JAK2V617F detection and dosage of serum erythropoietin: first steps of the diagnostic work-up for patients consulting for elevated hematocrit

The predictive values of common biological criteria for the diagnosis of polycythemia vera were studied in a cohort of patients with high hematocrit. We found JAK2V617F and erythropoietin assays were the most relevant first tests. Classification of patients according to their JAK2V617F status and erythropoietin levels facilitated the choice of further diagnostic investigations.

The discovery that more than 90% of people with polycythemia vera (PV) have the V617F mutation of JAK2 (JAK2V617F) calls for a re-assessment of the usefulness of Polycythemia Vera Study Group (PVSG) or World Health Organization (WHO) criteria for diagnosing PV. New guidelines have recently been proposed; however, they were not based on biological and clinical data from a large series of untreated patients. In the present study, in which the clinical and biological data of 419 untreated patients (168 with known JAK2 status) consulting for an elevated hematocrit were collected, we compared the PVSG, WHO and new PV criteria and tried to determine the most efficient diagnostic strategy. Males with a hematocrit (Hct) >50% and females with a Hct >48% were included after informed consent, following the guidelines of the ethical committee of "Région Bourgogne". The patients were evaluated before treatment, except for a small number who needed an emergency phlebotomy. The 57 patients with a Hct >60% (males) or >56% (females) were considered as having true erythrocytosis and red cell mass (RCM) was not measured. Standardized endogenous erythroid colony (EEC) assays, dosage of serum erythropoietin and detection of JAK2V617F by quantitative polymerase chain reaction in blood granulocytes were performed as described elsewhere.

The PVSG criteria were more stringent than the WHO criteria since 39 patients diagnosed as having PV by the WHO criteria were considered to have idiopathic erythrocytosis (IE) according to the PVSG. JAK2V617F was present in 13/13 WHO-positive/PVSG-negative PV patients who were tested. The WHO criteria were then used as a reference to evaluate the value of other PV markers in 168 patients for whom JAK2 status could be assessed. The positive and negative predictive values (PPV, NPV) of JAK2V617F with the WHO criteria as the reference were 92% and 97%, respectively. We used an alternate classification to evaluate the PPV and NPV of EEC, serum erythropoietin level and bone marrow histology (BMH). This classification was based on the WHO criteria minus the studied parameter which was replaced by JAK2V617F, considered as a major criterion. This modified classification allowed us to determine the PPV and NPV of EEC (95% and 83%, respectively), low (<3.3 IU/L) serum erythropoietin (86% and 89%, respectively), and bone marrow histology (91% and 77%, respectively). Although JAK2 status had excellent PPV and NPV, used singly none of the studied parameters was sufficient to establish or refute the diagnosis of PV. Among the 71 patients with the JAK2 mutation, six did not fulfil the WHO criteria (Table 1). Four had low erythropoietin levels: patients A86 and A98, initially classified as having apparent erythrocytosis, were thus classified as having idiopathic erythrocytosis.

Table 1. Characteristics of JAK2V617F-positive patients who did not have PV according to the WHO-PV criteria.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Diagnosis (WHO)</th>
<th>Hct (%)</th>
<th>RCM</th>
<th>Platelets (×10^12/L)</th>
<th>WBC (×10^9/L)</th>
<th>Epo (IU/L)</th>
<th>BMH</th>
<th>Splenomegaly</th>
<th>JAK2V617F (%)</th>
<th>EEC</th>
<th>Revised diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A86</td>
<td>F</td>
<td>AE</td>
<td>55.7</td>
<td>1.14</td>
<td>389</td>
<td>9.2</td>
<td>2.2</td>
<td>+</td>
<td>no</td>
<td>79</td>
<td>PV</td>
<td></td>
</tr>
<tr>
<td>A98</td>
<td>F</td>
<td>AE</td>
<td>53.2</td>
<td>1.20</td>
<td>408</td>
<td>8.8</td>
<td>1.8</td>
<td>+</td>
<td>no</td>
<td>65</td>
<td>PV</td>
<td></td>
</tr>
<tr>
<td>N89</td>
<td>F</td>
<td>IE</td>
<td>68.3</td>
<td>2.02</td>
<td>168</td>
<td>9.25</td>
<td>0.6</td>
<td>nd</td>
<td>no</td>
<td>32</td>
<td>PV</td>
<td></td>
</tr>
<tr>
<td>B164</td>
<td>M</td>
<td>IE</td>
<td>62.0</td>
<td>2.06</td>
<td>327</td>
<td>9.1</td>
<td>0.6</td>
<td>nd</td>
<td>nd</td>
<td>43</td>
<td>PV</td>
<td></td>
</tr>
<tr>
<td>B153</td>
<td>M</td>
<td>IE</td>
<td>52.9</td>
<td>1.33</td>
<td>127</td>
<td>6.2</td>
<td>1.8</td>
<td>no</td>
<td>19</td>
<td>IE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D109</td>
<td>M</td>
<td>IE</td>
<td>59.1</td>
<td>1.26</td>
<td>327</td>
<td>10.2</td>
<td>6.4</td>
<td>nd</td>
<td>no</td>
<td>14</td>
<td>IE</td>
<td></td>
</tr>
</tbody>
</table>

Hct: hematocrit; RCM: red cell mass; WBC: white blood cell count; Epo: erythropoietin; EEC: endogenous erythroid colony assay; BMH: bone marrow histology in favor of PV; BMH−: bone marrow histology; IE: idiopathic erythrocytosis; AE: apparent erythrocytosis; nd: no data; not in favor of PV; splenomegaly (palpable and/or confirmed with ultrasound); revised diagnosis was obtained according to our strategy as described in the text.
erythrocytosis, a very high hematocrit, the presence of JAK2V617F and the absence of a cause of secondary erythrocytosis led to the diagnosis of PV. Two other JAK2V617F-positive patients had normal erythropoietin levels. They do not have any WHO criteria of PV and show no signs of evolution in the absence of treatment 2 years after diagnosis. For these reasons, despite their JAK2V617F positivity, these patients were considered to have idiopathic erythrocytosis. Ninety-seven patients did not have the JAK2 mutation. Three of them fulfilled the WHO criteria of PV, with EEC. One had low erythropoietin levels and two had normal levels, but the assays were done after phlebotomy.

In line with recent reports, we show, in a large cohort of patients, that the presence of JAK2V617F has the best PPV and NPV for the diagnosis of PV. However, one cannot rely on a single test to make or reject the diagnosis of PV. Indeed, our study confirms that the absence of JAK2V617F does not exclude the diagnosis of PV and that, as previously reported, JAK2V617F can be found in patients not fulfilling PV criteria who are, therefore, classified as having idiopathic erythrocytosis.

We then asked whether a combination of tests could reach a PPV and/or NPV of 100%. This was possible only by combining JAK2 status, erythropoietin levels and EEC status and retaining the diagnosis when two of these three criteria were in favor of PV. Since EEC is not routinely available in every hospital, we evaluated the interest of first subgrouping patients according to their JAK2 status and erythropoietin levels to guide further explorations (Figure 1). This strategy allowed a diagnosis of PV to be affirmed in 61/72 patients finally classified as having PV (group 1) and definitively excluded PV for 11 patients with erythropoietin >17.7 IU/L (group 5). For patients in other groups, a confirmatory test (mainly EEC) should be performed if JAK2V617F is detected or if serum erythropoietin concentration is low (groups 2 and 3). In groups 4 and 5 (normal/high erythropoietin), RCM or a careful search for a cause of secondary erythrocytosis should be the first steps. This strategy prioritizes the most relevant investigations, thus saving time and money.

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Key words: erythrocytosis, polycythemia vera, JAK2V617F, erythropoietin, myeloproliferative disorders.

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References

8. Teffera A, Pandarani A. Mutation screening for JAK2V617F: when to order the test and how to interpret the results. Leuk Res 2006;30:739-44.