Influence of the JAK2 V617F mutation and inherited thrombophilia on the thrombotic risk among patients with essential thrombocythemia

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ABSTRACT

It is uncertain whether the JAK2 V617F mutation increases the thrombotic risk in patients with essential thrombocythemia, and it is unknown whether inherited thrombophilia is an additive risk factor in mutated subjects. We studied 132 patients with essential thrombocythemia, 38 of them (29%) with a history of thrombosis. The JAK2 mutation was present in 83 (63%), and inherited thrombophilia in 7. The mutated patients <60 years had a relative risk (RR) for thrombosis at any time of 3.83 (95%CI 1.27-11.49) in comparison with wild-type patients; in those with both the mutation and thrombophilia the RR was 2.23 (95%CI 1.57-3.18) and 7.66 (95%CI 2.66-22.03) in comparison with mutated or wild-type patients without thrombophilia, respectively. During the follow-up, only the homozygotes for JAK2 V617F were more prone to thrombosis (RR 17.25, 95%CI 2.33-127.4). Among the patients >60 years, no increase in RR was associated with the JAK2 mutation. In conclusion, in the younger patients with ET the thrombotic risk is higher in the JAK2 V617F-mutated and is further increased by the presence of inherited thrombophilia.

Key words: essential thrombocythemia, thrombosis, JAK2 V617F mutation, inherited thrombophilia.

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Introduction

Essential thrombocythemia (ET) is complicated by thrombosis with a rate as high as 50%;[1] advanced age and a prior history of thrombosis are the two most important risk factors for vascular complications.[1-4] Some studies have addressed whether inherited thrombophilia is an additive risk factor in such a setting, but results remained elusive.[5-7] In a cohort of 304 patients with polycythemia vera (PV) and ET, 16% of those with venous thromboembolism carried factor V (FV) Leiden.[5] However, this seems simply to mirror the association of FV Leiden with venous thrombosis in the general population, without interaction with the thrombotic risk due to myeloproliferative neoplasms.[8] In contrast, in a cohort of 214 patients with PV and ET the risk for venous thromboembolism associated with the prothrombin (PT) G20210A was claimed to be higher than that expected.[7]

The JAK2 V617F mutation is detectable in the large majority of patients with PV and in about half of patients with ET;[9] in the latter, the phenotype shows multiple features resembling PV, such as increased hemoglobin and white blood cells.[10,11] The JAK2 mutation is associated with enhanced platelet and leukocyte activation as well as plasma hypercoagulability.[12,13] This, and the evidence that leukocytosis is associated with an enhanced hazard for thrombosis,[4,14,15] could be a plausible basis underlying in ET an increased risk for thrombosis in the patients with the mutation in comparison with those without.

A meta-analysis of 2,436 patients with ET estimated that the JAK2 mutation was associated with a 1.8-fold increased risk for thrombosis.[16] In contrast, in a recently published cohort of 657 patients with ET, the JAK2 mutation did not influence the risk for thrombosis.[15] Such discrepancies could be explained, in part, by different mutational loads in the patients investigated, related to whether the individuals harbored the mutation in the homozygous status (i.e. >50% mutant allele burden), who are more prone to overall thrombosis[17] and to arterial thrombosis.[15] The effect of the combined carriership of the JAK2 V617F mutation and the inherited thrombophilia on the thrombotic risk is unknown. In the present study, we tackled this issue by assessing in a cohort of patients with ET the risk of thrombosis according to the JAK2 V617F mutational load and to the presence of inherited thrombophilia.

Design and Methods

Patients and laboratory methods

We conducted a retrospective cohort study among patients

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with ET diagnosed according to either previous[18] or updated[9] WHO criteria. All patients underwent laboratory screening for thrombophilia. Genomic DNA was extracted from peripheral blood granulocytes by standard procedures and was archived. All patients gave informed consent for future investigations on the archived DNA. According to these criteria, 132 out of a total of 159 patients referred to our center between 2002 and 2007 who had a stored or fresh DNA sample available for JAK2 mutational analysis were recruited. This study was approved by the institutional review board.

The JAK2 V617 mutation was detected by allele-specific polymerase chain reaction according to Baxter *et al.*[19] The sequencing analysis was carried out according to Wolanskyj *et al.*[20] Heterozygous or homozygous status was defined as a mutant allele burden ≤50% or > 50%, respectively. Screening for thrombophilia included measuring antithrombin and protein C functional activities, free protein S antigen, and fasting homocysteine; searching for the FV Leiden, for the PT G20210A, and for antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies, and anti-β2glycoprotein I).[8,21]

All the assays were performed blinded to the diagnosis and clinical history of the patients.

Study end-points

The events of interest were thromboses that occurred as inaugural manifestation of ET or during follow-up. Inaugural thromboses encompass the events occurring within the two years preceding the diagnosis, translating the knowledge that 75% of thromboses which indicate PV occur within this interval of time.[22] The recorded events were cerebrovascular disease [ischemic stroke or transient ischemic attack (TIA)], acute coronary syndrome (acute myocardial infarction or unstable angina pectoris), peripheral arterial thrombosis, retinal artery or vein occlusion, thrombosis of deep veins (including cerebral and splanchnic veins), and pulmonary embolism. Splanchnic venous thrombosis includes occlusion of hepatic, portal, mesenteric, and splenic veins. Moreover, superficial vein thromboses diagnosed by ultrasound objective methods were also computed. Diagnosis of first or recurrent major thrombosis was accepted only if objectively proven according to previously published criteria.[23]

Clinical characteristics

The clinical characteristics of the investigated patients are shown in Table 1. Thirty-eight (28.7%) suffered from thrombosis, arterial in two-thirds of the cases and venous in one-third. Thrombosis was inaugural in 34 cases and occurred during the follow-up in 10 (4 first thromboses and 6 recurrences). Recurrences were myocardial infarction in one case, splenic infarction in one, superficial vein thrombosis in 2, pulmonary embolism in one, and cerebral venous thrombosis in one. After diagnosis of ET, the patients with thrombosis of splanchnic or cerebral veins received long-term oral anticoagulation. All the remaining patients received antiplatelet agents, independently of the occurrence of a previous thrombosis. Forty-nine patients were prescribed cytoreduction: hydroxyurea in 32 cases, interferon in 11, and anagrelide in 6. We computed a total observation time of 454 years (median 3.2, range 1-6).

Statistical methods

Differences between groups were estimated by the Fisher's exact test, the χ^2 test, and the Mann-Whitney test employed when appropriate (statistical significance $p<0.05$). The relative risk (RR) for thrombosis with the 95% confidence interval (95%CI) was estimated by a 2 x 2 contingency table.

Results and Discussion

Screening for the JAK2 V617F mutation

The mutation was found in 62.9% of the patients, 8 of whom (6.1%) were homozygous (Table 2). In agreement with current knowledge, the mutated patients had higher Hb level ($p=0.01$), a higher leukocyte count ($p=0.003$), and a higher rate of splenomegaly ($p=0.04$), particularly in homozygotes ($p=0.01$), in respect to those without mutation; the platelet count was similar in both groups ($p=0.38$) (Table 2). No patient had Hb levels meeting the WHO criteria for diagnosis of PV,[9] or had subnormal serum erythropoietin levels. All patients with splanchnic venous throm-

Table 1. Patients' characteristics.

Sex (M / F) – N. (% males)	46 / 86 (35)
Median age at diagnosis – years (range)	53 (20-92)
Inherited thrombophilia – N. (%)	7 (5.3)
History of thrombosis prior to two years before diagnosis of ET – N. (%)	7 (5.3)
First thrombosis at diagnosis or during the two years preceding diagnosis of ET – N. (%)	34 (25.7)
First thrombosis during follow-up after diagnosis – N. (%)	4 (3.0)
Total first ET-related thrombosis – N. (%)	38 (28.7)
Median age at first thrombosis – years (range)	47 (25-84)
First arterial ET-related thrombosis – N. (%)	25 (18.9)
Total acute coronary syndromes – N. (%)	6 (4.5)
Myocardial infarction – N.	5
Unstable angina – N.	1
Total cerebrovascular disease – N. (%)	15 (11.4)
Ischemic stroke – N.	7
Transient ischemic attack – N.	8
Peripheral arterial occlusion – N. (%)	4 (3.0)
First venous ET-related thrombosis – N. (%)	13 (9.8)
Total venous thromboembolism at usual sites – N. (%)	3 (2.3)
Deep venous thrombosis of the limbs and/or pulmonary embolism – N.	2
Superficial vein thrombosis – N.	1
Total venous thrombosis at unusual sites – N. (%)	9 (6.8)
Hepatic venous thrombosis – N.	2
Portal-mesenteric venous thrombosis – N.	4
Splenic vein thrombosis – N.	2
Cerebral venous thrombosis – N.	1
Retinal venous thrombosis – N. (%)	1 (0.7)
Recurrent ET- related thrombosis – N. (% of patients with ET-related thrombosis)	6 (15.7)

bosis, in whom hemodilution can occur, had no increase in the red cell mass evaluated by ^{51}Cr-labeled erythrocytes.

Screening for thrombophilia

Four patients were heterozygous for FV Leiden (3%), and 3 heterozygous for PT G20210A (2.3%). This is quite comparable to the prevalence of those polymorphisms among Caucasian individuals.[8]

Risk of thrombosis in the patient groups

The distribution of thromboses according to the presence of the JAK2 mutation is shown in Table 2. The rate of ET-related first thrombosis was higher in the patients with the mutation (30/83, 36.1%), in comparison with those without (8/49, 16.3%, p=0.01). The rate of first thrombosis was significantly higher both in homozygotes (5/8, 62.5%, p=0.01) and heterozygotes (25/75, 33.3%, p=0.03). The RR for thrombosis was increased 2-fold in heterozygotes, and 3.8-fold in homozygotes as compared with patients without the mutation (Table 2). A separate analysis showed that the RR was statistically significant only with regard to arterial thrombosis and to the homozygous status (RR 4.9 in comparison with wild-type patients) (Table 2). The analysis restricted to thrombotic inaugural manifestations provided a similar estimate, whereas during the follow-up the RR for thrombosis was 6.1-fold increased among homozygotes, but was not influenced by the heterozygous state (Table 2). During follow-up, the incidence of thrombosis was 2.2 percent patient-years: 1.8 among the wild-type individuals, 1.6 among the heterozygotes, and 7.9 among the homozygotes. Thrombosis was a first event in 4 patients (receiving hydroxyurea [n=2] or interferon [n=2]) and a recur-

Table 2. Patients' characteristics according to the JAK2 mutational status. ET-related thrombotic events are recorded, after the exclusion of those that occurred prior to two years before the diagnosis. Statistically significant values of the relative risk for thrombosis are in bold.

	JAK2 V617F allele burden %			
	0	1 – 100	1 – 50	> 50
N. (% of total patients)	49 (37.1)	83 (62.9)	75 (56.8)	8 (6.1)
Hb, gr/dL at diagnosis, median (range)	13.5 (11.0-16.7)	14. 3 (11.1-16.8)	14. 3 (11.1-16.8)	11.8 (11.5-13.8)
WBC $\times 10^9$/L at diagnosis, median (range)	8.21 (3.10-16.16)	9.69 (3.33-18.02)	9.53 (3.33-18.02)	12.6 (6.98-14.92)
Platelets $\times 10^9$/L at diagnosis, median (range)	813 (480-2300)	690 (430-1461)	690 (430-1461)	900 (500-1000)
Splenomegaly, N. (%)	8 (16.3)	27 (32.0)	22 (29.3)	5 (62.5)
Inherited thrombophilia, N. (%)	1 (2.0)	6 (7.2)	5 (6.7)	1 (12.5)
First arterial thrombosis, N. (%) with inherited thrombophilia, N.	5 (10.2) 0	20 (24.1) 4	16 (21.3) 3	4 (50.0) 1
First venous thrombosis, N. (%) - with inherited thrombophilia, N.	3 (6.1) 0	10 (12.0) 1	9 (12.0) 1	1 (12.5) 0
Inaugural first thrombosis, N. (%)	7 (14.2)	27 (32.5)	23 (30.6)	4 (50.0)
Thrombosis during follow-up, N. (%) first thrombosis recurrent thrombosis	3 (6.1) 1 2	7 (8.4) 3 4	4 (5.3) 2 2	3 (37.5) 1 2
Relative risk for thrombosis (95%CI)				
Overall first thrombosis at any time	1 (reference)	**2.21 (1.10 - 4.43)**	**2.04 (1.00-4.15)**	**3.82 (1.66-8.78)**
a) < 60 years	1 (reference)	**3.83 (1.27-11.49)**	**3.43(1.12-10.44)**	**7.66 (2.66-22.03)**
b) > 60 years	1 (reference)	1.14 (0.42-3.03)	1.12 (0.41-3.05)	1.30 (0.20-8.44)
First arterial thrombosis at any time	1 (reference)	2.36 (0.94-5.89)	2.09 (0.81-5.34)	**4.90 (1.66-14.46)**
a) < 60 years	1 (reference)	3.28(0.80-13.44)	2.72 (0.64-11.52)	**8.62 (2.04-36.42)**
b) > 60 years	1 (reference)	1.69 (0.49-5.80)	1.64 (0.46-5.75)	2.16 (0.29-16.07)
First venous thrombosis at any time	1 (reference)	1.96 (0.56-6.80)	1.96 (0.55-6.88)	2.04 (0.24-17.30)
a) < 60 years	1 (reference)	4.92 (0.66-36.52)	4.84 (0.64-36.28)	5.75 (0.44-74.46)
b) > 60 years	1 (reference)	0.31 (0.03-3.32)	0.35 (0.03-3.67)	0
Inaugural thrombosis	1 (reference)	**2.27 (1.07-4.83)**	**2.14 (0.99-4.61)**	**3.50 (1.32-9.28)**
a) < 60 years	1 (reference)	**3.46 (1.14-10.49)**	**3.22 (1.05-9.88)**	**5.75 (1.73-19.05)**
b) > 60 years	1 (reference)	1.26 (0.42-3.79)	1.23 (0.40-3.77)	1.62 (0.23-11.11)
Thrombosis during follow-up	1 (reference)	1.37 (0.37-5.08)	0.87 (0.20-3.72)	**6.12 (1.48-25.22)**
a) < 60 years	1 (reference)	3.28 (0.42-25.67)	1.81 (0.20-16.45)	**17.25 (2.33-127.4)**
b) > 60 years	1 (reference)	0.31 (0.03-3.32)	0.35 (0.03-3.67)	0
*Recurrent thrombosis**	1 (reference)	0.64 (0.15-2.66)	0.30 (0.05-1.78)	1.75 (0.37-8.06)
a) < 60 years	1 (reference)	0.63 (0.10-3.90)	0.37 (0.04-2.94)	2.00 (0.33-11.97)
b) > 60 years	1 (reference)	0	0	0

**risk estimated for patients with inaugural ET-related thrombosis.*

Table 3. Relative risk for thrombosis at any time in the overall patients and after stratification by age according to the presence of the JAK2 V617F mutation and thrombophilia. Statistically significant values of the relative risk for thrombosis are in bold.

		JAK2 V617F		Wild type
Thrombophilia		Yes	No	No
RR for thrombosis (95% CI)	All patients	**5.00 (2.41-10.34)**	**1.94 (0.96-3.96)**	1 (reference)
		2.56 (1.58-4.15)	1 (reference)	–
RR for thrombosis (95% CI)	Age <60 years	**7.66 (2.66-22.03)**	**3.43 (1.12-10.44)**	1 (reference)
		2.23 (1.57-3.18)	1 (reference)	–
RR for thrombosis (95% CI)	Age >60 years	2.50 (0.50-12.29)	1.02 (0.37-2.78)	1 (reference)
		2.43 (0.53-11.12)	1 (reference)	–

rence in 6 (receiving hydroxyurea [n=3] or interferon [n=1], or no cytoreduction [n=2]); overall, after diagnosis of ET only 2 events out of 10 occurred in the absence of cytoreduction. The prevalence of inherited thrombophilia did not differ among the patient groups (JAK2 V617F absent *vs.* heterozygous *vs.* homozygous) (p=0.34). Overall, the RR for thrombosis associated with thrombophilia was 2.70 (95% CI 1.55- 4.70). Among the patients with the JAK2 mutation, those with thrombophilia had a RR of 2.56 (1.58- 4.15) in comparison with those without thrombophilia. Among patients without thrombophilia those with the JAK2 mutation had an RR of 1.94 (95% CI 0.96-3.96) in comparison with those without the mutation. The carriers of both the JAK2 mutation and inherited thrombophilia had an RR of 5.0 (95% CI 2.41-10.34) in comparison with patients with neither the mutation nor thrombophilia, suggesting an additive interaction between the two risk factors (Table 3). Such an increase in the RR was significant only as regards the inaugural thromboses (RR 4.57, 95%CI 1.88-11.10). During the follow-up the carriers of both the JAK2 mutation and inherited thrombophilia had an RR of 8.16 (95%CI 0.58-114.40) in comparison with patients with neither the mutation and thrombophilia, without achieving statistical significance likely due to the small number of events. None of them had a recurrent thrombosis.

The JAK2 mutation was associated with an increased RR for thrombosis in the patients <60 years (RR 3.83, 95%CI 1.27-11.49). The analysis of this patient group highlights the findings obtained in the overall cohort, confirming that the homozygous state for the JAK2 mutation is highly associated with an enhanced thrombotic risk (Table 2). The patients <60 years with both the mutation and thrombophilia had an RR of 2.23 (95%CI 1.57-3.18) and 7.66 (95%CI 2.66-22.03) in comparison with mutated or wild-type patients without thrombophilia, respectively (Table 3). In contrast, neither carriership of heterozygous nor homozygous JAK2 mutation produced any increase in the thrombotic risk among the patients >60 years, even in the presence of thrombophilia (Tables 2 and 3). In the present study, the magnitude of the increase in risk for thrombosis among the overall JAK2 V617F-positive patients (2.2-fold), and among homozygotes in particular (3.8-fold), was consistent with previous reports.[16,17] Moreover, the excess in arterial thromboses among homozygotes is in agreement with a recent investigation.[15] At the time of diagnosis, the increase in risk for inaugural thrombosis among heterozygotes and homozygotes was substantially unchanged in respect to the overall estimate. In contrast, during the follow-up the risk for thrombosis was increased only for homozygotes (6.1-fold in respect to the wild-type patients).

Inherited thrombophilia produced a limited impact on the overall risk for thrombosis at any time, which was 2.7-fold increased among the carriers with respect to the non-carriers. This small increase in risk is in agreement with the mild clinical penetrance associated with heterozygosity of FV Leiden or PT G20210A.[8] However, in the presence of both the JAK2 mutation and inherited thrombophilia, the risk was 5-fold increased in comparison with non-carriers of either alteration, suggesting an additive interaction.

After stratification of the patients according to age, the presence of the JAK2 mutation was associated with a 3.8-fold enhanced risk for thrombosis only in those <60 years, but not in those >60 years. In the group of the younger patients with the JAK2 mutation, the presence of thrombophilia increased the risk for thrombosis at any time 2.2-fold in comparison with mutated patients without thrombophilia and 7.7-fold in comparison with wild-type patients without thrombophilia. We acknowledge that the results of our study are based on a small number of individuals carrying both inherited thrombophilia and the JAK2 mutation. Indeed, the characteristics of our cohort reflect the current knowledge concerning ET as regarding the rate of thrombosis, the rate of the JAK2 V617F mutation and its homozygosity, and the phenotype associated with the mutation. Finally, the prevalence of inherited thrombophilia was similar to that found in the general population. Therefore, our results are unlikely to be biased and can be generalized. In conclusion, in the younger patients with ET the thrombotic risk is higher in the JAK2 V617F-mutated and is further increased by the presence of inherited thrombophilia. Accordingly, it can be suggested that the knowledge of the JAK2 mutation (especially in the homozygous state) and of thrombophilia could allow a further risk stratification among the low-risk patients <60 years without history of thrombosis. The magnitude of the thrombotic risk in such patients and the final opportunity of employing such criteria for risk stratification and for tailored therapeutic measures during the follow-up must be confirmed by prospective trials.

Authorship and Disclosures

VDS conceived and designed the study and was responsible for the statistical analysis, the final interpretation of the data, and the final drafting of the manuscript; VDS, ER, TZ were responsible for the recruitment and clinical management of the patients, and the evaluation of the thrombotic events; TZ, AC, CL were responsible for the laboratory screening for thrombophilia; PC, AF, CL were responsible for the laboratory screening for JAK2 V617F mutation and for sequencing analysis; TZ, ER, AC were responsible for the final database collecting the laboratory and clinical data; GL as senior author critically revised the paper and gave important intellectual contribution. All authors were involved in the final revision of the article, interpretation of the data and final approval of the version to be published.

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