Coagulopathy and Thrombosis as a Result of Severe COVID-19 Infection: A Microvascular Focus

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Abstract

Coronavirus disease of 2019 (COVID-19) is the clinical manifestation of the respiratory infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). While primarily recognized as a respiratory disease, it is clear that COVID-19 is a systemic illness impacting multiple organ systems. One defining clinical feature of COVID-19 has been the high incidence of thrombotic events. The underlying processes and risk factors for the occurrence of thrombotic events in COVID-19 remain inadequately understood. While severe bacterial, viral, or fungal infections are well recognized to activate the coagulation system, COVID-19-associated coagulopathy is likely to have unique mechanistic features. Inflammatory-driven processes are likely primary drivers of coagulopathy in COVID-19, but the exact mechanisms linking inflammation to dysregulated hemostasis and thrombosis are yet to be delineated. Cumulative findings of microvascular thrombosis has raised question if the endothelium and microvasculature should be a point of investigative focus. von Willebrand factor (VWF) and its protease, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13), play important role in the maintenance of microvascular hemostasis. In inflammatory conditions, imbalanced VWF-ADAMTS-13 characterized by elevated VWF levels and inhibited and/or reduced activity of ADAMTS-13 has been reported. Also, an imbalance between ADAMTS-13 activity and VWF antigen is associated with organ dysfunction and death in patients with systemic inflammation. A thorough understanding of VWF-ADAMTS-13 interactions during early and advanced phases of COVID-19 could help better define the pathophysiology, guide thromboprophylaxis and treatment, and improve clinical prognosis.
Introduction

Coronavirus disease of 2019 (COVID-19) is a respiratory illness caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is an enveloped, positive-sense single-stranded ribonucleic acid virus belonging to the Coronaviridae family. The COVID-19 outbreak started in Wuhan, China, in late 2019 and rapidly spread to the rest of the world. On March 11, 2020, the World Health Organization declared COVID-19 outbreak as pandemic. As of June 24, 2020, the global number of COVID-19 cases stood at 9.26 million with 478,000 deaths (Source: Johns Hopkins Coronavirus Resource Center, https://coronavirus.jhu.edu/). Disease course is markedly different between individuals while some are completely asymptomatic, others develop mild symptoms including mild fever, loss of taste or smell, dry cough, sore throat, shortness of breath, and myalgia. In susceptible individuals, the disease progresses to pneumonia, hypoxemia, acute respiratory distress, and multiorgan dysfunction that may lead to death. The predominance of asymptomatic or mild infections has contributed to the rapid spread of COVID-19 compared with earlier coronavirus outbreaks of SARS and Middle East respiratory syndrome in 2002 and 2012, respectively.

Consumptive Coagulopathy and the High Incidence of Thrombosis in COVID-19 Patients

Altered coagulation is a common feature of acute systemic diseases, specifically to those affecting primarily the respiratory system. Based on studies in patients with acute respiratory distress syndrome (ARDS), the coexistence of disseminated intravascular coagulation (DIC) with subsequent consumption of procoagulation proteins and platelets has been consistently described. This in turn leads to the formation of microthrombi in the vascular bed of organs resulting from excess coagulation byproducts and suppression of endogenous anticoagulation factors. The coexistence of consumptive coagulopathy and thrombosis are the result of a common pathologic pathway; however, the exact mechanisms that tilt the balance toward thrombosis in COVID-19 are less well understood. In this sense, some features of the coagulopathy associated with COVID-19 may be not unique to this disease; however, the magnitude of the thrombotic response and its impact on mortality suggests the presence of additional mechanisms, beyond what is known for similar respiratory acute inflammatory diseases.

Several studies have linked coagulation abnormalities to severe COVID-19 illness. (Table 1). In a study evaluating 449 severe COVID-19 patients, Tang et al reported positive correlation of 28-day mortality with fibrin degradation product (FDP), D-dimers and prothrombin time (PT), and negative correlation with platelet count. Laboratory parameters were recorded at the time of onset of severe COVID-19 in the study. In an earlier study comprising 183 patients, Tang et al reported elevated D-dimer levels and FDP levels and prolonged PT and activated partial thromboplastin times (aPTTs) at the time of admission in nonsurvivors compared with survivors. In the same study, significantly lower levels of fibrinogen and antithrombin levels were observed during the late hospitalization in nonsurvivors. Huang et al reported higher D-dimers and prolonged PT at the time of admission in intensive care unit (ICU) patients compared with non-ICU patients in a study of 41 patients. Wang et al reported elevated PT in a study of 138 patients. In the same study, elevated levels of D-dimers were found in ICU patients compared with non-ICU patients as well as in survivors compared with nonsurvivors in a subgroup of patients with a definitive outcome. In a study of 94 COVID-19 patients, Han et al reported lower antithrombin and higher D-dimers, FDP, and fibrinogen levels compared with healthy controls. Zhou et al reported an association of elevated D-dimers with in-hospital death in a study of 191 patients. Also, elevated PT and decreased platelet counts were observed in nonsurvivors compared with survivors. Elevated levels of D-dimers were reported by Richardson et al among 5,700 patients in the New York City area. Ranucci et al reported a procoagulant profile in 16 patients characterized by increased clot strength by viscoelastography, elevated D-dimer levels, and hyperfibrinogenemia. A meta-analysis of 9 studies encompassing 1,779 patients with severe disease has identified significantly lower platelet counts. A subgroup analysis based on survival has identified even lower platelet counts in nonsurvivors in this study. Llitjos et al and Helms et al reported elevated D-dimer and fibrinogen levels in 26 and 150 ICU-admitted patients, respectively. Overall, elevated PT, increased D-dimer and fibrinogen levels, and thrombocytopenia are frequently reported in COVID-19 patients. However, bleeding events requiring therapeutic intervention are not reported.

Multiple studies have reported a higher incidence of thrombotic events, particularly pulmonary embolism, as a frequent complication in COVID-19 patients. Llitjos et al reported overall rate of 68% venous thromboembolism (VTE) in severe COVID-19 patients admitted to ICU. In this study, VTE incidence was found to be significantly higher in patients treated with prophylactic anticoagulation compared with those treated with therapeutic anticoagulation. Helms et al reported 64 clinically relevant thrombotic complications in 150 ICU-admitted patients. Importantly, the incidence of thrombotic complications in COVID-19 ARDS patients was significantly higher than non-COVID-19 ARDS patients in this study. Ackermann et al compared lung sections of COVID-19 patients with those died from ARDS secondary to influenza A (H1N1) infection and found relatively higher: (1) endothelial cell injury, (2) alveolar microthrombi (ninefold), and (3) intussusceptive angiogenesis in COVID-19 lung sections. Similarly, higher incidence of thromboembolic complications in ICU-admitted COVID-19 patients was also reported by Klok et al (31%), Lodigiani et al (27.6%), Middeldorp et al (47%), Nahum et al (79%), and Cui et al (25%). For comparison, in a study by Zhang et al, the reported cumulative incidence of VTE in ICU-admitted patients receiving guideline-recommended thromboprophylaxis was 9.55% (95% confidence interval: 6.55–13.81).
### Table 1  Studies (multiple patients) reporting abnormal coagulopathy in COVID-19

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study, number of patients</th>
<th>Findings/Significance</th>
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<tbody>
<tr>
<td><strong>Clinical features of COVID-19 patients, coagulation parameters included</strong></td>
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<tr>
<td>Huang et al(^{13})</td>
<td>Prospective, 41 patients</td>
<td>Prothrombin time and D-dimer levels on admission were higher in patients that required ICU treatment</td>
</tr>
<tr>
<td>Zhou et al(^{16})</td>
<td>Retrospective, 191 COVID-19 patients</td>
<td>Increased D-dimer on admission is associated with poor prognosis</td>
</tr>
<tr>
<td>Guan et al(^{44})</td>
<td>Retrospective, 1,099 COVID-19 patients</td>
<td>Thrombocytopenia in 36.2%</td>
</tr>
<tr>
<td>Goyal et al(^{36})</td>
<td>Retrospective, 393 COVID-19 patients</td>
<td>Thrombocytopenia in 27%</td>
</tr>
<tr>
<td>Zhu et al(^{45})</td>
<td>Meta-analysis</td>
<td>Elevated D-dimer in ~37.2% of patients</td>
</tr>
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<td><strong>Studies on coagulation parameters</strong></td>
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<tr>
<td>Ranucci et al(^{18})</td>
<td>Prospective, 16 ARDS COVID-19 patients</td>
<td>Patients showed a procoagulant profile (clot strength, platelet, fibrinogen, D-dimers, hyperfibrinogemania)</td>
</tr>
<tr>
<td>Tang et al(^{12})</td>
<td>Retrospective, 183 COVID-19 patients</td>
<td>Nonsurvivors had significantly higher D-dimer and fibrin degradation product (FDP) levels, longer prothrombin time, and activated partial thromboplastin time compared with survivors on admission. 71.4% of nonsurvivors and 0.6% survivors met the criteria of DIC during their hospital stay</td>
</tr>
<tr>
<td>Lippi et al(^{19})</td>
<td>Meta-analysis</td>
<td>Low platelet count associated with increased risk of severe disease and mortality in patients with COVID-19</td>
</tr>
<tr>
<td>Zhang et al(^{29})</td>
<td>Retrospective, 343 COVID-19 patients</td>
<td>Patients with D-dimer levels $&gt;2.0 \mu$g/mL had a higher incidence of mortality when comparing to those who with D-dimer levels $&lt;2.0 \mu$g/mL.</td>
</tr>
<tr>
<td>Escher et al(^{108,109})</td>
<td>Case study, 1 patient and 3 more in the follow-up publication</td>
<td>Continual increase of D-dimers, elevated FVIII activity, and normal platelet counts</td>
</tr>
<tr>
<td>Bowles et al(^{112})</td>
<td>216 COVID-19 patients 34 tested for lupus anticoagulant</td>
<td>91% of patients tested positive for lupus anticoagulant. All lupus anticoagulant-positive specimens had a prolonged aPTT. Increased aPTT should not be a reason to withhold anticoagulation therapy</td>
</tr>
<tr>
<td>Lorenzo-Villalba et al(^{115})</td>
<td>Case reports, 3 patients</td>
<td>Severe thrombocytopenia during COVID-19 infection associated with either cutaneous purpura or mucosal bleeding</td>
</tr>
<tr>
<td>Yin et al(^{116})</td>
<td>Retrospective, 449 COVID-19 and 104 non-COVID severe pneumonia</td>
<td>Patients with severe pneumonia induced by SARS-CoV-2 had higher platelet count than those induced by non-SARS-CoV-2. Patients infected by SARS-CoV-2 may benefit from anticoagulant treatment, if they have markedly elevated D-dimer</td>
</tr>
<tr>
<td>Tabatabai et al(^{48})</td>
<td>Case series, 10 patients</td>
<td>Elevated FVIII activity and low normal antithrombin and functional Protein C activity</td>
</tr>
<tr>
<td><strong>Thrombosis in the COVID-19 patients</strong></td>
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<tr>
<td>Middeldorp et al(^{24})</td>
<td>Retrospective, 198 patients</td>
<td>The cumulative incidences of VTE at 7, 14, and 21 days were 16%, 33%, and 42%, respectively. VTE was higher in the ICU and was associated with death</td>
</tr>
<tr>
<td>Nahum et al(^{25})</td>
<td>Prospective, 34 patients</td>
<td>Deep vein thrombosis was found in 22 patients (65%) at admission and in 27 patients (79%) when the venous ultrasonograms performed 48 hours after ICU admission were included. D-dimers and fibrinogen were also increased</td>
</tr>
<tr>
<td>Cui et al(^{26})</td>
<td>Retrospective, 81 severe COVID-19 patients</td>
<td>Incidence of VTE at 25%. D-dimer increase has a predictive value</td>
</tr>
<tr>
<td>Klok et al(^{22\text{a}})</td>
<td>Retrospective, 184 patients, no control group</td>
<td>31% cumulative incidence of symptomatic acute pulmonary embolism (PE), deep vein thrombosis, ischemic stroke, myocardial infarction, or systemic arterial embolism in COVID-19 patients</td>
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<tr>
<td>Study</td>
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<tr>
<td>Zhang et al.</td>
<td>Prospective, 281 ICU COVID-19 patients</td>
<td>Cumulative incidence of VTE at 28 days was 9.55%, despite all patients receiving thromboprophylaxis</td>
</tr>
<tr>
<td>Demelo-Rodríguez et al</td>
<td>Prospective, 156 COVID-19 patients</td>
<td>D-dimer levels &gt; 1,570 ng/mL were associated with asymptomatic DVT</td>
</tr>
<tr>
<td>Grandmaison et al</td>
<td>Cross-sectional study, 58 COVID-19 patients, 29 in the ICU and 29 in the medicine ward</td>
<td>In the ICU, VTEs were found in 17 (58.6%) of the 29 patients In the medicine ward, VTEs were found in 6 (20.7%) patients</td>
</tr>
<tr>
<td>Fraissé et al.</td>
<td>Retrospective, 92 ICU COVID-19 patients</td>
<td>High rate of thrombotic events (TEs) in ICU COVID-19 patients highlighting the necessity for thromboprophylaxis and TE screening. Hemorrhagic events (HEs) were also observed in patients on full-dose anticoagulation</td>
</tr>
<tr>
<td>Jian et al.</td>
<td>Retrospective, 3,218 COVID-19 patients</td>
<td>Acute stroke was the most common neuroimaging finding, present in 1.1% of hospitalized COVID-19 patients</td>
</tr>
<tr>
<td>Desborough et al.</td>
<td>Retrospective, 66 patients</td>
<td>10 patients had at least one proven episode of thromboembolism. Major bleeding occurred in seven cases</td>
</tr>
<tr>
<td>Akel et al.</td>
<td>Case reports, 6 patients</td>
<td>Patients did not have any hypercoagulable risk factors yet presented with pulmonary embolism</td>
</tr>
<tr>
<td>Kashi et al.</td>
<td>Case reports, 7 patients</td>
<td>Arterial thrombosis</td>
</tr>
<tr>
<td>Lax et al.</td>
<td>Prospective autopsy study, 11 deceased COVID-19 patients</td>
<td>Death may be caused by the thrombosis observed in segmental and subsegmental pulmonary arterial vessels despite the use of prophylactic anticoagulation</td>
</tr>
<tr>
<td>Thomas et al.</td>
<td>Retrospective, 63 COVID-19 patients</td>
<td>High thrombotic risk in patients with COVID-19</td>
</tr>
<tr>
<td>Gomez-Arbelaez et al</td>
<td>Case reports, 4 patients</td>
<td>Aortic thrombosis and associated ischemic complications in patients with severe SARS-CoV-2 infection</td>
</tr>
</tbody>
</table>

**Anticoagulation treatment in COVID-19 patients**

<table>
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<tr>
<td>Tang et al.</td>
<td>Retrospective, 449 severe COVID-19 patients, 99 received heparin</td>
<td>Anticoagulant therapy is associated with better prognosis in severe COVID-19 patients with sepsis induced coagulopathy or markedly elevated D-dimer</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>3 case reports</td>
<td>Treatment with tissue plasminogen activator lead to improvement in the respiratory status</td>
</tr>
<tr>
<td>Ayerbe et al.</td>
<td>2,075 COVID-19 patients, admitted in 17 hospitals in Spain</td>
<td>Heparin had been used in 1,734 patients. Heparin was associated with lower mortality</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>Retrospective, 1,099 COVID-19 patients</td>
<td>High risk of venous thromboembolism, also high risk of bleeding</td>
</tr>
<tr>
<td>Artifoni et al.</td>
<td>Retrospective, 62 patients</td>
<td>16 patients developed VTE, 7 patients developed PE Very high negative predictive value of baseline D-dimer level for VTE and PE</td>
</tr>
<tr>
<td>Russo et al.</td>
<td>Retrospective, 192 COVID-19 patients</td>
<td>Preadmission antithrombotic therapy, both antiplatelet and anticoagulant, does not seem to show a protective effect in severe forms of COVID-19 with ARDS at presentation and rapidly evolving toward death</td>
</tr>
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</table>

**Link between SARS-CoV-2 and thrombosis**

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<tr>
<td>Ackermann et al.</td>
<td>7 lung autopsies from COVID-19 patients and 7 from ARDS</td>
<td>Vascular angiogenesis distinguished the pulmonary pathobiology of COVID-19 from that of equally severe influenza virus infection</td>
</tr>
<tr>
<td>Maier et al.</td>
<td>Case studies 15 COVID-19 patients with hyperviscosity</td>
<td>Possible causal relationship between hyperviscosity and thrombotic complications in COVID-19</td>
</tr>
</tbody>
</table>

(Continued)
A high incidence of DIC diagnosed by D-dimer, fibrinogen, and antithrombin III levels has become a focus for the initiation of anticoagulation therapy in severe COVID-19 patients, with some studies relying on D-dimers alone. A retrospective analysis of 183 patients performed by Tang et al suggested that more than 70% of severe COVID-19 patients who succumb to the infection demonstrate increased risk of thrombosis, further this group suggests that all of these patients meet the International Society on Thrombosis and Haemostasis definition of DIC. Subsequently, Tang et al reported an equivalent 28-day mortality rate (30%) in 99 patients receiving low molecular weight or unfractionated heparin for 7 days compared with 350 nonheparin treated patients or those receiving a less than 7-day course of therapy. A case series reported by Wang et al detailed the use and outcome following tissue plasminogen activator (tPA) in three patients with ARDS and coagulopathy consistent with DIC. Intravenous dosing with tPA indicated a potential benefit in each of the three cases of COVID-19. However, this study also warns of both unrelated effects and high risk of severe bleeding secondary to off-label tPA use. Several of the studies in coagulopathic COVID-19 patients suspected of DIC rely heavily on analysis of fibrin degradation and D-dimer levels, which are expected to be increased during DIC, arterial and venous thromboses, strokes, and thrombotic microangiopathies. However, D-dimers are a nonspecific indicator of thrombosis in severe COVID-19 patients with pulmonary injury. Fibrin accumulation and lysis continuously occur during nonthrombotic inflammation as well as tissue necrosis, and therefore, significant D-dimer elevations also accumulate during cancers and infections, consistent with inflammatory processes that coincide with the progression of severe COVID-19-related macrophage activation syndrome. Therefore, we suggest that more comprehensive and robust assays be used to evaluate changes in hemostasis. For example, to date the use of thrombin, plasmin, or simultaneous thrombin/plasmin generation assays have not been reported within the context of hemostasis management of COVID-19 patients. Since their introduction thrombin and plasmin generation assays have not been reported within the context of hemostasis management of COVID-19 patients. In some studies, a high proportion of patients were still hospitalized at the end of the reporting period; conclusions and clinical courses therefore were based on incomplete information, and completion of these patients’ clinical course could alter the final conclusions. The picture of coagulopathy in COVID-19 is complex. Specific, sensitive, and temporal assessments of coagulation and fibrinolysis should be established and further work is needed to untangle the roles of the host inflammatory response, preexisting thrombotic risk, and prehospitalization pharmacologic regimens in the optimal management of coagulopathy in the setting of COVID-19.

### Inflammation, Liver Injury, and Hypoxia in COVID-19 Patients

The risk of hospitalization, morbidity, and mortality from COVID-19 is highest for older patients with preexisting conditions such as hypertension, diabetes, cardiovascular disease, and obesity. Increased inflammation is commonly observed in COVID-19 patients, while severe cases are characterized by immune dysregulation and hyperinflammation, with a markedly increased serum interleukin (IL)-6. Cytokine release syndrome has also been reported in COVID-19 patients and correlates with adverse

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</thead>
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<tr>
<td>Huisman et al¹⁰⁵</td>
<td>12 COVID-19 patients</td>
<td>Low ADAMTS-13 activity, increased VWF levels and factor VIII levels</td>
</tr>
<tr>
<td>Galeano-Valle et al¹¹¹</td>
<td>Prospective study, 24 patients</td>
<td>Prevalence of antiphospholipid antibodies in COVID-19 and venous thrombosis was low</td>
</tr>
<tr>
<td>Magro et al¹³²</td>
<td>Case reports, 5 severe COVID-19 cases</td>
<td>Procoagulant state is associated with systemic complement activation</td>
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Abbreviations: ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; aPTT, activated partial thromboplastin time; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease of 2019; DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis; FVIII, factor VIII; ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VTE, venous thromboembolism; VWF, von Willebrand factor.
clinical outcomes. The presence of several inflammatory markers such as C-reactive protein, procalcitonin, ferritin, and fibrinogen are often reported in COVID-19 patients. Further, multiple studies reported elevated levels of the proinflammatory cytokine IL-6 in severe cases of COVID-19, probably in response to overwhelming systemic inflammation, was also observed in several studies. The role of IL-6, in particular, is considered central in the pathogenesis of COVID-19 complications and therefore tocilizumab, an IL-6 inhibitor, is being used in ongoing clinical trials to prevent catastrophic inflammation.

Liver injury during COVID-19 infections was described in multiple studies, including elevated levels of alanine aminotransferase, aspartate aminotransferase, and bilirubin. The liver is the primary source of plasma proteins, particularly those involved in hemostasis. Thus, the occurrence of liver injury may contribute further to derangements of key hemostasis proteins and contributes to coagulopathy. Similarly, hypoxemia observed in COVID-19 patients induces prothrombotic conditions through upregulation of plasminogen activator inhibitor and stimulation of endothelial synthesis of procoagulants, including tissue factor and VWF. Thus, multiple clinical characteristics observed in COVID-19 patients contribute to altered coagulation and lead to increased incidence of thrombosis. However, the early onset of coagulopathy—before systemic organic effects occur—suggests proinflammatory conditions as the primary driving cause of thrombotic events in COVID-19 patients.

### VWF/ADAMTS-13 in Hemostasis and Thrombosis

VWF and its cleaving protease, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13), play an important role in hemostasis particularly within the microvasculature. VWF is a large multimeric glycoprotein primarily expressed by endothelial cells and platelets. Endothelial cells show both basal secretion and regulated release of VWF stored in Weibel-Palade bodies in response to various stimuli. On the other hand, platelets secrete VWF stored in α-granules only upon activation. ADAMTS-13 is expressed both by hepatic stellate cells and endothelial cells; the relative contribution of hepatic and microvascular expression is not clear. ADAMTS-13 regulates the biological activity of VWF by cleaving prothrombotic ultra-large VWF multimers (>10,000 kDa) secreted from endothelial cells into hemostatically active high molecular weight multimers (<10,000 kDa) under shear stress conditions. Severe deficiency of ADAMTS-13 results in accumulation of ultra-large VWF multimers leading to microvascular thrombosis and consumptive thromboctopenia, a condition termed thrombotic thrombocytopenic purpura (TTP). In the event of vascular injury, VWF facilitates binding of platelets to subendothelium through its interactions with glycoprotein Ib and collagen, thereby inducing thrombus formation. A reciprocal relationship exists between VWF and ADAMTS-13 levels where elevated circulatory VWF antigen levels are associated with concomitant decrease in ADAMTS-13 activity and vice versa. Abnormal VWF/ADAMTS-13 ratios are implicated in arterial thrombosis, ischemic stroke, pediatric stroke, and perioperative thrombosis in infants. In addition, abnormal VWF/ADAMTS-13 metabolism has been positively associated with myocardial infarction in young women. It is worth highlighting that in the case of perioperative thrombosis, elevated VWF even in the absence of significant deficiency of ADAMTS-13 was associated with thrombosis. Severe hypoxia and acidosis likely caused a higher increase in VWF during cardiac surgery and were at higher risk of thrombosis.

### Table 2: Studies reporting elevated inflammatory markers in COVID-19

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<th>Study</th>
<th>Patient group (number of patients) comparison</th>
<th>Elevated inflammatory markers</th>
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<tr>
<td>Huang et al</td>
<td>ICU (13) vs. non-ICU (28)</td>
<td>Procalcitonin, IL-1β, IFN-γ, IP-10, and MCP-1</td>
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<tr>
<td>Wang et al</td>
<td>ICU (36) vs. non-ICU (102)</td>
<td>Procalcitonin</td>
</tr>
<tr>
<td>Zhou et al</td>
<td>Nonsurvivor (54) vs. survivor (137)</td>
<td>Procalcitonin, ferritin, and IL-6</td>
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<tr>
<td>Richardson et al</td>
<td>Relative to reference range (3066)</td>
<td>Procalcitonin, ferritin, and CRP</td>
</tr>
<tr>
<td>Ruan et al</td>
<td>Nonsurvivor (68) vs. survivor (82)</td>
<td>CRP and IL-6</td>
</tr>
<tr>
<td>Giamarellos-Bourboulis et al</td>
<td>Dysregulated (21) vs. intermediate state (26) of immune activation</td>
<td>CRP and IL-6</td>
</tr>
<tr>
<td>Chen et al</td>
<td>Severe (≥9) vs. moderate (≥7)</td>
<td>CRP, ferritin, IL-6, and TNF-α</td>
</tr>
<tr>
<td>Han et al</td>
<td>COVID-19 patients (102) vs. controls (45)</td>
<td>CRP, IL-6, TNF-α, and IFN-γ</td>
</tr>
<tr>
<td>Du et al</td>
<td>Mild pneumonia (124) vs. no pneumonia (54) (pediatric patients)</td>
<td>Procalcitonin, IL-6, TNF-α, and IFN-γ</td>
</tr>
<tr>
<td>Wang et al</td>
<td>SpO2 &lt;90% (≥36) vs. SpO2 ≥90% (≥7)</td>
<td>CRP and IL-6</td>
</tr>
<tr>
<td>Tan et al</td>
<td>Severe (25) vs. mild/moderate (31)</td>
<td>CRP and IL-6</td>
</tr>
<tr>
<td>Tabatabai et al</td>
<td>Relative to reference range (10)</td>
<td>Fibrinogen, CRP, and ferritin</td>
</tr>
</tbody>
</table>

Abbreviations: COVID-19, coronavirus disease of 2019; CRP, C-reactive protein; ICU, intensive care unit; IFN-α, interferon-α; IL-β, interleukin-1β; IL-6, Interleukin-6; IP-10, interferon-γ induced protein 10; MCP-1, monocyte chemotactic protein-1; SpO2, blood oxygen saturation level; TNF-α, tumor necrosis factor-α.
Elevated levels of VWF are found in several inflammatory and metabolic disorders including diabetes, obesity, and sickle cell disease.\textsuperscript{77} In patients with systemic inflammatory response syndrome, active VWF predicted 28-day mortality.\textsuperscript{78} VWF is an acute-phase response protein released by activated endothelial cells in response to inflammatory stimuli.\textsuperscript{77} Inflammatory cytokines, IL-8 and tumor necrosis factor-α induced the release of VWF from human umbilical vein endothelial cells.\textsuperscript{79} VWF released in inflammation binds to NETs released from activated neutrophils and recruits platelets and leukocytes to promote thrombosis.\textsuperscript{77} ADAMTS-13 deficiency in inflammatory conditions was demonstrated to promote VWF-dependent leukocyte adhesion and extravasation in mice.\textsuperscript{80}

In patients with systemic inflammation, ADAMTS-13 activity decreases proportional to the inflammatory response; an imbalance between ADAMTS-13 activity and VWF antigen is associated with organ dysfunction and death.\textsuperscript{51,82} Dysregulated host response to infection including inflammation can result in septic shock. In septic shock, ADAMTS-13 activity was significantly lower\textsuperscript{83–86} and elevated ratio of VWF propeptide (VWFpp) that is secreted along with ultra-large VWF multimers in to blood stream and ADAMTS-13 was associated with disease severity.\textsuperscript{86} In patients with DIC, ADAMTS-13 activity decreased with DIC score\textsuperscript{87} and VWFpp/ADAMTS-13 ratio was significantly elevated in nonsurvivors compared with survivors.\textsuperscript{88} An interesting observation is that smoking, which is associated with adverse outcomes in COVID-19 patients,\textsuperscript{89} was also found to be associated with decreased plasma ADAMTS-13 levels in a study of 3,244 individuals.\textsuperscript{90} Increased expression of angiotensin-converting enzyme 2, the entry receptor for SARS-CoV-2, in the small airway epithelia of smokers was suggested as the potential mechanism for increased risk of severe COVID-19 in smokers.\textsuperscript{91} Smoking is also associated with increased inflammatory markers.\textsuperscript{92}

The imbalance between ADAMTS-13 and VWF in heightened inflammation could be a result of inhibition and/or deficiency of ADAMTS-13 activity.\textsuperscript{93} The inhibition of VWF cleavage by ADAMTS-13 in inflammatory conditions was suggested to be mediated by several mechanisms: (1) thrombospordin-1 released from α-granules of activated platelets by binding to the A2-A3 domain of VWF\textsuperscript{94,95}; (2) α-defensins released from neutrophils by binding to the A2 domain of VWF\textsuperscript{96}; and (3) oxidation of Met 1606 residue in the ADAMTS-13 cleavage site of VWF.\textsuperscript{97} Moreover, nonphysiological high concentrations of IL-6 have been shown to inhibit cleavage of VWF by ADAMTS-13 in vitro under shear flow conditions.\textsuperscript{79} Granulocyte elastases, plasmin, and thrombin that are elevated in inflammatory conditions lower ADAMTS-13 activity through its proteolytic cleavage.\textsuperscript{98,99}

**VWF-ADAMTS-13 Interactions in COVID-19**

Despite playing an important role in the maintenance of hemo-stasis and the occurrence of micro- and macrovascular thrombosis, VWF-ADAMTS-13 interactions have not received much investigative attention in the evaluation of COVID-19 pathophysiology, specifically in relation to elevated incidence of VTE. Importantly, reduced ADAMTS-13 activity has been shown to correlate with increased inflammation in multiple systems\textsuperscript{100–102} while IL-6 has been shown to inhibit the cleavage of ultra-large VWF strings by ADAMTS-13 under flowing conditions.\textsuperscript{79,103} The authors could find only five studies evaluating both VWF and ADAMTS-13 levels in COVID-19 patients in literature\textsuperscript{104–108} (\textit{Table 3}). Majority of these studies reported lower ADAMTS-13 activity concurrent with higher VWF in COVID-19 patients.\textsuperscript{104–107} In one of these studies, Bazzan et al\textsuperscript{104} reported lower ADAMTS-13 levels in 88 COVID-19 patients compared with healthy controls (48.71 ± 18.7% vs. healthy control, 108 ± 9.1%; normal value 60–130%). Within patient cohort, lower ADAMTS-13 and higher VWF levels were found in nonsurvivors (9/88) compared with survivors. Further, lower than 30% ADAMTS-13 activity were significantly associated with mortality in survivor analysis. Huisman et al\textsuperscript{105} observed low ADAMTS-13 activity levels (0.48 ± 0.14 IU/mL against a reference range of 0.61–1.31) in parallel with elevated VWF antigen and activity (≈ fourfold) in 12 ICU-admitted patients. A similar reduction in ADAMTS-13 and increased VWF levels was also reported by Adam et al\textsuperscript{106} and Latimer et al\textsuperscript{107} in four adult and 1 pediatric patients, respectively. On the other hand, Escher et al\textsuperscript{108} observed normal to lower-normal ADAMTS-13 levels concurrently with >2.5-fold increase in VWF antigen and activity in 3 ICU-admitted patients. Two other studies,\textsuperscript{7,109} reported VWF measurements alone, observing >threefold increase in both VWF antigen and activity. From the limited number of studies so far, it appears that COVID-19 infection may be characterized by markedly elevated VWF levels and below normal ADAMTS-13 activity. However, the current literature is limited by the small number of studies and variable timing of VWF/ADAMTS-13 measurements in relation to disease onset. Further evaluation of VWF and ADAMTS-13 interactions in large patient cohorts are warranted to more confidently understand their contributions to COVID-19 pathogenesis.

A secondary mechanism potentially contributing to ADAMTS-13 deficiency relates to the antiphospholipid antibody generation during SARS-CoV-2 infection.\textsuperscript{7,110–112} Anti-phospholipid antibodies have been inconsistently reported in all cases of COVID\textsuperscript{19,7,111,112} but strongly associated to prolong aPTT as reported by Bowles et al.\textsuperscript{112} Patients with antiphospholipid syndrome have been found to have abnormal ADAMTS-13 plasmatic activity further increasing the risk of thrombosis.\textsuperscript{113} The exact mechanisms by which antiphospholipid antibodies interfere with ADAMTS-13 cleaving activity are unclear. We speculate that antiphospholipid antibodies generated during active SARS-CoV-2 infection can potentially bind the spacer domain of ADAMTS-13 interfering with the recognition and proteolysis of VWF. Such a mechanism is similar to the binding of autoantibodies against ADAMTS-13 present in TTP resulting in clinical thrombosis.\textsuperscript{114}

Based on the limited available data, we propose a mechanistic model in which: (1) SARS-CoV-2 causes endothelial activation and damage leading to overwhelming VWF release and (2) proinflammatory mediators or antibodies during the severe phase of COVID-19 result in reduced cleavage of high molecular weight VWF by ADAMTS-13, ultimately leading to thrombosis, see \textit{ Fig. 1}. This concept should be confirmed by large patient cohorts that encompass mild and severe clinical courses of COVID-19 disease. A mechanistic understanding of thrombosis...
during COVID-19 infection is greatly needed to better guide thromboprophylaxis and treatment. The extent to which VWF-ADAMTS-13 interactions contribute to the pathophysiology of COVID-19 should be an important investigative focus.

**Funding**

This work was partly supported by funds from the Hemos tasis Branch/Division of Plasma Protein Therapeutics/ Office of Tissues and Advanced Therapies/Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration. This research was also supported by the Intramural Research Program of the National Library of Medicine at the NIH.

**Conflict of interest**

None declared.

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Thrombosis and Haemostasis Vol. 120 No. 12/2020 © 2020. Thieme.

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