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EDITORIAL COMMENT

Platelets and the Endothelium

Active Participants in Severe COVID-19 Infection*

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The coronavirus disease 2019 (COVID-19) pandemic currently gripping the planet is presenting the biggest acute public health crisis in generations, impacting not only physical well-being but also exerting huge social, economic, and organizational pressure, and diverting resources and attention from other life-threatening conditions. Although the initial phase of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is typically mild, a significant proportion of patients go on to experience severe COVID-19, characterized by pneumonitis and a systemic inflammatory response that can lead to multiorgan failure and death. Beyond prevention with public health interventions, key to tackling the disease is gaining an awareness and understanding of the underlying pathophysiology, in terms of the infection itself and the host response. Identifying prominent actors in these processes can help find candidate treatments.

It is now established that severe COVID-19 is associated with significant increases in a range of cytokines, including interleukin-6 (IL-6) (1). It is also clear that microthrombosis and macrothrombosis contribute significantly as pathological mechanisms. This can manifest as, for example, microvascular dysfunction, pulmonary emboli, or disseminated intravascular coagulation, and an increased risk of atherothrombotic events, such as acute myocardial infarction and ischemic stroke. Furthermore, a strong association between pre-existing cardiovascular disease and outcomes from severe COVID-19 raised hypotheses about shared mechanisms.

Now, in this issue of *JACC: Basic to Translational Science*, Canzano et al. (2) provide valuable and detailed insights into platelet and endothelial function during COVID-19, including stratification by severity of the illness. The authors, based in Milan, Italy, the early epicenter of the pandemic in Europe, should be congratulated for undertaking this work in such challenging conditions.

Through a series of elegant studies including direct measurements and incorporation of samples into in vitro models from patients with COVID-19, they paint a picture of profound dysregulation of platelets and the endothelium, fueled by an intense inflammatory response.

Four main processes of particular significance are identified. First, IL-6 seems to be a key cytokine driving platelet hyperreactivity in COVID-19 infection. Although in this study the mechanism for this observation was not fully determined, recent reports have suggested that IL-6 signaling may stimulate platelets via interaction with the platelet collagen receptor glycoprotein VI (3). Furthermore, a monoclonal antibody against IL-6, tocilizumab, seemed to abrogate these effects, and there is growing evidence this agent may improve clinical outcomes.

Second, platelet-derived microvesicles containing tissue factor seem critical. Tissue factor activates factor VII and therefore the extrinsic pathway of the coagulation cascade, resulting in generation of thrombin that cleaves soluble fibrinogen to insoluble fibrin and activates platelets via protease-associated receptors 1 and 4 (4). In this study, prophylactic or therapeutic levels of low-molecular-weight heparin were unable to adequately suppress thrombin

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generation, suggesting that more potent or broad antithrombotic therapy may be needed to halt this process.

Third, increased levels of platelet-leukocyte aggregates observed during COVID-19 infection underline the role that activated platelets play in directly stimulating inflammatory cell function. These form via platelet surface expression of P-selectin, contained within α-granules that fuse with the cell membrane on platelet activation, binding to leukocyte P-selectin glycoprotein ligand-1 (4).

Finally, endothelial dysfunction clearly plays a role. Depletion of nitric oxide (NO), an inhibitor of platelet activation and anti-inflammatory at normal physiological concentrations, was observed. There was also evidence of increased endothelial prostacyclin (PGI_2). Although the balance of benefits and harms of PGI_2 in acute inflammation remain to be fully explored, it is clear that, although it acts to inhibit platelet activation, PGI_2 also leads to vasodiliation that, if excessive, may contribute to circulatory collapse.

No doubt there are other responsible mechanisms, including such systemic factors as increased circulating catecholamines and serotonin, which may increase platelet reactivity. The fact that samples in this study were negative for the virus mean any direct effect of SARS-CoV-2 on platelets and the endothelium has not been assessed here. Although it remains contentious, there is some evidence from other work that the virus binding to platelet angiotensin converting enzyme-2 may contribute to activation.

What implications do these findings have for treatment of COVID-19? As well as direct targeting of the inflammatory response, it is logical to consider what antithrombotic therapy might be of benefit during COVID-19 infection. In the absence of confirmed venous thromboembolism, only heparin (low-molecular-weight heparin or unfractionated) at subtherapeutic doses is currently recommended (1), but the findings of this study may support the investigation of more intense routine anticoagulation. Considering the apparently prominent role of platelets in COVID-19, antiplatelet therapy may also be rational. The most common antiplatelet agents available are the cyclooxygenase inhibitor aspirin and oral $P2Y_{12}$ inhibitors, such as the thienopyridines clopidogrel and prasugrel, and the cyclopentyl-triazolopyrimidine ticagrelor (4). Among the $P2Y_{12}$ inhibitors, clopidogrel is less potent and reliable than prasugrel and ticagrelor. However, subtleties in the effects of each agent may hypothetically influence overall benefits and risk in severe COVID-19.

For example, there is convincing evidence that $P2Y_{12}$ inhibition significantly inhibits platelet P-selectin expression. Although Canzano et al. (2) also show that aspirin reduced platelet P-selectin expression in vitro, this has not been reliably shown in clinical studies outside the setting of COVID-19, and it remains clear that any ability of aspirin to inhibit this is much less than that of $P2Y_{12}$ inhibition.

Pleiotropic effects also need to be considered. Aspirin may counteractively potentiate the inflammatory response, crucially increasing plasma IL-6 during states, such as endotoxemia, in contrast to $P2Y_{12}$ inhibitors that reduce it (1). Ticagrelor, but not other $P2Y_{12}$ inhibitors, potentiates adenosine-induced stimulation of neutrophil chemotaxis and phagocytosis via local inhibition of adenosine uptake through equilibrative nucleoside transporter-1 on erythrocytes and platelets, whereas clopidogrel may have an effect of reducing circulating leukocyte count and cytokines, such as IL-6, compared with ticagrelor. However, both aspirin and ticagrelor, but not the other agents, may theoretically counteract endothelial NO depletion (5). Aspirin may increase NO availability via mechanisms, such as acetylation of endothelial NO synthase (eNOS); increasing the activity of dimethylarginine-dimethylaminohydrolase, responsible for degrading the endogenous eNOS inhibitor asymmetric dimethylarginine; and generation of 15-epi lipoxin A_4, which increases eNOS activity. Ticagrelor, but not other $P2Y_{12}$ inhibitors, may also increase NO generation, adenosine having the effect of increasing eNOS activity. High but not low doses of aspirin inhibit PGI_2 generation, whereas ticagrelor may theoretically upregulate it via an adenosine-dependent mechanism. How these divergent effects translate into influencing the course of severe COVID-19 remain undetermined.

Any benefits of initiation or intensification of antithrombotic therapy need to be balanced against associated increases in bleeding risk through study of clinical outcomes and data should soon be available from trials including regimens of antithrombotic therapy. Therapeutic anticoagulation is included in an arm of the ongoing REMAP-CAP (Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia) study (NCT02735707), which is also studying the effects of single antiplatelet therapy with either aspirin or a $P2Y_{12}$ inhibitor. Similarly, the RECOVERY (Randomized Evaluation of COVID-19 Therapy) trial (NCT04381936) is including aspirin as a treatment allocation. In practice, development of thrombocytopenia or deranged clotting parameters is common during severe COVID-19 and this may dissuade

clinicians from initiating antithrombotic therapy. However, because these abnormalities are typically caused by overconsumption of platelets and clotting factors, respectively, early initiation may be rational even in this difficult situation. Whether multiple versus single agent antithrombotic therapy is superior in COVID-19 remains largely unexplored.

Beyond COVID-19, how can one apply these findings? Such is the nature of human ingenuity in the face of adversity, global crises often lead to rapid progression in technology driven by necessity and targeted investment. Developments can typically be exploited for further benefit once the acute problem is overcome. Looking for any silver lining of the current predicament, perhaps through the intense research being carried out into COVID-19, we can hope that at least advances will be made of more broad relevance to other intense inflammatory states, such as sepsis, and even to more chronic conditions, such as atheroinflammation. What remains clear, however, is the logical way out of this unhappy global experience is the continued pursuit of scientific knowledge and fruitful collaboration toward a common goal of overcoming COVID-19.

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