Multicolor Flow Cytometry analysis of microparticles in Whole Blood reveals significant differences compared to analysis performed in Platelet Free Plasma

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Background. As potential biomarker, circulating cell-derived Microparticle (MP) characterization and count could provide diagnostic and/or prognostic information in human disease or may be used to monitor the response to treatment. The characterization of human MPs is strictly dependent on sample preparation and analysis. For this reason the ISTH SSC have provided guidelines for standardization of the study of MP by flow cytometry, one of the most commonly used technique for their enumeration and characterization. Whether the total number and the relative abundance of the different cell-derived MPs present in plasma sample or in whole blood (WB), the most physiological 'environment', is, to the best of our knowledge, still unknown. **Aims.** To characterize circulating MPs in WB, avoiding methodological manipulations, in order to have a complete picture of MPs in terms of number and phenotype. **Method.** Citrated WB from healthy volunteers (n=20) was analysed with a multiparametric flow cytometry assay by using BD FACSAria II^{TM}, a four laser equipped cell sorter. Violet Proliferation Dye (VPD) was used to discriminate MPs (VPD ^{dim}) from platelets (VPD ^{bright}) and 7AAD to exclude apoptotic bodies. The cell origin of the different MPs was assessed by using the following antibodies: CD135 (erythrocyte), CD41 (platelet), CD144/CD31 (endothelial cell), CD45 (leukocyte), CD66 (granulocyte) and CD14 (monocyte). The procoagulant phenotype of MPs was evaluated measuring tissue factor (TF, CD142) expression as well as assessing the presence of phosphatydilserine (PS) with Annexin V. Finally, MPs were counted by using BD Trucount tubes^{TM}. Results have been compared to those obtained with fresh Platelet Free Plasma (PFP) derived from the same donors and generated by conventional methods. **Results.** WB contains a number of MPs 30 fold higher than that found in PFP (120.000±40.000 vs 3.000±800 MPs/ul), evidencing that centrifugation steps induce a partial loss of them. Most of the MPs in WB originate from erythrocytes (60%), and the remaining are from platelets (20%), granulocytes (5%), endothelial cells (2%) and monocytes (3%). By contrast 35% of MPs in PFP derived from erythrocytes and 60% and equally distributed among those derived from platelets, granulocytes and endothelial cells. The majority of MPs both in WB and in PFP are Annexin V negative (90% and 70% respectively). TF^{+}/AnnexinV^{+} MPs are present in both type of samples representing 50% of the platelet-derived. **Conclusions.** The analysis of MPs in WB and PFP revealed important differences between the two biological samples. The high number of erythrocyte-derived MPs found in WB emphasizes their potential physiological role in health and disease as documented by recent published studies. Similarly, the role of the Annexin V^{-} MPs, which represent the majority both in WB and in PFP, deserve further investigation.