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

François Girodon, Eric Lippert, Pascal Mossuz, Irène Dobo, Nathalie Boiret-Dupré, Jean-François Lesesve, Sylvie Hermouet, Vincent Praloran

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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 *JAK2V*^{617F} and erythropoietin assays were the most relevant first tests. Classification of patients according to their *JAK2V*^{617F} status and erythropoietin levels facilitated the choice of further diagnostic investigations.

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The discovery that more than 90% of people with polycythemia vera (PV) have the V617F mutation of *JAK2* (*JAK2V*^{617F})[1-5] 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 large series of untreated patients.[6-8] 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 *JAK2V*^{617F} by quantitative polymerase chain reaction in blood granulocytes were performed as described elsewhere.[5,9]

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. *JAK2V*^{617F} 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

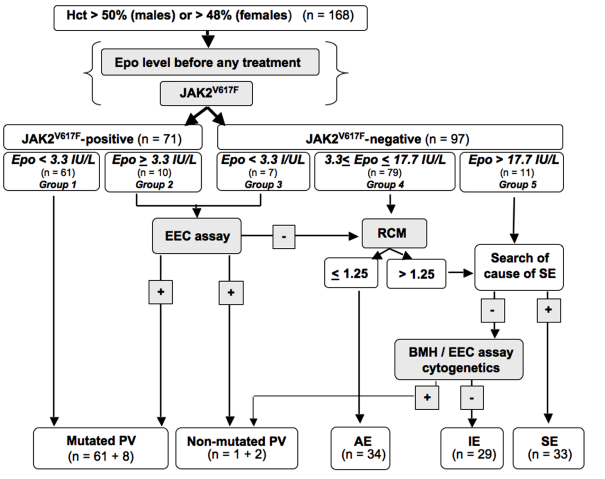

Figure 1. Diagnostic work-up of erythrocytoses with *JAK2*^{V617F} detection and serum erythropoietin (Epo) dosage as the first steps. Patients were classified in groups 1 (*JAK2*^{V617F}-positive, low serum Epo), 2 (*JAK2*^{V617F}-positive, normal/high Epo), 3 (*JAK2*^{V617F}-negative, low serum Epo), 4 (*JAK2*^{V617F}-negative, normal serum Epo) and 5 (*JAK2*^{V617F}-negative, high serum Epo). For each group, the most efficient exploration strategy is indicated. Hct: hematocrit; EEC: endogenous erythroid colony; RCM: red cell mass; PV: polycythemia vera; AE: apparent erythrocytosis; IE: idiopathic erythrocytosis; SE: secondary erythrocytosis.

(PPV, NPV) of *JAK2V*^{617F} 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 *JAK2V*^{617F}, 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* eventually developed a complete phenotype of PV with a RCM >1.25 and EEC formation. For patients N89 and B164 (who had only low serum erythropoietin and were thus classified as having *idiopathic*

Table 1. Characteristics of *JAK2*^{V617F}-positive patients who did not have PV according to the WHO-PV criteria.

Patient	*Sex*	*Diagnosis (WHO)*	*Hct (%)*	*RCM*	*Platelets (×10^9/L)*	*WBC (×10^9/L)*	*Epo (IU/L)*	*BMH*	*Splenomegaly*	*JAK2^{V617F} (%)*	*EEC*	*Revised diagnosis*
A86	F	AE	55.7	1.14	389	9.2	2.2	+	no	79	–	PV
A98	F	AE	53.2	1.20	408	8.8	1.8	+	no	65	–	PV
N89	F	IE	68.3	2.02	168	9.25	0.6	nd	no	32	–	PV
B164	M	IE	62.0	2.06	327	9.1	0.6	nd	nd	43	–	PV
B153	M	IE	52.9	1.33	127	6.2	9.7	–	no	19	–	IE
D109	M	IE	59.1	1.26	327	10.2	6.4	nd	no	14	–	IE

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 *JAK2V*^{617F} and the absence of a cause of secondary erythrocytosis led to the diagnosis of PV. Two other *JAK2V*^{617F} -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 *JAK2V*^{617F} 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 erythropoetin levels and two had normal levels, but the assays were done after phlebotomy.

In line with recent reports,[6-8] we show, in a large cohort of patients, that the presence of *JAK2V*^{617F} 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 *JAK2V*^{617F} does not exclude the diagnosis of PV and that, as previously reported,[10] *JAK2V*^{617F} 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).[9] For patients in other groups, a confirmatory test (mainly EEC) should be performed if *JAK2V*^{617F} 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.

François Girodon, Eric Lippert,° Pascal Mossuz,# Irène Dobo,@ Nathalie Boiret-Dupré,^ Jean-François Lesesve,§ Sylvie Hermouet,** Vincent Praloran°*

FG and EL contributed equally to this work

**Laboratoire d'Hématologie, CHU de Dijon, Dijon, France; °Laboratoire d'Hématologie, CHU de Bordeaux, Bordeaux, France; #Laboratoire d'Hématologie, CHU de Grenoble, Grenoble, France; @Laboratoire d'Hématologie, CHU d'Angers, Angers, France; ^Laboratoire d'Hématologie, CHU de Clermont-Ferrand, Clermont-Ferrand, France; § Laboratoire d'Hématologie, CHU de Nancy, Nancy, France; **Laboratoire d'Hématologie, CHU de Nantes, Nantes, France*

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Correspondence: François Girodon, Laboratoire d'Hématologie, Hôpital du Bocage, CHU de Dijon, Dijon, France. Phone: international +33.380293047. Fax: international +33.380293660. E-mail: francois.girodon@chu-dijon.fr

References

1. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005;434:1144-8.
2. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Cancer Genome Project. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054-61.
3. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352: 1779-90.
4. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia and myeloid metaplasia with myelofibrosis. Cancer Cell 2005;7:387-97.
5. Lippert E, Boissinot M, Kralovics R, Girodon F, Dobo I, Praloran V, et al. The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. Blood 2006;108:1865-7.
6. James C, Delhommeau F, Marzac C, Teyssandier I, Couedic JP, Giraudier S, et al. Detection of JAK2 V617F as a first intention diagnostic test for erythrocytosis. Leukemia 2006;20:350-3.
7. Tefferi A, Spivak JL. Polycythemia vera: scientific advances and current practice. Semin Hematol 2005; 42: 206-20.
8. Tefferi A, Pardanani A. Mutation screening for JAK2V617F: when to order the test and how to interpret the results. Leuk Res 2006;30:739-44.
9. Mossuz P, Girodon F, Donnard M, Latger-Cannard V, Dobo I, Boiret N, et al. Diagnostic value of serum erythropoietin level in patients with absolute erythrocytosis. Haematologica 2004;89:1194-8.
10. Percy MJ, Jones FG, Green AR, Reilly JT, McMullin MF. The incidence of the JAK2 V617F mutation in patients with idiopathic erythrocytosis. Haematologica 2006;91: 413-4.