The Impact of SARS-CoV-2 Infection on Blood Coagulation and Fibrinolytic Pathways: A Review of Prothrombotic Changes Caused by COVID-19

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Abstract

The cardinal pathology of coronavirus disease 2019 (COVID-19) is a primary infection of pulmonary tract cells by severe acute respiratory syndrome coronavirus 2, provoking a local inflammatory response, often accompanied by cytokine storm and acute respiratory distress syndrome, especially in patients with severe disease. Systemic propagation of the disease may associate with thrombotic events, including deep vein thrombosis, pulmonary embolism, and thrombotic microangiopathy, which are important causes of morbidity and mortality in patients with COVID-19. This narrative review describes current knowledge of the pathophysiological mechanisms of COVID-19-associated coagulopathy, with focus on prothrombotic changes in hemostatic mediators, including plasma levels of clotting factors, natural anticoagulants, components of fibrinolytic system, and platelets. It will also highlight the central role of endothelial cells in COVID-19-associated coagulopathy. This narrative review discusses also potential therapeutic strategies for managing thrombotic complications. Awareness by medical experts of contributors to the pathogenesis of thrombotic events in COVID-19 is imperative to develop therapeutics not limited to regular anticoagulants. Instituting cooperation among medical personnel and researchers may lessen this novel virus' impact now, and in the event of recurrence.

Keywords
- SARS-CoV-2
- COVID-19
- coagulopathy
- thrombosis

The latest formidable hazard to global health is the advancing outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the novel virus responsible for coronavirus disease-19 (COVID-19), identified in December 2019 in Wuhan, China. Three months after the advent of COVID-19, the Director General of the World Health Organization announced that COVID-19 had become a global pandemic.[1] At the time of writing, this outbreak is the gravest adversity worldwide, with nearly 200 million infected patients and more than 4 million deaths as of July 27, 2021.[2]

COVID-19 shows a broad spectrum of clinical manifestations, varying from asymptomatic, or mild, to upper respiratory tract signs, multiple organ dysfunction, cytokine storm, thrombotic complications manifested in most severe cases, and finally death.[3] Fever, dry cough, dyspnea, and myalgia are the most common manifestations, followed by viral pneumonia and type 1 respiratory failure in 10 to 15% of cases. Around a third of patients require intensive care unit (ICU) admission for acute respiratory distress syndrome (ARDS), occasionally with multiorgan failure.[4–6]

SARS-CoV-2 mostly uses the body's angiotensin-converting enzyme-2 (ACE-2) as a receptor for entering the host cells. In addition to ACE-2, SARS-CoV-2 may require transmembrane protease serine 2 (TMPRSS2) and basigin (CD147) to infect cells, as well as entering the cells by a microparticle-bearing pathway.[7,8] There is also some evidence indicating that coronaviruses tend to interact with acetylated sialic acid residues presented copiously on the membrane proteins of

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megakaryocytes and endothelial cells.[9,10] This narrative review deals with what is known about COVID-19-associated coagulopathy, focusing on current knowledge of alterations in blood coagulation and fibrinolysis mediators following SARS-CoV-2 infection and their association with disease mortality, especially in severely affected patients.

COVID-19 as a Novel Risk Factor of Thrombosis

A plethora of evidence, retrieved from a large variety of studies covering this pandemic, has highlighted a large incidence of hemostatic derangements in the form of hypercoagulable and hypofibrinolytic states, mostly in critically ill patients receiving intensive care support.[11–14] The virus does not seem to have innate prothrombotic effects; instead, the hemostatic disturbances are most likely a consequence of the profound hyperinflammatory response and endotheliopathy, even if a direct platelet-activating effect cannot be ruled out.[15,16] The prime etiology of morbidity and mortality in these patients is the synchronized activation of inflammatory responses and coagulation pathways (known as thromboinflammation).[17,18] The early clinical findings reported from Wuhan, China, indicated that patients with the severe form of disease suffered from acute lung disturbance and hypoxia.[19–22] Laboratory findings also showed that a large number of hospitalized patients have plasma hypercoagulability with significantly high levels of D-dimer, and mild prolongation of prothrombin time (PT) with slight thrombocytopenia.[23,24] The complementary reports from other countries demonstrated that extremely ill patients receiving intensive care support experienced thrombotic events, including deep vein thrombosis (DVT) and pulmonary embolism (PE).[25–30] Autopsy findings corroborated these findings, with reports of deteriorating PE following DVT in the lower extremities, and frequent reports of platelet-rich clots in the small arteries and capillaries of the lung.[31–35] PE has been the most common thrombotic event, followed by DVT and arterial thrombosis.[36,37]

Fundamental aspects of the underlying mechanisms of COVID-19-associated hemostatic disorders have not been precisely characterized. However, it is well appreciated that this condition results from a severe inflammatory response to the virus, one that is conceptualized in the literature as "thromboinflammation."[17] Thromboinflammation was also confirmed in SARS and Middle East respiratory syndrome (MERS), both of which are caused by coronaviruses.[38] Since the respiratory tract is the primary gateway for coronavirus, the inflammatory process initially involves the alveoli, progresses to cytokine storm, and triggers a localized hemostatic dysfunction with ensuing formation of microthrombi in the pulmonary vasculature. Autopsy findings support the notion of lung-originating coagulopathy in COVID-19 patients.[32,39] In patients with systemic inflammatory response syndrome, consequent to more severe illness, this condition might be followed by a generalized coagulopathy in the gastrointestinal tract or lower extremity, or as coronary or cerebrovascular ischemia.[40–43] Based on the high potential risk of thrombotic events, interim guidance from the International Society on Thrombosis and Haemostasis recommends thromboprophylaxis with heparin in all hospitalized patients, as long as anticoagulation does not impose additional bleeding risk.[35,44] When the diagnosis of thromboembolism is confirmed, anticoagulation with a therapeutic dose is recommended. However, major thrombotic events still occur, even in patients undergoing anticoagulation.

Pathophysiology of COVID-19-Associated Coagulopathy

Generalized infection, similar to what is seen in bacteria- or virus-induced sepsis, causes systemic inflammation and activation of clotting pathways, which can lead to sepsis-induced coagulopathy, a state known as thromboinflammation.[45] Vascular endothelial cells seem to be a hotspot in the cross-talk between inflammation and coagulation. The collaboration between coagulation pathways and endothelial cells is crucial for adequate hemostasis.[46] ARDS, frequently seen following sepsis-induced multiorgan failure, is indicated by disordered endothelial-cell integrity and alveolar damage, with fibrin being deposited inside the pulmonary vasculature and alveolar cavity.[47,48] In a SARS-CoV-2 infection, endotheliopathy primarily occurs in the lung; respiratory endothelial cells are directly infected by the virus via interaction of its spike glycoprotein and the host ACE-2 receptor, which is copiously expressed on endothelial cells.[42] After the virus has entered the respiratory tract cells, innate immune cells present pattern recognition receptors (PRRs) to identify exogenous pathogen-associated molecular patterns.[49] Endogenous damage-associated molecular patterns created by damaged cells are also consistently recognized by PRRs. All three factors of the old concept of Virchow's triad seem to be involved in the pathogenesis of thrombotic events in COVID-19 (►**Fig. 1**).

Pathophysiological mechanisms of COVID-19-associated coagulopathy comprise multiple systems and their interactions (►**Fig. 2**). Endothelial damage causes subendothelial collagen exposure and tissue factor (TF) decryption. TF release into the plasma along with von Willebrand factor (VWF) leads to activation and propagation of the coagulation cascade and platelet adhesion pathways.[50,51] Circulatory markers of endothelial damage, including VWF, soluble thrombomodulin, plasminogen activator inhibitor-1 (PAI-1), and angiopoietin 2, have been correlated to increased mortality in patients with COVID-19.[52–54] Therefore, innate inflammatory response and coagulation-cascade activation are triggered primarily in the lung and create localized microthrombi in the alveolar vasculature, which are generalized in the severe form of the disease. Inflammatory cytokines, as interleukin 6 (IL-6) and multiple acute-phase reactants, can cause endotheliitis. Alternative- and lectin-complement pathway activation also has been reported to exacerbate inflammation and endotheliitis. The renin–aldosterone–angiotensin system (RAAS) is involved through the interaction of ACE2, in host cells, and the virus spike protein.[55] Moreover, angiotensin II upregulates PAI-1 expression

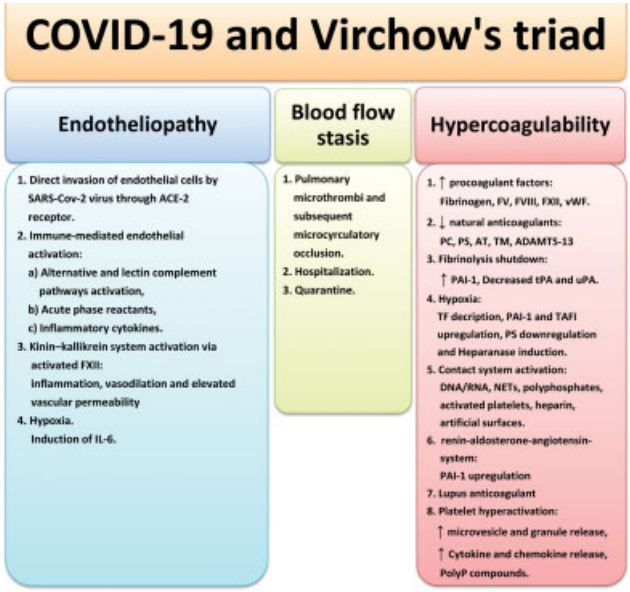

Fig. 1 Pathogenesis of COVID-19-associated coagulopathy. Attachment and entrance of SARS-Cov-2 virus by endothelial's ACE-2 receptor causes endotheliitis. Immune system hyperactivation, including alternative and lectin complement pathway activation, release of acute-phase reactants and cytokine storm further induce endotheliitis. Kinin–kallikrein system activation, with endpoint product of bradykinin, results in vasodilation and vascular permeability, and subsequent edema in many organs, as well as inflammation. Pulmonary occlusive microthrombosis decelerates microcirculation in the lung. Immobility follows hospitalization and quarantine. Elevated clotting factors, presence of lupus anticoagulant, reduced natural anticoagulants, along with fibrinolysis shutdown, create a hypercoagulable environment. In addition to inflammation and heparinization resistance via induction of IL-6 and heparanase respectively, hypoxia potentiates hypercoagulable and hypofibrinolytic conditions. Contact system activation occurs via negatively charged natural or artificial materials. The Renin–aldosterone–angiotensin system further potentiates the hypofibrinolytic state. Pathophysiological platelet hyperactivation includes elevated microvesicle, granule, cytokine, and chemokine release. Excretion of polyphosphate (polyP) compounds by activated platelet leads to activation of FXII. ACE-2, angiotensin-converting enzyme 2; ADAMTS-13, a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13; AT, antithrombin; F, factor; IL-6, interleukin-6; NETs, neutrophil extracellular traps; PAI-1, plasminogen activator inhibitor-1; PC, protein C; PS, protein S; SARS-Cov-2, severe acute respiratory syndrome coronavirus 2; TAFI, thrombin-activatable fibrinolysis inhibitor; TF, tissue factor; TM, thrombomodulin; tPA, tissue plasminogen activator; uPA, urokinase plasminogen activator; VWF, von Willebrand factor.

in endothelial cells,[56–58] and the release of PAI-1 from their α-granules.[59] Thus, deregulated RAAS may create a prothrombotic condition in patients by increasing PAI-1 levels. The contact system activation and the kallikrein–kinin systems are incorporated in the pathophysiology of COVID-19-associated coagulopathy and hyperinflammation.[60,61] Negatively charged surfaces such as nucleic acids, heparin, polyphosphate compounds, activated platelets, neutrophil extracellular traps, and artificial surfaces could localize factor XII (FXII), plasma prekallikrein (PK), and high-molecular-weight kininogen (HMWK) close together, whereupon reciprocal activation of FXII and PK occurs.[62] FXIIa triggers the intrinsic coagulation pathway and kallikrein drives inflammation by cleaving HMWK to bradykinin (BK) causing consequent vascular permeability.[62] An additional significant contributing mechanism to the coagulopathy is severe hypoxia due to COVID-19-associated lung injury.[63] Under severe hypoxic conditions, hypoxia-inducible factor-1 (HIF-1) upregulates PAI-1 and TF while downregulating protein S[64–66] (►**Fig. 2**).

HIF-2 also drives PAI-1 and suppresses TF pathway inhibitor.[67,68] Hypoxia also suppresses thrombomodulin expression on endothelial cells, with subsequent antithrombin inefficiency.[69–71] One of the notable explanations of anticoagulation inefficiency with heparin, in the management of COVID-19 patients with thrombosis, may be the fact that hypoxia induces heparanase activity.[72] Obesity is another risk factor of hypercoagulability, as obese patients have shown hypercoagulable and hypofibrinolytic changes, including elevated levels of FVII, FVIII, VWF, TF, fibrinogen, PAI-1, and thrombin-activatable fibrinolysis inhibitor (TAFI).[73–75] A role for lupus anticoagulant and antiphospholipid antibodies has been suggested for pathogenesis of COVID-19-associated coagulopathy.[52,76–80] In severely infected patients, immobility under conditions of quarantine or hospitalization is another predisposing factor of thrombosis.[81,82] Modification of the equilibrium between clot formation and degradation in favor of hypercoagulability and hypofibrinolysis, impaired endothelial function, platelet hyperactivation, and excessive immune response are emerging as major contributors to the thrombotic complications. Thus, using a multipurposed therapeutic approach beyond the regular anticoagulants can better prepare for fighting the disease (►**Table 1**). Of all proposed pathophysiological mechanisms of COVID-19-associated coagulopathy, this review will focus on prothrombotic blood coagulation and fibrinolytic changes in patients severely infected with SARS-CoV-2.

Clotting Factors

Increased levels of plasma clotting factors are reflective of hypercoagulability in COVID-19 patients. There are several studies regarding changes in the plasma levels of their clotting factors. Most reported results on levels of fibrinogen, VWF antigen, and factor VIII (FVIII) activity. A study assessing 24 patients admitted to the ICU showed increased fibrinogen levels in all patients, markedly increased FVIII activity (up to 460 U/dL), and VWF antigen in 11 (48%) patients.[83] While these findings were not consistent with acute disseminated intravascular coagulation (DIC), they supported the association of hypercoagulability with inflammation.[83] A case series of 10 severe COVID-19 cases showed a marked increase in FVIII activity and plasma fibrinogen concentration, but none developed symptomatic venous thromboembolism (VTE).[84] Significant immunohistochemical hyper-expression of FVIII has been highlighted in an autopsy study on lung specimens of two patients.[85] Another study, on 102 patients, indicated significant increases in plasma levels of fibrinogen, VWF, and FVIII in those needing respiratory support, compared with those with minimal or no respiratory support.[86]

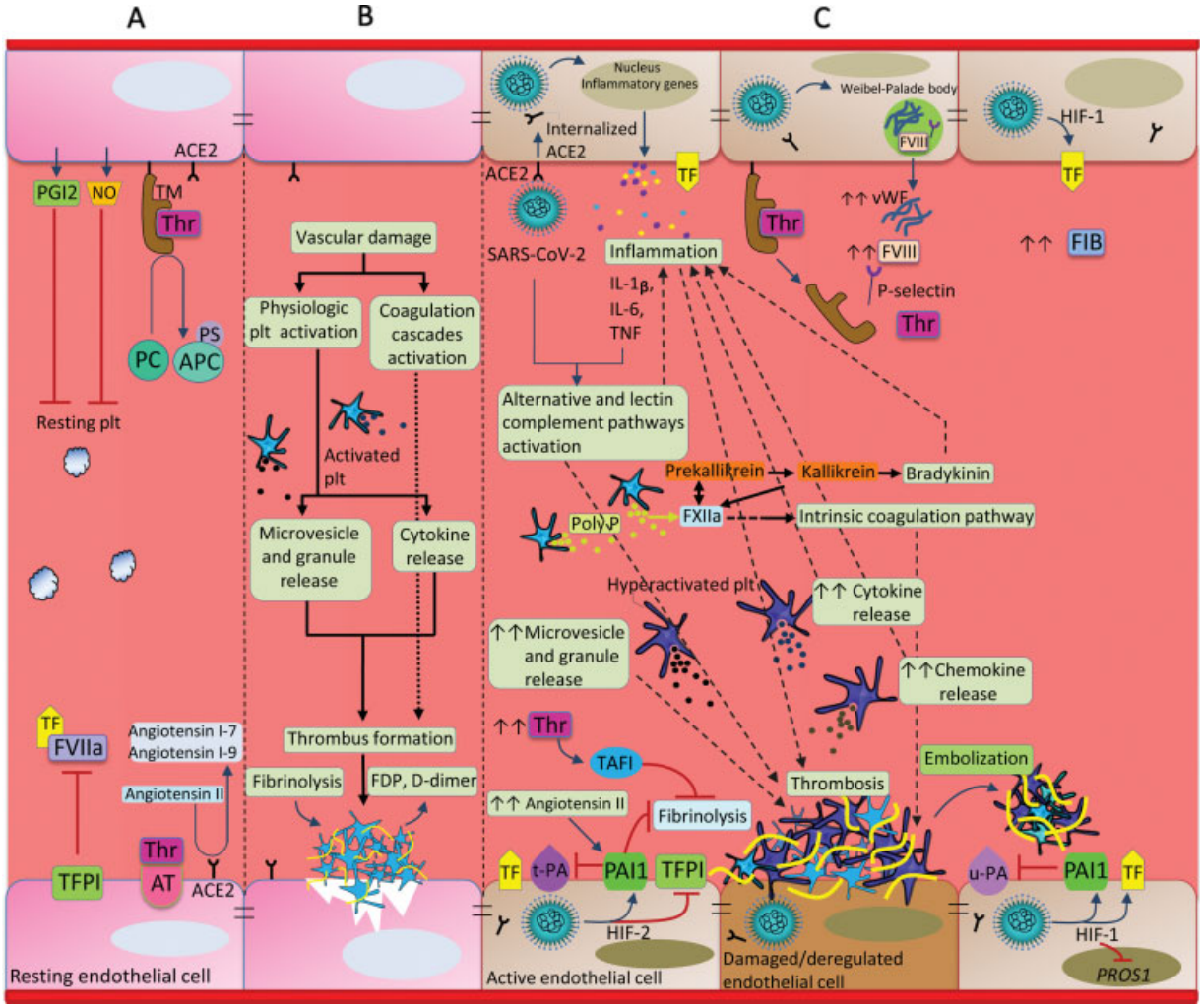

Fig. 2 COVID-19 pathogenesis. (**A**) In the absence of damage or pathogen, the endothelial cells and platelets maintain their resting modes and stay away from each other, hemostasis continues unaffected. Resting endothelial cells produce natural anti-inflammatories and anticoagulants, including nitric oxide (NO), prostaglandin I_2 (PGI_2), activated protein C (APC), thrombomodulin (TM), antithrombin (AT), and tissue factor pathway inhibitor (TFPI). The angiotensin-converting enzyme 2 (ACE2) receptor on the endothelial cell surface is typically involved in the unimpaired renin–aldosterone–angiotensin system through converting angiotensin II to angiotensin 1–9 and angiotensin 1–7, thus preventing the accumulation of angiotensin II. (**B**) In the presence of vascular damage, physiological hemostatic mechanisms are triggered until blood loss is completely stopped and the clot is dissolved. (**C**) Direct invasion by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) of the endothelial cell, via the ACE-2 receptor, causes activation of the endothelium, characterized by elevated circulatory levels of von Willebrand factor (VWF), factor VIII (FVIII), soluble TM, and plasminogen activator inhibitor 1 (PAI1). The inflammatory process related to COVID-19 that augments immune cell responses, production of inflammatory cytokines (such as IL-1β, IL-6, and TNF), and activation of the complement pathway further influences the endothelium and mediates endothelial damage and dysfunction. Inflammation reduces the bioavailability of PGI_2 and NO, leading to endothelial damage. In the hyperinflammatory and hypoxic condition, pathological hyperactivation of platelets and coagulation cascades, combined with endothelial dysfunction, lead to thrombus formation. The contact system activation via negatively charged surfaces, like polyphosphate (polyP) compounds, combined with kallikrein–kinin systems, accentuates the hypercoagulability and inflammatory condition through the production of activated FXII (FXIIa) and bradykinin, respectively. The hypoxia-inducible factor-1 (HIF-1) and -2 (HIF-2) upregulate PAI-1 and TF, as they downregulate the protein S gene (*PROS1*) and TFPI, leading to hypercoagulability and hypofibrinolysis. The PAI1 levels are elevated by accumulated levels of circulatory angiotensin II when ACE2 is internalized, following SARS-CoV-2 infection, and could not proteolyse the angiotensin II. The increased thrombin (Thr) generation drives thrombin-activatable fibrinolysis inhibitor (TAFI) to suppress PAI1. Despite the increased profibrinolytic factors of tissue (tPA) and urokinase (uPA) plasminogen activators, overwhelming levels of PAI1 and TAFI create the net result of fibrinolysis shutdown and lead to embolization of the platelet-rich fibrin clot. IL, interleukin; TNF, tissue factor necrosis.

Certain alveolar endothelial cells produce FVIII, a prothrombotic acute-phase reactant and marker of endothelial cell activation, as are fibrinogen and VWF, all three of which are frequently reported to be increased in the plasma. These data may support the notion that endotheliitis and subsequent pulmonary microthrombosis originate in the alveoli and are caused by SARS-CoV-2.[87,88] Moreover, relatively low levels of ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) in plasma in association with the high VWF antigen may lead to microangiopathic changes in critically ill patients.[89] von Meijenfeldt and colleagues compared hemostatic changes during hospitalization and at their follow-up 4 months after discharge. Factor V (FV), VWF, and fibrinogen were increased in

Table 1 Major contributors to COVID-19 pathogenesis and their related potential therapeutic approaches

Pathology	Laboratory features	Potential therapeutics
Coagulopathy	↑D-dimer, ↑Fibrinogen, factor VIII, von Willebrand factor, tissue factor ↑PAI-1 and tPA ↑Clot lysis resistance ↓Natural anticoagulants ↑Thrombin generation potential ↑Plasma kallikrein	Heparin (prophylactic or therapeutic doses) Fibrinolytic therapy with alteplase (tPA) or nebulized recombinant t-PA Wild-type activated protein C Wild-type protein S Recombinant antithrombin Recombinant thrombomodulin Plasma kallikrein inhibitors (lanadelumab and ecallantide)
Endotheliopathy	↑Factor VIII and von Willebrand factor Soluble thrombomodulin ↑PAI-1 ↑ Angiopoietin 2	Prostacyclin therapy (iloprost, epoprostenol, and treprostinil) Inhaled nitric oxide Phosphodiesterase 3 inhibitors (dipyridamole and cilostazol)
Thrombocytopathy	Borderline thrombocytopenia Platelet hyperactivation.	Prostacyclin therapy Inhaled nitric oxide Phosphodiesterase 3 inhibitors Aspirin P2Y 12 inhibitors
Excessive immune response and inflammation	↑Inflammatory markers (C- reactive protein, erythrocyte sedimentation rate, and ferritin) ↑Inflammatory cytokines (IL-1β, IL-6, and TNF-α) Macrophage activation, complement system, and NETosis ↑Bradykinin	Dexamethasone Tocilizumab (anti-IL-6) Eculizumab (C5 blocker) Anakinra (anti-IL-1β) Plasma exchange to reduce cytokine storm Prostacyclin therapy Inhaled nitric oxide Recombinant 3K3A-APC mutant[a]

Abbreviations: C5, complement component 5; COVID-19, coronavirus disease 2019; IL-1β, interleukin-1 β; IL-6, interleukin-6; NETosis, neutrophil extracellular trap formation; P2Y 12, the adenosine diphosphate receptor on platelets; PAI-1, plasminogen activator inhibitor-1; TNF-α, tumor necrosis factor α; tPA, tissue plasminogen activator.

[a]Activated protein C (APC) is a protease with anticoagulant and cytoprotective activities protecting cerebrovascular endothelium from ischemic injury. 3K3A-APC, a modified APC, maintains its full cytoprotective and anti-inflammatory effects while it confers approximately 90% lower anticoagulant activity than wild-type APC.

patients during hospitalization, but normalized at the follow-up. Factor II (prothrombin) (FII) was significantly elevated at follow-up, compared with control and hospitalization levels. Thrombinography results showed considerably raised thrombin-generating potential at follow-up, but were not associated with elevated prothrombin. FVIII levels were elevated during hospitalization and remained significantly higher than levels found in healthy controls. This increase in FVIII suggests a continued hypercoagulable state in survivors.[90] In a study on 20 critically ill patients admitted to ICU, two groups were studied, those who died within 24 hours after coagulation-profile sampling (terminal-stage group) and those who lived more than 3 days after sampling (nonterminal-stage group). In both groups FVIII activity was significantly higher than, and had approximately the same values as, the normal range. Other clotting factor activities including FII, FV, FVII, FIX, FX, FXI, and FXII remained in the normal range in both patient groups. In the terminal-stage group, FV and FVII activity was much lower than that in the nonterminal group. However, FV and FVII activities, confirmed by prolonged PT, were much lower in the terminal-stage group than in the nonterminal group.[91] In a cohort of 102 severely ill hospitalized patients there was a marked elevation of FV activity (34–248 IU/dL, median = 150) compared with the control group (22–161 IU/dL, median = 105; $p < 0.001$). FV activity was associated with the rate of VTE. Patients with elevated FV activity (>150 IU/dL) experienced significantly higher rates of VTE than those with FV activity ≤150 IU/dL (33 vs. 13%; $p = 0.03$). The VTE rate was lower among anticoagulated COVID-19 patients (21%, $n = 91$ vs. 36%, $n = 11$) and those with increased FV activity, (30%, $n = 44$ vs. 60%, $n = 5$) when compared with nonanticoagulated. However, these differences were not statistically significant. These findings do suggest that FV deserves further investigation for VTE and anticoagulation therapies. In the former study, fibrinogen and FVIII activity were also significantly higher; FX activity was slightly (or marginally) higher. Patients with concomitant increases in FV and FVIII activity had higher VTE rates than those with normal activity ($p = 0.048$).[92] FXII is probably the most involved, directly connected to the inflammatory responses via the BK-forming kallikrein–kinin system. Bronchoalveolar lavage fluids from 54 ARDS patients in a case–control study revealed FXII to be upregulated, with higher levels in ARDS fatalities, and was positively associated with tumor necrosis factor-α levels.[93] One of the autopsy findings has been the

accumulation of FXII in patients' lung tissue, with immunofluorescence analysis indicating FXII activation within the respiratory vascular walls and in clot-rich alveolar cavities. FXII is located at the central position of the contact activation system, with pivotal roles in triggering inflammatory responses, complement system, and coagulation cascade. Hence, FXII could be considered a valuable pharmacological target in the management of severely ill patients.[62]

Natural Anticoagulants

Protein S

Given the fact that protein S plays a dual role in anticoagulation and immunosuppression, it is hypothesized that a secondary deficiency of protein S, following SARS-CoV-2 infection, is strongly implicated in the underlying mechanisms of COVID-19-associated coagulopathy and cytokine storm. Unfortunately, in the context of COVID-19, there is scant empirical research into protein S level changes over the course of the disease. A study investigating the effect of baseline protein S activity of 91 patients, with survival as the main outcome, revealed protein S activity to be reduced in 65% of the patients; death was associated with lower activity of protein S (median 42 vs. 58%, $p < 0.001$).[94] Another study showed a significant decrease in protein S activity of patients compared with the control group.[91] Two other studies reported protein S activity slightly below the normal range.[83,92] The available COVID-19 literature around the role of protein S in coagulopathy and hyperinflammation crosstalk is more hypothetical than experimental. Increased plasma level of protein S-regulatory protein C4BP, caused by proinflammatory responses, leads to unavailability of free protein S for anticoagulant activities.[95] Development of autoantibody against protein S during adaptive immune responses can cause protein S insufficiency, as seen in varicella infection.[96] As a common consequence of respiratory distress in gravely ill COVID-19 patients, hypoxia downregulates *PROS1* gene expression.[64] Increased expression of IL-6 in stroke patients was associated with decreased protein S levels and a higher rate of VTE.[97] IL-6, as an early sensitive and specific predictor of a severe course of COVID-19,[19,98–101] also downregulates *PROS1* gene expression.[102] Recently proposed is the direct proteolysis of protein S by the virus via its own papain-like protease (PLpro).[103] Endotheliopathy as a common consequence of COVID-19 infection can reduce protein S levels, since endothelial cells are the major site of protein S production.[103] As the virus also affects two other sources of protein S synthesis or storage, platelets and hepatocytes, it can, theoretically, reduce the plasma level of protein S.[103] Given the critical roles played by protein S, it can potentially be considered as a therapeutic target in COVID-19 disease. However, whether secondary protein S deficiency causes both coagulopathy and hyperinflammation, for any reason, needs further investigation. More cohorts of patients are needed to determine whether protein S deficiency, if present, is itself a result of progressive, consumptive coagulopathy in a hyperinflammatory condition induced by the virus, and whether it exacerbates inflammation. In a hyperinflammatory state caused by virus-induced sepsis, the coagulation cascades are activated and progress to the consumption of all hemostatic mediators (even anticoagulants like protein S), a pathologic condition termed DIC. Since protein S is itself an anti-inflammatory protein, its consumption (deficiency) can potentially exacerbate inflammation.

Antithrombin

Concerning the changes in plasma levels of antithrombin, a systematic review and meta-analysis of 471 patients calculated the weighted mean difference (WMD) of antithrombin levels in these patients with or without severe illness.[104] It was shown that WMD of antithrombin levels in the gravely ill ($n = 197$, 41.8%), compared with those with a milder course, was –10 IU/dL (95% confidence interval: –3 to –17 IU/dL; I2: 86%). This study indicated that antithrombin levels are significantly reduced in severe illness.[104] Six cohorts showed antithrombin levels below the lower limit of the normal range, not in all, but in several their investigated subjects.[83,84,91,105–107] One of them found body mass index (BMI) to be significantly higher in patients with lower antithrombin values and documented an inverse correlation between antithrombin values and BMI (r: -0.33; $p = 0.0179$), suggesting that antithrombin may be the link between obesity and a poorer prognosis.[107] Two other COVID-19 cohorts found antithrombin levels significantly lower in nonsurvivors than in survivors.[107,108] Liao et al studied 308 patients retrospectively and found a significantly higher incidence of low antithrombin activity in critically ill and severely ill groups than in those with moderate disease.[109] In another study, reduced antithrombin values have been reported to be associated with nephritis.[110] However, five cohorts comparing the antithrombin activity between patients and a control group, and between ICU and non-ICU patients, showed that antithrombin values in most patients were within normal range, without any significant difference between the studied populations.[11,30,52,92,111] Altogether, these findings suggest that SARS-CoV-2-induced acute antithrombin deficiency caused by consumptive coagulopathy, or reduced production due to acute inflammation, justifies inclusion of antithrombin measurements as essential in the routine laboratory measures for monitoring COVID-19 patients.[112]

Antithrombin supplementation has also been a valuable measure for management of critically ill patients.[108,113] However, it is yet unknown why the acute antithrombin deficiency occurs in only some patients. Unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH), the two anticoagulants that are widely used in hospitals, require antithrombin to be efficacious. Another hypothesis posits that acute antithrombin deficiency may be responsible for inability to achieve an adequate anticoagulant effect following the usual doses of heparin therapy in some patients.[114] A multicenter cohort study on 150 SARS-CoV-2-infected patients evidenced thrombotic events in 43% of patients despite heparin therapy or prophylaxis. However, this reported failure of a response to heparin was not mechanistically related to antithrombin deficiency as the

majority had normal or even high levels of antithrombin.[30] Another cohort of 10 critically ill patients, who experienced thrombosis despite receiving prophylactic doses of LMWH, showed that all had reduced antithrombin levels. They were also nonresponders to the therapeutic doses of UFH. However, argatroban, a direct thrombin inhibitor that works independently of antithrombin, provided the patients with adequate anticoagulation.[115] Anakli et al also found that antithrombin supplementation using fresh frozen plasma, in severely ill patients experiencing COVID-19-associated coagulopathy, improves the anticoagulant effect of UFH and LMWH without the need to elevate heparin dosage.[108]

Fibrinolytic System

The imbalance between profibrinolytic and antifibrinolytic factors leads to pathologically upregulated (hyper-) or downregulated (hypo-) fibrinolysis. Fibrinolysis shutdown/ fibrinolysis resistance, which refers to a state of hypofibrinolysis, seems to be one of the leading pathophysiological mechanisms of the hypercoagulable state in COVID-19 disease. Viscoelastic hemostatic assays using thromboelastography (TEG) or rotational thromboelastometry (ROTEM) has provided evidence (such as elevated maximum clot firmness [MCF] and reduced maximum lysis) that patients severely infected with SARS-CoV-2 are in a hypofibrinolytic state.[11,12,116–118] In addition, "complete fibrinolysis shutdown" (lysis at 30 minutes of 0% on TEG) has also been reported in 57% of severely ill patients ($n = 44$).[118] A significant association between the hypofibrinolytic state and thrombotic complications was also evidenced by these studies. Moreover, the addition of exogenous tissue plasminogen activator (tPA) to the ROTEM sample of COVID-19 patients did not confer any sensitivity to the additive tPA.[11,119–121] Finally, two studies demonstrated that ICU patients who received therapeutic anticoagulation had significantly higher MCF and clot lysis time than the control groups.[122,123] The molecular mechanism of the hypofibrinolytic state in critical COVID-19 illness seems attributable primarily to the overexpression of PAI-1, the most potent antifibrinolytic mediator, from endothelial cells and activated platelets.[124–126] However, it seems that elevated PAI-1 is perhaps not the only cause of hypofibrinolysis. Studies on patients with interstitial lung disease have also evidenced an increase of TAFI and PAI-3 (protein C inhibitor) in the alveolar space.[127,128] Similar results were highlighted during the 2003 SARS-CoV epidemic.[129,130] Today, significantly increased PAI-1 values have been found in multiple studies on patients with COVID-19, with some studies reporting values up to fourfold higher in COVID-19 patients compared with control groups.[123,131] This overload of PAI-1 in SARS-CoV-2 infection can be further aggravated by raised angiotensin II levels in the blood of patients with COVID-19, with PAI-1 levels being upregulated in the endothelial cells, resulting in elevated circulatory PAI-1 levels.[56,58,123,127] Angiotensin 1–9 also activates platelets and stimulates the release of PAI-1 from α-granules.[59] Moreover, several studies have found elevation, in the plasma levels, of tPA, urokinase plasminogen activator (uPA), and TAFI in patients suffering from the severe form of COVID-19,[11,132] demonstrating the variable effects of infection on the fibrinolytic system. It seems that the overexpression of PAI-1 and TAFI overwhelms the local profibrinolytic effects of elevated tPA and uPA levels. Hence, the net result of these changes is a hypofibrinolytic state.

Platelets

Thrombocytopenia has been reported in approximately 55% of patients with SARS,[133,134] which, together with leukopenia, was the predominant laboratory feature.[135] The degree of thrombocytopenia and hypoxia was used as a prognostic model to estimate the mortality rate in the 2003 epidemic with an accuracy of 96.2%.[136] Similar studies corroborated these findings in SARS-CoV.[135,137–140] Thrombocytopenia and lymphopenia were introduced as a disease severity index and predictive factor of developing pneumonia and respiratory failure in MERS-CoV infection.[141,142] Other studies also endorsed these observations in the MERS-CoV epidemic.[143–145] A similar trend has been observed in SARS-CoV-2 infection; thrombocytopenia has been reported in 5 to 41.7% of patients, depending on disease severity,[23,146–148] and a meta-analysis of 1,779 COVID-19 patients demonstrated that thrombocytopenia correlated with a more than fivefold elevated risk of grave sickness.[149] Another meta-analysis of 7,613 patients demonstrated that the critically ill had a lower platelet count than those with nonsevere illness.[150] A temporary downward trend of platelet count could be clinically indicative of an aggravating thrombotic condition during hospitalization.[151] ►**Table 2** summarizes the changes in platelet indices in patients with COVID-19. Thrombocytopenia along with platelet hyperactivation (thrombocytopathy) contributes to the excessive thrombosis and deregulated immune response.

SARS-CoV-2-induced endothelial damage in the lung leads to platelet adhesion to the subendothelium of the pulmonary microvasculature via the aid of VWF's bridging function; subsequent platelet-rich thrombotic microangiopathy hinders viremic spread through the circulation.[147,152] In patients affected with the severe form of COVID-19, elevated total levels and binding capacity of VWF, with concomitant mild thrombocytopenia, suggests that infection triggers the process of platelet thrombosis, thereby activating the coagulation cascade. Consumption of platelets during the early stages of pulmonary endothelial damage is followed by a compensatory response of the bone marrow to restore the circulatory platelet count. This rebound thrombocytosis is accompanied by production of larger (increased mean platelet volume) and younger (increased immature platelet fraction) platelets with higher hemostatic potential.[146,153] Some pathophysiological mechanisms of virus-induced thrombocytopenia have been proposed so far, including generation of autoantibodies against platelets, viral invasion of the megakaryocytes via CD13 or CD66a, and endotheliitis-routed platelets and coagulation cascade activation with subsequent platelet consumption.[154] SARS-CoV-2 also changes the gene expression pattern of platelets, such as elevation in the basal expression of P-selectin.[155] The molecular basis of platelet hyperactivity seen in patients with COVID-19 may

Table 2 Platelet indices changes in COVID-19 patients

Parameters	In patients affected with SARS-Cov-2	Reference
Platelet count	Usually normal, until the advanced stages, when it reduces to moderately low levels.	149,150
MPV	Increased (at least in unwell patients with thrombocytopenia). Correlates with a decline in average platelet count. Can be used as an effective indicator of platelet activation and increased thrombosis risk.	158–160
IPF	Increased (at least in unwell patients with thrombocytopenia). Effective indicators of platelet activation and increased thrombosis risk. Predictor of a reduction in the total platelet count during coagulopathy. Reticulated platelets may reflect an increased platelet turnover in the setting of a normal platelet count and this aspect could be of critical importance in the early diagnosis of COVID-19.	158
PDW	Increased (at least in unwell patients with thrombocytopenia). A key marker of platelet activation in patients with COVID-19. Increased in venous thrombosis as well as in several hypercoagulable states, such as cardiovascular diseases.	160
PLR	Patients with severe COVID-19 have higher levels of PLR than patients with nonsevere disease. In COVID-19, PLR may be used as separate prognostic indicators of disease seriousness.	161,162
MPR	A high MPR level is an independent risk factor for severe pneumonia.	163
P-LCR	A high P-LCR is significantly associated with lower survival rates.	160,164,165
Platelet aggregation	SARS-CoV-2 infection results in faster platelet aggregation in response to low-dose agonists.	158

Abbreviations: IPF, immature platelet fraction; MPV, mean platelet volume; MRP, platelet mean volume/platelet count ratio; PDW, platelet distribution width; P-LCR, platelet larger cell ratio; PLR, platelet to lymphocyte ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

be associated with the increased MAPK pathway activation and thromboxane production.[155]

As the major and rapid compensators of platelet consumption or clearance, megakaryocytes possibly undergo pathological changes (megakaryocytopathy) during SARS-CoV-2 infection. One study revealed that the lung tissue of some patients with COVID-19 contains abnormal CD61+ megakaryocytes with nuclear hyperchromasia and atypia.[39] A case series of autopsy studies showed that megakaryocytes are present in the heart, kidneys, and especially lungs of patients with COVID-19.[156] Both studies have suggested that those megakaryocytes were actively producing platelets. However, the presence of these compensatory megakaryocytes, which possibly originated in the lungs, is inconsistent with the developing thrombocytopenia seen in some patients. A therapeutic application for antiplatelet drugs in patients has not yet been recommended for all patients. It is unclear whether targeting platelets during SARS-CoV-2 infection improves patient outcomes. Today, many randomized clinical trials are proposed on the use of prophylactic doses of antiplatelet agents in the management of patients with COVID-19 (NCT04365309, NCT04363840, NCT04410328; https://clinicaltrials.gov). However, in patients with mild thrombocytopenia, antiplatelet therapy would carry a greater risk of hemorrhage.[157]

Conclusion

COVID-19-associated coagulopathy is identified by a hypercoagulable state with a subsequent high rate of thrombotic microangiopathy, DVT and PE. Thrombotic events are the major cause of morbidity and mortality in extremely sick patients. The pathogenesis of COVID-19-associated thrombosis is likely to involve various cell types and elaborately interconnected processes including hemostasis, vascular integrity, and inflammation. It seems that endotheliopathy and thrombocytopathy may play a central role in the etiology of thrombotic microangiopathy in patients with COVID-19. Direct invasion of the endothelium and platelets by the virus can result in endotheliopathy and thrombocytopathy, respectively. These can also be due to the cellular response to the virus-induced inflammation, triggering immune cell response, complement activation, and cytokine storm. Although anticoagulation alone has yielded some satisfaction, we are still faced with dreadful thrombotic complications even in patients receiving anticoagulants. Thus, targeting a couple of main pathological processes with combinational therapeutic approaches, or treatment with pleiotropically acting drugs, is likely to be more efficacious than the approach of dealing exclusively with thrombosis in patients with COVID-19.

Conflict of Interest
None declared.

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