COVID-19 revolution: a new challenge for the internist

COVID-19 coagulopathy: towards the understanding of pathogenesis

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ABSTRACT

As the main title '*COVID-19 revolution: a new challenge for the internist*' states, the global coronavirus infection disease 2019 (COVID-19) pandemic represented a new challenge for the internists. This paper is part of a series of articles written during the difficult period of the ongoing global pandemic and published all together in this fourth issue of the *Italian Journal of Medicine*, with the aim of sharing the direct experiences of those who were the first to face this severe emergency, expressing each point of view in the management of COVID-19 in relation to other diseases. Each article is therefore the result of many efforts and a joint collaboration between many colleagues from the Departments of Internal Medicine or Emergency Medicine of several Italian hospitals, engaged in the front line during the pandemic. These preliminary studies therefore cover diagnostic tools available to health care personnel, epidemiological reflections, possible new therapeutic approaches, discharge and reintegration procedures to daily life, the involvement of the disease not only in the lung, aspects related to various comorbidities, such as: coagulopathies, vasculitis, vitamin D deficiency, gender differences, *etc.*. The goal is to offer a perspective, as broad as possible, of everything that has been done to initially face the pandemic in its first phase and provide the tools for an increasingly better approach, in the hope of not arriving unprepared to a possible second wave.

This paper in particular deals with the pathogenesis of COVID-19.

To date, the pathophysiology of coronavirus infection disease 2019 (COVID-19) remains partially unknown; however, increasing evidence suggests how a dysregulated hemostatic process could play an important role.

Several studies have reported abnormalities in coagulation parameters of COVID-19 patients, often characterized by increased levels of D-dimer and fibrinogen, mild thrombocytopenia, and slight or no change in partial thromboplastin time (PT) and activated partial thromboplastin time.[1] Furthermore, elevated D-dimer has been associated with disease severity and in-hospital mortality.[1-3] Tang *et al.* showed how d-dimer dynamics reflect disease evolution and how fibrinogen remains high, except for the final stages of disease in non-survivor patients who often develop disseminated intravascular coagulation. In survivor patients, disseminated intravascular coagulation (DIC) was an extremely rare event.[4]

COVID-19 associated coagulopathy (CAC) seems to be peculiar and different from bacterial sepsis-induced coagulopathy (SIC) and DIC where the increase of PT and the decrease of platelets count and fibrinogen is predominant. Moreover, in SIC/DIC, D-dimer levels do not rise in relation to disease severity due to the suppression of fibrinolysis (fibrinolysis shutdown).[5]

In the early stages of the disease, inflammation and thrombosis are localized within the lung, where severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry in the cell through angiotensin-converting enzyme 2 (ACE-2), largely expressed in type 2 pneumocyte and endothelial cell, causing endothelitis with a massive release of plasminogen activators.[6] Moreover, alveolar macrophages release urokinase-type plasminogen activator, upregulating local fibrinolysis in alveoli, that could explain the mismatched D-dimer elevation; with the progression of infection,

down-regulation of ACE-2 receptors causes an increase of angiotensin II, which induces vasoconstriction and hypercoagulability.[7]

Lung pathology shows capillary congestion, pneumocyte necrosis, interstitial hyaline membrane, intra-alveolar edema, type 2 pneumocyte hyperplasia with marked microvascular thrombosis, and hemorrhage. Carsana *et al.* observed fibrin thrombi in the small arterial vessels in 87% of cases and high levels of D-dimer in the blood; moreover, these microthrombi were often located along the areas of alveolar damage and associated with endothelial damage.[8] Such evidence may justify activation of the coagulation cascade strictly connected to the local inflammatory response.

Inflammatory burst seems to play a pivotal role in thrombi formation. The link between inflammation and thrombosis is well established, so much so that the term '*immunothrombosis*' has been coined to embrace this process.[9] During infections, there is a crosstalk between immune cells and coagulation aimed at limiting the dissemination of pathogens within the body.[10,11] A lot of mechanisms and pathways seem to be involved in this process of stress-induced physiological hemostasis. However, these same elements, in the event of a dysregulated response, could lead to intravascular thrombosis and organ damage.

Innate immune cells play a crucial role in clot formation.[9] The complement system is a critical mediator of innate immune response that, in addition to promoting inflammation and defending against bacteria and viruses, is closely connected with coagulation and fibrinolysis processes. Three pathways activate the complement cascade: the classical pathway triggered by the antibody-antigen complex, the alternative pathway started by surface antigens, and the lectin pathway primed by binding mannose residues on pathogens surface. All these pathways converge on the common pathway that, through the activation of c3 convertase, produces powerful anaphylatoxins (C3a, C5a), C3b-initiated pathogen opsonization, and formation of C5b-9 membrane attack complex, playing a pivotal role not only in the lysis of target cells but also in the regulation of the coagulation process through platelet activation, tissue factor (TF) induction, and endothelial cell activation by increasing the secretion of von Willebrand Factor (vWF) and p-selectin.[12] Evidence of complement activation in SARS-CoV-2 infection comes from autopsy studies, where C5b-9, C4b, and mannan-binding lectin serine protease-2 deposits have been found in the lung, indicating generalized activation of leptin and alternative pathways.[13] These findings may explain the picture of macro- and microvascular thrombosis that notoriously worsens the course and prognosis of SARS-CoV-2 infection.

Moreover, disproportioned complement activation in a well-known pathogenetic mechanism underlying thrombotic microangiopathy (TMA), as in the case of the atypical hemolytic uremic syndrome,[14] which is characterized by diffuse microvascular thrombi, similar to those observed in autopsy cases of COVID-19. Another possible connection with thrombotic microangiopathies is the increase of vWF caused by vascular injuries. Laboratory biomarkers were seen in COVID-19 as a decrease of hemoglobin, increase in lactate dehydrogenase and bilirubin resemble those in mild microangiopathic hemolytic anemia, confirmation a possible overlapping physiopathology between CAC and TMA.

However, the complement system, although playing a key role in the immunothrombosis process, is not the only actor. In innate immunity, the complement system is closely connected with the Toll-like receptors (TLRs) pathway, present on endothelial cells, platelets, antigen-presenting cells such as macrophages and dendritic cells. TLRs are implicated in the processes of phagocytosis and the production of cytokines such as tumor necrosis factor-a, interleukin (IL)-6, IL-12, and IL-1b and in the increase in TF exposure.[15]

The link between inflammation and thrombosis is also strengthened by the activation of neutrophils with the release of neutrophil extravascular traps (NETs), a web-like structure comprising DNA, histones, proteolytic enzymes, and other proteins[16] that, in addition to trapping circulating cells and pathogens,[17] can exert multiple procoagulant effects, causing activation of endothelial cells and platelets *via* TLR-2 or TLR-4 dependent signaling pathways.[18,19] While macrophages, monocytes, and lymphocytes are recruited into lung tissue by the local immune response provoked by SARS-CoV-2, neutrophils are poorly present, and this could be a crucial aspect of the dysregulation of innate immunity; in fact, the persistence of neutrophils in the bloodstream and their ability to form NETs may contribute to the thrombotic manifestation of this disease.[20]

Endothelial damage following inflammation causes the release of phospholipid membrane vesicles, called microparticles, which exhibit a wide range of inflammatory and coagulative events, such as stimulating cells to release inflammatory cytokines and express TF.[15]

All these elements tend to establish a vicious circle that amplifies the relationship between inflammation and thrombosis during infection.

A possible confirmation that thromboembolic complications are directly related to the degree of inflammation and, consequently, to disease severity, comes from a Dutch single-center cohort study, in which the frequency of events was significantly higher in patients admitted to intensive care units.[21] Furthermore, Intensive Care Unit patients received twice the dosage of low-molecular-weight heparin compared to patients admitted to the wards, which would suggest that once the thrombogenic stimulus has triggered, it

will become poorly responsive to conventional antithrombotic drugs.

It would be interesting to evaluate whether immunomodulating drugs, capable of reducing inflammatory burden, and complement activation, could play a role in the control of thrombotic events.

Pathophysiology of COVID-19 associated coagulopathy is complex, multifactorial, and still partly unknown. CAC mimics some features of DIC and TMA, but, in relation to its unique characteristics, it deserves to be defined as a new category of coagulopathy, and further knowledge is needed for appropriate management.

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