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Original Research Article

Microparticle detection to guide platelet management for the reduction of platelet refractoriness in children – A study proposal

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ABSTRACT

Microparticles have been shown to shed from a variety of viable cells as a consequence of inflammatory processes, activation or physical stress. Seventy to 90% of circulating microparticles are thought to be platelet-derived. The content of microparticles in blood collected from normal blood donors is highly variable and transfers into the final blood component. Elevated microparticle content (MPC) in donor blood might indicate an asymptomatic clinical condition of the donor which might affect the transfusion recipient, particularly pediatric patients. ThromboLUX is a new technology designed to routinely test biological samples for microparticle content. We compared MPC in platelet-rich plasma (PRP) of apheresis donors and the corresponding INTERCEPT-treated apheresis products (N = 24). The MPCs in donor and product samples were correlated ($r = 0.74$, $P < 0.001$). Microparticles were significantly reduced after plasma replacement and INTERCEPT treatment. These findings are supported by phase contrast microscopy. Platelet transfusions given to patients with fever or systemic inflammation are less efficacious. In addition, transfusing heterogeneous platelets – concentrates with high MPC and activated platelets – to patients whose immune systems are activated might tip them over a threshold and cause platelet refractoriness. Restricting prophylactic platelet transfusions to homogeneous products – concentrates with resting platelets and therefore low MPC – may reduce the risk of refractoriness in cancer patients, especially children with immature immunity. To test this hypothesis we introduce an evaluation protocol for platelet management, *i.e.*, keeping a split inventory of homogeneous and heterogeneous platelets, and using only homogeneous platelets for prophylaxis as a strategy to reduce refractoriness.

1. Introduction

Microparticles are an important factor in transfusion medicine [1–3]. These small vesicles found in blood plasma play complex and dynamic physiological roles as mediators of far-reaching intercellular communication by expressing a variety of membrane-associated proteins and transferring receptors, growth factors, and microRNA [4]. Microparticles can be markers of inflammation [5] and hyper-coagulation [6]. They are continuously released from various cell types; however, 70–90% of microparticles are derived from platelets or megakaryocytes [7,8]. In addition to responding to agonists, platelets generate microparticles in response to stress such as shear forces and cooling [9]. Plasma microparticles are known to be elevated or show altered characteristics *in vivo* as a consequence of various pathological conditions [10–14].

Elevated microparticle content in blood products might therefore indicate a condition in the donor that could adversely affect the recipient after product transfusion. In fresh platelet products, microparticle content may indicate the level of stress that platelets were exposed to in the donor or during product separation [15,16]. Even after removal of most microparticles, the remaining platelets from which the microparticles originated, might carry characteristics that reduce platelet recovery and/or survival by a direct effect on the recipient's immune system [15], endothelial and macrophage activation [17], or other mechanisms. In addition, storage lesion also generates microparticles with aging of the platelet product [15,18]. Furthermore, adult platelet doses are often manipulated to be appropriate for children, potentially causing stress to platelets and additional microparticle formation [19]. Microparti-

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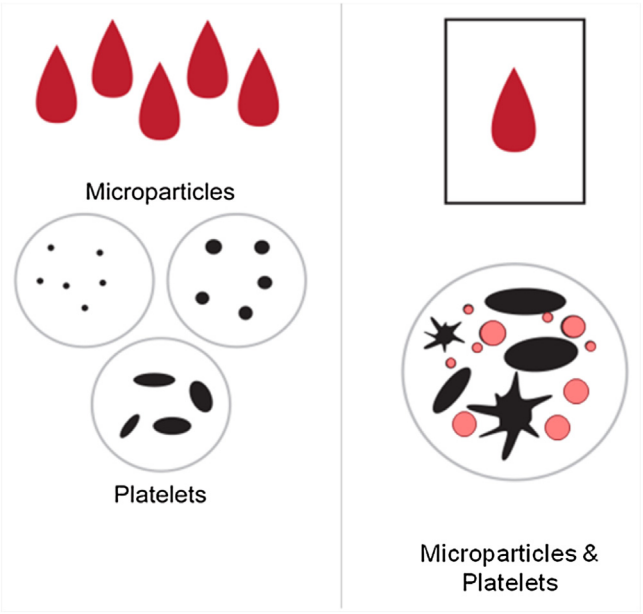

Fig. 1. Schematic comparison of current research tests of microparticles requiring separation from platelets (left) in contrast to ThromboLUX which can analyze the native sample (right). MP Factor, the relative concentration of microparticles to platelets, multiplied with the platelet count results in the absolute microparticle concentration.

cle content measured in donor samples might be a useful predictor of the microparticle content in the donated blood product especially when most of the plasma is replaced by an additive solution [20]. It is conceivable that the activated immune system of a febrile patient could be overwhelmed by the transfusion of heterogeneous platelets. As such, heterogeneous transfusions might cause platelet refractoriness. This might be particularly problematic in young children who do not yet have a fully developed immune system [21].

Several research technologies have been used for the measurement of microparticles in blood and other body fluids [15–17,22], including established methods such as flow cytometry and dynamic light scattering [23]. The measurement principle of the ThromboLUX microparticle assay is dynamic light scattering (DLS), which is based on measuring the Doppler shift of laser light scattered by particles moving with Brownian motion in suspension. The short duration of the test, small sample volume required and capability to use native samples make DLS ideally suited for routine screening of the composition of platelet transfusions. DLS screening is based on particle size and not the functional characterization of the detected particles. The optimized DLS setup in ThromboLUX allows microparticle enumeration to be performed in native platelet-rich plasma and platelet concentrate samples, *i.e.*, without removal of platelets, which is essential for routine applications (Fig. 1). The ThromboLUX microparticle assay was validated as a measure of microparticle content in platelet concentrates in comparison to flow cytometry [24].

For this work we hypothesized that the origin of the majority of microparticles in platelet concentrates is the donor, and that during the INTERCEPT process the microparticle content is reduced. We investigated the relationship between the microparticle content (MPC) of each donor's platelet-rich plasma (PRP) sample before apheresis and the MPC in the donor's corresponding apheresis platelet concentrate before and after INTERCEPT treatment. Based on our findings, we propose a study design comparing platelet utilization before and after implementation of platelet management. Using ThromboLUX-measured MPC the platelet inventory could be managed to optimize the prophylactic treatment of pediatric patients with homogeneous platelet transfusions. Such evaluations might validate that platelet management based on the ThromboLUX microparticle assay could effectively reduce the risk of pediatric patients developing platelet refractoriness.

2. Materials and methods

2.1. Sample preparation

All donor samples and blood products were collected with informed consent from normal volunteer donors under an institutionally approved human use protocol. The following samples were analyzed: (1) the leftover sample from the EDTA-anticoagulated peripheral blood obtained for cell count of the donor before donation, (2) a small aliquot of the donated apheresis platelet product (Trima Accel, Terumo BCT, Lakewood, CO, USA) before pathogen inactivation, and (3) a small aliquot of the donated platelet product after pathogen inactivation following the INTERCEPT process. The donor whole blood was centrifuged at 150*g* for 12 min at 22–24 °C and the supernatant PRP was collected into a microcentrifuge tube and kept agitated prior to analysis. The time interval between centrifugation and testing of the stored sample did not exceed 4 h.

Analysis of the platelet products was part of quality control. Product samples were either obtained from a pouch or from a tubing segment. The tubing segment was stored empty and filled and heat sealed off immediately prior to testing to ensure that a representative sample was obtained. A 5-cm segment provided enough sample to fill a ThromboLUX capillary for testing. Capillaries were filled with 100 μl of the samples following the ThromboLUX Test Kit instructions for use and analyzed according to the ThromboLUX Operator's Manual.

2.2. ThromboLUX-measured microparticle content

Analysis of dynamic light scattering and calculation of the ThromboLUX score has been described elsewhere [23]. ThromboLUX (LightIntegra Technology Inc., Vancouver, BC, Canada) measures the microparticle contribution to the total scattering intensity in the sample. The relative contributions of microparticles, platelets, and microaggregates to the dynamic light scattering signal were determined and particle size histograms were obtained. Microparticle content was calculated from the particle size distribution as particles with radii between 50 nm and 550 nm (Figs. 2B,D and 3B,D). Microparticle content reflects the relative contribution of microparticles to the scattering intensity compared to platelets and is therefore also a function of the platelet concentration of the sample. The absolute microparticle concentration is calculated by multiplying the ThromboLUX-reported microparticle factor (MP Factor) by the platelet count which is routinely obtained with a hematology analyzer. Exosome-sized particles with radii below 50 nm are also shown in the size distributions (Figs. 2B,D and 3B,D).

2.3. Phase contrast microscopy

Phase contrast microscopy was performed on fixed samples prepared by mixing 100 μl aliquots of donor PRP obtained before donation or platelet product with 100 μl paraformaldehyde (2% final concentration) at room temperature. Images were taken on a Nikon Eclipse 50i phase contrast microscope (100× oil-immersion objective NA = 1.25, DS-Fi1 digital camera).

2.4. Data analysis

Data analysis was aimed at determining the distribution of microparticles in platelet concentrates and the difference between