How to isolate and analyse microvesicles in human samples?

Editorial for the contribution of M-A. Azerad et al, entitled: Microvesicles and cancer

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In this edition of the Belgian Journal of Hematology, Azerad et al. discuss the definition, analysis and roles of microparticles/microvesicles (MVs) in cancer. Microvesicles are small spherical structures highly heterogenous both in size and in composition.[1,2] As stated by Azerad, the MV nomenclature is still a matter of debate since there is no consensus on size distribution due to inaccuracy and imprecision of size measurement.[1,3] As potential disease biomarkers, MV measurement and characterisation in biological fluids could reveal new diagnostic and/or prognostic information in human diseases.[4,5] Currently, the detection and quantification of MVs are hampered by their methods of isolation and their nanometric size.[6-8] Therefore, the validation and standardisation of sensitive characterisation techniques are needed. This is challenging since a wide range of pre-analytical variables including blood sampling, sample handling, plasma generation, and plasma freezing/storing are considered as major sources of variability and potential artefacts in MV analysis. Briefly, after gentle transport, 109mM citrated whole blood should be centrifuged at room temperature within 30min to 1h to isolate plasma, with a light brake only. A double centrifugation step at 2,500g is recommended to ensure removal of platelets and decrease platelet MV production during subsequent freeze/thawing. Samples processed fresh and those frozen prior to analysis should not be directly compared. In addition, frozen-thawed plasma should ideally be stored for an equal length of time and no more than one year. This information is often lacking and should be clearly highlighted.[9-11]

As indicated by Azerad., no 'gold standard' technique is recognized so far to characterize MVs. Each method has specific advantages and drawbacks. Inter-laboratory comparisons are currently not possible due to absence of appropriate biological MV preparations ('calibrators') with well defined characteristics (i.e. size distribution, concentration). Flow cytometry (FCM) remains the technique most used to quantify MVs and give insight into the cellular origin. FCM suffers from a lack of sensitivity for small size MVs (size<500nm) although recent improvements provided access to previously undetectable MVs (lower size limit: 200-300nm).[12] It is unknown if looking at smaller MVs will give additional biological information.[13] Submicron polystyrene beads are interesting tools in FCM to help in qualifying instruments and measure a reproducible part of the largest MVs.[14] However, absolute sizing of MVs using scatter parameters and polystyrene beads also presents drawbacks.[15-17] Interestingly, the use of specific protocols

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on different instruments decreases inter-instrument variability.[18] Whatever the instrument type, optimal scatter resolution may vary in-time and between individual instruments and should be regularly checked. Efforts should focus on the development and validation of biological calibrators to compare the sensitivity of different technologies and instruments.[15-17] Important advances in FCM confirm their interest as a competitive analytical method to measure MVs of smaller size in comparison with other technologies such as atomic force microscopy and nanoparticle tracking analysis.[6,19,20] These alternative technologies are promising options for the characterisation of small size MVs.[6,20]

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