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A Review of the Pathological Mechanisms and Clinical Implications of Coagulopathy in COVID-19

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Abstract:

Coronavirus disease-19 (COVID-19) is an ongoing global pandemic with approximately 15% of patients requiring mechanical ventilation and intensive care unit care. The mechanisms of end-organ damage and mortality in severe COVID are slowly being unraveled. COVID-related coagulopathy is a characteristic syndrome that plays a key role in multi-organ dysfunction and severe disease. Mechanistically, many components including endothelial cells, the coagulation system, complement system, cytokines, and NETosis are involved in the pathogenesis of this syndrome and provide potential therapeutic targets. We provide a focused review on the current understanding of COVID-19-related coagulopathy and its therapeutic implications.

Keywords:

Anticoagulation, coagulation, coagulopathy, COVID-19, cytokines, hematology, thrombosis

Introduction

The severe acute respiratory syndrome-coronavirus 2 (SARS-CoV2) is an RNA virus and the causative agent of an unprecedented public health crisis. The illness caused by this virus is called coronavirus disease-2019, or COVID-19, that has led to over 135 million cases and 2.9 million deaths worldwide as of April 10, 2021 (Source: Roser, Ritchie, Ortiz-Ospina *et al*. (2020) – 'Coronavirus Pandemic [COVID-19]. Published online at OurWorldInData.org.Retrieved from: "https://ourworldindata.org/coronavirus"). Infection with SARS-CoV2 has a highly variable presentation ranging from an asymptomatic carrier state to multi-organ dysfunction. The case fatality rate associated with COVID-19 has been estimated to range from 5% to 7%.[1] A significant proportion of infected patients are asymptomatic or have mild disease and approximately 14%–17% require critical care.[2,3] Mortality varies according to disease severity and approaches 50% for those requiring mechanical ventilation, rising sharply with concomitant multi-organ dysfunction.[2]

Efforts to describe the pathophysiologic mechanisms behind severe COVID-19 have revealed a key role of a thrombophilic coagulopathy in facilitating severe disease manifestations.[4] Micro- and macrovascular venous thromboses are now known to occur in most patients and a significant majority of patients demonstrate characteristic abnormalities on coagulation tests.[5] The extent of derangement of several coagulation markers has been found to directly correlate with a higher risk of intensive care unit (ICU) admission and mortality.[6]

Clinical and basic science data related to COVID-19 is a rapidly evolving field, and newer mechanisms describing the role of coagulopathy in COVID-19 are being described. The major incentive in understanding these mechanisms stems

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from the possibility of identifying novel pathways for intervention and prevention of severe disease and mortality. Over the past few months, significant modifications have occurred in many initial concepts related to COVID and many promising treatment options have been refuted. This narrative review was compiled with the aim of summarizing the current evidence underlining mechanisms of coagulopathy in COVID and treatment implications of the same. A precise summary of recommendations and controversies to the treatment of coagulopathy in COVID-19 is also provided.

Mechanisms and Clinical Relevance of Coagulopathy in COVID-19

Clinical and pathological evidence of coagulopathy

A prothrombotic state was initially noted in COVID-19 with the observation of diffuse alveolar damage and small-vessel thrombi in lung autopsies of patients dying of respiratory failure.[7] Several subsequent reports documented similar findings, incriminating endothelial damage, and microvascular thrombosis as the final mechanisms leading to end-organ damage.[8,9]

Thrombophilia was also noted clinically with the presence of venous thromboembolism (deep venous thrombosis [DVT] or pulmonary embolism [PE]) in a significant majority of patients.[10] Based on ultrasound Doppler screening, the prevalence of DVT was found to be as high as 69% in patients admitted with COVID-19.[11] A large meta-analysis including 28173 patients noted an overall VTE prevalence of 9.5%, which rose to 40% with ultrasound Doppler screening.[12] Coagulation abnormalities are noted in most patients admitted with COVID-19 and characteristically show an elevated D-dimer, fibrinogen, prothrombin time (PT), and a hypercoagulable phenotype on thromboelastography.[13,14] Of these, PT and D-dimer have been shown to have prognostic implications and must be documented for all patients at baseline. In a study including 213 patients, prolonged PT was noted in 18% of patients, and survival at day 11 in this group was much lower compared to those with a normal PT (90.4% ± 2.3% vs. 64.1% ± 7.7%).[15] Similarly, in an initial study from Wuhan, the elevation of PT above 16 s was shown to be associated with a higher mortality (odds ratio [OR]: 4.62 (1.29–16.50).[16]

Derangements in fibrinogen and D-dimer ostensibly act as surrogates of more severe coagulopathy and are also noted to correlate with a higher risk of mortality.[6,17] Elevations of D-dimer are noted to be much greater in patients with severe COVID-19 compared to those with mild disease.[18] In a large meta-analysis including 2911 patients from nine studies, elevated D-dimer at admission was significantly associated with all-cause mortality (RR: 4.77, 95% confidence interval [CI]: 3.02–7.54).[19] Similar results were obtained from another dataset from Wuhan, which included 1561 patients from 13 observational studies.[18] There are increasing data that a D-dimer cutoff value of 0.5 ug/ml identifies patients at a higher risk of mortality. Using this cutoff, the odds ratio for severe manifestations of COVID was 5.78 (95% CI: 2.16–15.44, $P < 0.001$) in the above study. A quantitative effect of D-dimer values has also been noted, with a markedly high OR for death with a D-dimer value of 1 mcg/ml compared to 0.5 ug/ml.(20·04 [6·52–61·56] vs. 1·96 [0·52–7·43]).[16]

It is important to note that the coagulopathy caused by COVID-19 is distinct from disseminated intravascular coagulation (DIC) and other viral hemorrhagic illnesses. Unlike DIC, there is a minimal elevation of prothrombin time (PT)/activated partial thromboplastin time (aPTT) and no reduction in fibrinogen. With increasing severity of illness, D-dimer continues to rise, but no reduction in fibrinogen is noted, which is different from DIC.[20] Unlike other viral illnesses causing coagulopathy, the risk of major bleeding in COVID-19 continues to be low and is estimated at approximately 2.3%.[4] Both the above manifestations appear to be distinct responses to the virus in question, likely mediated by a differential inflammatory response.[20]

Although the above modifications in coagulation parameters are described in most detail, several other changes are being gradually described. For instance, several characteristic changes have been observed on rotational thromboelastometry (ROTEM) between patients admitted to the medical wards and those requiring ICU care due to severe disease. Patients with more severe disease requiring ICU care demonstrated higher EXTEM-(clotting time and FIBTEM-maximum clot firmness values, indicating impaired hepatic synthesis of coagulation factors and higher fibrinogen levels, respectively.[21]

Another important study elegantly described differences in coagulation parameters and clotting factor levels among patients with COVID-19 in different clinical settings. D-dimer and F-VIII levels were universally elevated as expected. Interestingly, levels of several clotting factors were noted to differ among survivors and nonsurvivors. Although the differences in concentration are likely clinically insignificant, this re-affirms the unique nature of COVID-19 coagulopathy, there being no consumptive coagulopathy (unlike DIC).[22] Similar differences were also noted in patients who developed venous thrombosis. The clinical significance of altered levels of coagulation factors and thromboelastographic findings is expected to be elucidated with a further compilation of data.

Pathogenesis of Coagulopathy in COVID-19

Mechanisms involved in the pathogenesis of COVID-19 and organ dysfunction are gradually being unraveled and provide potential therapeutic targets. A summary of the currently understood mechanisms of coagulopathy in COVID-19 is summarized below.

Endothelial damage

Direct infection of pulmonary endothelial cells with SARS-CoV2 is the first step in the pathogenesis of severe COVID-19. SARS-CoV2 gains entry into pulmonary endothelial cells by directly binding to the cell surface angiotensin-converting enzyme 2 (ACE2) receptor through its spike protein.[23] Subsequently, the ACE2 receptor along with the virus is internalized into the endothelial cells, reducing ACE2 expression on the surface.[24] This disrupts the physiological role of ACE2 and reduces the conversion of angiotensin II to angiotensin, shifting the ACE/ACE2 balance toward a pro-inflammatory phenotype.[25] Reduced ACE2 activity increases vascular permeability by activation of the bradykinin-kallikrein system and leads to local recruitment of neutrophils and lymphocytes.[26] These initial events lead to disruption of the endothelial barrier followed by alveolar flooding and pulmonary dysfunction. Activation of coagulation pathways by these events leads to pulmonary microvascular thrombosis, causing further deterioration in pulmonary function.[27]

Disruption of the endothelial barrier plays a central role in the activation of coagulation through the tissue factor pathway.[28] Endothelial invasion and endotheliitis leads to disruption of normal homeostatic function of the endothelium, leading to a pro-thrombotic and pro-inflammatory phenotype. Endothelial disruption releases several proteins and cytokines which play a role in activating coagulation. These include vWF, P-selectin, CD40 L, and thrombomodulin, which are noted to be increased in the peripheral blood even in patients with nonsevere COVID-19.[29] P-selectin, fibrinogen, and CD40 L independently support platelet activation, leukocyte recruitment, and activation of coagulation through tissue factor pathway.[30-32] Thrombomodulin, which activates local anticoagulation by activation of protein C, is released after endothelial damage, and its circulating levels have been shown to correlate with disease severity and length of hospital stay in COVID-19.[33] Multi-organ involvement is also presumed to be mediated through ACE2 dependent cell entry into various tissues. ACE2 is highly expressed in the heart, lung, kidney, and gut. The virus is purported to gain entry into these cells through the ACE2 receptor, leading to local inflammation, microvascular thrombosis, and organ dysfunction.[34,35] SARS-CoV2 particles have been directly observed in the endothelial cells of the lung, kidney, heart, and liver with ensuing endotheliitis, providing support to this hypothesis.[36]

Inflammatory cytokines

Initial endothelial damage leads to cytokine secretion and complement activation, which serve the dual function of potentiating local inflammation and activating coagulation. Marked elevation of certain cytokines, mainly interleukin (IL)-6, IL-1, and tumor necrosis factor (TNF)-a, has been labeled as "cytokine storm" and is a key finding in severe COVID-19. These cytokines active downstream inflammatory pathways and play a supportive role in initiating coagulation.[37] IL-6 is involved in disease progression in COVID-19 by causing absolute lymphopenia and reduced cytotoxic T-cell function.[38] It additionally stimulates hepatic synthesis of FVIII and fibrinogen, leading to an increased risk of thrombosis.[39,40] The crucial role played by IL-6 is evident from a cohort of 501 patients where patients dying from severe COVID had significantly higher IL-6 levels compared to those who survived (OR: 1.008 [1.005-1.012]).[41] Several studies have suggested a cutoff value of 80–90 pg/ml to predict for a higher need of ventilation and poorer outcomes.[42] However, a standalone role of IL-6 and a single cutoff value predicting poorer outcomes has recently been questioned. Data from larger cohorts have shown a relatively modest elevation of IL-6 in COVID compared to cytokine storms with other etiologies.[43] The levels of IL-6 in COVID are lower by a factor of 10–200 x compared to those seen in cytokine storms in ARDS or CAR-T cell therapy.[44] It is thus suggested that a predictive model including other parameters including SpO2/FiO2 ratio, neutrophil-to-lymphocyte ratio, LDH level, IL-6 level, and age is more accurate for the prediction of mortality (AOC 0.94) in patients with severe COVID-19. In this model, IL-6 predicted mortality at a cutoff value of 163.4 pg/ml, with a sensitivity of 91.7% and specificity of 57.6%.[45] IL-6 inhibition has been exploited with the use of tocilizumab, which is gradually becoming less significant in treatment algorithms with an accrual of prospective data.

IL-1 and TNF also alter endothelial function by increasing tissue factor expression, shifting the hemostatic balance to a prothrombotic phenotype.[46] Elevation of TNF correlates with severe disease manifestations and risk of mortality.[47] Clinical data for specific inhibition of these cytokines are limited and investigational at present.

Complement activation and NETosis

The complement system provides a vital link between inflammation and coagulation in COVID. This premise is supported by multiple implicit findings. Products of complement activation including C3a, C3b, and C3dg are

observed to be present locally in lung tissues in the early stages of infection with SARS-CoV2. Mice with C3 knockout show diminished local and systemic inflammatory responses despite elevated systemic inflammatory markers.[48] Activated complement components are seen to be deposited in affected tissues along with viral particles and help to mediate local injury and thrombosis.[9] The coronavirus N protein can directly activate the complement system through the lectin pathway.[49]

Once complement is activated, it is inextricably linked to thrombosis through multiple mechanisms. Activated complement components increase tissue factor activity on endothelial cells.[50] MASP1 and MASP2 proteins, required for activation of the lectin pathway, can directly convert prothrombin to thrombin.[51] In addition, C5a–C9 membrane attack complex can directly lead to platelet activation. The complement system potentiates the thrombogenic potential of endothelial cells by local secretion of vWF.[52] Mechanistically, specific components that activate the coagulation cascade and can be directly targeted have are yet to be conclusively demonstrated in COVID-19.[53] The complement system appears to lead to thrombosis by acting as a link between initial inflammation and other cooperating mechanisms.

One significant new mechanism linking inflammation and coagulation in COVID is NET (neutrophil extracellular trap) formation. NETs are a form of programmed cell death, in which neutrophils extrude chromatin with histones and antipathogen proteins as part of innate immunity.[54] Neutrophilic infiltration and fibrin deposition consistent with NETosis have been noted in autopsies of patients with COVID-19. The same study demonstrated elevated levels of cf-DNA, MPO-DNA, and CItH3, which are markers of NET formation.[55] Soluble PF4 and RANTES, which activate NETosis, are also noted to be significantly increased in patients with severe COVID.[56] SARS-CoV2 has been shown to directly promote NET formation *in vitro* along with increased neutrophil reactive oxygen species, which mediate further tissue damage.[57] Activated complement components, specifically C3a and C5a, can directly stimulate NETosis, with C5a working best in the presence of interferon and TNF.[58,59] NETs have also been shown to contain C3, properdin, and factor B, reaffirming the link between complement activation and neutrophil activation. An intricate three-way relation between coagulation, NETosis, and complement system has been elegantly described. Once activated, NETosis, complement activation, and coagulation are mutually self-supporting and potentially explain the link between inflammation leading to coagulation and end-organ damage in COVID.[60]

Impaired fibrinolysis is another mechanism that may potentially explain a procoagulant state in COVID-19. A study including 40 critically ill patients with COVID-19 documented the presence of impaired fibrinolysis on viscoelastic rotational thromboelastometry (ROTEM), possibly explaining a new supportive mechanism for microvascular thrombosis.[61] The significance of this mechanism will become clear on larger datasets.

To summarize, COVID-19 leads to endothelial damage, which is followed by activation of coagulation through inflammatory cytokines, complement components, and NETosis. A hypercoagulable state contributes to microvascular thrombosis leading to end-organ dysfunction. Monitoring of coagulopathy with basic coagulation tests can identify patients at higher risk of having worse outcomes.

Implications for Management

With microvascular thrombosis being central to COVID-related end-organ damage, concerted efforts have been made to mitigate this pathway and have proven effective. Several approaches targeting more proximal pathways such as endothelial stabilization and cytokine inhibition are either in early phases or have been refuted to have a significant benefit. Basic coagulation tests in severe COVID have clinical relevance, and it is recommended that all patients have a PT/aPTT/fibrinogen and D-dimer at admission. The strongest prognostic data is available for D-dimer values, which may help to identify patients at a higher risk of worsening.

Prophylactic anticoagulation is recommended for all patients with the idea of preventing macro- and microvascular thromboses and reducing end-organ dysfunction and mortality. However, the benefit of anticoagulation in large studies has been extremely variable. The effect of prophylactic anticoagulation on mortality in COVID was reviewed in a meta-analysis including 6 studies with 6390 patients. No statistically significant reduction in mortality was noted in patients on anticoagulation compared to those without (mortality rate: 17.4% vs. 20.9%, [relative risk [RR]: 1.17], 95% CI: 0.87–1.57).[62] This study had significant heterogeneity in the use of anticoagulant agents and dosing strategies and may not be entirely representative. Similar findings were noted in a Cochrane review including 5929 patients from seven observational studies, with no mortality benefit noted on anticoagulation.[63] This review observed more than 20 upcoming randomized controlled trials (RCTs) testing this premise, which will hopefully provide definitive data.

However, a benefit of prophylactic anticoagulation has been observed in a subset of sicker patients in multiple studies. An observational retrospective study

from Mount Sinai including 2773 patients noted an improvement in survival after using prophylactic anticoagulation only in patients who were on ventilatory support.[64] An observational study from China including 449 patients also showed a benefit for selected patients with severe coagulopathy. An observational study from China including 449 patients also showed a benefit of anticoagulation for selected patients. Patients with a sepsis-induced coagulopathy score of more than 4 and high levels of D-dimer who received anticoagulation had a survival benefit at 28 days (40.0% vs. 64.2%, $P = 0.029$).[65] A cutoff value of D-dimer of more than six times the upper limit of normal has been shown to select patients who show a mortality benefit with prophylactic heparin (mortality rate 32.8% vs. 52.4%, $P = 0.017$).[66] Presumably, prophylactic anticoagulation targets the mechanism of end-organ damage in sicker patients and shows the greatest benefit in this subgroup.

Despite the evidence listed above, it is reasonable to provide universal prophylactic anticoagulation for two reasons. First, the risk of bleeding with severe COVID is low and the benefit of anticoagulation is yet to be disproved in an RCT; on the contrary, anticoagulation can potentially improve organ damage and prevent mortality. Second, reports of calamitous large-vessel thrombosis including PE and ischemic stroke have been reported in COVID-19, which can be prevented with effective anticoagulation.[67,68] The International Society on Thrombosis and Haemostasis (ISTH) guidelines recommend anticoagulation for all patients observing a low risk of bleeding and a potential benefit for patients with more severe coagulopathy.[69] Similar recommendations are echoed in the American Society of Hematology guidelines.

Data on the benefit of more proximal pathways including cytokine inhibition are much less encouraging. Initial data on the central role of IL-6 in COVID-19 prompted the use of IL-6 inhibition in patients with severe COVID, with several studies demonstrating a potentially lower risk of mortality.[70] However, this has been nullified with two randomized trials showing no benefit in terms of risk of severe disease or mortality with tocilizumab use.[71,72] Certain reports have even indicated a higher risk of thrombosis with tocilizumab despite reduction in levels of inflammatory cytokines.[73] Data on IL-1 blockade are in initial stages with no randomized trials available so far.[74]

Patients who develop clinical VTE (DVT or PE) must be initiated on therapeutic anticoagulation after clinical considerations. It is recommended that initial anticoagulation be started with a low molecular-weight heparin (LMWH), which limits staff exposure and can be rapidly reversed in case of bleeding.[75] Those at an especially high risk of bleeding should be initiated on unfractionated heparin and later switched to a longer-acting agent. For patients with low risk of bleeding and no expected drug interactions, apixaban or rivaroxaban can also be utilized. For patients with high-risk PE or hemodynamic deterioration, systemic or catheter-directed thrombosis must be initiated after objective documentation. Anticoagulant therapy is recommended for at least 3 months after discharge in patients with clinical evidence of VTE. A summary of guidelines for established VTE in COVID-19 is provided by a chest expert panel report.[75]

Several new investigational approaches to target COVID-related coagulopathy are being described at a rapid pace, and more data can be expected in the coming months. For instance, it is prudent to note that levels of antithrombin are reduced in patients with COVID, which can reduce the efficacy of unfractionated heparin (UFH) or LMWH and lead to thrombosis while on treatment.[76] It has been suggested that inhibition of protease-activated receptor type 1 with targeted agents like vorapaxar can bypass this phenomenon and provide effective anticoagulation.[77] A small case series demonstrated the utility of plasma exchange in reducing D-dimer and vWF levels in critically ill patients and needs further validation with prospective data.[78] Another interesting premise is to reduce the effect of post-COVID pulmonary compromise caused by fibrin deposition by using nebulized plasminogen. In an initial analysis, nebulized plasminogen has been demonstrated to safely reach smaller airways and may possibly have clinical use.[79]

The risk of venous thromboembolism in patients with COVID-19 appears to be elevated for 4–6 weeks after discharge and must be addressed. The incidence of postdischarge VTE has ranged from 0.5% to 2.5% in various studies.[80] However, the continuation of anticoagulation after discharge cannot be universally recommended due to a 1%–3% risk of bleeding in these patients. While an evidence-based approach to initiate postdischarge anticoagulation is not available, it is reasonable to individualize decisions to anticoagulated after considering the risks of thrombosis and bleeding. For instance, patients expected to have low mobility after discharge or those with an International Medical Prevention Registry on Venous Thromboembolism VTE risk score ≥ 4 can be considered candidates for short-term anticoagulation.[81] This approach is reflected in guidelines by both ISTH and National Institute of Health (COVID-19 Treatment Guidelines Panel, COVID-19 Treatment Guidelines, and National Institutes of Health, available at https://www. covid19treatmentguidelines.nih.gov/, Accessed April 10, 2021).

To summarize the clinical approach, it is recommended that coagulation parameters including PT/aPTT/Fibrinogen and D-dimer be obtained for all patients at baseline and prophylactic anticoagulation started universally in the absence of contraindications. A succinct algorithm for anticoagulation in COVID is provided in Mayo clinic guidelines.[82] For hospitalized patients, parenteral agents including UFH or LMWH are preferable. The agent of choice is enoxaparin at a dose of 40 mg once a day subcutaneously (S/C), increased to BD for patients more than 120 kg or body mass index >40.[83] As values of D-dimer appear to identify patients with mortality benefit with anticoagulation, a more aggressive approach with twice a day anticoagulation is reasonable. Cytokine blockade is still in an investigational approach; IL-6 blockade has not shown any benefit in randomized trials so far in terms of mortality or thrombotic risk.

Conclusions

To summarize, COVID-related inflammation and coagulopathy are intricately linked and responsible for severe disease manifestations. Prophylactic anticoagulation is an easy-to-use treatment modality that may prevent severe disease and mortality. The pathogenesis of COVID-19 is rapidly evolving and newer pathways are being described rapidly. Understanding of mechanistic pathways will enable us to understand newer targets for mitigating acute and chronic manifestations of COVID-19.

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There are no conflicts of interest.

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