Platelet Microparticles in Cardiopulmonary Bypass Surgery

Introduction

Excessive post-operative bleeding remains a significant problem in cardiopulmonary bypass surgery. 4% of patients require repeat sternotomy and many require blood transfusion for bleeding related complications. Understanding of the physiological processes underlying bleeding in cardiac surgery may help to identify patients at risk, and adjust management accordingly. Platelet microparticles (PMPs), released via exocytic budding from platelets are of interest in the physiology of bleeding.[1,2] Circulating PMP numbers are normally low, but are elevated in a variety of cardiovascular conditions.[3,4,5,6] PMPs are believed to have roles in inflammation,[7,8] vascular function,[1] angiogenesis[9,10] and cell signalling[1], but their main function is believed to be as a modulator of haemostasis.[1,2,] There is evidence that PMPs are pro-coagulant and that their expression is likely to be an important determinant of coagulation and bleeding.[1,2]

Evidence of the influence of cardiac surgery on PMP expression is conflicted.[11,12,13] No study has characterised changes in PMP expression at different stages of a CPB procedure. There is no evidence of a link between pre-operative or intra-operative PMP expression and post-operative blood loss. Determining the impact of cardiopulmonary bypass surgery on PMP expression may help us to understand the processes underlying perioperative bleeding. PMP expression may be predictive of severe bleeding and, in future, may provide a focus for the development of new methods of haemodynamic assessment, and help to reduce the risk of severe post-operative bleeding.

The study aims to characterise the production of PMPs at different stages of cardiac surgery, in terms of their number and surface protein expression, and to identify any relationship between PMP expression and the extent of post-operative bleeding. We hypothesise that a change in PMP number and/or a change in the expression of surface proteins will occur during surgery, and that reduced PMP expression will relate to severe post-operative bleeding.

Methods

We recruited 62 patients having first time cardiopulmonary bypass surgery. We excluded patients with any history of anaemia or haematological disease and female patients that were pregnant, breast-feeding or within six months of giving birth. 6mL samples of venous and arterial blood were collected at 9 sample points (t0-t8) outlined in the table below.

Sample(s)	Sampling Point
t0	Post anaesthesia, pre-operative
t1	Intra-operative, before heparin administration.
t2	Intra-operative, after heparin administration.
t3	On bypass, before aortic cross clamping.
t4	On bypass, 10 minutes after aortic cross clamping.
t5	On bypass, 10 minutes after the removal of aortic cross clamp.
t6	Off bypass, after protamine administration
t7	24 hours post-operatively
t8	5 Days post-operatively (Venous sample only)

Microparticle number and surface antigen expression were analysed by flow cytometry. 30µL of each PPP sample was stained with fluorescent antibodies for four platelet markers: GPIIb/IIIa (CD41), GPIb/IX (CD42), Integrin β3 (CD61) and P-selectin (CD62). Chest drains are routinely sited during cardiac surgery and the volume of fluid collected by these chest drains was used as a measure of blood loss. The drainage volume was recorded hourly for the first 12 hours post-operatively. Cumulative blood loss per kg of body weight was used as

a measure of bleeding. We assessed immediate, early and late bleeding at 1, 4 and 12 hours post-operatively. A secondary measure of bleeding was the receipt of blood products. Patients were grouped into those who had received any blood products and those who had not.

Results

The changes in total PMP counts throughout a CPB procedure are illustrated below. Changes in arterial total PMP counts broadly mirror those of venous counts. Each PMP subpopulation expressing individual markers showed similar trends in expression to the total PMP count. Each sequential pair of samples was compared by Wilcoxon signed-ranks test. A Bonferroni correction was used due to the large number of tests performed. Significance was defined as $p<0.003$. A significant decrease in PMP counts occurred from t2-t3 in both arterial and venous samples, ($p<0.001$). From t7-t8, there was a significant increase in PMPs ($p<0.001$). There were statistically significant increases in total PMP count from t4-5 in arterial samples only.

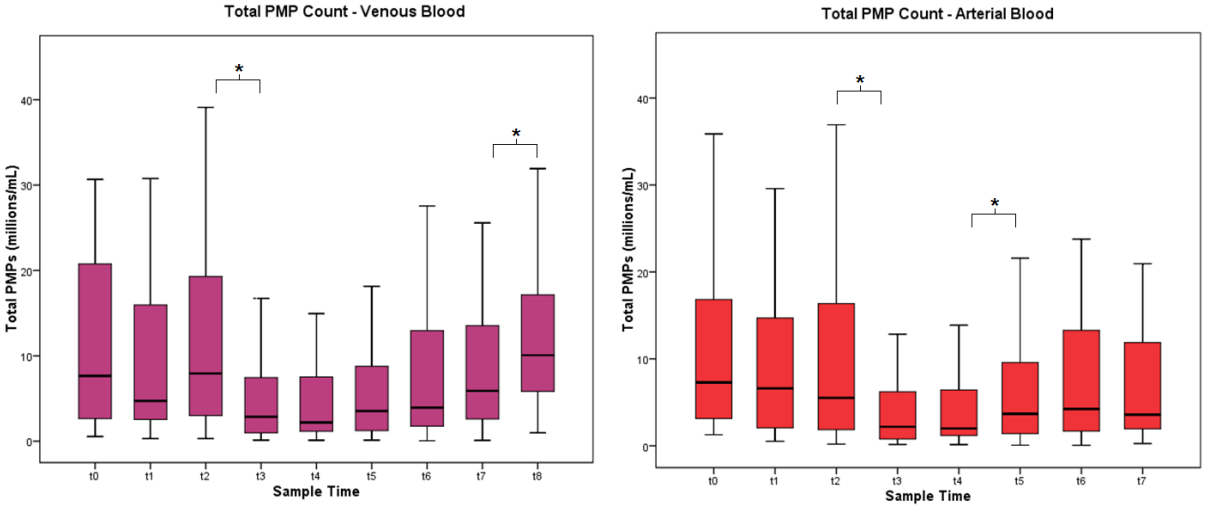

Figure 1: Box and whisker plots showing changes in total PMP counts in venous samples (left) and arterial samples (right). "Whiskers" represent the upper and lower quartile +/- 1.5x the IQR. Changes significant at the level of p=<0.003 are marked with an asterisk. Outliers have been excluded for clarity.

There were no significant correlations between total PMP counts and post-operative chest drain fluid volume per kg at 1hour, 4 hours or 12 hours. Correlations with individual PMP markers were also assessed. We found that no individual PMP population count was consistently significantly correlated chest drain fluid volume. There were also no significant differences in either total or marker specific PMP counts between those that had received blood products and those who did not at any of the time points considered (t0-6).

Conclusion

We demonstrate that PMP numbers are affected during CPB procedures. Results suggest that the primary cause of this change is CPB itself, rather than surgical trauma or any other intra-operative event. There is significant potential for error in the study of microparticles. More of the apparent trends in PMP counts may have proved significant had a larger experimental sample size been used and the impact of other CPB events, such as surgical trauma, aortic cross clamping, and heparin or protamine administration could be appraised with greater certainty. Trends in PMP numbers were consistent between arterial and venous samples and across the four PMP markers we considered. It is well documented that the study of microparticles suffers from lack of standardisation in methodology. We conclude that the use of any of these markers would be equally valid in a standardised method for counting microparticles. Evidence of an association between PMP counts and perioperative bleeding in CPB surgery was not found. The absence of any significant associations between preoperative PMPs and bleeding, suggests PMP

counts are unlikely to be of value in assessing bleeding risk in prospective CPB surgery patients.

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