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Arteriosclerosis, Thrombosis, and Vascular Biology

LETTER TO THE EDITOR

Letter by Brambilla et al Regarding Article, "Patients With COVID-19 Have Elevated Levels of Circulating Extracellular Vesicle Tissue Factor Activity That Is Associated With Severity and Mortality—Brief Report"

Marta Brambilla, Paola Canzano, Alessia Becchetti, Elena Tremoli, Marina Camera

To the Editor:

We read with great interest the work recently published by Rosell et al[1] suggesting that increased levels of TF (tissue factor)-positive extracellular vesicles (EVs) may drive thrombosis in patients with coronavirus disease 2019 (COVID-19). The results of the study, conducted on 100 COVID-19 patients and 28 healthy subjects, show indeed that the capacity of EVs isolated from patient plasma to generate factor Xa is significantly higher than that of EVs isolated from healthy subjects. Furthermore, TF-dependent activity is associated with disease severity, thus leading the authors to propose EV TF activity as a prognostic marker in patients with COVID-19.

Over the years, several studies have described the potential value of EVs as biomarkers and therapeutic targets.[2,3] It is worth mentioning, however, that the ideal biomarkers should be useful not only in assessing the risk of negative outcome but should also prompt the adoption of strategies to treat the disease. To do that, biomarkers should reflect the pathological state and also provide information on the dysfunctional cellular compartment.

Unfortunately, the authors do not provide any detail on EV characterization, justifying this gap with their previous failure to evaluate circulating TF-positive EVs by flow cytometry.[4] It should be considered on this regard that flow cytometry analysis of EVs has been largely improved in the last 10 years mainly through the development of highly sensitive instruments.[5] This has allowed a major breakthrough in identifying the molecular composition of vesicles, making them not only ideal candidates as disease biomarkers but also a useful tool for the clinician in view of an increasingly personalized pharmacological intervention.[6–8] Their potential ability to provide information on pathophysiological processes has prompted several scientific societies, including the International Society of Extracellular Vesicles, International Society for Advancement of Cytometry, and International Society for Thrombosis and Haemostasis Vascular Biology Scientific Standardization Committee to draw up guidelines for EV analysis by flow cytometry.[9–12] Further standardization studies by EV flow cytometry working groups are currently in progress to upgrade EV characterization from sample preparation to data analysis, ensuring that results can be compared and shared among different laboratories.[10,13] While waiting for these further indications, the frameworks available today propose standard operating procedures to improve the interpretation and reproducibility of results. This has minimized the preanalytical and methodological variability also in terms of TF-positive EV analysis, and indeed, during the years, several authors have published data on TF-positive EVs.[14–16]

Compliance to these recommendations has recently allowed us to characterize the signature of circulating EVs in COVID-19 patients through an in-depth analysis in terms of number, procoagulant phenotype, and cellular origin.[8] In line with Rosell et al, our data show that the total number of TF-positive EVs is significantly higher in COVID-19 patients than in healthy subjects or in coronary syndrome patients and correlates with their thrombin generation potential ($r=0.495$, $P=0.02$). Furthermore, in contrast to Rosell's speculation, we provide evidence that the most abundant TF-positive EVs comes from platelets, being 2-fold and 4-fold higher than those from endothelium and monocytes, respectively. As expected, this profile mirrors the pattern of TF expression in circulating cells, being the number of TF-positive platelets significantly higher in SARS-CoV2–infected patients than in healthy subjects. Of note, TF-positive platelets and procoagulant EVs both correlate with the disease severity, being higher in COVID-19 patients requiring mechanical ventilation. The evidence of a sustained platelet activation, characterized not only by elevated TF

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expression and increased number of platelet-leukocyte aggregates but also by a high EV release, would support the effectiveness of a pharmacological intervention aimed at reducing the platelet activation state.[8]

In conclusion, information on the cellular origin of circulating EVs makes these biomarkers more useful from a clinical point of view since they pose the rationale for therapeutic intervention. On this regard, results of the currently ongoing randomized clinical trials testing the efficacy of antiplatelet therapy on COVID-19 patients will provide evidence on the usefulness of platelet-EV biomarkers in this disease.

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Disclosures

None.

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