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Childhood polycythemia vera and essential thrombocythemia: does their pathogenesis overlap with that of adult patients?

Luciana Teofili,[1] Robin Foà,[2] Fiorina Giona,[2] and Luigi Maria Larocca[3]

[1]*Department of Hematology, Catholic University;* [2]*Division of Hematology, Department of Cellular Biotechnologies and Hematology, "La Sapienza" University;* [3]*Department of Pathology, Catholic University, Rome, Italy.*
E-mail: llarocca@rm.unicatt.it. DOI: 10.3324/haematol.12002

The incidence of polycythemia vera (PV) and essential thrombocythemia (ET) in children and adolescents is extremely low. The annual incidence of PV in patients aged less than 20 years is estimated to be about 2 new cases every 10 million people.[1] Similarly, the annual incidence of ET ranges from 1 to 4 new cases every 10 million people.[1-3] Therefore, on the whole it can be assumed that PV and ET are between 40 and 90 fold less frequent in children than in adults. The rarity of pediatric PV and ET has determined that, for many years, data on their clinical presentation and biologic features have been sparse and anecdotal.[4-10] Furthermore, it has been generally accepted that specific diagnostic criteria developed for adult patients with PV and ET should also apply to pediatric cases.[11, 12] Two recent reviews have extensively evaluated the pathogenesis of primary erythrocytosis and thrombocytosis (myeloproliferations independent of external influences) occurring in childhood.[13, 14] The authors indicate that, as in the adult population, pediatric age PV and ET can also present as sporadic diseases.[12,13] In addition, they underline that several cases of PV and ET reported in children are in fact familial diseases, caused by hereditary defects consisting of specific mutations of the *erythropoietin receptor*, *thrombopoietin* or *MPL* genes.[15-20]

To date, the discovery of specific genetic defects in adult Ph-negative myeloproliferative diseases (MPDs) has provided fresh insights into the understanding of the molecular pathogenesis of these disorders. Indeed, almost all patients with PV and about half of the patients with ET harbor a somatic point mutation in a highly conserved residue of the pseudokinase domain
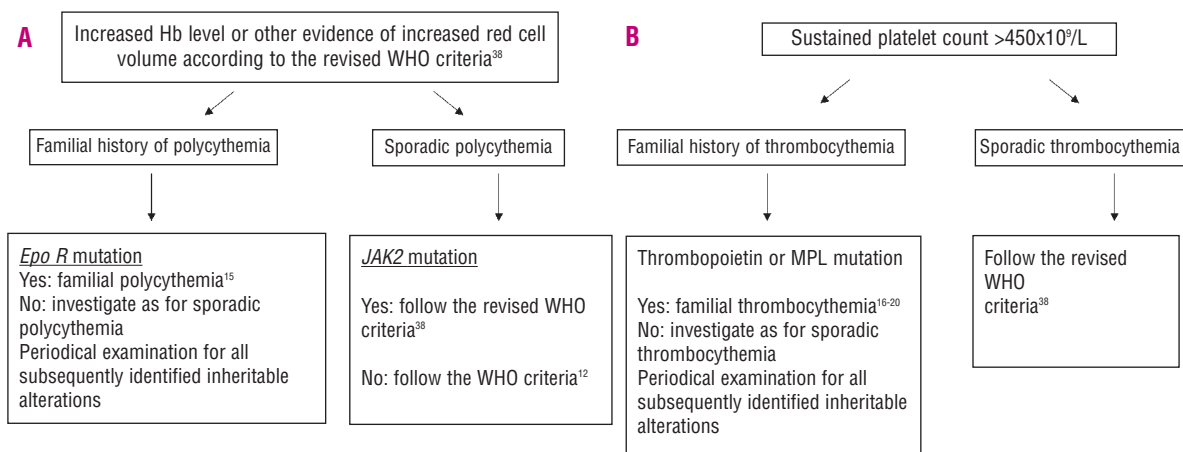
Figure 1. Proposed diagnostic algorithm for childhood polycythemia (A) and thrombocythemia (B).

of the *JAK2* tyrosine kinase, the *JAK2*[V617F] mutation.[21-25] Furthermore, in the rare PV patients who proved *JAK2*[V617F] negative, other functionally similar *JAK2* mutations involving the exon 12 have been described.[26] In addition to the presence of specific molecular alterations, the vast majority of female patients with PV, and a significant proportion of those with ET, show a clonal expansion of hematopoiesis, indicating the neoplastic nature of the myeloproliferation.[27] These specific MPD markers have been recently investigated in familial clusters of MPDs to explore whether *JAK2* mutations could also be present in the germ line, as reported for mutations of the *erythropoietin receptor*, *thrombopoietin* or *MPL* genes.[28-32] The results obtained have clarified that *JAK2*[V617F] is a somatic mutation secondarily acquired in this set of patients, occurring as frequently as in sporadic cases. In addition, like sporadic MPDs, these patients may also exhibit a clonal expansion of hematopoiesis.[28-32] Although the primary pathogenetic alteration underlying this type of familial MPD still has to be defined, unlike the mutations of the *erythropoietin receptor*, *thrombopoietin* and *MPL* genes[15-20] it does not appear to be hereditary.[28-32] Interestingly, members belonging to the same family can develop MPDs with different phenotypes.[28-32] This topic has been recently discussed in a well-presented review by Skoda and Prchal.[33]

The increasing knowledge accumulated over recent years in adult MPDs has prompted hematologists to reconsider pediatric PV and ET.[34-36] We have recently published a study carried out in 38 consecutive children with PV and ET,[34] diagnosed in accordance with the criteria in use at the time of their first evaluation.[11, 12] Among them, a patient with PV and 11 children with ET had a familial history of MPD. We have investigated the entire cohort of patients, including cases with a familial occurrence, for the presence of endogenous erythroid colony (EEC) growth, granulocyte *PRV-1*

RNA over-expression, *JAK2*[V617F] mutation and clonal hematopoiesis. Furthemore, in familial cases, mutations of the *erythropoietin receptor*, *thrombopoietin* and *MPL* genes were investigated. We found that 9 out of 11 patients with familial thrombocytosis showed an inherited activating mutation of MPL.[20] Indeed, the first important observation of this study was the high frequency of hereditary forms in this unselected series of patients. Although the exact prevalence of hereditary disorders in our patient population could be overestimated, since 12 patients belonged to 5 different families, their overall occurrence in pediatric patients is undoubtedly noteworthy. We observed that children with familial disorders did not exhibit the *JAK-2*[V617F] mutation, always had a polyclonal hematopoiesis, and rarely showed EEC growth and *PRV-1* RNA over-expression.[34] These data indicate that in children investigated for PV or ET, a careful screening for familial thrombocytosis and erythrocytosis is mandatory, since familial MPDs observed at this age are most likely due to inherited, although often unknown, defects. Importantly, the identification of specific hereditary defects could help these young patients to avoid more invasive diagnostic approaches, such as a bone marrow biopsy. Among 26 children with non-familial MPD, the prevalence of the *JAK2*[V617F] mutation was similar in PV (37%) and ET (38%), while a significant proportion of female patients had a clonal hematopoiesis.[34] The incidence of *JAK-2*[V617F] mutation in children with PV was significantly lower than in adult patients with PV investigated as control group (92% *vs.* 37%).[34] By contrast, the difference in the proportion of *JAK2*[V617F] mutated patients between adults and children with ET appeared less pronounced (58% *vs.* 38%).[34] In a previous study published in Blood by Randi *et al.*,[35] of 20 children with sporadic ET diagnosed according to the PVSG criteria 11 were examined for the presence of the *JAK2*[V617F] mutation and for the clonality of hematopoiesis. The

authors found that only 4 of them (25%) exhibited the $JAK2^{V617F}$ mutation and that the hematopoiesis was clonal in 4 out of the 15 female patients (28.5%). These findings differed significantly from the detection of 60% of $JAK2^{V617F}$ mutated patients and of 45% of clonal patients among the 47 adults with ET.[35] More recently, El-Monheim *et al.* evaluated the presence of the $JAK2^{V617F}$ mutation in 9 children with primary thrombocytosis.[36] While the bone marrow histology was suggestive of ET in all patients, the $JAK2^{V617F}$ mutation was detected only in 1 patient.[36] On the whole, the data that have emerged from these studies indicate that children with PV and ET harbor the $JAK2^{V617F}$ mutation much less frequently than adults.[34-36] Although it could be speculated that children showing a wild type $JAK2$ may become $JAK2^{V617F}$ positive at a later stage, the presence of EEC growth and of *PRV-1* RNA over-expression in patients who proved negative for the $JAK2^{V617F}$ mutation[34] suggests that, in pediatric MPDs, other molecular defects, functionally similar to the $JAK2^{V617F}$ mutation, could affect the $JAK2$ dependent signaling pathway.

At the time of writing, we have been investigating 47 consecutive children, of whom 19 had a familial MPD (5 PV and 14 with ET). We identified the *MPL* activating mutation in 3 further patients with familial thrombocytosis, while no hereditary genetic defects were found in the new cases of familial PV. In addition, all children with PV were investigated for the exon 12 $JAK2$ mutations,[26] but no case carrying these mutations was found.

On the whole, the findings obtained by our group confirm that pediatric erythrocytosis and thrombocy-tosis are heterogeneous diseases, including both sporadic and hereditary disorders. In adult patients, the presence of $JAK2$ mutations plays a pivotal role in the classification of MPDs[37] and offers the opportunity to modify the current diagnostic approach, based on the exclusion of a secondary myeloproliferation[38]. In contrast, pediatric cases often recognize different pathogenetic mechanisms. We have recently demonstrated that the proposed diagnostic guidelines of MPDs based on the detection of $JAK2$ mutations[38] are not appropriate for pediatric patients[39]. Indeed, in the case of childhood MPDs, it should first be established if the disease is congenital or acquired. For this purpose, the first step of the diagnostic screening should investigate the possible presence of a familial occurrence suggestive of the congenital origin of MPD. In fact, this set of patients has a very low probability of carrying $JAK2$ mutations while they could exhibit hereditary mutations of the *erythropoietin receptor*, *thrombopoietin* or *MPL* genes. In addition, as recently suggested,[34-36] $JAK2$ mutations are detectable only in a minority of children with non-familial PV and ET. For the diagnosis of all other cases, the extensive investigation of complementary MPD markers, such as clonality of hematopoiesis, EEC growth or *PRV-1* RNA over-expression should be performed. In line with these observations, we propose a specific diagnostic approach for childhood MPDs (see Figure 1). Meanwhile, it is hoped that studies investigating alternative pathogenetic mechanisms in adult patients with wild type $JAK2$ MPDs will help to clarify the genetic alterations underlying childhood PV and ET.

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Acquired thrombotic thrombocytopenic purpura: ADAMTS13 activity, anti-ADAMTS13 autoantibodies and risk of recurrent disease

Bernhard Lämmle,[1] Johanna A. Kremer Hovinga,[1] and James N. George[2]

[1]*Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital and University of Bern, Switzerland and [2]Hematology-Oncology Section, Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA. E-mail: bernhard.laemmle@insel.ch. DOI: 10.3324/haematol.12701*

Thrombotic thrombocytopenic purpura (TTP), first described by Moschcowitz in 1924,[1] has long been known as a generally fatal acute disease occurring in previously healthy subjects[2]. Despite its rarity, over the past eight decades it has attracted the interest of many researchers and clinicians whose many hypotheses as to its pathophysiological mechanisms have been widely discussed (for reviews see refs. #3-7). The outcome of affected patients has been dramatically improved by the largely empirical introduction of plasma therapy in the 1970s,[8] and a prospective study by the Canadian Apheresis Study Group demonstrated the superiority of plasma exchange with fresh frozen plasma (FFP) replacement over simple FFP infusion.[9] However, in spite of the use of plasma exchange and FFP replacement (and corticosteroids in many patients), the mortality from acute TTP is still high even today, with some 10-20% of patients dying from the acute disease episode.[5]

In 1982, Moake et al.[10] suggested that unusually large von Willebrand factor multimers (ULVWF), observed in plasma during the remission phase in 4 patients with a chronically relapsing form of TTP, were responsible for the recurring platelet clumping in the microvasculature of their patients. They hypothesized that a deficiency of a VWF depolymerase was causing the persistence in plasma of these highly adhesive ULVWF multimers.[10] In 1996, such a "depolymerase", initially named von Willebrand factor-cleaving protease, was partially purified from plasma[11,12] and later shown to be a member of the ADAMTS (A disintegrin and metalloprotease with thrombospondin type 1 repeats) family of proteases, denoted ADAMTS13.[13-16] In 1997, a complete deficiency of VWF-cleaving protease (ADAMTS13) activity was reported by Furlan et al.[17] in 4 patients, including 2 brothers, with chronic relapsing TTP, and in 1998, two independent retrospective studies showed that 20/24[18] and 37/37[19] patients with a clinical diagnosis of acute