The importance of studying red blood cells microparticles

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Microparticles (MP) may be generated during the in vitro storage of red blood cells but also in certain clinical conditions. Grisendi G. and her colleagues from the University of Modena and Reggio Emilia present a new multiparametric staining assay for detecting red blood cell microparticles (RMP) from human red blood cells in vitro using flow cytometry.

The new methodology, in which carboxyfluorescein diacetate succinimidyl ester (CFSE) staining is shown to detect phosphatidylserine (PS)-negative MP that fail to react with annexin V, is very promising and has relevant screening implications for blood product shelf life. There is a concern that prolonged storage of red blood cells may impair product quality, leading to MP generation which may increase procoagulant activity and nitric oxide depletion. Earlier, it was shown that most MP from erythrocytes generated in vitro through stimulation with Ca²⁺ ionophore express PS on their surface and trigger thrombin generation via the intrinsic pathway of coagulation.

It is possible that spontaneously generated RMP stored in blood bank conditions are of a different constitution and may have a different physiological function. In addition, the exact constitution may depend on the specific storage conditions as well as the storage time. Other studies have shown high percentages of PS-positive RMP after red blood cell storage under blood bank conditions. In addition to increased procoagulant activity of RMP, which can be attributed to PS expression, RMP were found to have an altered membrane composition compared to RBC which leads to differences in chemokine binding affinity. It was hypothesised that these RMP trigger increased release of local inflammatory cytokines.

From a clinical prospective the advent of improved methodology for RMP identification may be relevant in specific conditions. RMP generation has been demonstrated to play a key role in the pathobiology of sickle cell anaemia and to explain vaso-occlusive crises. Moreover, a recent study has shown that MP could sustain protein C and protein S consumption. These molecules are well known naturally occurring anticoagulants, which have been found to decrease during crises in children with sickle cell anaemia.

In this respect the recent development of improved methods for identifying and therefore studying RMP is certainly very welcome. If different subsets of MP and RMP can be accurately identified, there is a promising prospect for their use as biomarkers not only for quality control in blood bank products, but also for improved understanding of disease pathogenesis. Apart from the fact that many studies have shown that the numbers and properties of MP may be altered by disease, flow cytometry of MP in a diagnostic setting is challenging because of the very small size of MP and because MP can easily be generated due to pre-analytical handling and storage conditions. Over the past few years several collaborative efforts have been taken to standardise flow cytometric measurements by standardisation of instrument settings between laboratories, using standardisation beads. Although standardisation remains challenging, different groups have shown consistent results performing repeated measurements of MP using carefully maintained conditions within their laboratories. Recently, the numbers of circulating PS-positive MP from healthy volunteers, measured using an older model of flow cytometer, were compared with assays detecting procoagulant properties of MP and a significant correlation was found. However, one would expect this correlation to be lost if MP membrane properties are altered in vitro or in vivo as was observed in patients with acute myeloid leukaemia and in patients with chronic renal failure, in whom disease-related PS-positive MP displayed abnormal coagulant properties.

In order to add diagnostic value to MP measurements, the scientific community needs to standardise not only instrument settings but also pre-analytical conditions. We, therefore, need more tools to differentiate between MP. The study by Grisendi et al. highlights current efforts to connect membrane composition to the function and to the evolution of MP.

How are CFSE-positive/PS-negative MP generated? What are the specific pathogenic properties? Can we "steer" MP production in blood bank products by additives? Can we use these markers to discriminate MP derived from activation from MP derived from pre-analytical handling such as freezing?

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References

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