

Multifactorial pathogenesis of COVID-19-related coagulopathy: Can defibrotide have a role in the early phases of coagulation disorders?

Thrombotic complications emerged as an important issue in patients with coronavirus disease 2019 (COVID-19). Consolidated reports regarding the clinical and laboratory findings in COVID-19 patients reveal thrombocytopenia, elevated D-dimer, prolonged prothrombin time, disseminated intravascular coagulation (DIC), and pulmonary intravascular coagulation (PIC).^{1,2} Recently, some authors^{3,4} emphasized the potential role of antiphospholipid antibodies in the pathogenesis of thrombotic events in patients with severe COVID-19. However, some articles recently published in *Journal of Thrombosis and Hemostasis*^{5,6} have pointed out some doubts affirming that the high levels of C-reactive protein (CRP) observed in COVID-19 patients may lead to false positive results and that the lack of immunoglobulin G (IgG) and/or immunoglobulin M (IgM) titers, as in the case series by Zhang et al,³ could have precluded the evaluation of the role of antiphospholipid antibodies in the pathogenesis of the thrombotic sequelae. Moreover, as noted by Tang,⁶ two of the three cases mentioned in the letter by Zhang et al³ also seem to meet the International Society on Haemostasis and Thrombosis criteria for DIC, and therefore, causality between antiphospholipid antibodies and thrombosis in those patients cannot be confirmed.

Given the evolution by stage of COVID-19, it is difficult to establish the exact moment of the onset of the thrombotic phenomena. However, it is conceivable that these events originate during the transition from resistance to tolerance phase, even if an activation of platelet hyper-aggregation phenomena may be present in the very early stages of COVID-19.

In our opinion, macrophages are the key cells in inducing these phenomena. It is known that macrophage activation as well as macrophage viral infection itself determines the production of immunomodulating cytokines and reactive oxygen species (ROS) from the early stages of the infection.⁷ Traditionally, macrophages have been assumed to be the source of the majority of the ROS in the vessel walls, and undoubtedly these cells play an important role in vessel pathology.⁸ In addition, macrophages infected with severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) produce the procoagulant prothrombinase, which is important for both the

initiation and localization of fibrin deposition.⁹ No less important is the ability of macrophages to induce the liver synthesis of fibrinogen and thromboplastin. Also, the direct damage inflicted on endothelial cells by cytokines, particularly IL-6, and ROS is probably crucial. As a protective mechanism, the endothelium functions relatively independent of the mitochondrial pathway of energy supply, and the synthesis of adenosine triphosphate (ATP) in activated endothelium occurs mainly via a glycolytic pathway.¹⁰ Similarly, mitochondrial modulation of free radicals, calcium homeostasis, and iron-sulfur clusters in endothelial cells controls the response to inflammation, oxidative stress, and apoptotic stimulus.¹⁰ Accordingly, depending on specific tissue needs and local stresses, endothelial cells are capable of evoking either antithrombotic or prothrombotic events. Healthy endothelial cells express antiplatelet and anticoagulant agents that prevent platelet aggregation and fibrin formation. In case of damage, endothelial cells trigger fibrin formation, as well as platelet adhesion and aggregation.¹¹

Indeed, damage to the endothelial barrier results in uncovering of subendothelial matrix components, of which collagen and tissue factor (TF) are considered to be the most active hemostatic components.¹¹ Consequently, endothelial damage can trigger the activation of platelets (eg, via the collagen receptor glycoprotein VI) and the TF-dependent generation of thrombin and fibrin (via the extrinsic coagulation pathway of factor VII).

Additionally, endothelial damage is associated with the disruption of the "glycocalyx," a structure that covers the endothelium surface and regulates thrombus formation, vascular permeability, and inflammation, thus activating the synthesis of adhesion factors that recruit platelets and leukocytes into the endothelium.¹¹

Macrophage-derived ROS directly inactivate the endothelium-derived nitric oxide, act as cell-signaling molecules, and promote protein dysfunction, events that contribute to the initiation and progression of endothelial dysfunction. The major vascular damaging ROS is the superoxide anion (O_2^-), which leads to consequent accumulation of H_2O_2 and inactivation of nitric oxide (NO), the main vascular relaxing factor, thus playing a critical role in the modulation of vascular remodeling. Finally, the reaction product of O_2^- and NO, peroxynitrite, is a strong oxidant molecule, which can oxidize proteins, lipids, and nucleic acids, and thus, cause cell damage.⁸

In addition to ROS, proinflammatory cytokines, particularly interleukin (IL)-6, upregulate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway, which is also associated with the activation of the apoptotic pathway in the endothelial cells by mediating mitochondrial damage and endoplasmic reticulum stress.¹¹

Another factor, the macrophage stimulating 1 (Mst1), associated with inflammatory response, can activate mitochondria-dependent endothelial cell death through the inhibition of mitophagy, and represents the primary trigger of venous endothelial cell apoptosis. Mst1 also regulates the levels of oxidative stress and mitochondrial performance through inhibition of the expression of transcription factor NF-E2 p45-related factor 2 (Nrf2).¹² Nrf2 regulates the expression of phase II detoxifying enzymes including NADPH, NAD(P)H quinone oxidoreductase 1, glutathione peroxidase, ferritin, heme oxygenase-1, and antioxidant genes that protect cells from various injuries via their anti-inflammatory effects.¹³ Thus, Mst1 and NF- κ B signaling pathways have been found to be related to the endothelial cell damage associated with inflammatory response.

Therefore, it is more likely that the observed thrombotic phenomena in COVID-19 patients, partly attributed to antiphospholipid antibodies, rather configures a picture similar to that of hepatic veno-occlusive disease (VOD), also called sinusoidal obstruction syndrome (SOS). In fact, the autoptic examination of lung tissue so far obtained from COVID-19 infected individuals¹⁴ show patterns similar to the early alveolar epithelial and lung endothelial injury observed in the histopathological examination of lung lesions in VOD/SOS syndrome. VOD/SOS is a potentially life-threatening complication observed after hematopoietic stem cell transplantation (HSCT),¹⁵ with a complex pathophysiologic cascade which is initiated by a toxic injury from the conditioning regimen, with subsequent endothelial cell damage, and a prothrombotic-hypofibrinolytic state leading to central venous occlusion and sinusoidal obstruction. This is associated with an increase in von Willebrand factor expression and platelet adhesion. Moreover, vascular endothelial cells constitute a target for the blood-borne executors of the immune system, B and T cells. Furthermore, pro-inflammatory and pro-apoptotic changes contribute to endothelial damage after conditioning. Endothelial lesions after HSCT are not limited to the sinusoids but can lead to different endothelial syndromes, including VOD/SOS, capillary leak syndrome, engraftment syndrome, transplant associated microangiopathy, or diffuse alveolar hemorrhage. These changes facilitate the egress of red blood cells, leucocytes, and cellular debris and dissect the endothelial cell lining. Finally, the damaged sinusoidal lining embolizes downstream and obstructs sinusoidal flow. In the early stage, histological examination shows thickening of the subintimal zone, which leads to a narrowing of the venular lumen and an increased resistance to blood flow.¹⁵

Defibrotide, which is recommended for the management of VOD/SOS, has a favorable risk/benefit profile, was the first drug approved in the United States for treatment of hepatic VOD/SOS with renal or pulmonary dysfunction following HSCT and in Europe for treatment of severe hepatic VOD/SOS post-HSCT. Thus, all the

above reported evidence not only further support a similarity between the early thrombotic disorders in COVID-19 and VOD/SOS, but also allow us to hypothesize a role of defibrotide in the initial stages of the disease before the use of low molecular weight heparin.

That defibrotide can influence conditions with inflammation and oxidoreductive stress has long been known. Several studies since the 1990s have shown that in addition to the profibrinolytic action of defibrotide, which is due to stimulation of the tissue plasminogen activator and inhibition of plasminogen activator inhibitor in the blood stream, defibrotide also exerts an antithrombotic action through alternative mechanisms that include a peculiar antioxidant and anti-inflammatory capacity.¹⁶⁻¹⁸ Therefore, it is capable of interfering with the procoagulant state associated with inflammation. In fact, it has been reported that defibrotide inhibits the synthesis of proinflammatory cytokines by activated macrophages and mononuclear cells, in particular of IL-6 and tumor necrosis factor *in vivo*.¹⁹ Defibrotide also inhibits the generation of O₂⁻ by activated monocytes/macrophages. Other effects ascribed to defibrotide are an increase in nitric oxide generation and nitric oxide synthase (NOS) activity. Defibrotide is very efficient in attenuating the generation of ROS and is capable of restoring endothelial NOS levels in the face of oxidative stress.¹⁸ Additionally, *in vitro* or *ex vivo* studies have demonstrated that defibrotide blocks TF expression induced by inflammatory mediators, promotes expression of thrombomodulin by endothelial cells, attenuates prothrombinase activity, and reduces thrombin-induced platelet aggregation and platelets/leukocyte endothelial cell interactions in the vascular bed.²⁰

Notably, Myers et al²¹ showed that the use of high-dose methylprednisolone, similar to defibrotide, is effective for the treatment of VOD/SOS, thus supporting the role of macrophage-related inflammation in the pathogenesis of the microembolism observed in VOD/SOS as well as COVID-19. The involvement of macrophages in these events is confirmed by the ability of defibrotide to inhibit heparanase, which is a key factor required for the activation and function of macrophages.²²

Therefore, the latest evidence suggests that the COVID-19 coagulopathy is an evolving phenomenon in which endothelial damage, platelet aggregation, and other events as systemic inflammation favoring coagulation would contribute to the severity of this disease.

We believe that early anticoagulation and early thrombolytic agents may achieve better prognosis in these patients. Additionally, recent reports suggest that defibrotide could be the drug of choice for its pro-fibrinolytic, antithrombotic, thrombolytic, as well as anti-inflammatory and antioxidant functions that may help to restore endothelial function.

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CONFLICTS OF INTEREST

The authors have no competing interest to declare.

AUTHOR CONTRIBUTIONS

AM, CM, GC, and GLN contributed to study design, data analysis, literature review, manuscript drafting, and final editing.

KEYWORDS

COVID-19, defibrotide, inflammation, macrophages, oxidative stress, pulmonary intravascular coagulation

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