

Philadelphia-negative Chronic Myeloproliferative Disorders in Children

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Abstract

Philadelphia-negative chronic myeloproliferative disorders (Ph-MPDs) are clonal haematopoietic disorders typically found in median-advanced age. The recently discovered acquired *JAK2V617F* mutation and other somatic mutations in *JAK2*, *TPO*, *MPL* and *EPO-R* in the myeloid cells of these patients are now considered specific biological markers, and their presence is considered a main diagnostic criterion of these diseases. Ph-MPD are extremely rare in children and need to be distinguished from other more common causes of thrombocytosis and erythrocytosis. Essential thrombocythaemia (ET) is the less rare Ph-MPD, while polycythaemia vera (PV) and primary myelofibrosis (PMF) are occasional findings in paediatrics. In this group of patients the incidence of haemorrhagic and thrombotic complications, as well as transformation into MF and acute leukaemia, seem to be rarer than in adults. Moreover, the biological markers found in adults with Ph-MPDs are not as common in paediatric cases, and familial forms are relatively frequent. Therefore, the tests used in adults are not exhaustive for most children. Because gene mutations are detectable in a minority of paediatric cases, new markers are needed to better understand such cases. Therapy is devoted to reducing symptoms, not improving collateral drug effects. New drugs targeting the *JAK2* molecule are under development and open new possibilities in the treatment of these rare diseases.

Keywords

Philadelphia-negative myeloproliferative disorders, essential thrombocythaemia, polycythaemia vera, primary myelofibrosis, *JAK2V617F*

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Polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF) are classified as Philadelphia-negative chronic myeloproliferative disorders (Ph-MPDs), clonal haematopoietic disorders^{1,2} that result in overproduction of mature myeloid cells and that are characterised by an increased risk of thrombotic and/or haemorrhagic complications³ and a possible evolution into MF and/or acute leukaemia.⁴ In PV there is a prevalent increase of the red cell line often associated with high granulocyte and platelet numbers, while ET is associated with an isolated elevated platelet count. PMF is characterised by the presence of a high reticuline rate in bone marrow. Primary Ph-MPDs typically occur in patients of advanced age and are very rarely seen in the paediatric setting, where they should be considered in differential diagnosis among various conditions.

Erythrocytosis, Thrombocytosis and Myelofibrosis

The expansion of the erythrocyte compartment in the peripheral blood may be absolute, as defined by the increase of total red cell mass; by contrast, relative erythrocytoses are caused by a severe reduction of the plasma volume. Absolute secondary erythrocytosis is driven by an increase in the erythropoietin (EPO) level,⁵ which may be due to either a physiological response to tissue hypoxia or abnormal EPO production, or a deregulation of the oxygen-dependent EPO synthesis. Other congenital forms of secondary

erythrocytosis may be due to abnormal haemoglobin with high oxygen affinity,⁶ to 2,3-bisphosphoglycerate deficiency⁷ or to altered oxygen-sensing pathway genes (*HIF-1- α* , *PHD2*, *VHL*).^{8–11} Another congenital primary erythrocytosis in which the erythropoietic compartment is expanded independently of EPO level is primary familial and congenital polycythaemia (PFCP), caused by a number of mutations in the cytoplasmic domain of the erythropoietin receptor (*EPO-R*) gene.¹² PV is a primary erythrocytosis with a thrombotic risk mainly related to rheological alterations. In adults, PV has an incidence of 2/100,000 persons/year¹³ and is slightly more common in males. Along with hereditary erythrocytosis, PV is very rare in children. The most common causes of erythrocytosis in children are secondary forms, such as renal diseases (cancers, transplantations, malformations), EPO-producing tumours (haemangioblastoma, hepatic adenoma) and Down's syndrome.¹⁴

Thrombocytosis in children is usually reactive to different conditions, such as chronic and acute inflammatory diseases, iron deficiency, asplenic states, neoplasms and use of drugs (steroids and vincristine).^{15,16} Primary thrombocytosis can be either isolated (ET) or the accompanying feature of other MPD (chronic myelogenous leukaemia, PV, PMF and hypereosinophilic syndrome). ET is characterised by a sustained increase of platelet count over the normal level; in adults it is twice as common in females than in males and has an incidence of 1.5/100000 persons/year.¹³ While reactive

Table 1: The Italian Association of Paediatric Haematology-Oncology Experience of Paediatric Essential Thrombocythaemia

Number of patients	90
Male/female	28/62
Median age at diagnosis (range)	6.75 years (1 month – 17 years)
Median follow-up duration (range)	5 (2–11 years)
Clinical symptoms and signs	None: 49% Headache: 19% Splenomegaly: 18% Vein thrombosis: 4% Nose bleeding: 2% Various: 8%
Mean platelets x 10 ⁹ /l (range)	1,260 (611–4,020)
WBC x 10 ⁹ /l	11.75
JAK2V617F/WT	16/65 (24%)
EEC-positive	17/28 (60%)
Monoclonality	15/32 (46%)
MPL/TPO mutation	None
Treatment adopted	None: 31% Aspirin: 32% Anagrelide: 29% IFN- α : 19% Hydroxyurea: 32%

All patients are Italian. No cases of familial essential thrombocythaemia are reported. IFN = interferon; WBC = white blood cell.

thrombocytosis is very frequent in children, the incidence of ET is considered to be very low.¹⁷

Bone marrow fibrosis occurs rarely as an evolution of paediatric malignancies, rheumatic diseases, sickle cell anaemia, infectious diseases, vitamin D deficiency rickets and severe combined immunodeficiency. PMF is the most rare Ph-MPD (estimated incidence 0.4–0.7/100,000 persons/year)¹⁸ and usually affects elderly people, reducing life expectancy.¹⁹ Fewer than 3% of cases occur in patients under 30 years of age, and PMF is only anecdotal in childhood.

Diagnosis of Polycythaemia Vera, Essential Thrombocythaemia and Primary Myelofibrosis in Adults

Multiple research groups have recently reported the occurrence in Ph-MPD of a somatic, acquired mutation (valine-617 to phenylalanine-V617F) in the JAK2 kinase auto-inhibitory domain.^{20–24} JAK2 is a protein involved in signalling transduction, and the V617F mutation determines a constitutive activation of JAK2, which continuously stimulates STAT5,^{20,22,23} leading to a proliferation of normally maturing cells.

Currently, such a mutation is considered a specific biological marker for Ph-MPD, playing a major role in pathogenesis. In fact, it is present in almost all patients affected by PV and in about half of those with ET and MF.²¹ Expression of mutant JAK2V617F has been suggested to induce kinase activation in haematopoietic cell lines, and homozygous JAK2 mutation is associated with pronounced trilinear megakaryocyte, erythroid and granulocyte myeloproliferation.^{20,22,23} JAK2V617F-mutated patients display different clinical and laboratory findings, including higher white blood cell (WBC) count, haemoglobin concentration and haematocrit and lower platelet count compared with wild-type patients.²⁵ ET and PV JAK2-mutated patients exhibit a higher risk of cardiovascular events and a more frequent evolution

into secondary MF.^{26,27} However, no clear dose-dependent correlation has been found in ET between the burden of the JAK2 allele and clinical symptoms;²⁸ in general, both PV and ET mutated cases have a worse prognosis than wild-type patients.^{27,29} Other mutations have been found in patients with Ph-MPD: somatic gain-of-function mutations affecting JAK2 exon 12 are identified in 25% of PV patients without the classic V617F mutation³⁰ and isolated elevated haematocrit without WBC or platelet increase. About 1% of ET and 5% of PMF cases carry acquired mutations in the thrombopoietin-receptor gene (MPLW515K/L).³¹

The discovery of JAK2 and other gene mutations is so important that the World Health Organization (WHO) updated the 2001 criteria for Ph-MPD in 2008.³² The diagnosis of PV is now established in patients with the two following criteria: increased haemoglobin or haematocrit or elevated red blood cell (RBC) mass and the presence of JAK2V617F or a similar mutation. In the few cases without any mutation, a minor criterion is needed (bone marrow trilineage myeloproliferation, subnormal serum EPO level or spontaneous endometrial epithelial cell [EEC] growth).

Since the JAK2V617 mutation is found in only about 50% of ET, it is diagnosed in the presence of the following conditions:

- a sustained platelet count over 450x10⁹/l;
- large and mature megakaryocyte proliferation in the bone marrow;
- no evidence of PV, PMF or other causes of reactive/secondary thrombocytosis; and
- a clonal marker such as JAK2V617F.

Clinical Picture and Treatment of Myeloproliferative Disorders in Adults

PV patients have a classic polyglobulic face with peripheral acrocyanosis, which is not present in ET and PMF. Most patients with Ph-MPD have splenomegaly and some also have liver enlargement. In all Ph-MPD patients, thrombotic and haemorrhagic events are quite common; primary events involve both arteries and veins, and secondary events are either spontaneous or complications of trauma or surgical procedures. These diseases can transit from one clinical phenotype to another and sometimes it is very difficult to establish which Ph-MPD is involved (undefined MPD). Both PV and ET can transform in myelofibrosis and all Ph-MPD can evolve into an acute leukaemia.

The treatment of Ph-MPD is aimed at reducing the thrombotic risk and controlling cell counts without increasing the risk of transformations. The haematocrit control in PV is obtained with phlebotomies. At present, a hematocrit lower than 45% is considered the goal of therapy.³³ The use of low-dose aspirin³⁴ is effective in controlling both primary and secondary thrombosis in PV, and on this basis aspirin is also commonly used in ET.³⁵ Cytoreductive drugs are able to reduce platelet and WBC counts and thrombotic complications,³⁶ and in PMF has been described to control spleen enlargement.^{14,37}

Myeloproliferative Disorders in Children

A few cases of PV in childhood have been published,^{14,38–41} all presenting with plethora and very high haematocrit levels. In 10 children JAK2V617F, in two JAK2 exon 12 mutations and in five JAK2 wild-type have been described.⁴²

No guidelines are available regarding the optimal management of PV in children. Almost all authors used phlebotomies to maintain the haematocrit below 50%; the use of low-dose aspirin is scarcely reported in spite of the demonstrated utility in adults.⁴³

A great interest in Ph-MPD in children has emerged recently, with a particular emphasis on ET. In fact, it affects not only those of median-advanced age but also young people,^{44,45} being diagnosed in those below 40 years of age in about 20% of cases⁴⁶ and, rarely, in children. By contrast, PV and MF are only anecdotal in paediatrics.

An increased platelet count over $500\text{--}600 \times 10^9/l$ is extremely common in children, to the extent that a secondary/reactive thrombocytosis is found in 5–6% of hospitalised children.^{15,16} Some other paediatric thrombocytoses are found in children from kindreds with familial thrombocytosis.⁴⁷ By contrast, it has been estimated that 0.09/100,000 children/year⁴⁸ are affected by ET. This means that ET in childhood is 60 times less common than in adults.⁴⁶

Most children with ET reported in the literature^{17,49–59} are characterised by young age, very high platelet counts and a quite severe clinical picture, with about 20% having haemorrhagic or thrombotic complications. Many children presented with headache. Rare cases of transformation are also reported, possibly related to drug use.^{49,60–62} Some reported cases belong to families with thrombocythaemia.⁶³ Platelet functional studies did not give evidence of significant alterations as in adults: serum EPO was found to be within normal limits and no patients with spontaneous EEC growth *in vitro*⁵¹ or mutation in the *TPO* or *MPL* genes were found.⁶⁴

On the whole, this picture suggests severe disease, but our opinion is that the reported cases focused more on complicated patients and the mild features could have been underestimated. In fact, we recently⁶⁵ collected a retrospective cohort of 90 children (under 16 years of age), all diagnosed as being affected by primary ET in agreement with the criteria in use at the time of the first diagnosis, followed in 14 centres of the Italian Association of Paediatric Haematology-Oncology (AIEOP) (see *Table 1*). The main clinical features were heterogeneous and different from those seen in adult ET. In fact, children seem to have a milder clinical picture, even with very high platelet counts; headache is confirmed to be the most common symptom. Vascular complications are infrequent even at long follow-ups and do not correlate with a high leukocyte count at diagnosis.^{66,67}

It is important to note that the myeloproliferative origin of ET in the paediatric age-group is confirmed only in a minority of children;^{68,69} in fact, about one-quarter of children with ET carry the *JAK2V617F* mutation, and no mutations of *MPL515* were found. However, it seems to be related to thrombosis, mainly in unusual veins, as in ET in adults.²⁶ In addition to these cases, the AIEOP found a high frequency of familiar cases that have suggested the use of specific criteria for the diagnosis of ET in children.⁴⁷

There are no guidelines for the treatment of paediatric ET. The usual aims for starting therapy were reported to be the resolution of symptoms and/or the reduction of platelet count at least to a level of $500\text{--}600 \times 10^9/l$.^{70,71} Low-dose aspirin (50–100mg/daily) has been used to control symptoms with no serious haemorrhagic risk. Hydroxyurea, interferon (IFN)- α and anagrelide were used

as cytoreductive agents. Each of these drugs is effective at reducing platelets, but they need to be used continuously and have significant collateral effects. Therefore, the necessity for cytoreduction needs to be confirmed.

PMF is extremely rare in childhood, as is secondary myelofibrosis.⁴³ Numerous neonatal cases suggest a congenital pathogenesis.⁷² Therefore, the clinical and laboratory findings suggest that PMF presenting in infancy may represent a distinct entity compared with the disease in adults, and a conservative approach to clinical management has been recommended.⁷² The treatment of paediatric patients with PMF is largely palliative, and therapeutic interventions are mostly used in patients with symptoms.³⁷

Final Considerations

On the whole, the findings show that PV, ET and PMF in childhood are heterogeneous diseases. While adults with Ph-MPD are now assessed using biological and histological diagnostic criteria, the same tests are not exhaustive for most children. Because gene mutations are detectable only in a minority of paediatric cases, new markers have to be found.

Considering that a relevant number of cases have been found to occur in families,⁴¹ the real number of paediatric sporadic Ph-MPD cases is not clear.

We do not know whether anti-aggregation is necessary in children, nor whether the commonly high platelets counts are dangerous for them. It is not clear whether the use of cytoreduction is necessary and what the long-term drug toxicities are.^{43,73}

The new biological insights are improving the therapeutic options for Ph-MPD. In fact, new drugs targeting the *JAK2* molecule are being developed, but no data are yet available regarding the safety and usefulness of these drugs in children.

The application of biological tests to all children with a clinical diagnosis of Ph-MPD, as well as the long-term observation of patients, will allow the proposal and validation of diagnostic and therapeutic guidelines specific for the paediatric community. Therefore, in our view, Ph-MPD in children represents an interesting field in which the combined use of biology and clinical observation may significantly improve diagnostic and therapeutic behaviour by the joint effort of both adult and paediatric haematology. ■



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