ISTH SSC Vascular Biology Project 5

Comparison of the sensitivity and the specificity of assays to measure TF-EVs in plasma samples

Vienna, the 10th of June 2019

Object: The Scientific Subcommittee on Vascular Biology initiates a new collaborative project in order to compare the sensitivity and the specificity of current available assays to measure extracellular vesicle-associated tissue factor activity or tissue factor antigen of extracellular vesicles (TF-EVs) in human plasma samples.

Dear Colleagues,

The clinical and scientific interest in extracellular vesicles (EVs) is growing exponentially. Different subtypes of EVs, such as leukocyte-, endothelial-, or tumor-derived EVs, can trigger coagulation by exposing tissue factor (TF). Although TF was initially thought to be exclusively present outside the vasculature ('envelope model'), there is increasing evidence that highly procoagulant TF exposing EVs can be present in the circulation after medical interventions and in various clinical conditions, such as sepsis or cancer.

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There are at least two reasons why the standardization of assays that determine EV-associated TF activity is timely and relevant. Firstly, various in-house and commercially available assays have been developed to measure EV-TF activity, but hitherto the results of these methods cannot be compared easily. Secondly, a EV-TF dependent factor Xa generation assay and an EV-TF dependent plasma clotting assay have been developed and applied in clinical trials. These assays have shown promising results for the prediction of VTE in pancreatic cancer patients, but further investigations are warranted to prove their clinical utility.

During the 64th Annual SSC Meeting of the ISTH (Dublin, Ireland) last year, a round table bringing together international specialists, addressed the questions of TF-EV methodologies. A proposal emerged to organize a new collaborative project to compare the current available antigenic and functional assays as a first step towards standardization.

As described in Annex 1 below, aliquots from about 12 different platelet-depleted plasma samples will be sent to participating labs. TF-EVs will be measured using methodologies registered by the participating labs. Each lab will use its own reagents with the notable exception of a common TF standard for functional assays which will be distributed with the plasma samples. These samples will include blind samples from

healthy donors and positive controls (samples prepared from blood stimulated with lipopolysaccharide [LPS] or, alternatively, samples spiked with TF-EVs from various sources). Each sample will be requested to be measured in triplicate meaning that each participating lab will have to cover the costs for reagents of around 36 (+ calibration) measurements. The final outcome will be reported to the SSC on Vascular Biology of the ISTH, and will be submitted for publication to the Journal of Thrombosis and Haemostasis.

If you wish to participate, kindly complete the online registration form following the link: https://docs.google.com/forms/d/e/1FAIpQLSecG9WXUpIvVZqF-82aHjfhcwEIPghzATGOarmCDop1X50q5w/viewform?usp=pp_url

If you need more information or if you encounter difficulties to register, please contact isthworkshop3@gmail.com. Registrations will remain open until July 23, 2019.

Thank you in advance for your input.

With best regards

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Co-Chair (extracellular vesicles) of the vascular biology ISTH SSC

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Chairman of the vascular biology ISTH SSC

Annex 1: Project design

SSC-VB Project 5 : Comparison of the sensitivity and the specificity of assays to measure TF-EVs in plasma samples.

Objectives

Core labs

- **prepare**, according to ISTH recommendations, **aliquots from 12 different samples** (platelet free plasma) originating from healthy donors (non stimulated / LPS-stimulated / spiked with TF-EVs from different sources).

- **characterize samples** (stability and homogeneity between frozen aliquots) with at least 4 different methods related to the characterization of EV size, antigen, and procoagulant activity (1. NTA or TRPS; 2. FCM, 3. PS-dependent functional assay, 4. TF-dependent functional assay)

Participating labs

- **describe in details the method(s)** they are using in their lab (functional and antigenic-based methods
- declare which of these methods they intend to include in the project
- **test samples** (in triplicates) prepared and sent by the core lab, using their own reagents and protocols
- **send the data** to the core lab

Project 5 : results analysis by the core labs

a. **Sensitivity** will be evaluated measuring the discriminative capability of assays between:
- Platelet free plasma (PFP) from blood stimulated or not with LPS
- PFP spiked with different concentrations of TF-MVs from tumoral cell line/monocytes/human saliva....

b. **Specificity** will be evaluated measuring the signal of:
- PFP spiked with the same concentration of KO-TF MVs vs TF-MVs
- PFP from blood with activators or inhibitors of contact phase.

c. Other performances to be tested: **reproducibility (CV), linearity**....

Proposed timeline

- Lab registration and detailed description of methods to measure TF-MVs
- Sample preparation and characterization

first year

- Sample shipment
- Sample analysis, data collection and analysis according a scoring strategy integrating the analytical performances (*Tripodi et al J Thromb Haemost 2004, 2008*)

second year