Response by Mackman et al to Letter 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"

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LETTER TO THE EDITOR

Response by Mackman et al to Letter 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"

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In Response:

We would like to thank Brambilla et al for their interest in our article[1] showing that patients with coronavirus disease 2019 (COVID-19) have elevated levels of EVTF (extracellular vesicle tissue factor) activity and that EVTF activity is prognostic for mortality.

A prognostic marker is defined as a marker that is associated with outcome of a disease. EVTF activity fits this definition without a need to define the source of the TF-positive EVs.

Is it better to measure levels of TF activity or TF protein as a marker of thrombotic risk? As we described in our review in 2010,[2] it is better to measure TF activity rather than TF protein because TF protein can be present in an inactive or encrypted state.[3] Importantly, levels of TF antigen and activity may not correlate. For instance, one study reported detectable TF protein on EVs in the plasma of patients with glioblastoma multiforme[4]; however, no EVTF activity was detected in these samples.[5] This casts considerable doubt on studies that only measure TF protein.

The scientific endeavor relies on the use of reproducible assays developed in different labs. For example, our EVTF assay has been used by numerous labs to measure levels of EVTF activity in clinical samples with highly reproducible results.[6,7] Is there a similar reliable method that is used by the field to measure levels of TF on EVs by flow cytometry? The answer is no. Our lab has found that we can easily detect TF-positive EVs by flow cytometry in the culture supernatant of LPS (lipopolysaccharide) stimulated THP-1 cells but not in plasma of LPS stimulated whole blood, despite the fact that these samples have abundant EVTF activity.[8] Something in plasma appears to block antibody recognition of TF. Other groups with expertise in both TF and flow cytometry have also failed to detect TF-positive EVs in plasma by flow cytometry (B. Osterud, F. Dignat-George, personnel communication). We agree with Brambilla et al that there have been several sporadic reports of TF protein-positive EVs in plasma samples from clinical samples by difference groups.[9] However, none of these studies present positive and negative controls, and they often use commercial anti-TF antibodies that may or may not recognize TF.[10,11] Therefore, it remains unclear what is being measured in these studies.

Do platelets express TF? This is a controversial topic. We concluded in 2013 that platelets can splice TF pre-mRNA to mRNA, but there was no convincing evidence that platelets express TF protein.[12] A more recent review summarizes the possible reasons for erroneous detection of platelet TF antigen and activity.[10] The evidence that platelets express TF and release TF-positive EVs is largely based on flow cytometry studies.[10] Simply showing that platelets from clinical samples contain TF cannot be used to conclude that they express it because platelets have been show to efficiently bind TF-positive EVs from other cells, such as monocytes.[13] In their recent article, Canzano et al[14] showed that level of TF protein-positive EVs derived from platelets increased 2.3-fold in patients with COVID-19 compared with healthy controls. However, levels of TF protein-positive EVs derived from erythrocytes also increased by 2.1-fold in patients with COVID-19 compared with healthy controls.[14] Based on this data, one would conclude that both platelets and erythrocytes express TF and release TF-positive EVs.

Further studies are needed to determine the cellular source of circulating TF-positive EVs in patients with COVID-19 using validated techniques.

ARTICLE INFORMATION

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Disclosures

None.

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