The effects of JAK inhibitor therapy upon novel markers of thrombosis In myeloproliferative neoplasms

Thrombosis is a relatively common and potentially life-threatening complication of myeloproliferative neoplasms (MPN). The introduction of JAK inhibitors (JAKi) has had a profound effect upon the therapeutic landscape for MPN, however, potential effects of these drugs upon thrombotic risk is largely unknown. Our objective was to study, in a MPN patient cohort, the in vivo effects of these agents on novel markers of thrombosis including platelet-neutrophil aggregates, platelet-monocytes aggregates, monocyte tissue factor expression and platelet activation as compared with conventional therapy.

The therapeutic management of both polycythaemia vera (PV) and essential thrombocythaemia (ET) is directed by perceived thrombotic risk. In the largest epidemiologic study in PV, the European Collaboration on Low-dose Aspirin (ECLAP), the cumulative rate of nonfatal thrombosis was shown to be 3.8 events per 100 persons per year, with no difference between arterial and venous thrombosis.[1] In prospective studies in ET, the rate of fatal and nonfatal thrombotic events ranged from 2% to 4% patient-years.[2,3] Moreover, in primary myelofibrosis (PMF), the prevalence of major thrombosis was assessed in 707 patients sequentially followed in four large European institutions where the overall cumulative rate of cardiovascular death and nonfatal thrombotic complications was 2.23 events per 100 persons per year.[4] Collectively, these studies highlight that MPN-associated thrombosis is a significant clinical problem. As regards conventional risk factors, age at presentation, elevated haematocrit and a previous history of thrombosis are consistent prognostic factors for recurrent thrombosis in MPN.[1,5-7] More recently, leucocyte count and *JAK2* V617F allele burden have also been described as contributing to this thrombotic risk profile.[1,8-10]

Previous studies have identified that more platelets from MPN patients circulate in an activated form as determined by the surface expression of P-selectin and tissue factor.[11] Accordingly, in ET and PV patients, thrombin generation induced by platelets is enhanced and associated with platelet activation, particularly in carriers of the *JAK2* V617F mutation.[12] Furthermore, both platelet activation status and tissue factor expression have been shown to be elevated in MPN patients compared to controls, and postulated to predict for thrombosis.[13]

Cytoreduction with hydroxycarbamide has been shown to decrease thrombotic risk in the high risk ET population, whereas the effects of more novel agents on thrombotic risk in the MPN population are as yet unclear.[14] We note with interest the recently published article concerning the safety and efficacy of the JAK1/2 inhibitor ruxolitinib (Novartis Pharmaceuticals, Switzerland) in polycythaemia vera compared to standard therapy for patients who were intolerant or resistant to hydroxycarbamide.[15] In addition to demonstrating superiority as regards haematocrit control, reduced spleen size and decreased symptom burden, it was also noted that thromboembolic events occurred in only one patient receiving ruxolitinib whereas six patients receiving standard therapy had a thromboembolic event. We postulated that treatment with the JAK inhibitors ruxolitinib or SAR302503 (Fedratinib, Sanofi Pharmaceuticals, Paris (now withdrawn)) may affect key surrogate markers for thrombosis in patients with MPN and hence studied the in vivo effects of these agents on the presence of platelet-neutrophil aggregates, platelet-monocytes aggregates, monocyte tissue factor expression and platelet activation .

A total of 29 adult patients with myeloproliferative neoplasms were recruited into this study; 15 were receiving JAKi therapy (13 patients with MF and 2 patients with PV) and 14 (9 patients with MF and 5 patients with PV) were receiving conventional treatments or an observant approach (10 patients on hydroxycarbamide, 1 on interferon-α and 3 observation only). Among the 15 MPN patients receiving JAKi, the median age was 64 years with a median disease duration of just over 6 years. A total of 9 out of 15 (60%) patients were *JAK2* V617F positive, 1 patient out of 15 (7%) had the *MPL* W515L mutation, 2 out of 15 (14%) patients had a mutation in CALR and 3 out of 15 (20%) patients had a prior history of thrombosis (2 deep venous thromboses, 1 arterial thrombosis). Regarding JAKi agents, four patients received SAR302503 and 11 patients received ruxolitinib. At the time of sample collection, 8 patients were receiving aspirin and 1 was receiving warfarin. No statistical difference between the two cohorts in terms of

Table 1. Comparison between novel markers of thrombosis pre- and post JAK inhibitor therapy.

	Cell surface markers	% expression pre JAKi median values	Range	% expression post JAKi median values	Range	*P*
Platelet monocyte aggregates	CD14/CD62p	30.9	(0.4-72)	18.2	(2.9-70.9)	0.1763
	CD14/CD42b	60.2	(44.8-90.8)	54.4	(33.5-89.1)	0.1763
Platelet neutrophil aggregates	CD15/CD62p	8.2	(2.1-27.6)	5.7	(1.4-55.7)	0.5016
	CD15/CD42b	65.4	(2.4-97.8)	75.9	(4.1-94.4)	0.3575
Platelet activation markers	CD62p	12.2	(6.1-38.5)	9.6	(4.2-66.9)	0.9697
	CD63	27.7	(1.4-74.9)	22.2	(1.3-97.2)	0.9460
Monocyte tissue factor expression	CD14/CD142	66.3	(37-97.7)	56.9	(12.5-91.1)	0.0645
Neutrophil activation markers	CD15/CD63	70.6	(12.5-95)	63.1	(9.1-96.5)	0.3203
White cell count		7.25	(1.0-42.9)	8.55	(1.3 - 53.4)	0.4357
Platelet count		137.5	(53-445)	103.5	(60-673)	0.4725
Monocyte count		0.4	(0.0-1.7)	0.5	(0.2-2.7)	0.0080

disease stage or thrombotic risk was apparent. Ethical approval for this study was obtained from the National Research Ethics Service Committee. All patients gave informed consent prior to sample collection and storage.

Baseline citrated whole blood samples were taken prior to commencing the JAKi therapy and subsequent samples were collected after at least one month of uninterrupted therapy, and for those patients on standard therapies samples were taken at one time point whilst they were on stable therapy. Flow cytometric analysis was performed on a Beckman Coulter Navios (Brea, California, USA) instrument according to the manufacturer's instructions, and data were analysed using inbuilt Beckman Coulter Navios software. Samples were stained with anti-CD142 PE, anti-CD14 PC7, anti-CD15 PC5, anti-CD63 FITC, anti-CD62p and anti-FITC CD42b PE. Platelet-neutrophil aggregates were defined as $CD15^+/CD42b^+$ and $CD15^+/CD62p^+$ populations. Platelet-monocyte aggregates were defined as $CD14^+/CD42b^+$ and $CD14/CD62p^+$populations. Platelet activation was defined by expression of CD63 and CD62p. Monocyte tissue factor expression was defined as co-expression of $CD14^+$ and $CD142^+$ and activated neutrophils were defined as $CD15/CD63^+$. Statistical analysis was performed using GraphPad Prism version 6 (La Jolla, CA, USA). Data are presented as median (interquartile range) and compared using Wilcoxon signed-rank test. *P* values <0.05 were considered statistically significant.

No statistically significant difference in platelet-monocyte aggregates, platelet-neutrophil aggregates, platelet activation markers, neutrophil activation markers or monocyte tissue factor expression (as defined above) was apparent between myeloproliferative neoplasm patients on conventional therapy and MPN patients selected to receive JAKi treatment (data not shown). When comparing paired samples at baseline and one month post-JAKi therapy, slight decreases in platelet monocyte aggregates, as defined by $CD14^+/CD42b^+$ (P=0.17) and $CD14/CD62p^+$ (p=0.17), and neutrophil activation, as defined by $CD15^+/CD63^+$ (P=0.3), was evident following JAKi therapy, but these results failed to reach statistical significance (Table 1). Interestingly, a near significant trend towards a decrease in monocyte tissue factor expression (as defined by a $CD14/CD142^+$ population; P=0.06 was noted following JAKi therapy). In parallel, a significant increase in monocyte count with JAKi treatment (P=0.008) was evident but no significant change with respect to neutrophil, total leucocyte and platelet count occurred. No apparent difference existed between the effects of ruxolitinib compared to SAR302503 on any of these markers, although the study was not powered to detect such. No thrombotic events occurred during the study period.

There are likely to be multiple risk markers of thrombosis in MPN, many of which may as yet remain unidentified. This is an area of investigation that is currently evolving and identification of risk markers, which are applicable to clinical practice, may further define risk groups by which to stratify treatment. Given the expanding role of JAKi therapy within the MPN arena, it will be of key importance to delineate the effects of these agents upon thrombotic risk. To date, this has not been addressed in a systematic fashion. In this pilot study, which represents the first report of the effect of JAKi on such in vivo factors, we were unable to detect a statistically significant effect of JAKi therapy upon these novel markers for thrombosis, although a near significant trend towards attenuated monocyte tissue factor expression was noted despite an increase in monocyte count. Of particular relevance, previous work has demonstrated that monocyte activation markers have been found to be significantly higher in ET patients with a history of thrombosis than in patients without thrombosis, supporting a role for monocyte activation in thrombosis associated with this MPN subtype.[16] The effects of JAKi therapy on monocyte activation status and tissue factor expression are interesting and may underpin the potential of JAK inhibitors to moderate thrombosis risk.

Conclusions from this pilot study are somewhat limited due to a number of factors which include small sample size, heterogeneity within the cohort and a relatively short interval between baseline and follow up testing of patients receiving JAKi therapy. However, our findings certainly indicate a potential biological basis for a reduction in thrombosis with JAKi and that further longitudinal clinical studies in a larger cohort of MPN patients receiving JAKi therapy is required to address a fundamental biological question; i.e. whether JAK inhibitors such as ruxolitinib can truly impact upon thrombotic risk. The demonstration of a reduction in thrombosis rate would be of key importance in addressing first-line use of these novel agents in PV and ET.

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