SSC Subcommittee Project/Collaborative Project

NAME OF PROJECT: Identification of markers that can separate procoagulant platelets from apoptotic platelets

Subcommittee: Platelet physiology SSC in collaboration with the Vascular Biology SSC

- **Person responsible**:

  Chair: Marie Lordkipanidzé (Platelet Physiology SSC) and Johannes Thaler (Vascular Biology SSC)

  PI: Emma Josefsson (Vascular Biology SSC) and Sofia Ramström (Platelet Physiology SSC)

- **Design:**

  Expert survey and focus group discussion, with a request to contribute from the ISTH community.

- **Aim/Objective/Rationale (Needs assessment / Reason)**

  We propose a new project to identify methods used to assess procoagulant platelet markers/methods, with emphasis on combinations of markers/methods that can separate procoagulant platelets from apoptotic platelets.

  There is a need for harmonization in this area since many of the markers/methods used to assess procoagulant platelets are not specific (in isolation) but are also associated with platelet apoptosis including: platelet phosphatidylserine (PS) exposure (assessed by Annexin V / lactadherin binding), FXa/FVa binding, thrombin generation, increased membrane permeability, mitochondrial depolarization and microvesiculation.

- **Background**

  Procoagulant platelets is a subpopulation of highly activated platelets that express coagulation-promoting activity by PS exposure and membrane ballooning, which may be shed as microvesicles (reviewed by Agbani & Poole *Blood* 2017). A high and sustained level of cytosolic calcium is required to drive the procoagulant phenotype. Procoagulant platelets are important for clot stabilization during normal haemostasis and high levels of these platelets correlate with transient ischemic attack and stroke (Prodan CI et al., *Int J Stroke* 2008, Prodan CI et al., *Transl Res* 2011).

  PS exposure facilitates binding of coagulation factors Va and Xa and promotes thrombin generation. Fibrinogen gets converted to fibrin and leads to clot stabilization. TMEM16F (ANO6) is a Ca^{2+} dependent scramblase identified in this pathway (Suzuki et al., *Nature* 2010). A mutation in TMEM16F has been found in Scott Syndrome patients (Castoldi E et al., *Blood* 2011). Scott

Syndrome is a rare bleeding disorder with a defect in phospholipid scrambling activity, where activated platelets from Scott Syndrome patients have decreased PS exposure and deficient thrombin generation. TMEM16F KO mice have prolonged bleeding times and platelets from these mice showed deficiency in Ca^{2+} dependent PS exposure and procoagulant activity (Yang H et al *Cel*l 2012, Baig AA et al., *Arterioscler Thromb Vasc Biol* 2016). Cyclophilin D is an essential regulator of the mitochondrial permeability transition pore (MPTP). Cyclophilin D KO murine platelets have a defect in dual agonist induced formation of the MPTP, decreased PS exposure and generate less thrombin (Jobe *Blood* 2008).

In previously published literature, procoagulant platelets have been given many names including: Sustained calcium-induced platelets (SCIP), Ballooned non-spread platelets (BNS), Ballooned and procoagulant spread (BAPS), Collagen and thrombin activated platelets (COATED), Highly activated platelets, High density bubble-shaped platelets (HDBS), Fibrinogen capped platelets (FIB-CAP), GSAO+ necrotic platelets. Harmonization of methods and nomenclature, as to which of these can be considered the same, would thus be of value and is a potential output of this project.

- **Method**

Survey and moderated virtual panel discussion (zoom or other forum) with 5-10 invited experts. Moderated by Emma Josefsson and Sofia Ramström. Marker-methods identified will be summarised and posted on ISTH website for further input from the scientific community.

- **Expected timeline:**

o Launch: July 2021

o Duration: 1 year

- Selection of primary and secondary panel members from individuals having expressed interest (August 2021)
  a. Decision for inclusion in focus group will depend on expertise, experience, geographic distribution and gender.
  b. If more than one person from the same institution / lab has applied, we will ask them to delegate one single individual to represent their group.
- Online survey sent out to primary panel (September 2021)
- Focus group meeting(s) (October 2021)
  a. 6-8 people per meeting group. Discuss 4-5 questions.
- Draft themes / statements from the focus group meeting on areas of agreement and areas of uncertainty (November-December 2021)
- Circulate draft to primary and secondary panel for review and suggestions (January-March 2022)
- White paper to be made available to ISTH members for comments (April 2022)
- Presentation at ISTH congress 2022 (July 2022)

o Finalization/analysis: 2022

o Reporting: ISTH London meeting, 2022

- **Expected outcomes** (ie. publications):

o Publication type (SSC Communication, Guidance document or original article):

SSC Communication