Kanematsu Report – DAVID CONNOR

Introduction
Patients with acquired thrombocytopenia, namely immune thrombocytopenic purpura (ITP) and myelodysplasia (MDS) have an increased bleeding risk associated with their thrombo-cytopenia, however standard platelet functional testing methods such as platelet aggregation are unable to assess the bleeding risk. Flow cytometry can be performed even in extreme thrombocytopenia and can assess platelet response to agonist stimulation. Previous studies have demonstrated the utility flow cytometry to assess bleeding in a paediatric population with ITP or MDS, but this utility has not been demonstrated in adults.

Aims:
The aims of this study were:
1. To measure levels of platelet function and platelet reactivity in patients with acquired thrombocytopenia secondary to immune thrombocytopenia (ITP) and myelodysplasia (MDS)
2. To correlate changes of platelet reactivity with severity of bleeding in patients with acquired thrombocytopenia secondary to ITP and MDS

Methods:
Patients with either ITP or MDS were recruited. Flow cytometry was performed for platelet glycoproteins (CD9, CD31, CD41, CD42b, CD61 and CD110) and platelet activation markers (CD62p, CD63, PAC-1) in both resting and TRAP, CRP and ADP stimulated whole blood samples and compared to normal individuals. Bleeding scores for each patient was recorded. Laboratory staff were blinded to patients bleeding score.

Results:
Platelet function and Platelet Reactivity
Patients with MDS demonstrated significantly decreased expression of all platelet activation markers in CRP and TRAP stimulated platelets, whereas patients with ITP demonstrated significantly decreased expression of platelet activation markers in TRAP simulated platelets only (Figure 1). In addition, significantly higher expression of CD41 and CD42b, but not CD9, CD31 or CD61 was found on platelets in patients with ITP than in normal controls.

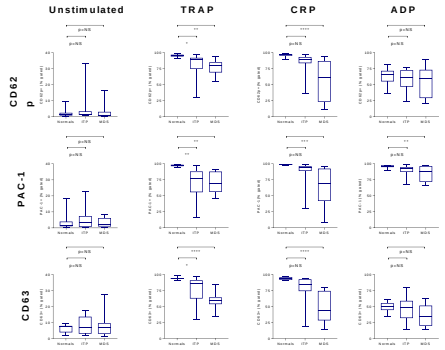

Figure 1: Expression of platelet activation markers in TRAP, CRP or ADP stimulated platelets from normal donors or patients with either ITP or MDS. * p<0.05, **p<0.01, **p<0.001, ***p<0.0001.

Correlation to Bleeding Scores
Higher bleeding scores were significantly associated with decreased platelet reactivity as measured by PAC-1 binding for TRAP and ADP Stimulation and for CD63 expression for CRP binding (Table 1).

Table 1: Association of WHO bleeding scores with platelet reactivity.
Results are p-values as assessed by an ordinary one-way ANOVA.

	Unstimulated	TRAP	CRP	ADP
CD62p	0.2697	0.0974	0.1430	0.9348
PAC-1	0.6613	**0.0042**	0.0941	**0.0229**
CD63	0.5612	0.1280	**0.0409**	0.3348

Conclusions:
Patients with MDS and ITP demonstrate decreased platelet reactivity as measured by flow cytometry. Decreased platelet reactivity is associated with higher bleeding scores and demonstrated the utility of flow cytometric analysis for assessing bleeding risk in patients with thrombocytopenia

Publications arising from this grant.
Publications / Book Chapters
Pasalic L, Pennings GJ, Connor D, Campbell H, Kritharides L and Chen V. Flow Cytometry Protocols for Assessment of Platelet Function in Whole Blood. In Haemostasis and Thrombosis: Methods and Protocols: Methods in Molecular Biology. Favaloro EJ and Lippi G, eds. 2017. Springer Science and Business Media, New York, USA. pp369-89

Conference Reports
Connor D, Thorp B, Joseph JE. Using flow cytometry to improve the risk assessment and management of bleeding in subjects with acquired thrombocytopenia. ISTH2019 Congress. International Society of Thrombosis and Haemostasis, Melbourne Australia.

We also plan to submit this work for publication in the second half of 2019.

Otherwork arising from this grant.
Since the establishment of these assays for assessing bleeding risk in patients with thrombocytopenic, we have established a collaboration between 4 major metropolitan hospitals for the investigation of patients with suspected inherited platelet function or inherited platelet number disorders. The flow cytometric investigation of platelet function and platelet glycoprotein forms an essential component of this group.

David Connor
27th June, 2019.