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Brief report

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Although approximately 95% of patients with polycythemia vera (PV) harbor the V617F mutation in \textit{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 \textit{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 \textit{JAK2} exon 12 mutations-positive PV is similar to that of \textit{JAK2} (V617F)-positive PV. (Blood. 2011;117:10:2813-2816)

Introduction

Polycythemia vera (PV) is a myeloproliferative neoplasm associated with somatic mutations of \textit{JAK2} and, in few instances, of \textit{LNK}1 and characterized by increased red blood cell production.2 The clinical course of this myeloproliferative neoplasm typically includes a prepolycytemic phase characterized by borderline erythrocytosis and thrombocytosis, an overt polycytemic phase with trilineage hyperplasia, and eventually post-PV myelofibrosis, an overt polycytemic phase that is mainly characterized by isolated erythrocytosis and thrombocytosis, and in few instances, of additional somatic mutations. Approximately 95% of patients with PV carry the unique V617F mutation in \textit{JAK2} exon 14,4-6 whereas several mutations in \textit{JAK2} exon 12 have been described in the minority of \textit{JAK2} (V617F)-negative subjects.7-10 In some patients, \textit{JAK2} (V617F) and \textit{JAK2} exon 12 can coexist as 2 separate clones.11

The initial study by Scott et al7 led to the conclusion that \textit{JAK2} exon 12 mutations define a distinctive myeloproliferative syndrome that is primarily 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, \textit{JAK2} exon 12 mutations were detected in 4% of the cases.4 However, it is currently unclear whether patients with \textit{JAK2} exon 12 mutations have a distinct clinical course compared with \textit{JAK2} (V617F)-positive patients.19 \textit{JAK2} (V617F) is preferentially found in subjects with a common constitutional \textit{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 \textit{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 \textit{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 \textit{JAK2} (V617F)-positive PV3 from the Pavia center have been...
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. The International Working Group on Myeloproliferative Neoplasms Research and Treatment criteria were applied to define transformation into post-PV MF 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, allele-specific polymerase chain reaction assays, and high resolution melting and melting curve analysis using wild-type specific hybridization probes. In the control group, the granulocyte JAK2 (V617F) mutation was assessed using a quantitative polymerase chain reaction-based allelic discrimination assay.

**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 chi² 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 tests 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 exon 12 mutations and demonstrated its strength to routine detection of these highly variable mutations.

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 × 10⁹/L), 13 of 106 (12%) with erythrocytosis and thrombocytosis (platelet count > 400 × 10⁹/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.

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.

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 × 10⁶/100 patient/years for thrombosis (11 patients), 0.5 × 10⁶ patient/years (3 patients) for hemorrhage, 0.5 × 10⁶ patient/years for post-PV MF (2 patients), 0.7 × 10⁶ patient/years for AML (5 patients), and 1.3 × 10⁶ 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Age, y (n = 106)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>52</td>
</tr>
<tr>
<td>Range</td>
<td>15-92</td>
</tr>
<tr>
<td>Sex (n = 106)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>57 (54%)</td>
</tr>
<tr>
<td>Female</td>
<td>49 (46%)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL (n = 106)</td>
<td>19.3</td>
</tr>
<tr>
<td>Range</td>
<td>16.6-25.0</td>
</tr>
<tr>
<td>WBC count, × 10⁹/L (n = 106)</td>
<td>7.6</td>
</tr>
<tr>
<td>Range</td>
<td>4.2-21.0</td>
</tr>
<tr>
<td>Platelets, × 10⁹/L (n = 106)</td>
<td>293</td>
</tr>
<tr>
<td>Range</td>
<td>132-1590</td>
</tr>
<tr>
<td>Cardiovascular risk factor* (n = 89)</td>
<td>55 (61%)</td>
</tr>
<tr>
<td>Splenomegaly (n = 73)†</td>
<td>17 (23%)</td>
</tr>
<tr>
<td>Prior thrombosis (n = 86)</td>
<td>13 (15%)</td>
</tr>
<tr>
<td>Subnormal serum erythropoietin (n = 77)</td>
<td>73 (95%)</td>
</tr>
<tr>
<td>Endogenous erythroid colonies (n = 42)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>35 (83%)</td>
</tr>
<tr>
<td>Absent</td>
<td>7 (17%)</td>
</tr>
<tr>
<td>Bone marrow histology/ trephine (n = 42)</td>
<td></td>
</tr>
<tr>
<td>Normal cellularity (age-related†)</td>
<td>7 (17%)</td>
</tr>
<tr>
<td>Increased cellularity (age-related‡)</td>
<td>35 (83%)</td>
</tr>
<tr>
<td>Karyotype (n = 45)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>40 (89%)</td>
</tr>
<tr>
<td>Abnormal‡</td>
<td>5 (11%)</td>
</tr>
<tr>
<td>Familial cases</td>
<td>3 (3%)</td>
</tr>
</tbody>
</table>

*Smoking, arterial hypertension, diabetes, hypercholesterolemia and hyperglyc- eridemia/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.
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.

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


Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


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