

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 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, ADAMTS13 play important role in the maintenance of microvascular hemostasis. In inflammatory conditions, imbalanced VWF-ADAMTS13 characterized by elevated VWF levels and inhibited and/or reduced activity of ADAMTS13 has been reported. Also, an imbalance between ADAMTS13 activity and VWF antigen is associated with organ dysfunction and death in patients with systemic inflammation. A thorough understanding of VWF-ADAMTS13 interactions during early and advanced phases of COVID-19 could help better define the pathophysiology, guide thromboprophylaxis and treatment and improve clinical prognosis.

Keywords

COVID-19

Thrombosis

Inflammation

ADAMTS13

Von Willebrand Factor

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 RNA virus belonging to *Coronaviridae* family (1). The COVID-19 outbreak started in Wuhan, China, in late 2019 and rapidly spread to 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 (2-4). In susceptible individuals, the disease progresses to pneumonia, hypoxemia, acute respiratory distress, multi-organ dysfunction that may lead to death (3). The predominance of asymptomatic or mild infections has contributed to the rapid spread of COVID-19 compared to earlier coronavirus outbreaks of severe acquired respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) in 2002 and 2012 respectively (4, 5).

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, the coexistence of disseminated intravascular coagulation with subsequent consumption of pro-coagulation proteins and platelets has been consistently described (6). This in turn leads to the formation of micro-thrombi in the vascular bed of organs resulting from

excess coagulation byproducts and suppression of endogenous anti-coagulation factors (7). The coexistence of consumptive coagulopathy and thrombosis are the result of a common pathologic pathway; however the exact mechanisms that tilts the balance towards thrombosis in COVID-19 are less well understood (8). 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 (9, 10) (Table 1). In a study evaluating 449 severe COVID-19 patients, Tang *et al.* (11) reported positive correlation of 28-day mortality with fibrin degradation product, 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 of 183 patients, Tang *et al.* (12) reported elevated D-dimer levels and fibrin degradation product (FDP) levels and prolonged PT and activated partial thromboplastin times (aPTT) at the time of admission in non-survivors compared to survivors. In the same study, significantly lower levels of fibrinogen and anti-thrombin levels were observed during the late hospitalization in non-survivors. Huang *et al.* (13) reported higher D-dimers and prolonged PT at the time of admission in ICU-patients compared to non-ICU patients in a study of 41 patients. Wang *et al.* (14) reported elevated PT in a study of 138 patients. In the same study, elevated levels of D-dimers were found in ICU-patients compared to non-ICU patients as well as in survivors compared to non-survivors in a sub-group of patients with a definitive outcome. In a study of 94 COVID-19 patients, Han *et al.* (15) reported lower antithrombin and higher D-dimers, FDP and fibrinogen levels compared to healthy controls. Zhou *et al.* (16) 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 non-survivors compared to survivors. Elevated levels of D-dimers were reported by Richardson *et al.* (17) among 5700 patients in the New York City area. Ranucci *et al.* (18) reported a procoagulant profile in sixteen patients characterized by increased clot strength by viscoelastography, elevated D-dimer levels and hyperfibrinogenemia. A meta-analysis of 9 studies encompassing 1779 patients with severe disease has identified significantly lower platelet counts (19). A sub-group analysis based on survival has identified even lower platelet counts in non-survivors in this study. Llitzos *et al.* (20) and Helms *et al.* (7) 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 (Table 1). Llitzos *et al.* (20) reported overall rate of 69% 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 to those treated with therapeutic anticoagulation. Helms *et al.* (7) reported 64 clinically relevant thrombotic complications in 150 ICU-admitted patients. Importantly, the incidence of thrombotic complications in COVID-19 acute respiratory distress syndrome (ARDS) patients was significantly higher than non-COVID-19 ARDS patients in this study. Ackermann *et al.* (21) compared lung sections of COVID-19 patients with those died from acute respiratory distress syndrome (ARDS) secondary to influenza A(H1N1) infection and found relatively higher; 1) endothelial cell injury, 2) alveolar

microthrombi (9-fold) 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%) (22), Lodigiani *et al.* (27.6%) (23), Middeldorp *et al.* (47%) (24), Nahum *et al.* (79%) (25) and Cui *et al.* (25%) (26). 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) (27).

A high incidence of disseminated intravascular coagulation (DIC) diagnosed by D-dimer, fibrinogen and antithrombin III levels has become a focus for the initiation of anti-coagulation therapy in severe COVID-19 patients (28), with some studies relying on D-dimers alone (11, 29). A retrospective analysis of 183 patients performed by Tang *et al.* (12) 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 (ISTH) definition of DIC. Subsequently Tang *et al.* (11) reported an equivalent 28-day mortality rate (30%) in 99 patients receiving low molecular weight or unfractionated heparin for 7 days compared to 350 non-heparin treated patients or those receiving a less than 7-day course of therapy. A case series reported by Wang *et al.* (28) detailed the use and outcome following tissue plasminogen activator (tPA) in 3 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 (30). However, D-Dimers are a non-specific indicator of thrombosis in severe COVID-19 patients with pulmonary injury. Fibrin accumulation and lysis continuously occur during non-thrombotic inflammation as well as tissue necrosis and therefore, significant D-dimer elevations also accumulate during cancers (31) and infections, consistent with inflammatory processes that coincide with the progression of severe COVID-19 related macrophage activation syndrome (32). 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 been highly informative regarding the assessment of hemorrhage, coagulation and fibrinolysis (33, 34). Assessment of impairment of these systems would provide a useful and appropriate guidance needed for and monitoring of therapeutic interventions in the unique coagulopathies associated with COVID-19 (33, 34). Because, patients are often on unfractionated or low molecular weight heparin and plasminogen activator inhibitor 1, VWF, plasminogen, fibrinogen and FVIII are all reported to be elevated in SARS infection (35), careful modification of these assays may be warranted to optimize the concentrations of added tPA, tissue factor and thrombomodulin.

These studies present a heterogeneous picture that is difficult to evaluate in the aggregate. Inclusion criteria for patients varied across these studies, making direct comparisons between the studies difficult. Further, the studies used different regimens of thromboprophylaxis, which could impact outcomes. 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, pre-existing thrombotic risk, and pre-hospitalization 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 (13, 14, 16, 17, 36, 37). A common theme of all these co-morbidities is their association with vascular inflammation and endothelial dysfunction (38, 39). Pro-inflammatory conditions affect hemostasis by blocking of fibrinolysis and induction of prothrombotic conditions through activation of endothelial cells and innate immune cells via release of several factors including tissue factor, von Willebrand Factor (VWF) and neutrophil extracellular traps (NETs) that promote thrombosis (40). Induction of pro-inflammatory conditions was reported in the pathophysiology of several viral diseases including influenza and SARS (41). Increased inflammation is commonly observed in COVID-19 patients, while severe cases are characterized by immune dysregulation and hyper-inflammation, with a markedly increased serum IL-6 (42). Cytokine release syndrome (CRS) has also been reported in COVID-19 patients and correlates with adverse clinical outcomes (43). The presence of several inflammatory markers such as C-reactive protein (CRP), procalcitonin, ferritin, and fibrinogen are often reported in COVID-19 patients (13, 14, 16, 17, 36, 37, 44-48) (Table 2). Further, multiple studies reported elevated levels of the pro-inflammatory cytokine IL-6 in severe cases of COVID-19 (16, 37, 42, 47, 49-53) (Table 2). A concurrent increase in the levels of anti-inflammatory cytokine IL-10, 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 (54), and therefore tocilizumab, an IL-6 inhibitor, is being used in ongoing clinical trials to prevent catastrophic inflammation (55-58).

Liver injury during COVID-19 infections was described in multiple studies, including elevated levels of alanine aminotransferase, aspartate aminotransferase and bilirubin (14, 16, 17, 36, 44, 47). 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 (59). Similarly, hypoxemia observed in COVID-19 patients induces prothrombotic conditions through upregulation of plasminogen activator inhibitor and stimulation of endothelial synthesis of pro-coagulants, including tissue factor and VWF (60-63). 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 pro-inflammatory conditions as the primary driving cause of thrombotic events in COVID-19 patients.

VWF-ADAMTS13 in Hemostasis and Thrombosis

VWF and its cleaving protease, ADAMTS13, play an important role in hemostasis particularly within the microvasculature (64). 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 alpha-granules only upon activation (65).

ADAMTS13 is expressed both by hepatic stellate cells and endothelial cells ; the relative contribution of hepatic and microvascular expression is not clear (66). ADAMTS13 regulates the

biological activity of VWF by cleaving pro-thrombotic 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 (67). Severe deficiency of ADAMTS13 results in accumulation of ultra-large VWF multimers leading to microvascular thrombosis and consumptive thrombocytopenia, a condition termed thrombotic thrombocytopenic purpura (TTP) (64). In the event of vascular injury, VWF facilitates binding of platelets to sub-endothelium through its interactions with glycoprotein Ib and collagen, thereby inducing thrombus formation (64). A reciprocal relationship exists between VWF and ADAMTS13 levels where elevated circulatory VWF antigen levels are associated with concomitant decrease in ADAMTS13 activity and vice versa (68-70). Abnormal VWF-ADAMTS13 ratios are implicated in arterial thrombosis (71), ischemic stroke (72, 73) pediatric stroke (74) and perioperative thrombosis in infants (75). In addition abnormal VWF/ADAMTS13 metabolism has been positively associated with myocardial infarction in young women (76). It is worth highlighting that in the case of perioperative thrombosis, elevated VWF even in the absence of significant deficiency of ADAMTS13 was associated with thrombosis (75). Severe hypoxia and acidosis likely caused a higher increase in VWF during cardiac surgery and were at higher risk of thrombosis (75).

Elevated levels of VWF are found in several inflammatory and metabolic disorders including diabetes, obesity and sickle cell disease (77). In patients with systemic inflammatory response syndrome, active VWF predicted 28-day mortality (78). VWF is an acute-phase response protein released by activated endothelial cells in response to inflammatory stimuli (77). Inflammatory cytokines, IL-8 and TNF- α induced the release of VWF from human umbilical vein endothelial (HUVEC) cells (79). VWF released in inflammation binds to NETs released from activated neutrophils and recruits platelets and leukocytes to promote thrombosis (77).

ADAMTS13 deficiency in inflammatory conditions was demonstrated to promote VWF dependent leukocyte adhesion and extravasation in mice (80).

In patients with systemic inflammation, ADAMTS13 activity decreases proportional to the inflammatory response; an imbalance between ADAMTS13 activity and VWF antigen is associated with organ dysfunction and death (81, 82). Dysregulated host response to infection including inflammation can result in septic shock. In septic shock, ADAMTS13 activity was significantly lower (83-85) and elevated ratio of VWF pro-peptide (VWFpp) that is secreted along with ultra-large VWF multimers in to blood stream and ADAMTS13 was associated with disease severity (86). In patients with DIC, ADAMTS13 activity decreased with DIC score (87) and VWFpp/ADAMTS13 ratio was significantly elevated in non-survivors compared to survivors (88). An interesting observation is that smoking, which is associated with adverse outcomes in COVID-19 patients (89) was also found to be associated with decreased plasma ADAMTS13 levels in a study of 3244 individuals (90). Increased expression of ACE-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 (91). Smoking is also associated with increased inflammatory markers (92).

The imbalance between ADAMTS13 and VWF in heightened inflammation could be a result of inhibition and/or deficiency of ADAMTS13 activity (93). The inhibition of VWF cleavage by ADAMTS13 in inflammatory conditions was suggested to be mediated by several mechanisms: 1) thrombospondin-1 released from α -granules of activated platelets by binding to A2-A3 domain of VWF (94, 95); 2) α -defensins released from neutrophils by binding to A2 domain of VWF (96); and 3) oxidation of Met 1606 residue in the ADAMTS13 cleavage site of VWF (97). Moreover, non-physiological high concentrations of IL-6 have been shown to inhibit

cleavage of VWF by ADAMTS13 *in vitro* under shear flow conditions (79). Granulocyte elastases, plasmin, and thrombin that are elevated in inflammatory conditions lower ADAMTS13 activity through its proteolytic cleavage (98, 99).

VWF-ADAMTS13 Interactions in COVID-19

Despite playing an important role in the maintenance of hemostasis and the occurrence of micro and macrovascular thrombosis, VWF-ADAMTS13 interactions have not received much investigative attention in the evaluation of COVID-19 pathophysiology, specifically in relation to elevated incidence of VTE. Importantly, reduced ADAMTS13 activity has been shown to correlate with increased inflammation in multiple systems (100-102), while IL-6 has been shown to inhibit the cleavage of ultra-large VWF strings by ADAMTS13 under flowing conditions (79, 103). The authors could find only five studies evaluating both VWF and ADAMTS13 levels in COVID-19 patients in literature (104-108) (Table 3). Majority of these studies reported lower ADAMTS13 activity concurrent with higher VWF in COVID-19 patients (104-107). In one of these studies, Bazzan *et al.* (104) reported lower ADAMTS13 levels in 88 COVID-19 patients compared to healthy controls ($48.71 \pm 18.7\%$ vs HC, $108 \pm 9.1\%$; normal value 60–130%). Within patient cohort, lower ADAMTS13 and higher VWF levels were found non-survivors (9/88) compared to survivors. Further, lower than 30% ADAMTS13 activity were significantly associated with mortality in survivor analysis. Huisman *et al.* (105), observed low ADAMTS13 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 (~4 fold) in 12 ICU-admitted patients. A similar reduction in ADAMTS13 and increased VWF levels was also reported by reported by Adam *et al.* (106) and Latimer *et al.* (107) in 4 adult and 1 pediatric patients respectively. On the other hand, Escher *et al.* (108), observed normal to lower-normal ADAMTS13 levels concurrently with >2.5 fold

increase in VWF antigen and activity in 3 ICU-admitted patients. Two other studies (7, 109) reported VWF measurements alone, observing >3-fold 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 ADAMTS13 activity. However, the current literature is limited by the small number of studies and variable timing of VWF/ADAMTS13 measurements in relation to disease onset. Further evaluation of VWF and ADAMTS13 interactions in large patient cohorts are warranted to more confidently understand their contributions to COVID-19 pathogenesis.

A secondary mechanism potentially contributing to ADAMTS13 deficiency relates to the anti-phospholipid antibody generation during SARS-CoV-2 infection (7, 110-112). Anti-phospholipid antibodies have been inconsistently reported in all cases of COVID 19 (7, 111, 112), but strongly associated to prolong aPTT as reported by Bowles *et al.* (112) .Patients with anti-phospholipid syndrome have been found to have abnormal ADAMTS13 plasmatic activity further increasing the risk of thrombosis (113). The exact mechanisms by which anti-phospholipid antibodies interfere with ADAMTS13 cleaving activity are unclear. We speculate that anti-phospholipid antibodies generated during active SARS-CoV-2 infection can potentially bind the spacer domain of ADAMTS13 interfering with the recognition and proteolysis of VWF. Such a mechanism is similar to the binding of autoantibodies against ADAMTS13 present in thrombotic thrombocytopenic purpura resulting in clinical thrombosis (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) pro-inflammatory mediators or antibodies during the severe phase of COVID-19 result in reduced cleavage of high molecular weight VWF by ADAMTS13, ultimately leading to

thrombosis, see Figure 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-ADAMTS13 interactions contribute to the pathophysiology of COVID-19 should be an important investigative focus.

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Conflict of interest

Dr. Buehler reports personal fees from Kalocyte Inc., outside the submitted work. The rest of the authors has nothing to disclose.

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Figure Legends

Figure 1: VWF-ADAMTS13 metabolism in inflammation. A) During normal homeostasis, ADAMTS13 regulates the activity of VWF by cleaving pro-thrombotic ultra-large VWF multimers released from endothelial cells in to hemostatically active high molecular weight multimers. B) In inflammatory disorders, pro-inflammatory cytokines (e.g. IL-8 and TNF- α) stimulate excess release of VWF stored in Weibel-Palade bodies of endothelial cells. VWF interacts with neutrophil extracellular traps (NETs) released from neutrophils to provide a scaffold for platelet adhesion and thrombus formation. C) In inflammation, cleavage of VWF by ADAMTS13 is prevented by multiple mechanisms that either inhibit or reduce the proteolytic activity of ADAMTS13.

Tables

Table 1: Studies (multiple patients) reporting abnormal coagulopathy in COVID-19

Study	Type of study, number of patients	Findings /Significance
Clinical features of COVID-19 patients, coagulation parameters included		
Huang <i>et al.</i> (13)	Prospective, 41 patients	Prothrombin time and D-dimer levels on admission were higher in patients that required ICU treatment
Zhou <i>et al.</i> (16)	Retrospective, 191 COVID-19 patients	Increased d-dimer on admission is associated with poor prognosis
Guan <i>et al.</i> (44)	Retrospective, 1099 COVID-19 patients	Thrombocytopenia in 36.2%
Goyal <i>et al.</i> (36)	Retrospective, 393 COVID-19 patients	Thrombocytopenia in 27%
Zhu <i>et al.</i> (45)	Metanalysis	Elevated D-dimer in about 37.2% of patients
Studies on coagulation parameters		
Ranucci <i>et al.</i> (18)	Prospective, 16 ARDS COVID-19 patients	Patients showed a pro-coagulant profile (clot strength, platelet, fibrinogen, d-dimers, hyperfibrinogenemia)
Tang <i>et al.</i> (12)	Retrospective, 183 COVID-19 patients	Non-survivors had significantly higher D-dimer and fibrin degradation product (FDP) levels, longer prothrombin time and activated partial thromboplastin time compared to survivors on admission. 71.4% of non-survivors and 0.6% survivors met the criteria of DIC during their hospital stay

Lippi <i>et al.</i> (19)	Metanalysis	Low platelet count associated with increased risk of severe disease and mortality in patients with COVID-19
Zhang <i>et al.</i> (29)	Retrospective, 343 COVID-19 patients	Patients with D-dimer levels ≥ 2.0 $\mu\text{g/ml}$ had a higher incidence of mortality when comparing to those who with D-dimer levels < 2.0 $\mu\text{g/ml}$
Escher <i>et al.</i> (108, 109)	Case study, 1 patient and 3 more in the follow up publication	Continual increase of D-dimers, Elevated FVIII activity and normal platelet counts.
Bowles <i>et al.</i> (112)	216 COVID-19 patients 34 tested for lupus anticoagulant	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
Lorenzo-Villalba <i>et al.</i> (115)	Case reports, 3 patients	Severe thrombocytopenia during COVID-19 infection associated with either cutaneous purpura or mucosal bleeding
Yin <i>et al.</i> (116)	Retrospective, 449 COVID-19 and 104 non-COVID severe pneumonia	Patients with severe pneumonia induced by SARS-CoV2 had higher platelet count than those induced by non-SARS-CoV2. Patients infected by SARS-CoV2 may benefit from anticoagulant treatment, if they have markedly elevated D-dimer
Tabatabai <i>et al.</i> (48)	Case series, 10 patients	Elevated FVIII activity and low normal antithrombin and functional protein C activity

Thrombosis in the COVID-19 patients

Middeldorp <i>et al.</i> (24)	Retrospective, 198 patients	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
Nahum <i>et al.</i> (25)	Prospective, 34 patients	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

Cui <i>et al.</i> (26)	Retrospective, 81 severe COVID-19 patients	Incidence of VTE at 25%. D-dimer increase has a predictive value
Klok <i>et al.</i> (22)	Retrospective, 184 patients, no control group	31% cumulative incidence of symptomatic acute pulmonary embolism (PE), deep-vein thrombosis, ischemic stroke, myocardial infarction or systemic arterial embolism in COVID-19 patients
Zhang <i>et al.</i> (27)	Prospective, 281 ICU COVID-19 patients	Cumulative incidence of VTE at 28 days was 9.55%, despite all patients receiving thromboprophylaxis
Demelo-Rodriguez <i>et al.</i> (117)	Prospective, 156 COVID-19 patients	D-dimer levels > 1570 ng/ml were associated with asymptomatic DVT
Grandmaison <i>et al.</i> (118)	Cross-sectional study, 58 COVID-19 patients, 29 in the ICU and 29 in the medicine ward	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
Fraissé <i>et al.</i> (119)	Retrospective, 92 ICU COVID-19 patients	high rate of thrombotic events (TE) in ICU COVID-19 patients highlighting the necessity for thromboprophylaxis and TE screening. Hemorrhagic events (HE) we also observed in patients on full-dose anticoagulation
Jain <i>et al.</i> (120)	Retrospective, 3218 COVID-19 patients	Acute stroke was the most common neuroimaging finding, present in 1.1% of hospitalized COVID-19 patients.
Desborough <i>et al.</i> (121)	Retrospective, 66 patients	10 patients had at least one proven episode of thromboembolism. Major bleeding occurred in seven cases
Akel <i>et al.</i> (122)	Case reports, 6 patients	Patients didn't have any hypercoagulable risk factors yet presented with pulmonary embolism
Kashi <i>et al.</i> (123)	Case reports, 7 patients	Arterial thrombosis

Lax <i>et al.</i> (124)	Prospective autopsy study, 11 deceased COVID-19 patients	Death may be caused by the thrombosis observed in segmental and subsegmental pulmonary arterial vessels despite the use of prophylactic anticoagulation
Thomas <i>et al.</i> (125)	Retrospective, 63 COVID-19 patients	High thrombotic risk in patients with COVID-19
Gomez-Arbelaez <i>et al.</i> (126)	Case reports, 4 patients	Aortic thrombosis and associated ischemic complications in patients with severe SARS-CoV-2 infection

Anticoagulation treatment in COVID-19 patients

Tang <i>et al.</i> (11)	Retrospective, 449 severe COVID-19 patients, 99 received heparin	Anticoagulant therapy is associated with better prognosis in severe COVID-19 patients with sepsis induced coagulopathy or markedly elevated D-dimer
Wang <i>et al.</i> (28)	3 case reports	Treatment with tissue plasminogen activator lead to improvement in the respiratory status
Ayerbe <i>et al.</i> (127)	2075 COVID-19 patients, admitted in 17 hospitals in Spain	Heparin had been used in 1734 patients. Heparin was associated with lower mortality
Wang <i>et al.</i> (128)	Retrospective, 1099 COVID-19 patients	High risk of venous thromboembolism, also high risk of bleeding
Artifoni <i>et al.</i> (129)	Retrospective, 62 patients	16 patients developed VTE, 7 patients developed PE Very high negative predictive value of baseline D-dimer level for VTE and PE
Russo <i>et al.</i> (130)	Retrospective, 192 COVID-19 patients	Pre-admission 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.

Link between SARS-CoV-2 and thrombosis

Ackermann <i>et al.</i> (21)	7 lung autopsies from COVID-19 patients and 7 from ARDS	Vascular angiogenesis distinguished the pulmonary pathobiology of COVID-19 from that of equally severe influenza virus infection
	Case studies	
Maier <i>et al.</i> (131)	15 COVID-19 patients with hyperviscosity	Possible causal relationship between hyperviscosity and thrombotic complications in COVID-19
Huisman <i>et al.</i> (105)	12 COVID-19 patients	Low ADAMTS13 activity, increased vWF levels and factor VIII levels
Galeano-Valle <i>et al.</i> (111)	Prospective study, 24 patients	Prevalence of antiphospholipid antibodies in COVID-19 and venous thrombosis was low
Magro <i>et al.</i> (132)	Case reports, 5 severe COVID-19 cases	Procoagulant state is associated with systemic complement activation

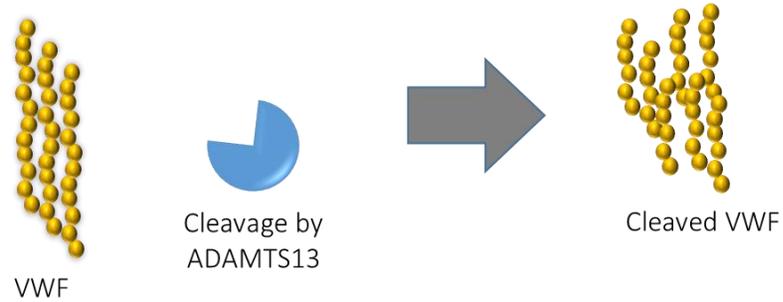
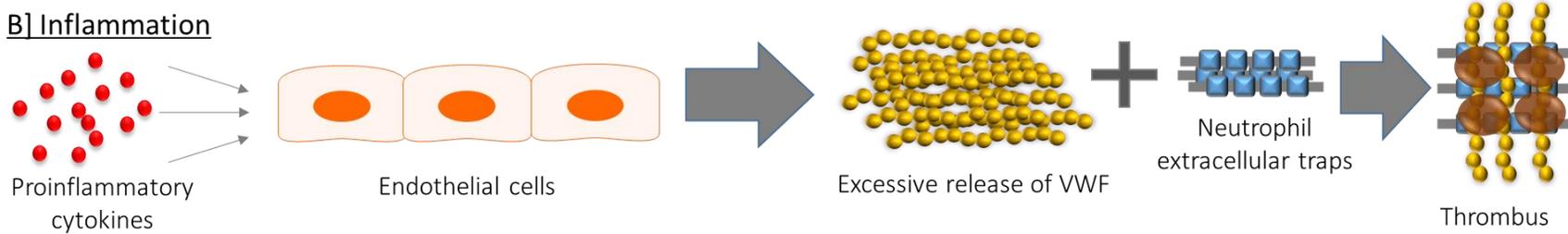
Table 2: Studies reporting elevated inflammatory markers in COVID-19

Study	Patient group (number of patients) comparison	Elevated inflammatory markers
Huang <i>et al.</i> (13)	ICU (13) vs non-ICU (28)	Procalcitonin, IL-1 β , IFN- γ , IP10, and MCP1
Wang <i>et al.</i> (14)	ICU (36) vs non-ICU (102)	Procalcitonin
Zhou <i>et al.</i> (16)	Non-survivor (54) vs Survivor (137)	Procalcitonin, Ferritin and IL-6
Richardson <i>et al.</i> (17)	Relative to reference range (3066)	Procalcitonin, Ferritin and CRP
Ruan <i>et al.</i> (37)	Non-survivor (68) vs Survivor (82)	CRP and IL-6
Bourbolis <i>et al.</i> (42)	Dysregulated (21) vs intermediate state (26) of immune activation	CRP and IL-6
Chen <i>et al.</i> (47)	Severe (≥ 9) vs moderate (≥ 7)	CRP, Ferritin, IL-6 and TNF- α
Han <i>et al.</i> (49)	COVID-19 Patients (102) vs controls (45)	CRP, IL-6, TNF- α and IFN- γ
Du <i>et al.</i> (50)	Mild pneumonia (124) vs no pneumonia (54) (pediatric patients)	Procalcitonin, IL-6, TNF- α and IFN- γ
Wang <i>et al.</i> (52)	SpO ₂ $\geq 90\%$ (≥ 36) vs SpO ₂ $< 90\%$ (≥ 7)	CRP and IL-6
Tan <i>et al.</i> (53)	Severe (25) vs mild/moderate 31)	CRP and IL-6
Tabatabai <i>et al.</i> (48)	Relative to reference range (10)	Fibrinogen, CRP and Ferritin

Abbreviations: ICU – Intensive care unit, IL-1 β – Interleukin-1 β , IFN- γ – Interferon- γ , IP-10 – Interferon- γ induced protein 10, MCP-1 – Monocyte chemotactic protein-1, IL-6 – Interleukin-6, CRP – C-reactive protein, TNF- α – Tumor necrosis factor- α , COVID-19 – Corona virus disease of 2019, SpO₂ – Blood oxygen saturation level.

Table 3: Studies reporting ADAMTS13 and VWF levels in COVID-19

Study	Patient group (number of patients) comparison	Findings /Significance
Bazzan <i>et al.</i> (104)	Non-survivor (9) vs survivor (79)	Lower ADAMTS13 and elevated VWF levels in non-survivors compared to survivors. After survival analysis, Lower than 30% ADAMTS-13 levels were significantly associated with higher mortality
Huisman <i>et al.</i> (105)	Relative to reference range (12)	Lower ADAMTS13 and elevated VWF levels
Adam <i>et al.</i> (106)	Relative to reference range (4)	Lower ADAMTS13 and elevated VWF levels
Latimer <i>et al.</i> (107)	Relative to reference range (1 pediatric patient)	Lower ADAMTS13 and elevated VWF levels
Escher <i>et al.</i> (108, 109)	Case study, 1 patient and 3 more in the follow up publication	Massive elevation of VWF and normal to lower-normal ADAMTS13 activity. COVID-19 coagulopathy may be a distinct entity of highly prothrombotic alterations most probably an endothelial disease
Helms <i>et al.</i> (7)	Relative to reference range (150)	Elevated VWF levels

Figure 1**A) Hemostasis****B) Inflammation****C) Cleavage of VWF by ADAMTS13 is prevented by the following mechanisms**

- 1) Binding of thrombospondin-1 released from α -granules of activated platelets to A2-A3 domain of VWF harboring the proteolytic cleavage site of ADAMTS13
- 2) Binding of α -defensins released from neutrophils to A2 domain of VWF
- 3) Oxidation of Met 1606 residue in the ADAMTS13 cleavage site of VWF by reactive oxygen species
- 4) High concentrations of IL-6
- 5) Proteolytic cleavage of ADAMTS13 by granulocyte elastases, plasmin, and thrombin that are elevated in inflammatory conditions