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Arteriosclerosis, Thrombosis, and Vascular Biology

LETTER TO THE EDITOR

Letter by Brambilla et al Regarding Article, "Platelets Promote Thromboinflammation in SARS-CoV-2 Pneumonia"

Marta Brambilla, Paola Canzano, Elena Tremoli, Marina Camera

To the Editor:

We read with great interest the work recently published by Taus et al[1] highlighting the central role played by platelets in the thromboinflammation that characterizes coronavirus disease 2019 (COVID-19) pneumonia, being primed to spread proinflammatory and procoagulant activity.

The authors reported an increased potential of platelets to release cytokines, chemokines, and growth factors, highlighting their possible involvement in the local and systemic inflammation as well as in immune modulation. They also provided evidence that in patients with COVID-19 platelets are characterized by high expression levels of P-selectin, and this leads to an increased number of platelet-leukocyte aggregates. This feature has been consistently reported also in other studies published in the recent past months.[2–6] Despite this platelet activated phenotype, no other major alteration in the platelet-dependent hemostatic process (except for a reduced expression of collagen-induced fibrinogen receptor αIIbβ3) was reported. Finally, the authors addressed the platelet involvement in the COVID-19 associated procoagulant phenotype by separately analyzing the intrinsic and extrinsic pathway of blood coagulation. They stated that the platelet procoagulant contribution is mediated by an increased FXII activity that correlated with APTT. Conversely, the authors suggested that the extrinsic pathway of coagulation plays a minor role in the prothrombotic condition associated with the disease since in patients with COVID-19 they found a prolonged PT associated with normal FVII activity.

It should be considered on this regard, however, that PT cannot evaluate the contribution of the endogenous TF (tissue factor), the main trigger of the extrinsic coagulation also present in human platelets. Furthermore, a direct evaluation of the platelet-associated expression of TF nor of phosphatidylserine, the phospholipid required for the assembly of the coagulation factors, was not performed due to limitations of safety protocols.

Some recently published data come in help filling this gap. In particular, it has been shown that the procoagulant role of platelets in patients with COVID-19 is sustained more by TF than by phosphatidylserine exposure. We have indeed provided evidences, by means of a whole blood flow cytometry analysis approach, that in these patients the number of TF expressing platelets is more than twice greater than that measured in healthy subjects ($P<0.001$).[6] The protein is functionally active being able to sustain thrombin generation through the activation of the extrinsic coagulation pathway. Unlike the TF expressing platelets, the number of phosphatidylserine positive platelets in patients with COVID-19 was comparable to that measured in healthy donors.[6,7] Interestingly, incubating healthy volunteers' platelets with patients plasma led to an increase in TF expression in the platelets.[6]

It is worth mentioning in this context that the assessment of the contribution of platelet-associated TF to the prothrombotic status found in COVID-19, as well as in any other clinical settings, needs some technical advice. Indeed, TF-positive platelets are those with the highest mean platelet volume[8] and this is consistent both in healthy subjects as well as in pathological conditions.[9] This platelet fraction is easily lost during platelet-rich plasma preparation, if this is performed with high-speed centrifugation (>100 g) lasting longer than 10 minutes, as described in the article by Taus et al. Therefore, evaluation of TF-positive platelets in whole blood allows a more accurate analysis, avoiding the risk of losing platelets. If platelets need to be analyzed in platelet-rich plasma, it must be representative as much as possible of whole blood to obtain unbiased results; this can be evaluated by assessing the percentage of recovered platelets as well as the mean platelet volume.[8]

Finally, the contribution of the extrinsic pathway to thrombosis in patients with COVID-19 is also supported by recently published data showing a significant increase

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in circulating extracellular vesicle-TF activity that is associated with severity of disease and mortality.[10] Notably, these findings complement well with the evidence that the TF-positive microvesicles derive mainly from platelets and erythrocytes, further highlighting the role of platelet activation in the thrombotic events described in patients with COVID-19.[6]

In conclusion, we believe that the very interesting data reported by Taus et al could be reinterpreted with a different perspective taking also into account the recently proposed interdependency of the intrinsic and extrinsic pathway on the fibrin formation process in flowing blood.[11] Indeed, while not excluding a potential role of polyphosphates in the activation of FXII, as pointed out by the authors, it could be also hypothesized that the increased FXII activity observed is the result of the activation of the extrinsic pathway supported by increased TF-positive platelet levels measured in patients with COVID-19.

ARTICLE INFORMATION

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Disclosures

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