Is the absence of *JAK2*^{V617F} mutation a risk factor for bleeding in essential thrombocythemia? An analysis of 106 patients

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Background. *JAK2*^{V617F} mutation has been recognized as a possible thrombotic risk factor in essential thrombocythaemia (ET). It's role is probably due to an increased myeloid proliferation and white blood cells (WBC) activation. Only few data are available about the effect of *JAK2*^{V617F} on hemorrhagic risk. The aim of our study was to evaluate the influence of the mutational status on hemorrhagic complication.

Methods. We retrospectively analysed laboratory and clinical findings of 106 consecutive patients with ET to evaluate possible relationships between thrombosis, abnormal bleeding, peripheral blood count, overexpression of *PRV1* and *JAK2*^{V617F} mutational status.

Results. On univariate analysis we found: an association between *JAK2*^{V617F} mutation and thrombotic events before or at diagnosis ($p<0.003$, OR=4.44, 95% CI=1.74-12.4); no statistical correlation between the median value of *JAK2*^{V617F} burden and an increased risk of thrombosis ($p=0.4$, 95% CI= –22.8-10.4); significant relationships between mutated status and higher haematocrit, high WBC count and low platelet count; and a strong correlation between *JAK2*^{V617F} and *PRV1* overexpression ($p<0.0001$). Moreover, the presence of the *JAK2*^{V617F} mutation and a WBC count greater than $8.4x10^9$/L were found to be independent factors related to thrombotic complications in multivariable analysis ($p<0.006$, OR=3.85, 95% CI=1.3-11.9; and $p<0.002$, OR=2.8, 95% CI=1.08-7.03, respectively). The prognostic impact of *JAK2* mutation status and WBC count on thrombosis was evaluated in the whole cohort. Only new cases occurring in patients without previous thrombotic events were recorded for the analysis. The multivariable analysis showed a statistical correlation between the presence of the mutation and a WBC count greater than 8.12×10^6/L and an increased risk of thrombosis if no cytoreductive treatment was started at diagnosis (*JAK2*^{V617F} $p=0.02$; WBC $p=0.02$; OR=4.97; 95% CI=1.04-23.8). Finally, wild-type *JAK2* was associated with a higher haemorrhagic risk ($p=0.02$) in univariate analysis but only a platelet count greater than $1,022 \times 10^9$/L was associated with an increased risk of bleeding in the multivariable analysis.

Conclusion. Our data confirm the role of both JAK2^{V617F} as factor associated with an increased risk of thrombosis at the diagnosis and during follow-up in no treated patients. Moreover a WBC count over $8.4x10^9$/L[1] was also strictly associated to an increased risk of thrombosis. Regarding bleedings, our statistical analysis allows to exclude the mutation protective role on haemorrhage.

Key words: essential thrombocythaemia, *JAK2*^{V617F}, myeloproliferative disorders, thrombosis, bleeding.

Introduction

Essential thrombocythaemia (ET) is a myeloproliferative neoplasm characterised by sustained thrombocytosis and increased risks of haemorrhage and thrombosis[1-3]. According to different reports[1], 30% to 70% of patients with ET have the Janus kinase 2 mutation (*JAK2*^{V617F}), which involves the pseudokinase domain leading to constitutive

signalling[1]. Patients with the mutant allele tend to have higher haemoglobin levels, higher white blood cell (WBC) counts, greater bone marrow cellularity on trephine biopsy, and lower platelets counts than patients with wild-type *JAK2*[2-3]. Moreover, increased expression of polycythaemia rubra vera 1 (*PRV1*) was previously observed in patients with the *JAK2* mutation[4]. Indeed, *PRV1* was considered a specific molecular marker for myeloproliferative neoplasms before the *JAK2* mutation was found, although it is over-expressed in several reactive conditions, as well as in growth factor-stimulated granulocytosis, suggesting that it could be a marker of neutrophil activation, which is one of the possible perturbations of neutrophil function accounting for the increased incidence of thrombotic events in patients with the *JAK2* mutation[4-6].

Many investigators have found that the risk of thrombosis is higher in *JAK2*^{V617F}-positive ET patients than in *JAK2*^{V617F}-negative ones, which is probably because the *JAK2* mutation induces both myeloid proliferation and WBC activation[2-3,5,7]. Furthermore, one study showed that the *JAK2*^{V617F} mutation is related to an increased risk of thrombotic complications with increasing clone burden, although another recent study did not confirm this finding[3,6].

Conversely, some patients show haemorrhagic symptoms, which are typical of the so-called "platelet-type" ET, involving spontaneous bleeding from the skin and mucous membranes. Although perhaps seemingly illogical, the bleeding risk is increased by extreme thrombocytosis (platelet count >1,500x10^9/L)[8] possibly because of an acquired von Willebrand's disease[9]. This disorder appears to be an important contributor to the bleeding tendency in most myeloproliferative neoplasms, and particularly in ET patients, although its presence is not predictive of a bleeding diathesis[9]. In addition, the bleeding risk in patients with myeloproliferative neoplasms is increased by the use of antiplatelet drugs[8].

The aim of this study was to evaluate the influence of *JAK2* mutation status on the risks of haemorrhage and thrombosis.

Methods

Patients

We retrospectively analysed clinical and laboratory data of 106 consecutive patients with ET, at diagnosis and during follow-up at our Clinical Department. Ninety-eight patients were diagnosed as having ET according to the WHO[10] diagnostic criteria and eight according to the PVSG criteria, but these latter were included in the study because they also met the WHO diagnostic criteria. Informed consent was obtained from all patients enrolled into the study.

Molecular analyses

Peripheral blood granulocytes isolated by gradient centrifugation and ammonium chloride red cell lysis, were re-suspended in Nucleic Acid Purification Lysis Solution (Applied Biosystems, Foster City, CA, USA) with 10 U of RNAse inhibitor. Genomic DNA and total RNA were extracted from lysed cells on a semi-automated work station AB6100 following the manufacturer's instructions.

JAK2 mutation analysis. The presence of the *JAK2*^{V617F} mutation was investigated as described by Baxter *et al.*[11]. Briefly, 80 ng of DNA from the patients were used to amplify the mutated and unmutated exon 12 of *JAK2* in an allele-specific polymerase chain reaction (PCR). PCR products were separated on a 3% agarose gel, stained with ethidium bromide, and viewed under UV light. A 203 base-pair fragment indicates the presence of the 1849G>T mutation. In a subgroup of 42 patients, a quantitative real-time PCR-based allelic discrimination assay was used to detect the *JAK2*^{V617F} mutation employing TaqMan real-time technology on an AB7900. Genomic DNA was amplified in a 40-cycle PCR at an annealing temperature of 61°C. All reactions were carried out in a final volume of 25 μL containing 1x PCR Master Mix (Applied Biosystems), 900 nM of both forward and reverse primers and 100 nM of each probe. For each DNA sample a control gene was amplified to test the amount of DNA. Relative allele frequencies were calculated as described previously[12].

PRV1 quantitative analysis. Total RNA was reverse transcribed with random hexamer priming using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). *GAPDH* expression was employed as an endogenous control and a sample test was considered acceptable when the CT_{GAPDH} was less than 29. Triplicate measurements of *PRV1* and *GAPDH* amplification were conducted for each sample and relative quantification was calculated

Table I - Sequences of the real-time primers and probes

	5'for	5'rev	5'probe
JAK2	AAGCTTTCTCACAAGCATTTGGTTT	AGAAAGGCATTAGAAAGCCTGTAGTT	MGB JAK2(A)FAM 5'-TCCACAGAA ACATAC JAK2(C)VIC 5'-CTCCACAGA CACATAC
PRV1	GCTGTCCACCAAAATGAGCAT	TTCTCACGCGCAGAGAAGATC	TaqMan 5'FAM-TTCTTGTTGAACCAC ACCAGACAAATCGG-3'TAMRA
GAPDH	GCACCGTCAAGG CTGAGAAC	CGCCCCACTTGATTTTGG	TaqMan 5'FAM-AAGCTTGTCATCAATGGAAA TCCCATCACCATC -3'TAMRA

using the Δ CT method. cDNA pooled from ten donors (5 males and 5 females) was used as the calibrator sample. The sequences of the primers and probes are listed in table I.

Statistical analysis

Statistical analyses were performed using: (i) the chi-squared test for categorical variables or Fisher's exact test as necessary; (ii) the *t*-test for unpaired data for correlations between categorical and continuous variables; and (iii) logistic regression models to estimate the risk of thrombosis and bleeding according to *JAK2* mutation status, risk stratification, and WBC count.

The prognostic impact of the mutated status on cardiovascular events was studied prospectively, evaluating the frequency of cardiovascular events during a median follow-up of 24 months. Only new events occurring in patients not previously affected by any cardiovascular thrombotic disease were recorded for the multivariable analysis. The classical cardiovascular risk factors (e.g. hypertension, mellitus diabetes, smoking) and other putative risk factors such as WBC and platelet counts were included in the analysis.

Results

The patients' characteristics and the statistical correlations between clinical features and laboratory data are reported in table II.

The median follow-up of the 106 patients was 24 months (range, 1-276), and their median age was 63 years (range, 25 to 92 years). None of them was on cytoreductive treatment before diagnosis, whereas 14/106 were receiving antiplatelet drug treatment and 4/106 were on oral anticoagulant therapy. Sixty of the 106 patients had the *JAK2*^{V617F}. Eight patients without the *JAK2*^{V617F} mutation and with *PRV1* over-expression did not have thrombotic events or other causes of *PRV1* over-expression; no other point mutations in *JAK2* or *MPL* were analysed. No difference was observed in peripheral blood counts between these eight patients and the *JAK2/PRV1*-negative ones [mean platelet count, 862 ± 295 vs 745 ± 171 x10^9/L, respectively ($p<0.1$); WBC count, 8.352 ± 2.348 vs 8.074 ± 2.023 x10^9/L, respectively ($p< 0.7$)].

By the time of writing we had performed real-time *JAK2*^{V617F} quantification on 42 of the *JAK2*^{V617F} -positive patients, while the allele burden of the remaining 18 patients was not available. The value of the mutated *JAK2* burden ranged between 1 to 25% in 27/42 patients; from 26 to 50% in 7/42 patients, from 51 to 75% in 3/42 and from 76 to 100% in 5/42. Thus, eight out of the 42 patients (19% of the subgroup) showed a mutated *JAK2* burden of over 51% and were considered homozygous for the mutation. The median *JAK2* burden in the whole cohort was 14% (range, 1-100).

According to the Italian Guidelines on Diagnosis and Therapy of ET[8], we found no statistical difference in age, gender and thrombotic risk status between *JAK2*^{V617F} wild-type and mutated patients at diagnosis and during treatment. Compared to the patients with wild-type *JAK2*, the *JAK2*^{V617F}-positive patients had significantly higher mean WBC count and haematocrit and PRV1 over-expression ($p<0.0002$, $p<0.03$ and $p<0.0001$, respectively) and a significantly lower platelet count ($p<0.0006$). Thirty-five out of the 106 (33.2%) patients had thrombosis. Among these, 24 out of 35 were referred to us with a positive history of thrombosis (20 *JAK2*-positive and 4 *JAK2*-

Table II - Clinical, laboratory findings and therapeutic choice at diagnosis in a cohort of 106 ET patients stratified according to *JAK2*^{V617F} mutation status

Variable	*JAK2*^{V617F} Positive	*JAK2*^{V617F} Negative	p-value
Patients number	60	46	n.s.
Age, years-median (range)	62 (25-92)	68 (25-91)	n.s.
Gender (M/F)	28/32	17/29	n.s.
Laboratory data			
Haematocrit, mean±SD (%)	45.2±3.9	42.1±4.5	p<0.0002
WBC count, mean±SD (x10^9/L)	9.2±2.8	8.1±2.0	p<0.03
Platelet count, mean±SD (x10^9/L)	650±145	766±195	p<0.0006
***PRV1*, n (%)**			
Positive	35 (58.3)	8 (13.4)	p<0.0001
Negative	25 (41.7)	38 (86.6)	
High-risk ET patients, n (%)[1]	43 (72)	33 (72)	n.s.
Treatment			
Cytoreductive (after diagnosis)			
Hydroxyurea, n	42	31	n.s.
Anagrelide, n	1	2	
Antiplatelet drugs or oral anticoagulant therapy before diagnosis	8	10	
Thrombosis			
Arterial (at diagnosis)	18	4	p<0.003 (OR=4.102; 95% CI=1.7-10.4)
Venous (at diagnosis)	2	0	
Arterial (during follow-up)	5	0	
Venous (during follow-up)	3	3	
Bleeding (at diagnosis)	1	6	p = 0.02 (OR=0.12, 95% CI= 0.005-0.8)

[1] High-risk ET patients = age >60 years or previous thrombosis or major bleeding or platelet count >1,500x10^9/L.

negative) and 11 experienced thrombosis during follow-up (8 *JAK2*-positive and 3 *JAK2*-negative) while they were not on cytoreductive treatment. Thus, among the group of 35 patients with thrombosis, 28 were found to be *JAK2*^{V617F}-positive.

On univariate analysis the *JAK2*^{V617F} mutation was significantly associated with a higher risk of thrombosis prior to the diagnosis of ET (p<0.003 OR=4.44; 95% CI=1.74-12.4). Moreover, WBC count, haematocrit and haemoglobin values at diagnosis were significantly higher in patients with a history of thrombosis than in patients without a thrombotic history (p<0.0004, p<0.01 and p<0.02, respectively), regardless of *JAK2* mutation status (Table III). Only the *JAK2*^{V617F} mutation and WBC count over the baseline median (8.4 x 10^9/L) were found to be independent factors related to thrombotic complications in multivariable analysis (p<0.006, OR=3.85, 95% CI=1.3-11.9; p<0.002, OR=2.8, 95% CI=1.08 to 7.03, respectively). No statistical correlation was found between age and thrombotic risk (p=0.5).

The *JAK2*^{V617F} burden was known for 18/20 patients with thrombosis at diagnosis and for 33 patients who had not had a thrombotic event prior to referral to our Centre. The median values of *JAK2*^{V617F} burden in these patients were 32.08% ± 29.92% and 25.8% ± 28.77%, respectively. No statistical differences were found using univariate analysis (p=0.4; 95% CI= –22.8-10.4).

The analysis of the prognostic impact of *JAK2* mutation status on thrombotic risk was performed in a subgroup of 11 patients of whom eight were *JAK2*^{V617F}-positive (3 with venous and 5 with arterial

Table III - Laboratory findings in a cohort of 106 ET patients stratified according to occurrence of thrombotic events (at diagnosis and during follow-up): higher mean WBC count, haematocrit and haemoglobin concentration were positive predictive factors for the occurrence of thrombotic complications

Variable	Clinical Thrombotic Event		p Value
	Yes (n=35)	No (n=71)	
WBC, mean±SD (x10^9/L)	9.9 ± 3.0	8.1 ± 2.1	p<0.0004
Haemoglobin, mean±SD (g/dL)	15 ± 1.4	14.2 ± 1.6	p<0.01
Haematocrit, mean±SD (%)	45±3.7	43.2 ± 3.6	p<0.02

events) and three were *JAK2*^{V617F}-negative (all 3 with venous thrombosis). Multivariate analysis performed on this subgroup of patients showed a statistical correlation between presence of the mutation and WBC count over 8.12x10^9/L and an increased risk of thrombosis if no cytoreductive treatment was started at diagnosis (*JAK2*^{V617F} p=0.02; WBC p=0.02, OR=4.97, 95% CI=1.04-23.8). Among these patients only two had an allele burden below 10%, while the others all had values above this, including two patients with a burden over 70%.

Bleeding events were recorded at diagnosis in seven out of 106 patients. In three out of seven cases these were major events as defined by the Italian Guidelines on Essential Thrombocythemia[8] (requiring transfusion therapy, lowering haemoglobin concentration by more than 2 g/dL or threatening life or organ function). Six of the seven events occurred in the *JAK2*^{V617F}-negative subgroup of patients. At presentation the patients had no haemostatic abnormalities other than thrombocytosis (platelet count >1,100 x 10^9/L). The minor bleeds were all from various mucocutaneous sites. In all cases the platelet count was over 900 x 10^9/L. No patient was taking antiplatelet drugs at the time of the haemorrhagic event.

On univariate analysis performed on this subgroup of patients, we found a negative correlation between the presence of *JAK2*^{V617F} and the occurrence of bleeding (p=0.02, OR=0.12, 95% CI=0.005-0.8). To investigate this correlation better, we performed a multivariable analysis focusing on platelet count. The result of this analysis showed that only a median platelet count over 1,022x10^9/L was an independent risk factor for bleeding complications (p<0.005, OR=1.0073; 95% CI=1.0022-1.0124).

Discussion

Our study confirms previously published data about the potential effect of the *JAK2*^{V617F} mutation on ET phenotype[2,3].

The *JAK2*^{V617F}-positive ET patients showed similarities to patients with polycythaemia vera with regards to both peripheral blood counts and overexpression of *PRV1*. Campbell *et al.* hypothesised that *JAK2*^{V617F}-positive thrombocythaemia and polycythaemia could be better viewed as a continuum, rather than two distinct entities, in which the clinical phenotype varies possibly due to the different amount of *JAK2* mutated alleles. This viewpoint suggests that the effects of the V617F mutation on erythropoiesis and myelopoiesis could account for clinical heterogeneity observed in ET patients [2,13,14].

In our study we found that the *JAK2*^{V617F} mutation was independently related to an increased risk of thrombotic complications, in agreement with some other investigators[2,3]. A possible explanation of this correlation could be that *JAK2*^{V617F}-positive ET patients have higher peripheral blood WBC counts, which are quite closely related to an increased risk of thrombosis, as shown by our results (OR=2.8, 95% CI=1.08-7.03) and by other studies[2,3,15-19]. Several pathophysiological mechanisms may explain the thrombogenic role of increased WBC in myeloproliferative neoplasms. It has been shown that neutrophils circulate in an activated state in these disorders[16,17], and this was confirmed in our cohort by the enhanced expression of *PRV1*. Such activation makes neutrophils able to bind to platelets in a dynamic adhesive process, which reflects the activation of both platelets and the leucocytes[16,17]. Thus, the process triggers the expression of tissue factors as well as endothelial activation and

damage[16,17]. In addition, leucocytosis may contribute to inflammatory processes in atherosclerotic plaques, in this way increasing the probability of vascular events.

The prognostic impact of *JAK2*^{V617F} mutation status was evaluated in the whole cohort: 8/60 patients in the *JAK2*^{V617F} group had thrombotic complications whereas 3/46 in the *JAK2* wild-type group did so. The multivariable analysis showed that presence of the *JAK2* mutation, especially if associated with leucocytosis, is a strong predictor of a subsequent thrombotic event. If these findings are confirmed in larger series, it could be important to consider mutation status and WBC count in the risk assessment scale.

In contrast to other investigators[7,8], we did not find a significant association between age and incidence of thrombotic events. There are at least two possible explanations for this lack of association in our study: (i) it may be related to cytoreductive and antiplatelet treatment, which all patients over 60 years of age started at diagnosis, highlighting the importance of therapy in mitigating the role of age, as shown in other studies[7,8,14]; (ii) it could be related to a sampling bias. We believe that the former hypothesis better explains the results obtained with the application of the available guidelines in lowering the incidence of thrombotic complications in high-risk patients[20].

Neither haematocrit nor haemoglobin concentration was confirmed to be a factor related to thrombosis when multivariable analysis was performed, as reported elsewhere[17]. The higher haematocrit found in the group with the *JAK2* mutation could be explained, at least in part, by the different male-to-female ratios in the two subgroups (28/32 and 17/29 in the *JAK2*-positive and -negative groups, respectively).

Few data have been published on the influence of *JAK2*^{V617F} on bleeding risk in myeloproliferative disorders[15]. Tefferi *et al*. found no correlation between heterozygous and homozygous *JAK2* mutation status and the occurrence of bleeding complications in patients with polycythaemia vera[15].

In our study there was a discordance between the results of univariate and multivariable analyses. The former suggested a protective effect of the *JAK2* mutation on bleeding risk, while in multivariable analyses we found that only a platelet count above 1,022 x10^9/L was a predictor of haemorrhagic risk. These findings may be due to a more frequent detection of very high platelet counts among patients with wild-type *JAK2*.

In conclusion, our data confirm that *JAK2*^{V617F} is a factor associated with an increased risk of thrombosis both at the diagnosis and during follow-up in untreated patients. Moreover, a WBC count over 8.4 x10^9/L was strongly associated with an increased risk of thrombosis, as recently shown by Barbui *et al*[8]. Our statistical analysis indicates that *JAK2*^{V617F} does not have a protective role on haemorrhagic risk. Nevertheless larger studies are needed to determine the effective role of *JAK2*^{V617F} in myeloproliferative disorders, especially with regards to bleeding complications.

References

1) Tefferi A, Gilliland DJ. The JAK2^{V617F} tyrosine kinase mutation in myeloproliferative disorders: status report and immediate implications for disease classification and diagnosis. Mayo Clin Proc 2005; **80**: 947-58.
2) Campbell PJ, Scott LM, Buck G, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2^{V617F} mutation status: a prospective study. Lancet 2005: **366**; 1945-53.
3) Finazzi G, Rambaldi A, Guerini V, et al. Risk of thrombosis in patients with essential thrombocythemia and polycythemia vera according to JAK2^{V617F} mutation status. Haematologica 2007; **92**: 135-6.
4) Goerttler PS, Steimle C, März E, et al. The JAK2 ^{V617F} mutation, PRV-1 overexpression, and EEC formation define a similar cohort of MPD patients. Blood 2005; **15**: 2862-4.
5) Passamonti F, Pietra D, Malabarba L, et al. Clinical significance of neutrophil CD177 mRNA expression in Ph-negative chronic myeloproliferative disorders. Br J Haematol 2004; **126**: 650-6.
6) Antonioli E, Guglielmelli P, Poli G, et al. Influence of JAK2^{V617F} allele burden on phenotype in essential thrombocythemia. Haematologica 2008; **93**: 41.
7) Wolansky AP, Lasho TL, Schwager SM, et al. JAK2^{V617F} mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance. Br J Haematol 2005; **131**: 208-13.
8) Barbui T, Barosi G, Grossi A, et al. Practice guidelines for the therapy of essential thrombocythemia. A statement from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. Haematologica 2004; **89**: 215-32.
9) Schafer A. Molecular basis of diagnosis and treatment of polycythemia vera and essential thrombocythemia. Blood 2006; **107**: 4214-22.
10) Vardiman JW, Harris NL, Brunning RD. The World

Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002; **100**: 2292-302.

11) Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005; **365**:1054-61.
12) Germer S, Holland MJ, Higuchi R. High-throughput SNP allele-frequency determination in pooled DNA samples by kinetic PCR. Genome Res 2000; **10**: 258-66.
13) Poodt J, Fijnheer N, Walsh IB, Hermans MH. A sensitive and reliable semiquantitative real time PCR assay to detect JAK2^{V617F} in blood. Hemat Oncol 2006; **24**: 227-33.
14) Klippel S, Strunck E, Temerinac S, et al. Quantification of PRV-1 mRNA distinguishes polycythemia vera from secondary erythrocytosis. Blood 2003; **102**: 3569-74.
15) Tefferi A, Lasho TL, Schvager SM, et al. The clinical phenotype of wild-type, heterozygous and homozygous JAK2^{V617F} polycythemia vera. Cancer 2006; **106**: 631-5.
16) Arellano-Rodrigo E, Alvarez-Larran A, Reverter JC, et al. Increased platelet and leukocyte activation as contributing mechanisms for thrombosis in essential thrombocythemia and correlation with the JAK2 mutational status. Haematologica 2006; **91**: 169-75.
17) Carobbio A, Finazzi G, Guerini V, et al. Leukocytosis is a risk factor for thrombosis in essential thrombocythemia: interaction with treatment, standard risk factors and JAK2 mutation status. Blood 2006; **109**: 2310-3.
18) Carobbio A, Antonioli E, Guglielmelli P, et al. Leukocytosis and risk stratification assessment in essential thrombocythemia. J Clin Oncol 2008; **26**: 3135-7.
19) Cheung B, Radia D, Pantelidis P, et al. The presence of the JAK2^{V617F} mutation is associated with a higher haemoglobin and increased risk of thrombosis in essential thrombocythaemia. Br J Haematol 2005; **132**: 244-5.
20) Radaelli F, Colombi M, Calori R, et al. Analysis of risk factors predicting thrombotic and/or haemorrhagic complications in 306 patients with essential thrombocythemia. Hematol Oncol 2007; **25**: 115.

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