Measurement of Procoagulant Microvesicles Generated within Ageing Platelet Concentrates

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Introduction

Many studies have shown that microvesicles (MV) are generated during storage of Platelet concentrates (PCs)

The influence of the concentrate preparation method on MV generation and their characteristics have not been fully explored

The aim of this study was to measure the generation of MV within ageing concentrates using a variety of different technologies

Methods

• PCs produced by apheresis (n=20) or from whole blood using the Buffy coat (n=20) method were stored for up to 7 days under standard blood bank conditions

• Platelet free plasma (PFP) samples were prepared on days 1, 2, 5 and 7 by double centrifugation at 1700g for 20 minutes and 13,000g for 2 minutes.

• Samples were either measured freshly or after storage at -70°C

• All PFP Samples were analyzed by calibrated automated thrombography (Thrombinscope) (using PRP and PPP reagent) and Annexin V capture ELISA (Hyphen – Biomed)

• A subset of apheresis PCs (n = 3) were also analysed by nanoparticle tracking analysis (Nanosight) and flow cytometry by measuring CD61/Lactadherin positive MVs

• PFP Samples were also filtered using 200nm filtration device (Ceveron MFU 500, Pathway diagnostics)

1) Thrombin generation ability

The ability of PFP samples to support thrombin generation, (with PRP reagent containing no exogenous phospholipid) increased significantly during storage in fresh and frozen/thawed PFP samples from apheresis but not buffy coat concentrates

2) Annexin – V capture assay

There was a significant increase in PS positive MV during storage in both type of PCs

3) Nanoparticle tracking analysis

There was a marked increase in MVs as analyzed by the Nanosight, which was reduced after filtration.

4) Flow Cytometry

Flow cytometry shows an increase in MVs during storage by scattergrams or by the number of CD61/Lactadherin positive events. Filtration removed the majority of the signal.

Conclusions

• The number of platelet derived procoagulant MVs in platelet concentrates increases during storage, particularly in apheresis derived platelet concentrates

• Freezing under the PFP isolation conditions used in this study does not significantly influence ETP results

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