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JAK2V617F allele burden and thrombosis: a direct comparison in essential thrombocythemia and polycythemia vera

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Abstract

OBJECTIVE—A direct comparison of the incidence and risk factors of major thrombosis in essential thrombocythemia (ET) and polycythemia vera (PV) according to their respective JAK2V617F allele burden is the object of this study.

METHODS—We compared the rate (%/pts/yr) of major thrombosis in 867 ET patients (57% JAK2V617F) with that of 415 PV patients (all JAK2V617F) and examined risk factors.

RESULTS—Patients with ET wild type, ET V617F and PV showed a rate of thrombosis of 1.4, 2.1, and 2.7 %/pt/yr respectively. This latter was found to progressively increase according to the time of diagnosis. The actuarial probability of arterial and venous thrombosis in the first 5 years of diagnosis was roughly similar in the three groups. While, in the subsequent periods, the curves of mutated ET patients diverged from wild type and after 10–15 years, ET mutated arm approached PV.

CONCLUSION—These findings support the concept of a continuum between ET JAK2 mutated and PV not only in reference to the hematological phenotype but also in terms of vascular events

Keywords

JAK2V617F allele burden; rate of thrombosis; essential thrombocythemia; polycythemia vera

INTRODUCTION

Patients with essential thrombocythemia (ET) can be categorized as either JAK2V617F mutated (V617F+) or wild type (V617F–)^{1, 2}. Mutated patients display multiple features resembling polycythemia vera (PV), with significantly higher hemoglobin level and neutrophil counts, lower platelet count, more pronounced bone marrow erythropoiesis and granulopoiesis and higher tendency to transform to PV^{3, 4}. This has suggested that mutated ET patients and

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PV are phenotypic expression of the same pathological process in which the effects of the V617F mutation on erythropoiesis are constrained by physiological mechanisms, including erythropoietin suppression and depleted iron stores, or by genetic modifiers, either acquired or constitutional⁵.

Remarkably, the presence of leukocytosis⁶ and V617F mutation and/or the burden of mutated allele have been related to the rate of thrombosis both in ET and PV⁷ but no data are available on direct comparability of the two diseases in terms of vascular complications.

Aim of this paper was to see whether ET patients harboring JAK2V617F mutation had a rate of major thrombosis comparable to PV patients depending on V617F allele burden. A positive result would support the hypothesis that ET and PV may be viewed as a continuum not only for the hematological phenotype but also in terms of vascular complications and may strengthen the prognostic role of allele burden mutation.

MATERIALS AND METHODS

A total of 1282 patients with ET (n=867) and PV (n=415) followed in two Italian Institutions (Ospedali Riuniti Bergamo, Italy and Policlinico Careggi, Florence, Italy) constituted the study sample. ET patients were selected from the previously reported cohort of 1063 patients⁸ on the basis of JAK2V617F allele burden that was tested in patients from Bergamo and Florence centers. Permission was obtained from local IRBs to review the medical records.

All patients were diagnosed by the PVSG, and from 2002 by WHO criteria. Patients were at low- or high-risk for thrombosis according to standard risk factors (age ≥ 60 years and/or a previous major thrombotic event). Low risk patients were not given cytoreductive therapy whereas hydroxyurea (HU) was prescribed in the great majority of high-risk patients. Low dose aspirin was prescribed according to the judgement of physicians in charge. A small group of elderly patients (n=11, 1%) was given busulfan. Cytoreductive therapy was aimed to reduce platelet number to $< 600 \times 10^9/L$ and in the majority of PV patients, these drugs were associated with phlebotomies, to maintain hematocrit level to less than 45%.

Vascular events included ischemic stroke, cerebral transient ischemic attack (TIA), acute myocardial infarction (AMI), peripheral arterial thrombosis (PAT) and venous thromboembolism (VTE). Diagnostic procedures were previously reported⁹. Follow-up of patients was updated until the occurrence of vascular event or at the last visit and patients developing disease transformation were truncated at the time of evolution.

JAK2V617F mutation was tested in all 1282 ET and PV patients while JAK2V617F allele burden was quantitated, at different times from diagnosis (see result section), in 719 cases representing 79,4% of mutated patients. Quantitative Real Time PCR (QRT-PCR) assay was performed as previously published⁹.

Statistical methods

Continuous variables were compared using the extension of the Wilcoxon rank-sum non-parametric test for trend across ordered groups and categorical variables were analysed using the Pearson extended chi-square and Fisher exact tests to see differences across the three groups in Table 1.

Thrombotic episodes occurring in follow-up were calculated as rates % patient/year (pt/yr). Thrombosis-free survival curves adjusted for patients' age at diagnosis were calculated by the Kaplan-Meier method and the test for trend of the survivor function across ordered groups was

used to compare JAK2 wild type ET, JAK2 mutated ET and PV patients. Patients were divided in groups according to the time from diagnosis to JAK2 mutation and allele burden assay.

JAK2V617F allele burden and other meaningful covariates (leukocytosis) were examined as risk factors for thrombosis by using multivariable Cox proportional hazard model performed at diagnosis and after selecting patients who remained asymptomatic up to 5 years from diagnosis. The cut-off values chosen for WBC count were the quartiles of the whole distribution at diagnosis. Estimates were adjusted for disease (PV versus ET) and presence of standard risk factors in both models.

RESULTS

Patients were divided in three groups: ET wild type, ET JAK2V617F and PV (all JAK2V617F). Table 1 reports their main clinical and laboratory findings. The groups were significantly different for age and gender distribution. As expected, hemoglobin, hematocrit and leukocyte levels progressively increased across groups, whereas platelet number were lower. JAK2V617F allele burden greater than 50% was found in 45% of PV and 6% of ET patients, respectively. The values between 26 and 50% were almost equally distributed in ET and PV patients (45% versus 36%). Note that only 298 (45%) of patients were tested for JAK2V617F allele burden assay at presentation or within two years from diagnosis

Vascular events occurred during follow-up are shown in Table 2. The cumulative incidence of total major arterial and venous thrombosis was 146 out of 1282 patients (11%). Annual rates of thrombosis were 1.4% patients/year in ET JAK2 wild type, 2.1% in ET mutated and 2.7% in PV ($p=0.001$).

Thrombosis-free survival is presented in Figure 1. In the first 5 years from diagnosis, there was no significant difference among the three groups; subsequently, the curves of mutated patients started to diverge from ET wild type and, in a long-term prevision, the ET mutated curve approached PV (test for trend $p=0.05$).

To see whether this pattern could be associated with changes of JAK2V617F allele burden, we divided patients in groups according to the time from diagnosis to the date of quantitative JAK2 assay (Figure 2). In the majority of ET cases (57%), the allele burden at presentation or within two years from diagnosis was between 1–25% and this percentage lowered progressively according to the duration of disease in favour of groups between 26–50% and over 50%. This progressive change was also seen in PV patients in whom the initial allele burden values between 1–25% were found in only 24% of cases, while the categories 25–50% and over 50% were more represented.

The impact of JAK2V617F allele values on the incidence of thrombosis was analyzed in two multivariable Cox models (Table 3). The first included whole population at diagnosis and the second only patients who remained thrombosis-free for 5 years from diagnosis ($N=605$, 80 major vascular events). The two groups showed different prognostic factors for thrombosis. At diagnosis, PV (versus ET), standard risk factors (age above 60 years and/or previous thrombosis) and leukocytosis were independently associated with an increased risk of events while after 5 years from diagnosis JAK2 allele burden became the only independent factor for predicting subsequent thrombotic events ($HR=1.74$, 95% CI 1.00–3.50, $p=.01$; and $HR=2.89$, 95% CI 1.47–5.61, $p=.002$, respectively). It is worth noting that after 5 years of diagnosis the median leukocyte counts lowered from 9.1 to $7.6 \times 10^9/L$ due to the effect of HU therapy. This analysis was extended by comparing the rate of thrombosis in patients with the same allele burden (26–50%) but different disease (ET vs. PV), at different times from diagnosis. Results shown in Figure 3 stress that in the first 5 years from diagnosis the rate of thrombosis is

influenced by the hematological phenotype, despite similar allele burden, whereas this is not true in the later period.

DISCUSSION

This is a retrospective analysis of a large cohort of 1282 unselected patients with ET and PV diagnosed from 1981 to 2008. In JAK2 mutated ET patients, a higher rate of thrombosis, compared to wild type, was documented. This finding is in keeping with other experiences^{10, 11} and three recent metanalyses^{11,12,13} where the odds ratio of thrombosis was almost double in mutated vs. non mutated ET. We previously reported that the thrombosis rate in mutated ET patients was similar to that observed in PV.¹⁴ However, that study was restricted to the mutational status and did not include JAK2 allele burden measurements. Moreover, the number of patients was limited and the analysis considered the sum of thrombotic episodes occurring at presentation and during follow-up. We confirm here in a larger patient population that the rate of thrombotic complications in mutated ET patients is intermediate between ET wild-type and PV in the first 15 years of diagnosis.

Considering JAK2V617F allele burden, recent reports have indicated that in PV and ET, considered separately, the rate of vascular events progressively increased according to the amount of mutated alleles^{3,15,16} but a direct comparison of the two diseases was not done. Thus, whether the same allele burden values could produce comparable rates of events in ET and PV remained undetected. In the present analysis, we have found that the frequency of thrombosis progressively increased according to the amount of allele burden both in ET and PV. The highest rate of vascular complications was seen in groups with allele burden over 50% found in the majority of PV and in a small proportion of ET.

Time distribution of vascular events in our three groups showed an actuarial probability of arterial and venous thrombosis in the first 5 years of diagnosis roughly similar. In the subsequent periods, the curves diverged from wild type and after 10–15 years ET mutated curve approached PV. Based on multivariable analysis, our interpretation is that this pattern is due to the burden of JAK2 mutant clones that, in analogy to BCR/ABL clones in chronic myeloid leukemia, can manifest a survival advantage enabling them to expand after many years of diagnosis.

This process appears slower in ET since around 5% of mutated alleles were acquired over 5 years period. In the first 2 years of diagnosis, 38% of ET patients had JAK2 allele burden between 26–50% whereas the percentage was 55% after 10 years ($p=0.02$). In PV, this change seems more pronounced, as shown by the percentage of patients with allele burden more than 50% that moved from 34% to 66% after 10 years of diagnosis ($p<.001$). From these data, one could assume an allele accumulation over time, but we are aware that this interpretation is based on findings not collected from the same patients tested at multiple time points. The only way to support the hypothesis of allele burden accumulation would be to collect prospective data over many years. In primary myelofibrosis an even more significant progress over time of JAK2V617F allele burden has been reported by the GIMEMA group.¹⁷ These investigators showed in a prospective evaluation that heterozygous cases progressed to a homozygous V617F status at the calculated rate of 10% patients-year in the period from diagnosis to 8 or more years of disease duration.

Three previous papers faced the question of clonal evolution in ET and PV. The first was published by Gale et al.¹⁸, who in a long-term serial analysis of X chromosome inactivation patterns and JAK2V617F mutant levels in patients with ET showed that minor mutant-positive clones can remain stable for many years. The second by Tefferi et al.¹⁹ who observed a time dependent increase in JAK2V617F allele burden in PV whereas a similar phenomenon was

not shown in ET. The third by Theodorides et al.²⁰ who demonstrated in a 5 year longitudinal study little change in the allelic ratios of JAK2 mutations of PV and ET. Several variables may account for these discrepancies, including therapy, study design, length of observational period and number of patients.

We have explored, in two multivariable models, the clinical relevance of allele burden in terms of vascular complications. Considering patients at diagnosis, we confirmed that standard risk factors (age above 60 years and/or previous thrombosis) and leukocytosis were significantly associated with an increased risk of events in follow-up.^{8,9,21} PV versus ET at diagnosis was also an independent predictor of thrombosis and the reason could be ascribed to a more pronounced myeloproliferation including also an expansion of red cell mass. On the contrary, after 5 years from diagnosis, multivariable model showed that these factors lost their prognostic value very likely in relation to a good control of myeloproliferation by cytoreductive therapy. At this time, the strongest and unique risk factor for subsequent thrombosis was JAK2V617F allele burden so that PV or ET phenotype did not influence the rate of subsequent events. Values between 26 and 50% and above 51% were predictors of subsequent events independently on standard risk factors and diagnosis of PV versus ET.. Interestingly enough is that ET genotype progressively approached PV even though hematological phenotype remained that of ET entity in the great majority of patients. In fact, in this series, only 5% of ET cases transformed and met the criteria of PV diagnosis.

In conclusion, these findings support the concept of a continuum between ET JAK2 mutated and PV not only in reference to the hematological phenotype but also in terms of vascular events. In addition, they draw attention on the accumulation of allele burden over long-time and its possible role in predicting thrombotic complications.

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FIGURE 1. Thrombosis-Free Survival by disease and JAK2V617F status

Curves plotted using the Kaplan Meier method, adjusting for age at diagnosis. P-value associated to the test for trend across ordered groups is quoted.

FIGURE 2. JAK2V617F allele burden distribution by the time of the test
ET patients (n=390) and PV patients (n=329)

FIGURE 3.

Rate of thrombosis in patients with 26–50% of JAK2 V617F allele burden according to time.

Features of 1,282 patients

TABLE 1

	ET JAK2 wild type	ET JAK2V617F	PV JAK2V617F	P
Patients number	376	491	415	
Laboratory features				
Age (years, median, range)	49 (8 – 88)	56 (12 – 93)	61 (17 – 91)	<.0001 [†]
Gender, F/M (number, %)	249/125 (67/33)	338/155 (69/31)	191/222 (46/54)	<.0001 [†]
Hemoglobin (g/dL, median, range)	13.6 (9.7 – 15)	14.5 (8.9 – 15)	17.6 (13 – 24)	<.0001 [†]
Hematocrit (% median, range)	41 (30 – 51.5)	43 (30 – 54)	53.8 (40 – 71.5)	<.0001 [†]
White Blood Cells ($\times 10^9/L$, median, range)	8.3 (3.3 – 26)	9.1 (4 – 35)	10.5 (4.9 – 49.7)	<.0001 [†]
Platelets ($\times 10^9/L$, median, range)	817 (407 – 2269)	733 (304 – 2149)	487 (112 – 1639)	<.0001 [†]
Quantitative JAK2 ^{V617F} (%) *				
0	376 (100)	-	-	
1–25		190 (49)	64 (19)	
26–50		177 (45)	118 (36)	
>50		23 (6)	147 (45)	<.0001 [†]
Years from diagnosis to assay (median, range)	-	2.26 (0 – 24.8)	1.32 (0 – 24.5)	0.060 [†]
Evolution (number, %)				
PV	26 (7)	51 (10.5)	28 (7)	0.004 [†]
MF	1 (0.5) §	23 (5)	-	0.842 [†]
AML	24 (6)	25 (5)	25 (6)	0.568 [†]
	1 (0.5)	3 (0.5)	3 (1)	
Clinical features				
High-risk patients (number, %)				
Previous Thrombosis (number, %)				
IMA	136 (36)	285 (58)	248 (60)	<.0001 [†]
Ictus/TIA	61 (16)	132 (27)	70 (17)	0.003 [†]
PAT	12 (3)	40 (8)	10 (2)	
VTE	20 (5)	36 (7)	26 (7)	
	8 (2)	15 (3)	1 (0)	
	21 (6)	41 (9)	33 (8)	

* Percentages calculated respectively on 390 and 329 ET and PV JAK2V617F patients, for whom quantitative determination was carried out

[†] Wilcoxon rank-sum non-parametric test for trend
[‡] Pearson extended chi-square test
[¶] Fisher exact test
[§] JAK2 persistently negative

TABLE 2

Vascular events in follow-up in the whole series of 1,282 patients

	ET JAK2 wild type	ET JAK2V617F	PV JAK2V617F
Patients number	376	491	415
Follow-up (years, median, range)	5.0 (0 – 27)	3.7 (0 – 25)	3.8 (0 – 26)
Thrombosis in the follow-up (number, %)	34 (9)	54 (11)	58 (14)
IMA	9 (2)	9 (2)	13 (3)
Ictus/TIA	13 (4)	15 (3)	17 (4)
PAT	3 (1)	6 (1)	3 (1)
VTE	9 (2)	24 (5)	25 (6)
Rate of thrombosis (%/patients/year)	1.4	2.1	2.7
Relative Risk (95% CI)	1	1.5 (1.00–2.31)	2.3 (1.50–3.49)

TABLE 3

Multivariable analyses for the prediction of thrombosis in follow-up

<i>At diagnosis</i>	HR	95% (CI)	p
Disease (PV vs ET)	1.55	1.02 – 2.36	0.04
Standard risk factors *	1.89	1.34 – 2.66	0.001
JAK2 ^{V617F} (1–25%)	0.98	0.46 – 1.63	0.73
JAK2 ^{V617F} (26–50%)	1.12	0.79 – 1.87	0.56
JAK2 ^{V617F} (>50%)	1.19	0.81 – 2.01	0.52
WBC 7.5 – 9.1 ($\times 10^9/L$) [†]	1.52	0.88 – 2.61	0.13
WBC 9.1 – 11.3 ($\times 10^9/L$) [†]	1.67	0.92 – 2.70	0.09
WBC ≥ 11.3 ($\times 10^9/L$) [†]	1.93	1.01 – 2.77	0.04
<i>At 5 years from diagnosis</i>			
Disease (PV vs ET)	1.54	0.80 – 2.97	0.19
Standard risk factors *	1.19	0.71 – 2.01	0.51
JAK2 ^{V617F} (1–25%)	1.61	0.75 – 3.44	0.22
JAK2 ^{V617F} (26–50%)	1.74	1.00 – 3.50	0.01
JAK2 ^{V617F} (>50%)	2.89	1.47 – 5.61	0.002

* Aged 60 years or older and/or previous thrombosis

Reference categories: ET, age below 60 years and asymptomatic, JAK2 wild type, WBC less than $7.5 (\times 10^9/L)$.[†] Quartiles of the WBC count distribution at diagnosis used as cut-off; multivariable model using the PVSG cut-off for leukocytosis at $10 \times 10^9/L$ produced an HR of 1.826 with 95% confidence interval 1.065–3.133 (p=0.0287)