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Coagulopathy, thromboembolic complications, and the use of heparin in COVID-19 pneumonia

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ABSTRACT

The SARS-CoV-2 (COVID-19) is causing a pandemic and potentially fatal disease of global public health concern. Viral infections are known to be associated with coagulation impairment; thus, thrombosis, hemorrhage, or both may occur. Understanding the pathophysiologic mechanisms underlying the development of coagulation disorders during viral infection is essential for the development of therapeutic strategies. Coagulopathy in COVID-19 infection is emerging as a precipitant factor for severe respiratory complications and death. An increase in coagulation markers, such as fibrinogen and D-dimer, has been found in severe COVID-19 cases. Heparin, clinically used as an anticoagulant, also has anti-inflammatory properties, including binding of inflammatory cytokines, inhibition of neutrophil chemotaxis, and protection of endothelial cells, and a potential antiviral effect. We hypothesized that low-molecular-weight heparin may attenuate cytokine storm in COVID-19 patients; therefore, low-molecular-weight heparin could be a valid adjunctive therapeutic drug for the treatment of COVID-19 pneumopathy. In this paper, we review potential mechanisms involved in coagulation impairment after viral infection and the possible role of heparin in the treatment of COVID-19 patients. (J Vasc Surg: Venous and Lym Dis 2020;8:711-6.)

Keywords: COVID-19; Pneumonia; Thrombosis; Coagulopathy; D-dimer; Low-molecular-weight heparin

Recent studies have shown that coagulopathy can occur during COVID-19 disease. Thromboembolic manifestations such as pulmonary embolism[1-3] and disseminated intravascular coagulation (DIC)[4] have been reported and have resulted in poor prognosis for the patient.

Our hypothesis is that heparin anticoagulation can be useful in COVID-19. Support of this comes from published mechanisms of coagulation impairment after viral infection. The purpose of this publication is to present this rationale.

THE INTERACTIONS BETWEEN INFLAMMATION AND COAGULATION

A correlation between inflammation and coagulation has been widely demonstrated. Inflammation can lead to altered coagulation, with a consequent imbalance between the procoagulant and anticoagulant states.[5] Several inflammatory cytokines, such as interleukin 6, interleukin 8, and tumor necrosis factor α, promote a procoagulant state through the expression of tissue factor by a mechanism that includes the activation of endothelial cells, platelets, and leukocytes.[6] Endothelial cell activation is characterized by the endothelial expression of cell surface adhesion molecules, such as vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and E-selectin.[7] The platelets are primarily associated with the initiation of coagulation cascades. Platelet activation is stimulated by bound platelet secretion products and local prothrombotic factors. Briefly, after activation, platelets change shape and release the contents of their granules, which contain several molecules, such as inflammatory mediators, hemostatic factors, angiogenic/antiangiogenic factors, growth factors, and protease.[8] During leukocyte activation, the synthesis and release of numerous inflammatory molecules, such as leukotrienes, prostaglandins, bradykinin, free oxygen radicals, and cytokines, occur.[9] Therefore, interaction between endothelial cells, platelets, and leukocytes is a crucial element in the immune response after inflammatory stimuli.

Furthermore, an increased release of histones and nucleosomes (DNA + histones), toxic elements to the endothelium, has been shown in sepsis and other inflammatory conditions. In contrast, activated protein C inactivates histones, protecting the endothelium.[10] In response to the infection, extracellular DNA fibers extruded by neutrophils (neutrophil extracellular traps

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[NETs]) are produced to allow neutrophils to trap and to kill external microorganisms. NETs stimulate the formation and deposition of fibrin to trap microorganisms and to control infection.[11] NETs also cause platelet adhesion, and a link with deep venous thrombosis occurrence has been shown in some experimental models.[12]

D-dimer is an epitope resulting from the plasmin degradation of cross-linked fibrin. D-dimer is elevated in several conditions, such as thrombosis, DIC, and inflammation.[13] Notably, D-dimer can promote the development of inflammation by activating neutrophils and monocytes, inducing the secretion of some inflammatory cytokines.[14,15]

THROMBOCYTOPENIA AND VIRAL INFECTION

In several viral infections, the impairment of platelet function, decrease in production, or platelet destruction has been documented. Thrombocytopenia can occur in both hemorrhagic and nonhemorrhagic viral infections, and it is frequently caused by autoimmune antibodies against platelets. Other mechanisms include increased platelet adhesion and activation, resulting in platelet consumption and bone marrow infection that directly affects megakaryocytes and thus platelet production.[5] In SARS-CoV infection, thrombocytopenia caused by autoantibodies, high levels of von Willebrand factor in the blood,[16] and activation of the coagulation cascade with final generation of fibrin have been described.[17-20]

In COVID-19 disease, thrombocytopenia at hospital admission does not seem to be a consistent factor; in a study of 41 patients, it was found in only 2 patients.[21] However, in a recent meta-analysis of nine studies including nearly 400 patients, the platelet count was significantly lower in patients with more severe COVID-19; also, thrombocytopenia was associated with more than fivefold increased risk of severe COVID-19 disease.[22]

MARKERS OF IMPAIRED COAGULATION IN VIRAL INFECTION

A procoagulative state can be evidenced through an increase in the levels of coagulation proteins. Increased levels of fibrinogen, D-dimer, thrombin-antithrombin complexes, plasmin-α_2-antiplasmin complexes, and thrombomodulin have been found in respiratory tract infections, influenza, and SARS-CoV infection. Furthermore, an increased level of the plasminogen 1 activator inhibitor, suggestive of impaired fibrinolysis, was also reported.[5]

Alterations in laboratory parameters compatible with DIC criteria have been described by Tang et al[4] in 15 deaths for COVID-19. DIC develops as a result of monocyte and endothelial cell activation due to cytokine release after injury, with expression of tissue factor and secretion of von Willebrand factor. The final result is the circulation of free thrombin that can activate platelets and stimulate fibrinolysis with elevated levels of D-dimer and fibrinogen degradation products. Therefore, in the final stage of disease, high levels of D-dimer and products of the degradation of fibrinogen have been reported; markedly elevated D-dimer levels also correlated with more severe disease and mortality.[4,23,24]

VIRAL INFECTION AND COAGULOPATHY

Either bleeding or thrombosis has been described as a complication in several viral infections. An exaggerated response to infection can become complicated up to DIC with the formation of microvascular thrombi in various organs.[25] Respiratory tract infections increase the risk of deep venous thrombosis and pulmonary embolism.[26] In the H1N1 influenza epidemic, both thrombotic and hemorrhagic complications, such as deep venous thrombosis, pulmonary embolism hemorrhage with hemoptysis, hematemesis, petechial rash, and a case of diffuse petechial cerebral hemorrhage, have been reported.[5] The occurrence of DIC, pulmonary hemorrhage, and thrombocytopenia has been reported in avian influenza (H5N1) in several patients.[27] In the severe acute respiratory syndrome induced by coronavirus, vascular endothelial damage in small and medium-sized pulmonary vessels, DIC, deep venous thrombosis, and pulmonary thromboembolism have been described.[18-20]

Tang et al[4] reported a consumption coagulopathy in advanced stages of COVID-19 pneumopathy with elevated levels of D-dimer and fibrinogen degradation products. Extremely increased D-dimer results from plasmin-associated hyperactive fibrinolysis, and plasminogen/plasmin system involvement in COVID-19 disease has been investigated in a recent paper.[28] Enhanced susceptibility to SARS-CoV-2 infection and fatality have been reported in patients with pre-existing comorbidities including hypertension, diabetes, coronary heart disease, cerebrovascular disease, chronic obstructive pulmonary disease, and chronic renal disease.[23] Elevated plasminogen/plasmin is common in people with several underlying medical conditions, and plasmin has been found to enhance the virulence and infectivity of SARS-CoV-2 virus by cleaving its spike proteins.[28]

Older age has been reported as a poor prognosticator for COVID-19 survival,[24] and many factors could be related. First, several diseases coexist in the elderly, predisposing to endothelial dysfunction and coagulation impairment, as discussed before. Second, immune response is different between young and older patients, and it is known that type I interferon responses in men decrease with age.[29] Exacerbated innate host response to SARS-CoV in aged nonhuman primates has been demonstrated with an increase in differential expression of genes associated with inflammation, whereas expression of type I interferon β was reduced.[30] Also, a deficiency in control of viral

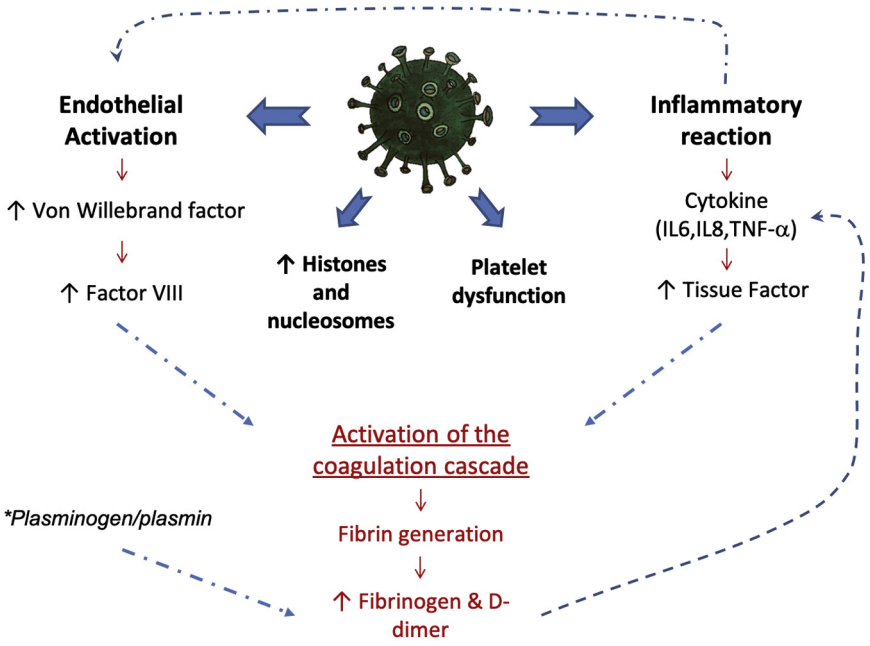

Fig 1. Mechanisms of coagulation impairment in COVID-19 infection. *Elevated levels of plasminogen/plasmin are found in several medical conditions, including hypertension, diabetes, cardiovascular disease, cerebrovascular disease, and chronic renal disease. *IL6*, Interleukin 6; *IL8*, interleukin 8; *TNF-α*, tumor necrosis factor α.

replication could be hypothesized as age-dependent defects in T-cell and B-cell function have been described.[31]

Potential mechanisms of impaired coagulation in COVID-19 infection are summarized in Fig 1.

HISTOPATHOLOGIC FINDINGS

Fibrin accumulation in the lung is a hallmark of acute lung injury and acute respiratory distress syndrome. Fibrin clots in the alveoli have been described in SARS-CoV infection in humans and mice. This coagulation response is hypothesized to protect the host by sealing the alveoli, preventing edema and alveolar hemorrhages but limiting the exchange of oxygen.[32] The first published autopsy report of deaths due to severe COVID-19 infection revealed diffuse alveolar damage with fibrin exudation in alveoli, modest infiltration of immune cells in vessel walls, and hyaline thrombi in small vessels and also in other organs and tissues.[33] Similar findings have recently been reported by American pathologists who described a thrombotic microangiopathy in lung associated with foci of alveolar hemorrhage.[34]

Moreover, a generalized thrombotic microvascular injury has been documented by Magro et al[35] in a series of lung and skin biopsies. Apart from the histologic changes described by the other authors, they found deposits of terminal complement components in the microvasculature, consistent with sustained, systemic activation of complement pathways. Therefore, in a subset of severe COVID-19 infections, a vasculitis-like syndrome with an associated procoagulant state can occur.

RATIONALE FOR THE USE OF HEPARINS IN COVID-19 INFECTION

Heparins are anticoagulant drugs currently used for the prophylaxis and therapy of venous thromboembolism and are classified according to their molecular weight.[36] Heparin reversibly binds antithrombin III and amplifies its subsequent inhibitory effect on activated factor X and thrombin (factor Xa).[37,38] Only unfractionated heparin containing at least 18 saccharide sequences can influence the action of antithrombin on thrombin; however, unfractionated heparin fragments of any length containing a unique pentasaccharide sequence can inhibit the action of factor Xa.[39,40] This feature has been exploited by pharmacologic research for the development of low-molecular-weight heparins (LMWHs).[41]

Fondaparinux, a synthetic analogue of the pentasaccharide sequence, has a longer half-life than LMWH and does not interact with platelets.[42] Fondaparinux binds reversibly to antithrombin to produce an irreversible conformational change that enhances its reactivity with factor Xa. This results in inhibition and depletion of factor Xa, which in turn inhibits thrombin generation in the coagulating signal transduction pathway. Notably, this molecule is characterized by therapeutic coverage for 24 hours and noninterference with platelets. Currently, fondaparinux is indicated for the prophylaxis and treatment of venous thromboembolism.[43]

Heparin also exhibits anti-inflammatory properties.[44] Although still to be fully clarified, some of the proposed mechanisms include binding with inflammatory cytokines, inhibition of neutrophil chemotaxis

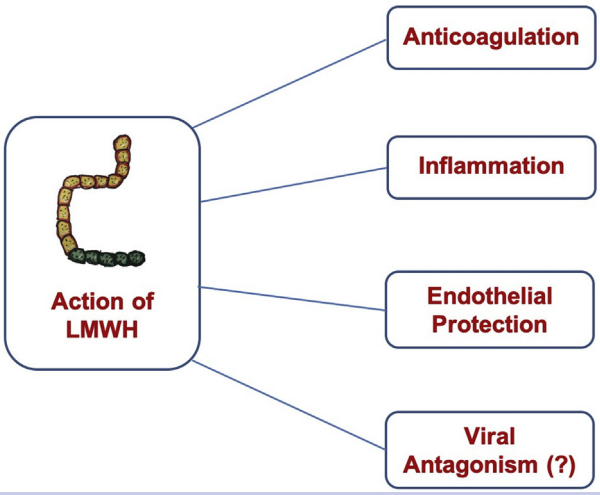

Fig 2. Potential effects of low-molecular-weight heparin (*LMWH*) in COVID-19 disease.

and leukocyte migration, neutralization of complement factor C5a and sequestration of acute phase proteins such as P-selectin and L-selectin, and induction of cell apoptosis through tumor necrosis factor α and nuclear factor κB pathways.[45,46] As described before, histones released from damaged cells can injure endothelial cells,[47] and heparin has been shown to antagonize histones and thus "protect" the endothelium.[48,49] Another mechanism of heparin's action is characterized by its effects on histone methylation and on the mitogen-activated protein kinase and nuclear factor κB signal pathways.[50] Therefore, heparin can protect from microcirculatory dysfunction and possibly reduce organ damage.

Experimental models put in light a potential antiviral role of heparin; indeed, its polyanionic polysaccharide strands can bind to different proteins, and therefore heparin could act as an effective inhibitor of viral adhesion.[51] Competition between heparin and herpes simplex virus for host cell surface glycoproteins has been described, and in zika virus infection, heparin prevented virus-induced cell death of human neural progenitor cells.[51,52] Furthermore, the addition of heparin to experimental cultured cells halved the infection rate from sputum of a patient with severe acute respiratory syndrome pneumonia.[53]

Surface plasmon resonance and circular dichroism, two spectroscopic techniques for molecular analysis, have been used in a recent work and showed that the binding domain of the Spike S1 SARS-CoV-2 protein receptor interacts with heparin.[54]

Potential mechanisms of LMWH action in COVID-19 disease are described in Fig 2.

Finally, in the recent report by Tang et al,[55] a favorable outcome was highlighted with the use of LMWH in patients with severe COVID-19 disease meeting sepsis-induced coagulopathy criteria (Table) or with markedly high D-dimer levels. There were 99 patients who were treated with heparin for at least 7 days; in almost all patients (n = 94), enoxaparin was used subcutaneously (40-60 mg enoxaparin/d); 5 patients received unfractionated heparin (10,000-15,000 units/d).

CONSIDERATIONS AND POSSIBLE THERAPEUTIC IMPLICATIONS

Growing evidence puts emphasis on involvement of the coagulation system due to inflammation in COVID-19 pneumopathy. Previously, in H1N1 patients who developed acute respiratory distress syndrome, empirical systemic anticoagulation significantly reduced venous thromboembolism incidence without increased hemorrhagic complications.[56] Therefore, although data are still numerically insufficient to establish what the appropriate therapeutic regimen may be, the addition of heparin can have a favorable impact on progression of COVID-19 infection.

The reported data suggested that in many cases, the infection may have an asymptomatic course.[57] The use of heparin is probably unnecessary in this population. However, in case of the onset and persistence of respiratory symptoms, even in patients in isolation at home, it is considered useful to start prophylaxis with LMWH or with fondaparinux if renal function is preserved (creatinine clearance >50 mL/min). Should the patient develop a progressive worsening of respiratory symptoms in association with an increase in coagulation markers, therapeutic or subtherapeutic doses of

Table. Sepsis-induced coagulopathy (*SIC*) score

Category	Parameter	0 point	1 point	2 points
Prothrombin time	PT-INR	≤1.2	>1.2	>1.4
Coagulation	Platelet count ($\times 10^9$/L)	≥150	<150	<100
Total SOFA	SOFA four items[a]	0	1	≥2
Total score for SIC ≥4				

PT-INR, Prothrombin time-international normalized ratio; *SOFA*, sequential organ failure assessment.
[a]Total SOFA is the sum of the four items (respiratory SOFA, cardiovascular SOFA, hepatic SOFA, renal SOFA). The SOFA score was included to confirm the presence of sepsis but does not reflect sepsis severity; therefore, the score for SOFA was limited to 2 points even if the SOFA score was more than 2. (Adapted from Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost 2020;18:1094-9.)

LMWH could be hypothesized, taking into account the clinical characteristics and hemorrhagic risk; however, a definitive recommendation cannot be provided because of the lack of consistent published data. In advanced states, as powerful intravascular generation of thrombin occurs, unfractionated heparin could have a role, especially in cases of severe renal failure (creatinine clearance <30 mL/min) because of impaired LMWH renal excretion. Furthermore, in these patients, careful monitoring of the coagulation parameters is necessary because of the possible development of DIC in the end stage of the disease.[4]

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