

## A standardized multi-colour flow cytometry approach to characterize the many faces of peripheral circulating microparticles

Paola Lanuti<sup>1,2</sup>, Eva Ercolino<sup>1,2</sup>, Giuseppina Bologna<sup>1,2</sup>, Giovanna Grifone<sup>3</sup>, Laura Pierdomenico<sup>1,2</sup>, Marco Marchisio<sup>1,2</sup> and Sebastiano Miscia<sup>1,2</sup>

<sup>1</sup> Section of Human Morphology, Department of Medicine and Aging Science (D.M.S.I.), University "G.d'Annunzio" of Chieti-Pescara, Chieti, Italy

<sup>2</sup> Center for Aging Sciences (Ce.S.I.), "Università G.d'Annunzio" Foundation, Chieti, Italy

<sup>3</sup> Institute of Molecular Genetics, National Research Council (CNR) Chieti, Italy

Microparticles (MP) are small vesicles (<1  $\mu\text{m}$  of diameter) released by several cell types and characterized by an integral plasma membrane expressing the phenotype of the cell from which they originate (Jayachandran et al., 2012). MP play a crucial role in a multitude of pathologies, including inflammatory and autoimmune disease, diabetes, atherosclerosis, malignancies and cardiovascular disease. The role, as potential biomarker, attributed to circulating MP has been emphasized by the recent literature. In such context, multicolour flow cytometry has great potential in the MP studies (Lanuti et al., 2012). Unfortunately, consensus guidelines on MP identification and enumeration has not been reached yet, due to their small sizes. We purpose to identify, characterize and count MP from whole blood by a seven-colors flow cytometry protocol, with the aim to standardize such method and to allow the identification of both the whole compartment and different MP subpopulations (i.e. endothelial-, platelet- and leukocyte-derived MP). We optimized a novel flow cytometry protocol, using peripheral whole blood stained by a combinations of seven different antigens/probes. Different panel combinations, anticoagulants and storage conditions were evaluated to set the best protocol with the aim to obtain reliable and reproducible MP counts. MP enumeration was carried out by a single platform method by using True-Count (BD Biosciences). Results demonstrated that the application of the newly optimized flow cytometry method here described, allows to obtain high reproducibility of MP enumeration, pointing out different MP subpopulations both in healthy donors and in different patients. This method may open new routes for the monitoring of MP numbers and phenotypes in different clinical setting.

### References

- [1] Lanuti et al. (2012) A novel flow cytometry approach to distinguish circulating endothelial cells from endothelial microparticles: relevance for the evaluation of endothelial dysfunctions. *Journal of Immunological Methods* 380: 16-22.
- [2] Jayachandran et al. (2012) Methodology for isolation, identification and characterization of microvesicles in peripheral blood. *Journal of Immunological Methods* 375: 207-214.

### Key words

Multicolor flow-cytometry, Microparticles, Standardization.