patients. Two recent articles have discussed the role of TF in thrombosis in COVID-19 patients.[116,117]

Contact Activation Pathway

Activation of the contact system leads to thrombin generation and upregulation of the kallikrein-kinin system.[118] Kallikrein induces the generation of bradykinin, which increases vascular permeability. In addition, bradykinin interacts with the renin-angiotensin system and increases inflammation, fibrinolysis, and complement

activation.[119] The effect of targeting the contact system has been studied in animal models of bacterial sepsis. An early study showed that administration of an anti-FXII (factor XII) antibody C6B7 prevented hypotension and extended the life of baboons challenged with *Escherichia coli* but did not prevent DIC.[120] In a second study, C6B7 reduced complement activation, neutrophil activation, and the fibrinolytic response (reduced tissue plasminogen activator and plasmin-α2-antiplasmin complexes) but increased PAI-1 (plasminogen activator inhibitor 1) in septic baboons.[121] More recently, the effect of blocking the contact pathway using an antibody 3G3 that prevents FXIIa activation of FXI (factor XI) was evaluated in a lethal *Staphylococcus aureus* baboon model.[122] Pretreatment of the baboons with 3G3 reduced the activation of coagulation, fibrin deposition in tissues, inflammation, neutrophil activation, complement activation, and increased survival.[122] An anti-FXII antibody 3F7 also reduced bradykinin generation and edema in mice.[123] Acquired ACE2 deficiency also leads to more bradykinin via an unknown mechanism, which would increase vascular permeability. A recent review discussed the potential benefits of targeting the contact activation pathway in COVID-19 patients.[124]

Fibrinolysis

The fibrinolytic system is activated in ARDS.[104,125,126] Elevated levels of PAI-1 in ARDS create a hypofibrinolytic state that leads to increased fibrin deposition within the vasculature and within the alveolar space. High plasma PAI-1 levels are associated with mortality in ARDS patients.[127,128] One study reported that the plasma PAI-1 level was higher in 16 SARS-CoV patients than 19 patients with other infectious pneumonias and healthy controls.[129] PAI-1 expression was increased in SARS-CoV–infected mice, and PAI-1$^{-/-}$ mice exhibited increased lung hemorrhage and increased mortality.[90] This study suggested that PAI-1–dependent inhibition of fibrinolysis is protective against intrapulmonary hemorrhage. A recent review described the fibrinolytic abnormalities associated with ARDS and discussed the use of thrombolytic drugs to treat COVID-19.[130] It was proposed that nebulized plasminogen activators could be used to degrade fibrin in the alveoli and improve oxygenation in COVID-19 patients.[130] Indeed, a recent study reported that intravenous administration of tissue plasminogen activator temporally improved the respiratory status of 3 patients with severe COVID-19 respiratory failure.[131]

Platelets

Platelets play an essential role in maintaining vascular integrity but also contribute to thrombosis. More recently, platelets have been found to participate in the immune response to viruses.[132] Interestingly, IAV particles were observed within platelets from patients with acute influenza infection.[133] In addition, IAV engulfment by platelets led to TLR7-dependent release of C3 and subsequent activation of neutrophils and neutrophil extracellular trap (NET) release.[133] Therefore, platelets participate in the host response to IAV infection. However, platelet activation during viral infection may also increase the risk of thrombosis. One study in COVID-19 patients found an association between thrombocytopenia and risk of in-hospital mortality.[134] A recent review discussed the potential role of platelets in thrombosis in COVID-19.[135]

Activation of the Endothelium

Under normal conditions, the endothelium maintains vascular integrity, limits binding and activation of immune cells and platelets, and inhibits coagulation by expression of anticoagulant proteins. However, during infection, the endothelium becomes activated, resulting in a loss of barrier function, expression of adhesion proteins that facilitate the recruitment of immune cells, release of VWF that allows binding of platelets, and expression of TF that activates the coagulation system. One study found that soluble ICAM-1 (intercellular adhesion molecule 1) and soluble VCAM-1 (vascular cell adhesion molecule 1) were associated with disease progression among hospitalized IAV(H1N1)pdm09 patients.[40] These biomarkers indicate that the endothelium is activated possibly by circulating inflammatory mediators. Although some IAV strains have been shown to replicate in human lung microvascular endothelial cells, only avian IAV H5N1 has been shown to infect lung microvascular endothelial cells in vivo.[136,137] Importantly, one study found that blocking replication of the highly pathogenic IAV strain H5N1 in the endothelium reduced systemic viral spread and mortality without affecting viral replication in the lungs of infected mice.[3] A recent study found that human capillary organoids derived from induced pluripotent stems cells could be infected with SARS-CoV-2, and this infection was blocked with recombinant, soluble human ACE2.[138] Interestingly, deceased COVID-19 patients had increased ACE2 expression in endothelial cells in the lungs compared with noninfected controls.[21] Two studies found evidence for direct infection of the endothelium by SARS-CoV-2 and diffuse endothelial inflammation in the lung, heart, kidney, and liver.[21,139] SARS-CoV-2 infection of endothelial cells may lead to apoptosis or pyroptosis. Recent reviews have discussed the potential role of the endothelium in COVID-19.[140,141]

Neutrophils and NETs

Hematopoietic changes are observed in SARS-CoV and MERS-CoV patients.[53] For instance, SARS-CoV patients often present with neutrophilia, which is associated with poor outcome.[79,84] Other studies have observed

neutrophilia in MERS-CoV–infected patients.[85–87] COVID-19 patients generally have increased numbers of circulating neutrophils, and an elevated neutrophil count has been associated with poor outcome.[43–47]

Neutrophils play a key role in clearing viruses in the lung by phagocytosing viral particles and by releasing NETs.[142–144] However, activated neutrophils can also damage host cells.[145–148] Neutrophils also play a key role in immunothrombosis—a term that has been used to describe the activation of coagulation that accompanies host innate immune defense.[149] Importantly, NETs may contribute to thrombosis and vascular occlusion.[150,151] There are several biomarkers used to measure the levels of NETs in plasma, including MPO (myeloperoxidase)-DNA complexes and citrullinated histone H3.[151] However, many of these assays have low specificity for NETs.[151] One study in IAV H1N1 and H7N9 patients reported elevated levels of MPO-DNA complexes at hospital admission that correlated with disease severity.[152] Similarly, serum from severe COVID-19 patients contained elevated levels of MPO-DNA complexes and citrullinated histone H3.[153] These results suggest that NETs may contribute to impairment of blood flow in the lungs of COVID-19 patients.[151,154]

Complement

The complement system plays a key role in the host immune response to viruses by opsonization of viral particles, recruitment of inflammatory cells, and lysis of infected cells.[155] However, complement activation can also damage host cells. SARS-CoV infection in mice activates the complement system.[156] $C3^{-/-}$ mice exhibited reduced recruitment of neutrophils and inflammatory monocytes into the lung and less respiratory dysfunction after SARS-CoV infection compared with controls.[156] Similarly, inhibition of the C5a receptor reduced lung injury in hDPP4 mice infected with MERS-CoV.[157] These results indicate that the complement system contributed to the lung pathology after SARS-CoV and MERS-CoV infection in mice. Importantly, significant deposits of terminal complement components have been noted in the lung microvasculature of COVID-19 patients.[33] Complement system inhibition with eculizumab, which binds to C5, might be beneficial for COVID-19—a hypothesis that is currently investigated in a clinical trial (https://www.clinicaltrials.gov; unique identifier: NCT04288713).[158] A recent review discussed complement as a target in COVID-19.[159]

CONCLUSIONS

Further studies are needed to understand the molecular basis of thrombosis in COVID-19 patients and how this contributes to morbidity and mortality. Measurement of additional circulating biomarkers of different systems, such coagulation, fibrinolysis, and complement, as well as markers of endothelial cell activation, will provide much needed information on the pathology of COVID-19. When optimizing antithrombotic treatment for COVID-19 patients, it is important to balance the risk of thrombosis and the risk of bleeding, especially as bleeding has been observed in the lungs of COVID-19 patients. It will be also interesting to know whether any of the proposed treatments for COVID-19 patients, such as blocking the IL-6 receptor or inhibiting complement activation, will reduce thrombosis.

ARTICLE INFORMATION

Received May 18, 2020; accepted June 29, 2020.

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Acknowledgments

We would like to acknowledge colleagues in the Division of Hematology (Drs Stephan Moll, Steven Grover, and Yohei Hisada), in the Division of Pulmonary and Critical Care Medicine (Drs C. Adrian Austin and Robert Hagan), and in the Department of Epidemiology (Dr Lisa Gralinski) at the University of North Carolina at Chapel Hill and Dr Alvin Schmaier (Case Western Reserve University) for helpful discussions and comments. Webinars from the International Society on Thrombosis and Haemostasis were also very helpful (https://academy.isth.org/isth/2020/covid-19/291581/marcel.levi.26.beverley.jane.hunt.thrombosis.thromboprophylaxis.26.coagulopathy.html?f=menu%3D8%2Abrowseby%3D8%2Asortby%3D2%2Alabel%3D19794; https://academy.isth.org/isth/2020/covid-19/293464/doctor.jerrold.h.levy.and.doctor.nicole.p.juffermans.html?f=menu%3D8%2Ac_id%3D293464%2Afeatured%3D16772).

Sources of Funding

Funding was from grants from the National Institutes of Health (HL119523, N.M. and HL142799, S.A.).

Disclosures

None.

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