Microparticle concentrations in donor blood, apheresis platelet concentrates and their potential importance for recipients

Microparticles in platelet concentrates are a key indicator of the level of platelet activation. This is a topic that is critically important to understand in more detail. Based on the literature between 10 and 25 percent of patients receiving prophylactic platelets become refractory. Our best explanation of refractoriness is an HLA mismatch, but according to the literature this can only explain a refractory rate of about 3-5 percent. This means there is a large gap in the rates of refractoriness we observe and the rates of refractoriness we can explain. Our goal is to start bridging this gap by improving the understanding of microparticle populations in platelet concentrates.
I’m an employee of LightIntegra Technology. LightIntegra is a spin off from Canadian Blood Services that was founded by Dr. Elisabeth Maurer. As a Canadian Blood Services scientist she was working with Dynamic Light Scattering as a tool to better understand platelet quality. From that work came a device called ThromboLUX which was the primary tool for collecting the data I’m presenting here today.
When discussing microparticles I’m referring to particles found in a platelet concentrate that are in the size range of 50 to 550nm in radius. These are known markers of inflammation and have been associated with many chronic conditions. Microparticles can potentially come from many sources like RBC, Endothelial cells and inorganic sources, however, it’s been shown that the vast majority of these particles are derived from platelets.

In the symbol on the right, and throughout this presentation, I have represented microparticle as small red dots.
This is a video from a differential interference contrast microscope. In it you can see a very clear difference in speed and movement pattern between the large, slow moving platelets and the small very fast moving microparticles. It is this speed difference that we exploit using Dynamic Light Scattering.

For the original video please contact dmillar@lightintegra.com
From the measured speed difference we are able to get a size distribution that might look something like this. There is a large and relatively narrow peak of platelets, and then a very minimal peak of microparticles. This is an example of a platelet unit that would likely be very good to give for prophylaxis because the platelets do not seem to be very activated and should circulate well in the recipient.
We’ve found about 1 in 6 platelet concentrates will look more like this. A very distinct microparticle peak with the platelet contribution significantly reduced and the average platelet size being slightly smaller than in the previous case. This would likely be a good unit to give to actively bleeding patients as the platelets appear to be much more activated and should be able to respond quickly.
With this new dynamic light scattering toy and the potential importance of microparticles we decided to ask three questions.

The first question is:
Do microparticles from the donor transfer through the apheresis process and into the platelet concentrate?
To help answer this, we had 54 donors give an apheresis unit. We then measured the microparticle levels in the unit. At the same time, we drew a small whole blood sample, spun it down and tested the microparticle levels in the platelet rich plasma. We found a fairly strong correlation which suggests that the microparticles in the donor do indeed transfer into the final product.

It’s important to note that there is not a 1 to 1 relationship in microparticle level between the platelet concentrate and the platelet rich plasma. This, however, is to be expected because with dynamic light scattering we are measuring the microparticle level relative to the platelet level. The platelet concentration in the product is 3 to 4 times what is found in the platelet rich plasma therefore it’s not that there are fewer microparticles in the product, there are simply more platelets.
DM [2]2  was this on TLUX or Flow!?  
Daniel Millar, 4/22/2016
The second question we asked is: What is the effect on microparticle population from processing done after collection of the platelet concentrate?

There were many options we could have investigated, we decided to focus on two of them:
1) Storage in plasma vs storage in a platelet additive solution (PAS)
2) Pathogen reduction
We conducted a large sample size comparison between apheresis platelets in plasma vs. apheresis platelets in a platelet additive solution (PAS). As expected, the removal of the plasma in the process of creating PAS stored platelets drastically reduced the concentration of microparticles. This may help to explain why we see lower rates of adverse events with PAS. We also looked at the count increments from the two groups. We found a difference in the means but this difference was not statistically significant and should be investigated further.
For the investigation on the effect of pathogen reduction we had 12 donors give a double donation. One unit from each donor was treated with Mirasol, the other was kept as control. On day 0 there was effectively no difference, however, by day 5 and day 7 there was a very significant difference in the microparticle concentrations. It appears some thing in the Mirasol process creates an environment that promotes microparticle generation.
The third and final question we asked is:
What is the impact of microparticles on the recipient?
This is of course a very complex question, to simplify it we created an autologous situation to minimize the effect of compounding factors. In this study we took a whole blood sample from 40 donors, spun it and tested the microparticle level in the Platelet Rich Plasma. Then we washed the platelets, radiolabeled them and transfused it back into the donor. This means we were not transfusing microparticles back in to the donor but rather platelets which had previously generated varying levels of microparticles.

We found an moderate inverse correlated between the platelet recovery and the percentage of microparticles, suggesting that platelets which have given off lots of microparticles may not circulate as much as platelets which have not give off many microparticles.
To summarize what we learned by asking these three questions:

1) It seems microparticles in the donor do transfer in to the product.
2) It seems that Mirasol creates an environment which promotes microparticle generation.
3) PAS removes microparticles by replacing a large portion of the plasma with additive solution, while the overall effect on count increment is a bit uncertain.
4) It seems high microparticle platelets do not recover as well in vivo as low microparticle platelets.
Thank you to the people and organizations that made these studies possible.
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References


