Commentary

Procoagulant activity of extracellular vesicles in plasma of patients with SARS-CoV-2 infection

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Extracellular vesicles (EVs) have been of special interest in recent years. Emerging evidence suggests that EVs play a key role in health and disease [1]. Released by all cell types, EVs circulate freely, are present in all body fluids and mediate intercellular communication locally and systemically. Further, EVs reprogram functions of circulating and tissue-bound recipient cells in physiological and pathological conditions [2]. EVs represent a heterogeneous population of differently sized nanovesicles with distinct biogenesis that carry diverse molecular and genetic cargo and deliver it to recipient cells [1,2]. Numbers of circulating EVs increase in inflammatory or infectious diseases, and their molecular/genetic content changes with disease progression. Therefore, EVs are being intensively evaluated as non-invasive liquid biomarkers of disease onset, progression and/or outcome [3].

EVs are prominently involved in SARS-CoV-2 infection [4]. A proteomic analysis of EVs isolated from plasma of COVID-19 patients identified several molecules involved in the immune response, inflammation, and activation of the coagulation and complement pathways, which are the main mechanisms of COVID-19-associated tissue damage and multiple organ dysfunctions [5]. SARS-CoV-2, unlike other related viruses, shows tropism for alveolar epithelial cells and endothelial cells, which express the human counter-receptor, angiotensin converting enzyme 2 (ACE2). The virus replicates within infected cells causing a severe respiratory syndrome accompanied by systemic inflammation, which may result in multi-organ damage and death [6]. The vascular and endothelial cell dysfunction associated with increased mortality appears to involve the coagulation pathway. Although procoagulant activity of circulating EVs has been known since 1967 [7], the role these EV play in the COVID-19-induced pathology has not been defined.

A report by Balbi and colleagues [8] shows that tissue factor (TF, CD142), a key initiator of the coagulation pathway, is present on the surface of circulating EVs in patients with SARS-CoV-2 infection. While surface TF expression in circulating EVs accumulating in high procoagulant pathological states was previously described [9], the Balbi et al. study, identifies TF as a prominent component of the antigenic signature characteristic for circulating EVs in COVID-19 patients and a potentially significant contributor to thrombotic episodes commonly seen in SARS-CoV-2 infections. EVs in sera of 33 COVID(-) and 34 COVID(+) patients were immunocaptured using a cocktail of 37 colored beads, each coated with an antibody specific for one of the 37 target antigens. Fluorescein-labeled antibodies specific for CD9/CD63/CD81 (i.e., tetraspanins) were used for detection of the EV-associated antigens by flow cytometry. A panel of 7/37 EV-associated antigens (CD142, CD133/1, CD209, CD86, CD69, CD49e and CD20) with the highest scores in EVs of COVID(+) patients was identified. This antigen profile discriminated COVID(+) from COVID(-) patients. Among the 7 antigens in the profile, TF (CD142) had the highest discriminating score. Importantly, expression levels of CD142 on the EV surface correlated with increased serum levels of TNF-alpha in COVID (+) patients. Further, EV-associated TF was biologically active in an assay measuring amidolytic activity of the TF/FVIIa complex, and antibodies neutralizing TF activity significantly reduced procoagulant activity of these EVs. The identification of the EV associated protein signature that reliably discriminates COVID(+) from COVID(-) patients is a significant achievement: TF in EVs emerges as a potential noninvasive biomarker of COVID-19 infection. Even more significant is the finding that TF in EVs from sera of COVID(+) patients was bioactive in ex vivo assays.

Another observation, linking the TF scores and activity of EVs in COVID(+) patients with serum levels of an inflammatory cytokine, TNF alpha, adds special significance to this study. It is known that TF expressed on cell surfaces is “cryptic” and has a low procoagulant activity. To acquire the full-fledged procoagulant activity, membrane-associated TF is “decripted” by an oxidoreductase, protein disulfide isomerase (PDI) [10]. In COVID (+) patients with elevated serum levels of IL-6, IL-8 and TNF-alpha, a “cytokine storm” results in vascular injury and endothelial cell (EC) damage. Activated platelets adhering to damaged ECs release PDI, which enhances the TF...
description, inducing a massive release from ECs of TF(+) EVs with high procoagulant activity. Balbi et al. demonstrated significant elevations in soluble TNF-alpha levels and in the concentration of circulating TF(+) EVs with strong procoagulant activity in COVID (+) relative to COVID(-) patients. The authors hypothesized that TNF-alpha in sera of COVID(+) patients binds to TNF receptors on the EC surface and induces activated ECs to release TF(+)EVs with strong pro-thrombotic activity. These TF-enriched EVs derived from virus-infected ECs might play a major role in vascular injury that characterizes COVID-19 infection.

In this study, Balbi et al. did not show that TF(+)EV derived from ECs directly contribute to vascular thrombosis in COVID-19 infection. Neither do the authors convincingly show that ECs rather than e.g., platelets, which are known to carry TF, are the source of TF(+) EVs. To do so, selective immune capture of EVs derived from ECs or from platelets would be necessary. The multiplex flow cytometry analysis of EVs from COVID(+) patients indicated that immune as well as non-immune cell types contributed to the total immunocaptured EV population. This emphasizes tremendous heterogeneity of the examined EVs. Based on expression of cell type-associated antigens, the majority of captured EVs originated from ECs or platelets. However, it remains unclear how many EV originating from ECs were TF(+)EVs. Nevertheless, multiplex flow cytometry on beads discriminated COVID(+) from COVID(-) patients by the EV protein profile that was significantly enriched in biologically-active TF only in COVID+ patients. Further, circulating EVs in COVID(+)+ patients with poor outcome carried significantly higher levels of CD142. The results suggest that TF(+)EVs not only have a diagnostic potential, but might also qualify as a non-invasive prognostic biomarker in SARS-CoV-2 infections. Future studies are necessary to confirm these promising results. Also, Balbi et al. provide a rationale for a future strategy of therapeutically targeting EVs in body fluids of COVID-19 patients. A depletion of pro-thrombotic TF(+)EVs and a pharmacologic blockade of TF activity in these EVs represent a potentially promising future therapeutic strategies.

Declaration of Competing Interest
The author has no conflicts of interest to disclose.

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