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Brief report

Molecular and clinical features of the myeloproliferative neoplasm associated with *JAK2* exon 12 mutations

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Although approximately 95% of patients with polycythemia vera (PV) harbor the V617F mutation in *JAK2* exon 14, several mutations in exon 12 have been described in the remaining patients. We conducted a European collaborative study to define the molecular and clinical features of patients harboring these mutations. Overall, 106 PVs were recruited and 17 different mutations identified. Irrespective of the mutation, two-thirds of patients had isolated erythrocytosis, whereas the remaining subjects had erythrocytosis plus leukocytosis and/or thrombocytosis. Compared with *JAK2* (V617F)-positive PV patients, those with exon 12 mutations had significantly higher hemoglobin level and lower platelet and leukocyte counts at diagnosis but similar incidences of thrombosis, myelofibrosis, leukemia, and death. In a multivariable analysis, age more than 60 years and prior thrombosis predicted thrombosis. These findings suggest that, despite the phenotypical difference, the outcome of *JAK2* exon 12 mutations-positive PV is similar to that of *JAK2* (V617F)-positive PV. (*Blood*. 2011;117(10):2813-2816)

Introduction

Polycythemia vera (PV) is a myeloproliferative neoplasm associated with somatic mutations of *JAK2* and, in few instances, of *LNK*[1] and characterized by increased red blood cell production.[2] The clinical course of this myeloproliferative neoplasm typically includes a prepolycythemic phase characterized by borderline erythrocytosis and thrombocytosis, an overt polycythemic phase with trilineage hyperplasia, and eventually post-PV myelofibrosis (MF).[3,4] Acute myeloid leukemia (AML) may occur as a result of additional somatic mutations. Approximately 95% of patients with PV carry the unique V617F mutation in *JAK2* exon 14,[4-6] whereas several mutations in *JAK2* exon 12 have been described in the minority of *JAK2* (V617F)-negative subjects.[7-16] In some patients, *JAK2* (V617F) and *JAK2* exon 12 can coexist as 2 separate clones.[17]

The initial study by Scott et al[7] led to the conclusion that *JAK2* exon 12 mutations define a distinctive myeloproliferative syndrome that is mainly characterized by isolated erythrocytosis and affects patients who receive a diagnosis of PV or idiopathic erythrocytosis. In a recent paper on 338 genotyped patients with PV, *JAK2* exon 12 mutations were detected in 4% of the cases.[4] However, it is currently unclear whether patients with *JAK2* exon 12 mutations have a distinct clinical course compared with *JAK2* (V617F)-positive patients.[18] *JAK2* (V617F) is preferentially found in subjects with a common constitutional *JAK2* haplotype, known as 46/1 or GGCC,[19-21] and this is true also for exon 12 mutations,[22] suggesting a common genetic background.

Because only small numbers of patients with *JAK2* exon 12 mutations have been reported by the various investigators, we initiated a collaborative study in Europe with the aim of collecting a large cohort of patients with this condition to define the molecular and clinical features of this myeloproliferative neoplasm.

Methods

We studied patients with PV associated to *JAK2* exon 12 mutations followed at 13 European centers. No intercenter differences in terms of demographics and blood cell counts were found. Thus, 320 consecutive patients with *JAK2* (V617F)-positive PV[4] from the Pavia center have been

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taken as controls. This study was approved by the Ethics Committee of the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. The procedures followed were in accordance with the Declaration of Helsinki of 1975, as revised in 2000, and samples were obtained after patients provided written informed consent.

Diagnostic criteria

The revised World Health Organization criteria were used for the diagnosis of PV.[2] The International Working Group on Myeloproliferative Neoplasms Research and Treatment criteria were applied to define transformation into post-PV MF,[23] and the World Health Organization criteria were adopted for diagnosis of AML.[2]

Various approaches were used for the detection of *JAK2* exon 12 mutations, including genomic DNA sequencing,[12] allele-specific polymerase chain reaction assays,[7,10,17] and high resolution melting[14] and melting curve analysis using wild-type specific hybridization probes.[15] In the control group, the granulocyte *JAK2* (V617F) mutation was assessed using a quantitative polymerase chain reaction-based allelic discrimination assay.[4]

Statistical analysis

An ad hoc database was developed for data collection and management. All statistical analyses were performed using Microsoft Excel 2000 and Statistica, Version 7.0 for Windows. The χ^2 of Fisher exact test was used to compare categorical variables among groups, whereas the Mann-Whitney U test was used to compare continuous variables among groups.

Results and discussion

In this multicenter study, we recruited 106 patients with *JAK2* exon 12-mutated PV. The most frequent mutation was N542-E543del (30%); the remaining are listed in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Because DNA sequencing can miss mutations with allele frequencies less than 10% to 15%, several more sensitive detection methods have been used in the study. Concerning diagnostic approaches, high resolution melting has been recently adopted and validated to detect *JAK2* exon 12 mutations and demonstrated its strength to routine detection of these highly variable mutations.[14]

Demographic and clinical data at diagnosis of 106 patients are reported in Table 1. Overall, 67 of 106 (64%) patients presented with isolated erythrocytosis, 16 of 106 (15%) with erythrocytosis and leukocytosis (white blood cell count $> 10 \times 10^9$/L), 13 of 106 (12%) with erythrocytosis and thrombocytosis (platelet count $> 400 \times 10^9$/L), and 10 of 106 (9%) displayed a trilineage pattern (erythrocytosis, leukocytosis, and thrombocytosis). With respect to the clinical phenotype at presentation, the Kruskal-Wallis test did not reveal any significant difference between the 5 most frequent mutations. Even grouping patients according to the detection method, no differences in clinical phenotype have been found. Two patients carrying also *JAK2* (V617F) have a heterogeneous phenotype.

The majority of patients had subnormal serum erythropoietin levels. Thus, in the absence of the *JAK2* (V617F) mutation, the presence of erythrocytosis and low serum erythropoietin merits investigation of exon 12 *JAK2* mutations. Bone marrow hypercellularity normalized for the patient's age was detected in 35 of 42 patients (83%). Interestingly, a recent pathologist-driven study suggested that bone marrow evaluation per se is not useful in the diagnostic approach to exon 12-mutated PV.[24]

Comparing the clinical phenotype at diagnosis of 106 PV patients with *JAK2* exon 12 mutations with that of 320 PV patients with *JAK2* (V617F),[4] Mann-Whitney U test showed that the former have a significantly higher hemoglobin level ($P < .001$), lower platelet count ($P < .001$), and leukocyte count ($P < .001$). Overall, isolated erythrocytosis was significantly more frequent in *JAK2* exon 12 mutated patients ($P < .001$), whereas the trilineage pattern was more frequent in *JAK2* (V617F)-positive patients ($P < .001$). We did not find differences in terms of age and sex.

Table 1. Demographic and clinical characteristics at diagnosis of 106 patients with *JAK2* exon 12-mutated polycythemia vera

Characteristic	Value
Age, y (n = 106)	
Median	52
Range	15-92
Sex (n = 106)	
Male	57 (54%)
Female	49 (46%)
Hemoglobin, g/dL (n = 106)	
Median	19.3
Range	16.6-25.0
WBC count, × 10^9/L (n = 106)	
Median	7.6
Range	4.2-21.0
Platelets, × 10^9/L (n = 106)	
Median	293
Range	132-1590
Cardiovascular risk factor* (n = 89)	55 (61%)
Splenomegaly (n = 73)†	17 (23%)
Prior thrombosis (n = 86)	13 (15%)
Subnormal serum erythropoietin (n = 77)	73 (95%)
Endogenous erythroid colonies (n = 42)	
Present	35 (83%)
Absent	7 (17%)
Bone marrow histology/trephine (n = 42)	
Normal cellularity (age-related‡)	7 (17%)
Increased cellularity (age-related‡)	35 (83%)
Karyotype (n = 45)	
Normal	40 (89%)
Abnormal§	5 (11%)
Familial cases	3 (3%)

*Smoking, arterial hypertension, diabetes, hypercholesterolemia and hypertriglyceridemia/dyslipidemia, and acquired or congenital thrombophilia were considered as cardiovascular risk factors.

†Splenomegaly was assessed by palpation.

‡According to EUMNET criteria.

§Abnormal as follows: 45, X,−Y; 46, XX, del(20p)/47, XX, del(20p),+14; 47, XY,+9; 46, XX, del(20q); 47, XX,+9.

The median follow-up of 106 patients was 3.9 years (range, 0-28 years). Sixty patients (56%) have been treated with cytotoxic therapy, whereas the remaining received only phlebotomy. The cumulative risk of events is reported in Figure 1. We found an incidence rate of 1.9×100 patient/years for thrombosis (11 patients), 0.5×100 patient/years (3 patients) for hemorrhage, 0.5×100 patient/years for post-PV MF (2 patients), 0.7×100 patient/years for AML (5 patients), and 1.3×100 patient/years for death (9 patients). Comparing these incidence rates with those obtained in *JAK2* (V617F)-positive PV, we found an incidence rate ratio of 0.8 (95% confidence interval [CI], 0.4-1.5; $P = .4$) for thrombosis, 0.3 (95% CI, 0.04-1.1; $P = .06$) for hemorrhage, 1.2 (95% CI, 0.3-5.7; $P = .8$) for post-PV MF, 1.5 (95% CI, 0.4-4.9; $P = .4$) for AML, and 1.8 (95% CI, 0.7-4.4; $P = .1$) for death.

To investigate prognostic factors for thrombosis, we tested by Cox proportional hazard regression different clinical parameters at diagnosis, such as age (continuous and > 60 years), sex, leukocyte

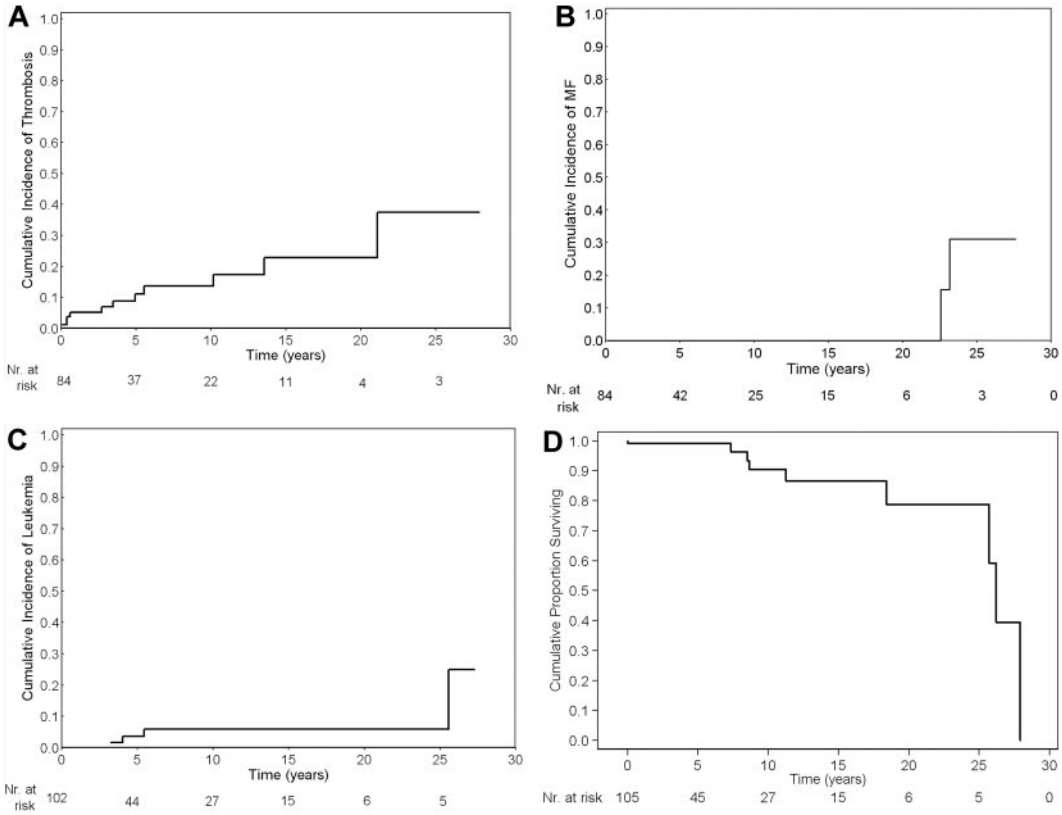

Figure 1. Cumulative risks of events. Cumulative risk of thrombosis (A), myelofibrosis (B), acute myeloid leukemia (C), and survival (D) in 106 patients with polycythemia vera carrying *JAK2* exon 12 mutations. For each graph, the number of patients at risk is reported.

count (continuous, > 10 and $> 15 \times 10^9$/L), platelet count (continuous, > 400 and $> 1000 \times 10^9$/L), hemoglobin level, and prior thrombosis. In a multivariate analysis, age more than 60 years (hazard ratio = 4.5; 95% CI, 1.2-17.1; $P = .028$) and prior thrombosis (hazard ratio = 3.9; 95% CI, 1.1-13.7; $P = .032$) independently predicted thrombosis. Increased leukocyte count had a borderline impact on post-PV MF occurrence ($P = .07$) as well as PV.[25]

In conclusion, this multicenter study indicates that PV associated with *JAK2* exon 12 mutations is mainly characterized by isolated erythrocytosis at clinical onset, irrespective of the type of mutation. This suggests that, in a clinical setting, it is not relevant to differentiate among mutations and, indeed, a screening method can be used for diagnosis. Despite the phenotypical differences, the clinical course appears to be very similar to that of *JAK2* (V617F)-positive PV, and current risk stratification of PV patients (advanced age and thrombosis) could be applied also in patients with *JAK2* exon 12 mutations.

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Authorship

Contribution: F.P. and M.C. designed research; F.P. interpreted results and wrote the paper; C.E., S.S., R.C.S., A.R.G., F.G., J.-J.K., M.F.M., M.R., C.B., A.M.V., E.L., H.G., E.R., T.H., T.L., C.A.O., and D.P. performed research and revised the manuscript; and C.P. performed statistical analysis.

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