Dear Colleagues,

The Scientific Subcommittee on Vascular Biology has set up a network aimed at standardizing the enumeration of cell-derived microparticles (MP). MP are sub-micron sized vesicles released from cell membranes in response to activation or apoptosis. They are generally defined as 0.1 to 1 μm membrane fragments exposing the anionic phospholipid phosphatidylserine (PS) and membrane antigens representative of their cellular origin. MP originating from several cell sources have been described in human plasma. Among them, platelet-derived MP (PMP) are believed to account for the majority of circulating MP in healthy subjects. It is now well recognized that MP behave as vectors of bioactive molecules playing a role in blood coagulation, inflammation, cell activation and cancer spread and metastasis. In clinical practice, circulating MP originating from blood and vascular cells are elevated in a variety of prothrombotic and inflammatory disorders, cardiovascular diseases, autoimmune disorders, infectious diseases, and malignancies. In these clinical settings, MP may give information about inaccessible tissues (tumors, endothelium, placenta, ...), correlate with disease activity, and have a prognostic value to identify patients with thrombotic or vascular risk. Moreover, they may be helpful for treatment monitoring. Although MP counts may provide useful diagnostic/prognostic information, assessment of their pathophysiological relevance in multicenter studies is hampered by methodological concerns and a lack of standardization. Among the different methodologies available to measure MP in biological samples, Flow Cytometry (FCM) is the most commonly used technique.

A first collaborative workshop was organized to define the inter-laboratory reproducibility of PMP counts using standard FCM. With a standardization strategy utilizing Megamix™ beads, the objectives of this first workshop were i) first, to establish the scatter resolution and the background noise level of the standard flow cytometers used in laboratories with respect to the strategy requirements; and ii) second, to define the inter-instrument reproducibility of PMP enumeration in human plasma. This study included 40 laboratories accounting for 59 flow cytometers. As a result, Megamix™ beads proved to be useful tools to allow instrument qualification and monitoring; however, differential behavior among FCM instrument sub-types impeded a universal standardization for PMP enumeration. Indeed, although PMP concentrations appeared consistent among instruments that measure forward scatter (FS/FSC) with a relatively wide solid angle, the initial strategy was inapplicable without substantial modifications among instruments measuring FS/FSC with a lower solid angle. Thus, this study indicates that standardization of PMP enumeration by FCM was feasible but was dependent on intrinsic characteristics of both the flow cytometer and the calibration tools.

Due to differential positioning of biological MP relative to plastic beads on different subgroups of flow cytometers (FCMr), the current ISTH protocol, developed on one subgroup, was not fully transposable to all types and brands of FCMr. Taking opportunity of a higher and more homogeneous resolution in side scatter (SS/SSC) as compared to forward scatter (FS/FSC) in one subgroup of FCMr, a modified strategy for standardization of MP counts by FCM has been recently proposed in order to provide optimized scatter-based reference levels for threshold and/or gating.
In practice, instruments showing a better resolution in FSC will use a FSC-dedicated set of beads to delineate the MP window of analysis while instruments showing a better resolution in SSC will use a SSC-dedicated set of beads. In preliminary studies, similar counts were obtained on these size-defined PMP subsets using different platforms, indicating the ability of this optimized standardization strategy to reach inter-instrument reproducibility even with various types of instruments. Moreover, this strategy is adapted to the new generation of high-sensitivity flow cytometers.

Based on this new strategy, we propose a new workshop to evaluate the inter-instrument reproducibility of PMP counts among different platforms. This exercise will proceed in two steps:

- In a first step, participating laboratories will received FSC- or SSC-optimized beads according to their instrument's characteristics and will validate the applicability of the strategy according to required performance levels in term of scatter resolution and background noise. This step will lead to acceptance or rejection of the tested instrument(s).
- In a second step, laboratories pre-selected based on their instrument(s) adequation with the standardization strategy will receive frozen aliquots of PFP samples prepared by the core laboratory and featuring defined levels of PMP subsets. These samples will be analyzed using common reagents and the standardized FCM protocol.

All reagents and plasma samples will be provided by the core laboratory free-of-charge. The number of participating laboratories will be limited to 30.

If you wish to participate, kindly complete the online registration form following the link: https://docs.google.com/spreadsheet/ccc?key=dGNjNDIaTDdMZmdxTUxZRnI5FjRy6E6MQ

If you need more information or if you encounter difficulties to register, please contact romaric.lacroix@univ-amu.fr. Registration will remain open until December 31th 2012.

We are looking forward to receiving your registration, and thank you in advance for your input.

With best wishes

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