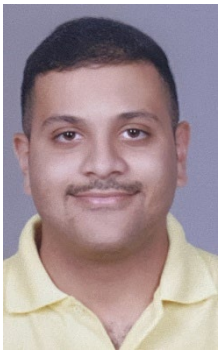
Summer Student Project Report 2024



Student
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Title of Project
Assessment of platelet function in capillary blood

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Lay Summary
Platelets are very important cells in the blood that help to stop bleeding but can also be responsible for driving heart disease and heart attacks. In both clinical and research settings it is important to be able to measure platelet function. Typical methods for measuring platelet function rely on taking venous blood, some tests the laboratory needs several tubes of blood to be taken. This summer project set out to explore whether using capillary blood taken from a single finger prick, like used for diabetic blood-sugar measurements, could provide a sample suitable for measuring platelet function. We compared platelet function in samples taken using the traditional venous blood sampling against capillary blood samples. Because of the small volumes a technique using flow cytometry was used and this was used to demonstrate that capillary blood is a suitable alternative to venous blood for analysis of platelet function. This is useful to understand as it could mean that much smaller and more accessible blood samples could be used to measure platelet function, this could have particular relevance to measuring platelet function in children or babies with suspected platelet function disorders.

What was the aim of your project?

The aim of my summer research project was to compare capillary blood vs. venous blood samples for the analysis of platelet function by flow cytometry.

How did you address the aim?

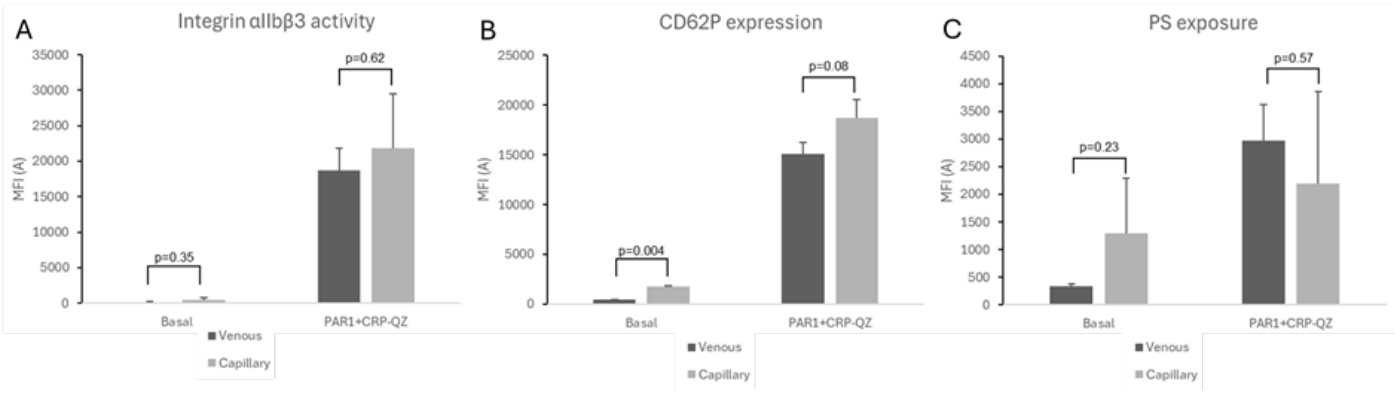
This comparative study used small blood samples for the capillary blood arm (90 µL), as a result flow cytometry was focussed on as the most appropriate technique to analyse platelet function in these samples. To reduce inconsistences between the two sampling techniques sodium citrate was used as the anticoagulant of choice for both methods. In this approach, several facets of platelet function were measured including basal, PAR and GPVI sensitivity to activation. From these samples PAC1 binding, CD62P expression, and annexin V binding were measured. These each cover independent aspects of platelet function; integrin αIIbβ3 activity, α-granule secretion and procoagulant activation.

What did you find out?

Capillary blood was shown to be a viable alternative for measuring platelet function, however this methodology likely needs further refinement as some uncertainties remain. Obtaining uncoagulated and uncontaminated capillary blood required careful sampling. With typical capillary blood sampling such as for glucose measurement the volume required is within $\leq$20 µL, however as 90 µL was sampled from a single lancet wound this required repeat palpation.

In the first instance the four-parameter platelet flow cytometry assay was set up and confirmed as a suitable method for this study. Once able to sample capillary blood consistently we sampled 3 donors for capillary blood and 3 for venous blood to perform a comparative assessment of platelet function. Several different conditions of platelet activation were measured however only basal (at rest) and a dual agonist combination of PAR1 peptide agonist SFLLRN (20 µM) + CRP-QZ (20 µg/mL) are shown here (Figure one).

Figure one



Using PAC1 binding to measure integrin αIIbβ3 activity we were able to show that venous and capillary blood were similar, with a slight trend towards increased activity in capillary blood (figure 1A). Measuring α-granule secretion using anti-CD62P we were able to demonstrate that capillary blood demonstrated significantly increased basal and stimulated activity compared to venous blood (figure 1B). Finally, comparing phosphatidylserine exposure with annexin V binding there were no significant differences, but there was a trend towards increased basal binding in capillary blood (figure 1C).

Taken together these data demonstrate that capillary blood sampling does allow measurement of platelet function by several different markers of activation, however, there remain some inconsistencies when compared to venous blood. This would suggest that venous blood remains the gold standard sampling method, while capillary blood may be a suitable alternative when the clinical situation indicates against venepuncture, but measurement of platelet function is an important parameter.

What did you learn from participating in this project?

Participating in this studentship has been a rewarding experience, allowing me to learn and refine my laboratory skills, which will be essential in my future career. I thoroughly enjoyed the hands-on experience, particularly using the flow cytometry to analyse the platelet activation markers such as PAC1, CD62P and annexin V, which gave me a deeper understanding of the complexities of platelet biology.

This experience has improved my technical skills in preparing and handling blood samples as well as analysing data from flow cytometry. Overall, the project has deepened my interest in platelet research and strengthened my ability to conduct independent scientific investigations and research. This project also sharpened my ability to follow detailed protocols and critically evaluate data.

How has this project affected your long-term goals?

This project has given me valuable insight into research as a career. The knowledge I gained during this project has made me appreciate the complexity and importance of platelet function. It gave me the opportunity to work closely with advanced techniques like flow cytometry which has deepened my appreciation for laboratory work and opened my eyes to the possibilities within the field of haematology and thrombosis research.

I am eager to gain even more research experience and explore the potential applications of innovative techniques for analysing platelet function. The insight I gained from this project inspired me to further investigate how such methods could serve as a valuable tool in research and potential clinical diagnostics. This project has solidified my interest in platelet research and has prompted me to consider furthering my education through a PhD.