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Molecular Pathophysiology of COVID-19-Associated Thrombosis: From Viral RNA Recognition to Cytokine Storm and Thromboembolism

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), has 79% genomic similarity to severe acute respiratory syndrome coronavirus (SARS-CoV), the cause of severe acute respiratory syndrome (SARS),. The SARS-CoV genome contains an extraordinary number of guanine-uracil (GU)-rich regions in its single-stranded ribonucleic acid (ssRNA) genome. GU-rich ssRNA fragments are a pathogen associated molecular pattern (PAMP) which activates innate immunity by binding to Toll-like receptor 8 (TLR8). The large number of GU-rich ssRNA fragments causes massive activation of innate immunity, resulting in high levels of proinflammatory cytokines, including interleukin-6 (IL-6). This cytokine upregulates synthesis of acute phase reactants. Acute phase reactants, particularly fibrinogen, increase blood viscosity, especially at low shear rates. Immunoglobulins also increase blood viscosity. Increased blood viscosity creates larger areas of lower shear in the vascular tree. Endothelial production of antithrombotic molecules such as nitric oxide and prostacyclin, inflow of fibrinolytic activity, and dispersion of activated

coagulation factors are decreased in these areas, promoting thrombosis. IL-6 also fosters formation of neutrophil extracellular traps (NETs), which could promote microthrombus formation. Thrombotic complications were also seen in SARS. The genomic similarity of SARS-CoV-2 to SARS-CoV suggests that the pathogenesis of thrombosis in these coronaviruses is hyperactivation of TLR8-mediated pathways, leading to high levels of IL-6, fibrinogen, blood viscosity, and NETs.

Thrombotic complications often occur in cases of coronavirus disease 2019 (COVID-19) [1]. Recently, Klock and colleagues found a 49% incidence of thrombosis in COVID-19 patients treated in intensive care units despite thromboprophylaxis with low molecular weight heparin [2]. These thrombi involve both large and small vessels [3,4,5]. Anticoagulation was associated with decreased mortality in COVID-19 patients in a study by Tang et al. [6]. We propose that thrombosis in COVID-19 is fostered by increased blood viscosity. This, we believe, is a consequence of cytokine storm syndrome which causes extreme elevations in the concentrations of acute phase reactants. Further, we propose that the high frequency of guanine-uracil (GU)-rich nucleotide sequences in the genome of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the single-stranded RNA (ssRNA) virus which causes COVID-19, hyperstimulates the innate immunity, ultimately resulting in thrombosis.

Innate immunity can be activated when molecules known as “pathogen-associated molecular patterns” (PAMPs) bind to Toll-like receptors (TLRs) [7]. These are a family of thirteen receptors, designated TLR1 through TLR13. GU-rich fragments of ssRNA are PAMPs.

Severe acute respiratory syndrome coronavirus (SARS-CoV) is the cause of severe acute respiratory syndrome (SARS), which originated in 2002. Its genome contains 904 GU-rich ssRNA segments in 130 regions, compared to only 130 segments in 16 regions in the human immunodeficiency virus-1 (HIV-1) genome [8]. The SARS-CoV-2 genome has 79% identity with that of SARS-CoV [9]. Thus, the high content of GU-rich regions in SAR-CoV-2 may cause hyperactivation of innate immunity resulting in a cytokine storm.

The pathogenicity of coronaviruses correlates with their ability to elicit host cytokine/chemokine responses [10,11]. Binding of GU-rich ssRNA to TLR8 increases synthesis of interleukin 6 (IL-6) and other cytokines in mice and in human peripheral blood mononuclear cells in vitro [8]. IL-6 plays a central role in cytokine storm syndrome in COVID-19 [12]. A longitudinal study of cytokine levels showed that IL-6 concentrations are elevated within three days of disease onset in patients with severe disease [13], demonstrating the contribution of innate immunity to the cytokine storm. Given that viral load remains substantial into the third week of symptoms [14], the TLR8 pathway could continue to generate IL-6 for a similar period of time. IL-6 levels stay elevated for at least 16 days after disease onset [13], enough time for acquired immunity to contribute to the cytokine storm. A majority of COVID-19 patients have detectable serum IgA in the first week of clinical symptoms, demonstrating the activity the acquired immunity [15].

A meta-analysis has shown that IL-6 concentrations are 2.9 times higher in patients with complicated COVID-19, defined as acute respiratory distress syndrome, admission to an intensive care unit, or “severe” or “critical” disease as defined by the Chinese New Coronavirus Pneumonia Prevention and Control Program score, compared to those with non-complicated disease (Coomes EA, Haghbayan H. Interleukin-6 in COVID-19: A systematic review and

meta-analysis. In preparation. doi.org/10.1101/2020.03.30.20048058). IL-6 upregulates hepatic synthesis of fibrinogen and other acute phase reactants [16]. Fibrinogen levels can be extraordinarily high in severely ill COVID-19 patients, reaching more than 9g/L (normal: 2 to 4 gm/L) [17]. Molecules with large diameters such as fibrinogen can act like "glue" between erythrocytes and augment erythrocyte aggregation [18]. This is the basis of the erythrocyte sedimentation rate (ESR). In areas of slow blood flow (i.e., low shear rates), progressive erythrocyte aggregation ("rouleaux formation") occurs, causing blood viscosity to increase exponentially [19]. When acquired immunity becomes active, elevated concentrations of immunoglobulins, particularly IgM and IgA, will also increase erythrocyte aggregation and blood viscosity [18].

The 19th-century German pathologist Rudolph Virchow noted that areas of sluggish blood flow are predisposed to thrombosis. Sluggish blood flow is nothing more than the expression of blood with high viscosity, which develops because blood is a non-Newtonian fluid, i.e., its viscosity varies with its shear rate. Areas of sluggish blood flow or low shear occur in veins and areas of changing arterial geometry. This is why atherothrombosis occurs preferentially near vascular bifurcations [20]. Muscle contraction is needed to minimize stasis of blood in leg veins and prevent thrombosis.

A decreased shear rate decreases shear-mediated endothelial production of antithrombotic molecules such as nitric oxide and prostacyclin, causing the phenomenon often called "endothelial dysfunction." A decreased shear rate also decreases dispersion of activated coagulation factors and decreases influx of fibrinolytic activity [20]. These factors make areas of low shear prone to thrombosis. By increasing blood viscosity, hyperfibrinogenemia, being part

of the acute phase reaction, is a fundamental link between inflammation and thrombotic complications [21].

To date, blood viscosity has not been studied in COVID-19 patients. However, a study of fifteen critically ill COVID-19 patients showed that all had elevated plasma viscosity [22]. Values ranged from 1.9 to 4.2 centipoise (cP) (normal: 1.4 to 1.8 cP). All patients with values greater than 3.5 cP had thrombotic complications. Blood viscosity would naturally be greater than plasma viscosity because hematocrit is the strongest determinant of blood viscosity. Normal blood viscosity is 3.26 ± 0.43 cP at a shear rate of 100/s [23].

Professor Holger Schmid-Schoenbein, a pioneer of hemorheology, likened the risk of thrombosis caused by increased blood viscosity to the accumulation of deadwood in a forest. Preventing a spark decreases the risk of a conflagration, but normalizing the risk requires removing the deadwood. Preventative measures in intensive care units decrease the risk of clotting, but the risk remains elevated until blood viscosity is reduced [24].

Another reason to suspect elevated blood viscosity as the cause of the high incidence of thrombosis in critically ill COVID-19 patients is the higher prevalence of thrombotic complications in elderly COVID-19 patients and those with hypertension, diabetes, obesity, and chronic obstructive pulmonary disease [20]; in precisely these patients elevated blood viscosity already exists. In patients with pre-existing elevated blood viscosity, COVID-19 may further increase blood viscosity sufficiently to cause thrombosis.

The acute phase reaction increases low shear blood viscosity and the risk of thrombosis. Therefore, an increased risk of thrombosis is seen with many infections including influenza, bacterial pneumonia, and malaria as well as immunization with Heplisav-B, a hepatitis B vaccine

[19]. Thrombophilia is also a feature of SARS. Thirty percent of critically ill SARS patients had venous thromboembolism in one study [25]. We have recently published an in-depth review dealing specifically with the role of blood viscosity in infectious diseases [19].

Patients hospitalized for pneumonia have an elevated risk of myocardial infarction for ten years [26]. This is probably because a clinically-inapparent mural thrombus which develops during the period of thrombophilia will organize into a lesion indistinguishable from an atherosclerotic plaque if the thrombus is not eliminated first by fibrinolytic activity. Exhaustion of fibrinolytic activity has been described in severe COVID-19 [27], increasing the chances of organization. This, coupled with the severe thrombophilia affecting critically ill COVID-19 patients, could increase their risk of myocardial infarction even more and for a longer time.

An increased risk of thrombosis can be suggested by an unexpectedly elevated ESR, which is an acceptable surrogate marker for blood viscosity at low shear rates if anemia is not present. Regarding anemia, a restrictive transfusion strategy is appropriate in these patients because transfused erythrocytes have decreased deformability, which increases blood viscosity.

Elevated levels of IL-6 also foster the development of neutrophil extracellular traps (NETs) [28]. These are net-like structures composed of neutrophil chromatin and cytoplasmic granule contents. Formation of NETs is another aspect of the immune response which becomes dysregulated in severe COVID-19 [29]. Intravascular NETs develop under conditions of low shear found in hepatic sinusoids and capillaries and can reach a diameter of tens of microns, much larger than a capillary [30]. They foster a focal prothrombotic environment because enzymes released from neutrophils inactivate anti-coagulant proteins such as antithrombin, thrombomodulin, protein C and tissue factor pathway inhibitor [31]. Further, platelets interact with NETs in COVID-19 [29]. Thus, NETs could easily serve as a nidus for microthrombus

formation. The tendency for microthrombosis could be accentuated by sluggish blood flow due to hyperviscosity. Uncontrolled NET formation together with hyperviscosity could account for the high incidence of microthrombi and capillary intussusception noted at autopsy of patients with pulmonary failure due to COVID-19 [32], a syndrome called “pulmonary intravascular coagulopathy” [33]. Significantly, these findings were much less frequent in patients who died of pulmonary failure due to influenza [32].

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