

## Brief report

# The revised WHO diagnostic criteria for Ph-negative myeloproliferative diseases are not appropriate for the diagnostic screening of childhood polycythemia vera and essential thrombocythemia

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In the proposed revised World Health Organization (WHO) criteria for the diagnosis of *BCR-ABL*<sup>-</sup> myeloproliferative diseases (MPDs), exclusion criteria have been replaced by the presence of *JAK2* mutations. We applied these criteria to 45 children with MPDs: 13 with polycythemia vera (PV) and 32 with essential thrombocythemia (ET). Among these 45 patients, 12 with ET and 5 with PV had a

familial history of MPD, and had been investigated for hereditary mutations of the erythropoietin receptor, thrombopoietin, or *MPL* genes. We found that the *JAK2*<sup>V617F</sup> mutation in children occurs less frequently than in adults, and that exon 12 *JAK2* mutations are absent. On the basis of the revised WHO criteria, a significant proportion of childhood PVs were misdiagnosed. Furthermore, all fa-

miliar ET, including patients carrying the hereditary *MPL*<sup>Ser505Asn</sup> activating mutation, were erroneously diagnosed as MPDs. Our observations suggest that childhood MPDs require a set of specific diagnostic criteria. (Blood. 2007;110:3384-3386)

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## Introduction

The recent discovery of specific genetic defects in Philadelphia chromosome (Ph)-negative myeloproliferative diseases (MPDs)<sup>1-7</sup> has given new insights in understanding the molecular pathogenesis of these disorders. They indicate the neoplastic nature of the disease and represent specific markers of MPDs that could modify the current diagnostic approach, based on the exclusion of a secondary myeloproliferation.<sup>8,9</sup> For this purpose, a panel of clinical investigators and pathologists with expertise in myeloproliferative disorders has recently revised the World Health Organization (WHO) criteria for the diagnosis of *BCR-ABL*<sup>-</sup> MPDs.<sup>10</sup> The novelty of the proposed diagnostic guidelines is that *JAK2* mutation analysis plays a pivotal role in the diagnosis of polycythemia vera (PV), while it complements bone marrow histology in essential thrombocythemia (ET) and idiopathic myelofibrosis (IM).<sup>10</sup>

MPDs in childhood are quite rare diseases.<sup>11,12</sup> Although pediatric and adult MPDs exhibit similar hematologic findings,<sup>13,14</sup> we and others have recently documented that the incidence of *JAK2*<sup>V617F</sup> mutations in childhood PV and ET is significantly lower than in adults.<sup>15-17</sup> Moreover, we could highlight that several pediatric cases of ET are in reality hereditary disorders, often resulting from the *MPL*<sup>Ser505Asn</sup> activating mutation.<sup>15</sup> In order to evaluate whether the revised WHO diagnostic criteria<sup>10</sup> are appropriate for the diagnostic screening of pediatric PV and ET, in the present study we have applied them to a series of 45 children with PV and ET, including sporadic and familial forms.

## Patients and methods

Patient samples were obtained following informed consent in accordance with the Declaration of Helsinki and with the approval from the Institutional Review Board of the Catholic University, Rome, Italy.

We evaluated 45 children with PV (n = 13) and ET (n = 32) consecutively observed between 1980 and April 2007 at the Division of Hematology of the University "La Sapienza" of Rome. All patients were diagnosed in accordance to the Polycythemia Vera Study Group (PVSG) and/or WHO criteria.<sup>8,9</sup> A total of 17 patients (12 with thrombocythemia and 5 with polycythemia) had a familial history of MPD. In all these patients, the disease appeared to be inherited, with the disease present in one of the parents of the affected children.

Peripheral blood samples were collected after informed consent. The granulocyte cell fraction was isolated and subjected to molecular analysis for the *JAK2*<sup>V617F</sup> mutation.<sup>15,18,19</sup> The exon 12 *JAK2* mutations were investigated in patients with polycythemia who proved negative for the *JAK2*<sup>V617F</sup> mutation, according to the method described by Scott et al.<sup>6</sup> In all patients with a familial history of polycythemia or thrombocythemia, a mutation analysis was carried out for the erythropoietin receptor gene (*Epo-R*; exon 8)<sup>20</sup> and for the thrombopoietin (*TPO*; 5' untranslated region)<sup>21</sup> and *MPL* (exon 10) genes.<sup>22</sup> All female patients were tested for clonality of hematopoiesis.<sup>15,18</sup> In addition, all patients were evaluated for endogenous erythroid colony (EEC) growth.<sup>18</sup> The methods used for *JAK2*, *Epo-R*, *TPO*, and *MPL* mutation analysis, hematopoiesis clonality, and EEC assays have been described in detail elsewhere.<sup>15,18,19</sup>

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**Table 1. Incidence of myeloproliferative markers and hereditary polycythemia markers in 13 children with PV**

Marker	Sporadic polycythemia	Familial polycythemia
No.	8	5
<i>JAK2</i> <sup>V617F</sup> mutation, no. patients (%)	3 (37)	0
Exon 12 <i>JAK2</i> mutations, no. patients (%)	0	0
<i>Epo-R</i> mutations, no. patients (%)	0	0
Low serum Epo, no. patients (%)	2 (25)	0
EEC growth, no. patients (%)	3 (37)	1 (20)
Clonal hematopoiesis, no. patients*	2/2	0/3
<b>PV diagnosis, no. patients (%)</b>	<b>5 (62)</b>	<b>1 (20)</b>
2 major + 1 minor, no. patients (%)	3 (37)	0
1 major + 2 minor, no. patients (%)	2 (25)	1 (20)

The number of patients fulfilling the proposed revised WHO diagnostic criteria for PV is shown in the lower part of the table.

\*Number of patients with clonal hematopoiesis among all female patients.

## Results and discussion

### Patients with polycythemia

The proposed revised WHO criteria for a diagnosis of PV require the fulfillment of both major criteria (increased red cell volume and presence of *JAK2*<sup>V617F</sup> mutation or other functionally similar *JAK2* mutations) and of 1 minor criterion (panmyelosis at bone marrow histology, low serum Epo level, or EEC growth)<sup>10</sup> In the absence of *JAK2* mutations, the presence of 2 additional minor criteria is necessary for a diagnosis of PV.<sup>10</sup> All evaluated patients had an increased red cell volume and showed a hypercellular bone marrow, as required.<sup>10</sup> However, the *JAK2*<sup>V617F</sup> mutation occurred rarely in children with sporadic polycythemia (37%), and it was not detectable in patients with familial disorders (Table 1). Furthermore, in contrast to adult patients,<sup>6</sup> no additional exon 12 *JAK2* mutations were detected in *JAK2*<sup>V617F</sup>-negative cases. The serum Epo level was normal in most patients with sporadic polycythemia and in all patients with familial polycythemia (Table 1). As a consequence, the presence of EECs might play a fundamental role in confirming or excluding a diagnosis of PV. In line with the low incidence of *JAK2* mutations, only 4 of the 13 investigated patients exhibited an EEC growth (3 with sporadic disorders and 1 with familial disorder). We failed to find *Epo-R* mutations among our patients with familial disorders (Table 1). On the whole, the proposed revised WHO criteria allowed a diagnosis of PV in 5 of 8 patients with sporadic polycythemia and in 1 girl with familial polycythemia (Table 1). Interestingly, this latter patient exhibited a polyclonal hematopoiesis in the human androgen receptor assay (HUMARA; Table 1).

### Patients with thrombocythemia

The proposed revised WHO criteria for a diagnosis of ET require the fulfillment of 4 criteria: (1) sustained platelet count of  $450 \times 10^9/L$  or more; (2) typical bone marrow histology; (3) exclusion of PV, IM, chronic myelogenous leukemia, myelodysplastic syndromes, or other myeloid neoplasms; and (4) presence of the *JAK2*<sup>V617F</sup> mutation or of another clonal marker, or, in the absence of a clonal marker, no evidence of reactive thrombocytosis.<sup>10</sup> All pediatric patients with both sporadic and familial thrombocythemia fulfilled the first 3 criteria. With regard to the presence of a clonal marker, the *JAK2*<sup>V617F</sup> mutation was found in 40% of

sporadic forms, while it was absent in all familial cases (Table 2). Furthermore, among the 18 female patients evaluated, hematopoiesis was clonal in 8 (all with sporadic thrombocythemia), while the other 10 patients appeared polyclonal (5 with sporadic and 5 with familial diseases; Table 2). Importantly, secondary causes of thrombocytosis listed in the proposed revised criteria<sup>10</sup> were ruled out in all 32 patients. In 10 of 12 patients with familial thrombocythemia (4 of 5 investigated families), we found the *MPL*<sup>Ser505Asn</sup> activating mutation, which is transmitted by dominant autosomal inheritance<sup>20</sup> (Table 2). Nevertheless, all patients fulfilled the proposed criteria, independently of whether they had a sporadic or familial disorder (Table 2).

The aim of this study was to assess the applicability of the proposed revised WHO criteria<sup>10</sup> in childhood PV and ET. For this purpose, we applied the criteria to an unselected series of 45 children with PV and ET, diagnosed in accordance with the criteria in use at the time of their first evaluation.<sup>8,9</sup> In more than one-third of cases, the disease appeared to be inherited. Although the exact prevalence of hereditary disorders in our patient population could be overestimated, since 17 patients with familial diseases belonged to 7 different families, their overall occurrence in pediatric patients is undoubtedly noteworthy. All children with familial disorders failed to exhibit *JAK2* mutations, had a polyclonal hematopoiesis, and frequently showed the inherited *MPL*<sup>Ser505Asn</sup> activating mutation. Nevertheless, 1 patient with familial PV and all patients with familial ET met the proposed revised WHO criteria.<sup>10</sup> These observations suggest that the diagnostic screening of pediatric patients should first investigate whether other family members show similar hematologic abnormalities. In such patients, a careful screening for specific hereditary mutations should be performed: their detection would allow to exclude the presence of *JAK2* mutations and to avoid more invasive diagnostic procedures such as a bone marrow biopsy. In particular, in order to prevent children with familial PV and ET from undergoing an inappropriate diagnostic process that may ultimately lead to an erroneous diagnosis of MPDs, guidelines for the diagnostic work-up of PV and ET in childhood should clearly state that in patients with a familial history of polycythemia and thrombocythemia hereditary mutations of *Epo-R*, *TPO*, or *MPL* must be excluded. Moreover, it should be recommended that all patients testing negative for these mutations be re-evaluated if new inheritable defects are described.

In our series of patients, the *JAK2*<sup>V617F</sup> mutation was found only in a proportion of children with sporadic MPDs. These findings confirm and expand similar results obtained in previous

**Table 2. Incidence of myeloproliferative markers and hereditary thrombocythemia markers in 32 children with ET**

Marker	Sporadic thrombocythemia	Familial thrombocythemia
No.	20	12
<i>JAK2</i> <sup>V617F</sup> mutation, no. patients (%)	8 (40)	0
Exon 12 <i>JAK2</i> mutations, no. patients (%)	0	0
<i>TPO</i> mutations, no. patients (%)	0	0
<i>MPL</i> mutations, no. patients (%)	0	10 (83)
EEC growth, no. patients (%)	10 (50)	3 (25)
Clonal hematopoiesis, no. patients*	8/13	0/5
ET diagnosis, no. patients (%)	20 (100)	12 (100)

The number of patients fulfilling the proposed revised WHO diagnostic criteria for ET is shown in the lower part of the table.

\*Number of patients with clonal hematopoiesis among all female patients.

studies.<sup>15-17</sup> In addition, we have found that no exon 12 *JAK2* mutations were detectable in *JAK2*<sup>V617F</sup>-negative patients with PV. We do not know if these patients will acquire *JAK2* mutations later or if their disease originates from different pathogenetic mechanisms. Whatever may be the explanation, a significant proportion of childhood PV would nonetheless be misdiagnosed by using the proposed revised WHO criteria. In these cases, the presence of several alternative positive criteria, as listed in the currently used WHO criteria,<sup>9</sup> should be considered. To this end, our findings indicate that the presence of a clonal hematopoiesis could represent a specific (albeit not sensitive) marker of MPD also in pediatric PV and ET.

Taken together, our observations suggest that childhood MPDs require a set of diagnostic criteria different from those proposed for adult MPDs. These appropriate diagnostic criteria should (1) exclude familial forms due to inherited molecular defects and (2) consider that pathogenetic alterations found in adult patients are detectable only in a minority of children. Otherwise, the application of diagnostic guidelines developed for adult patients to pediatric MPDs could, in the future, expose children to inappropriate molecularly tailored therapies.

## References

- Zhao R, Xing S, Li Z, et al. Identification of an acquired *JAK2* mutation in polycythemia vera. *J Biol Chem*. 2005;280:22788-22792.
- Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase *JAK2* in human myeloproliferative disorders. *Lancet*. 2005;365:1054-1061.
- James C, Ugo V, Le Couedic JP, et al. A unique clonal *JAK2* mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005;434:1144-1148.
- Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of *JAK2* in myeloproliferative disorders. *N Engl J Med*. 2005;352:1779-1790.
- Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase *JAK2* in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005;7:387-397.
- Scott LM, Tong W, Levine RL, et al. *JAK2* exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007;356:459-468.
- Pikman Y, Lee BH, Mercher T, et al. *MPLW515L* is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *Plos Med*. 2006;3:e270.
- Michiels JJ, Juvonen E. Proposal for revised diagnostic criteria of essential thrombocythemia and polycythemia vera by the Thrombocythemia Vera Study Group. *Semin Thromb Hemost*. 1997;23:339-347.
- Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100:2292-2302.
- Tefferi A, Thiele J, Orazi A, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. *Blood*. 2007;110:1092-1097.
- Hasle H. Incidence of essential thrombocythemia in children. *Br J Haematol*. 2000;110:751.
- Osgood EE. Polycythemia vera: age relationships and survival. *Blood*. 1965;26:243-256.
- Cario H. Childhood polycythemia/erythrocytoses: classification, diagnosis, clinical presentation, and treatment. *Ann Hematol*. 2005;84:137-145.
- Dame C, Sutor AH. Primary and secondary thrombocytosis in childhood. *Br J Haematol*. 2005;129:165-177.
- Teofili L, Giona F, Martini M, et al. Markers of myeloproliferative diseases in childhood polycythemia vera and essential thrombocythemia. *J Clin Oncol*. 2007;25:1048-1052.
- Randi ML, Putti MC, Scapin M, et al. Pediatric patients with essential thrombocythemia are mostly polyclonal and V617F/*JAK2* negative. *Blood*. 2006;108:3600-3602.
- El-Moneim AA, Kratz CP, Boll S, et al. Essential versus reactive thrombocythemia in children: retrospective analyses of 12 cases. *Pediatr Blood Cancer*. 2007;49:52-55.
- Teofili L, Martini M, Luongo M, et al. Overexpression of the polycythemia rubra vera-1 gene in essential thrombocythemia. *J Clin Oncol*. 2002;20:4249-4254.
- Teofili L, Martini M, Cenci T, et al. Different *STAT-3* and *STAT-5* phosphorylation discriminates among Ph-negative chronic myeloproliferative diseases and is independent of the V617F *JAK-2* mutation. *Blood*. 2007;110:354-359.
- Kralovics R, Indrak K, Stopka T, et al. Two new *EPO* receptor mutations: truncated *EPO* receptors are most frequently associated with primary familial and congenital polycythemia. *Blood*. 1997;90:2057-2061.
- Ghilardi N, Skoda RC. A single-base deletion in the thrombopoietin (*TPO*) gene causes familial essential thrombocythemia through a mechanism of more efficient translation of *TPO* mRNA. *Blood*. 1999;94:1480-1482.
- Ding J, Komatsu H, Wakita A, et al. Familial essential thrombocythemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. *Blood*. 2004;103:4198-4200.

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## Authorship

Contribution: L. Teofili contributed to the study design, analyzed data, and wrote the manuscript. F. Giona, G.P., and A.A. enrolled patients and recorded clinical data. M.M., T.C., and F. Guidi performed cell cultures and molecular analysis. L. Torti analyzed data. G.L. and R.F. critically reviewed the manuscript. L.M.L. designed the study and wrote the manuscript.

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