Procoagulant Platelet Response is Heightened in Essential Thrombocythaemia and Segregates According to JAK2V617F Mutation Status

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Introduction

Essential thrombocythemia (ET) is characterised by thrombocytosis and increased thrombotic risk although existing platelet function tests do not predict thrombosis. Procoagulant platelets are critical in thrombin generation and elevated levels are associated with thrombotic disorders.

We hypothesize that procoagulant platelet response will be increased in ET. We aim to characterise the procoagulant platelet response amongst patients with ET and to determine the effects of existing clinical and laboratory thrombotic risk factors on procoagulant platelets.

Methods

Patients with ET and healthy controls were recruited from Concord Hospital, Sydney, Australia and Singapore General Hospital, Singapore

Procoagulant platelets were measured by flow cytometry (identified by uptake of cell death marker GSAO and P-selectin expression) in whole blood (with various agonists stimulation): Unstimulated, ADP (5µM), Collagen (10mg/mL), Increasing doses of thrombin (0.5 – 5U/mL) with or without collagen (at fixed concentration of 10mg/mL).Reticulate platelet fraction and platelet aggregation were measured in a subset of subjects.

Results

1. Demographic characteristics and routine laboratory results of the

Demographic Characteristics	ET (n=57)	Controls (n=24)	P-value
Age (years)	**63.2 ± 14.8**	**53.4 ± 22.2**	**0.005**
Male gender, n (%)	**19 (33.3)**	**14 (58.3)**	**0.049**
Centre, n (%)			
Australia	45 (78.9)	20 (83.3)	
Singapore	12 (21.1)	4 (16.7)	
ET diagnosis, n (%)			
WHO criteria	34 (59.6)	-	
Non WHO criteria	23 (40.4)	-	
Prior thrombotic event, n (%)	18 (31.6)	-	
JAK2 V617F, n (%)	41 (71.9)	-	
Presence of cardiovascular risk factors, n (%)	28 (49.1)	-	
Antithrombotic therapy, n (%)			
Yes	53 (92.3)	-	
Aspirin only	42 (73.7)		
Cytoreductive therapy, n (%)			
Yes	42 (73.7)	-	
Hydroxyurea only	31 (54.4)		
IPSET score			
Low	8 (14.0)	-	
Intermediate	12 (21.1)	-	
High	37 (64.9)	-	
Laboratory Results			
Full blood count (on day of the assay)			
Haemoglobin (g/dL)	13.4 ± 1.4	13.5 ± 1.3	0.358
White blood cells (x10⁹/L)	**8.0 ± 3.1**	**6.2 ± 1.6**	**0.004**
Platelet (x10⁹/L)	**575 ± 247**	**234 ± 57**	**<0.001**
JAK2 V617F allele burden (at diagnosis) (%)^	19.8 ± 14.1	-	-

Table 1: Baseline demographic characteristics and laboratory results of the subjects with continuous variables are presented as mean ± SD. ^JAK2V617F allele burden (in white blood cells) results were available only in 29 patients from the Australian cohort.

2. Procoagulant platelet response is markedly increased in ET and segregates according to JAK2V617F mutation status

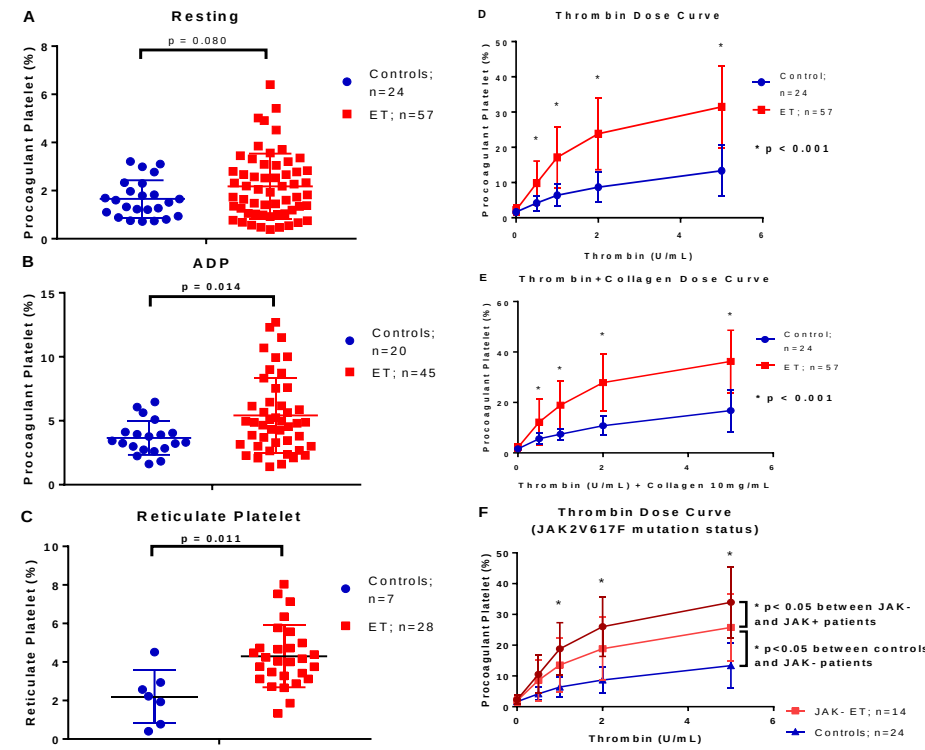

Fig. 1: (A) Resting procoagulant platelets in ET are not significantly higher than in controls. (B) ET patients have higher procoagulant platelet response with ADP stimulation. (C) Reticulate platelets are higher in ET. (D, E, F) ET patients have markedly higher procoagulant platelet response with thrombin± collagen stimulation. This response segregate with JAK2V617F mutation status with JAK+ ET having the greater reactivity.

3. Presence of JAK2V6a7F mutation significantly increased the procoagulant platelet response to thrombin stimulation

A

Factors	Unadjusted	
	B (95% CI)	p-value
Age	-0.07 (-0.27 to 0.13)	0.324
Male	4.30 (-1.71 to 10.30)	0.157
Thrombosis	4.96 (-1.36 to 11.27)	0.121
Presence of cardiovascular risk factors	-0.18 (-5.96 to 5.59)	0.949
Presence of JAK2V617F mutation	**7.59 (1.33 to 13.85)**	**0.019**
WHO ET diagnosis	-1.03 (-6.94 to 4.88)	0.727
Use of hydroxyurea	0.14 (-5.69 to 5.97)	0.961
Use of aspirin	2.83 (-5.53 to 11.18)	0.500
White cell count	0.59 (-0.34 to 1.51)	0.208
Platelet count	0.01 (0.00 to 0.03)	0.061
Reticulate platelet	0.88 (-2.29 to 4.05)	0.567
JAK2V617F allele burden	0.11 (-0.17 to 0.39)	0.498

B

Factors	Adjusted	
	B (95% CI)	p-value
Male	3.26 (-2.69 to 9.21)	0.276
Thrombosis	1.99 (-4.39 to 8.36)	0.534
Presence of JAK2V617F mutation	**6.87 (0.71 to 13.03)**	**0.030**
Platelet count	0.01 (0.00 to 0.02)	0.052

Table 2: (A) Amongst the patients, presence of JAK2V617F mutation is the only significant unadjusted predictors for procoagulant platelet response to 2U/mL thrombin. (B) JAK2V617F mutation remains a significant predictor for heightened procoagulant platelet response amongst the ET patients following 2U/mL of thrombin stimulation in this multivariate analysis model.

4. Trend of separation in procoagulant platelet response curves in patients with thrombosis and according to IPSET score.

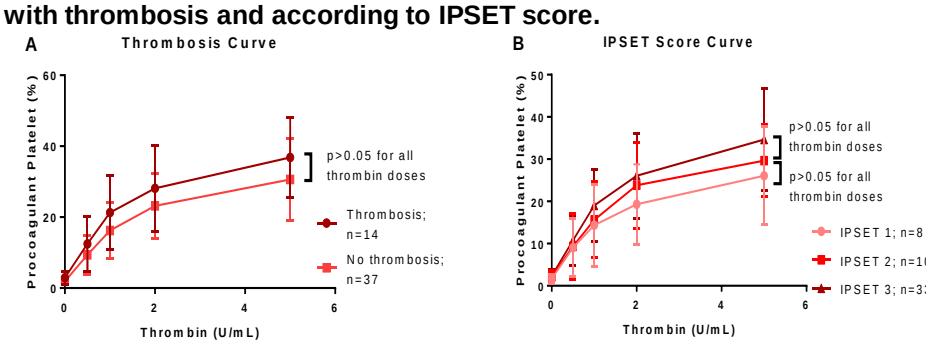

Fig. 2: (A) Thrombin dose curves in patients with thrombosis show a non-signficant trend towards greater procoagulant platelet response. (B) Procoagulant platelet response to thrombin in ET appear to segregate corresponding to patients' IPSET risk score. (A,B). Greater numbers are required to determine if this is signficant.

Conclusion

ET patients have heightened procoagulant platelet response particularly in the presence of strong agonists (thrombin±collagen stimulation).

This elevated response appears to be significantly influenced by the presence of JAK2V617F mutation while is independent of the presence other existing conventional thrombotic risk factors
→ Consistent with current clinical findings of increased thrombotic risk associated with JAK2V617F, our data suggest that this association could be mediated by the increased procoagulant platelet response and further mechanistic studies are warranted.

Procoagulant platelet response curves to thrombin start to separate according to thrombosis status and to IPSET risk groups
→ Future prospective studies required to elucidate the relationship between heightened procoagulant platelet response and thrombosis in ET.

Reference: Hua VM, Abeynaike L, Glaros E, et al. Necrotic platelets provide a procoagulant surface during thrombosis. *Blood*. 2015;126(26):2852-2862.