REVIEW
Erythrocytosis in Children and Adolescents—Classification, Characterization, and Consensus Recommendations for the Diagnostic Approach

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During recent years, the increasing knowledge of genetic and physiological changes in polycythemia vera (PV) and of different types of congenital erythrocytosis has led to fundamental changes in recommendations for the diagnostic approach to patients with erythrocytosis. Although widely accepted for adult patients this approach may not be appropriate with regard to children and adolescents affected by erythrocytosis. The "congenital erythrocyto-sis" working group established within the framework of the MPN&MPNr-EuroNet (COST action BM0902) addressed this question in a consensus finding process and developed a specific algorithm for the diagnosis of erythrocytosis in childhood and adolescence which is presented here. Pediatr Blood Cancer 2013;60:1734–1738.
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INTRODUCTION

Erythrocytosis is characterized by the expansion of the erythrocyte compartment in the peripheral blood and is reflected by an increase in the erythrocyte count, the hemoglobin content and the hematocrit above the age-, sex-, race-, and altitude-adjusted reference range. Absolute erythrocytosis as defined by an increase of the total red cell mass is distinct from relative erythrocytosis which is caused by a severe plasma volume reduction (e.g., due to diuretics or severe diarrhea). The third variant, apparent or "spurious" erythrocytosis is caused by additive alterations of both the red cell mass and the plasma volume within the respective normal ranges resulting in an increased hematocrit (e.g., smoker's polycythemia) [1].

Based on pathophysiology and erythrocytoses are usually classified either as primary or secondary erythrocytosis which can be of either congenital or acquired origin (Fig. 1) [2]. Primary erythrocytosis is a condition in which the erythropoietic compart-ment is expanded independently of extrinsic influences or responding inadequately to them due to a primary abnormality of the erythroid precursor cells within the bone marrow. Due to the negative physiological feedback, primary erythrocytosis is usually characterized by a low erythropoietin (Epo) concentration. So far, the only molecularly characterized congenital primary erythrocy-tosis is caused by erythropoietin receptor (*EPOR*) gene mutations (familial erythrocytosis type 1, ECYT1). The only known acquired form of a primary erythrocytosis occurs in polycythemia vera (PV), a clonal stem cell disease with trilineage myeloproliferation but predominance of erythropoiesis primarily affecting elderly patients.

Secondary erythrocytosis is driven by hormonal factors (predominantly by Epo) extrinsic to the erythroid compartment. The increased Epo secretion may represent a physiologic response to tissue hypoxia, an abnormal autonomous Epo production or a dysregulation of the oxygen-dependent modulation of Epo synthesis. The responsiveness of erythroid progenitors to circulat-ing cytokines is usually normal although subtypes of congenital secondary erythrocytosis may also display features of primary erythrocytosis (i.e., VHL-related erythrocytosis, ECYT2).

In the last decade knowledge of genetic and physiological changes in chronic myeloproliferative neoplasms including PV and of different types of congenital erythrocytosis has increased enormously. Particularly the fact that somatic *JAK2* mutations (mainly JAK2 p.V617F and *JAK2* exon12 mutations) can be detected in almost all patients with PV has resulted in the revision of the generally accepted diagnostic criteria for this disorder [3]. It has also led to fundamental changes in recommendations for the diagnostic approach to adult patients with erythrocytosis [4–6]. This approach focuses on the primary exclusion of PV based on *JAK2* analysis as the first step in the diagnostic evaluation of a patient with erythrocytosis and subsequent analysis of *JAK2*-mutation-negative patients includes bone marrow examination. Although, these recommendations are widely accepted for the diagnosis of erythrocytosis in adult patients this approach may not be appropriate with regard to children and adolescents affected by erythrocytosis.

The "congenital erythrocytosis" working group (WG 3) established within the framework of the COST (European Cooperation in Science and Technology) action BM0902 MPN&MPNr-EuroNet (see www.mpneuronet.eu) addressed this question in a consensus finding process based on the personal

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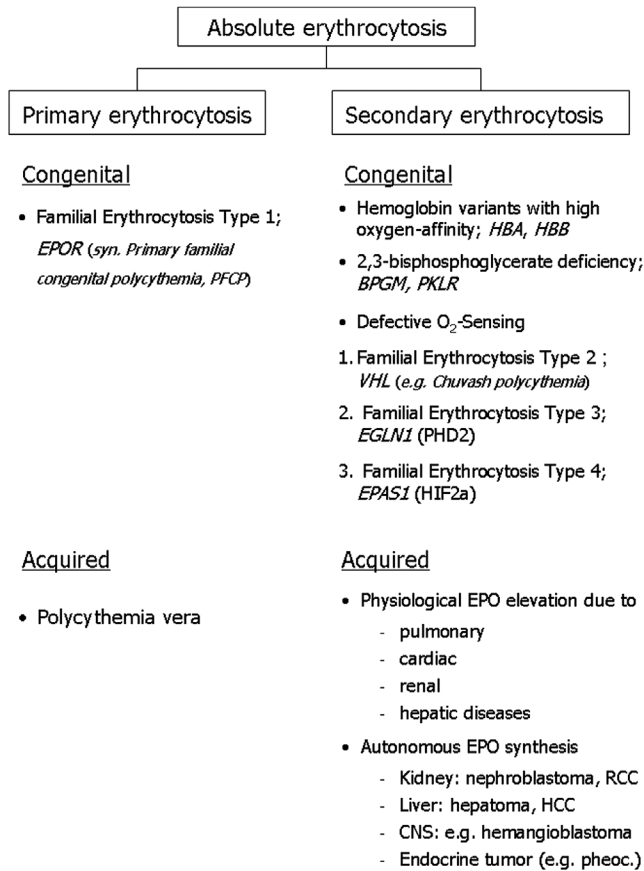
Fig. 1. Classification of absolute erythrocytosis.

experience of WG members and using published data on erythrocytosis in children and adolescents compared to data on adult patients.

METHODS

A panel of four central questions as a prerequisite to decide on recommendations to be included in diagnostic algorithms either in agreement or disagreement with established guidelines for adult patients were developed. At first, it had to be clarified whether different forms of erythrocytosis in children and adolescents do occur with a similar prevalence as in adults. The second question concerned the clinical, biochemical, and molecular characteristics of these disorders depending on the age of patients. At third, any age-specific aspects and consequences of diagnostic procedures had to be considered. And finally, it had to be concluded whether an alternative diagnostic algorithm would be regarded useful and should be proposed.

A systematic selective literature search using PubMed and other available search portals was performed by each author.

Finally, answers to the four panel questions and a draft of a specific diagnostic algorithm for pediatric patients with erythrocytosis were discussed after being circulated prior to the WG meeting at the 6th MPN&MPNr EuroNet meeting (Billund, Denmark, 24–26th October 2012) among 13 participants from eight countries.

RESULTS

Prevalence of Different Erythrocytosis Subtypes in Pediatric Patients and Adults

In contrast to congenital erythrocytosis, PV has a much lower incidence and prevalence in children and adolescents as compared to adults. The annual incidence of PV is about 20 per 1 million with a median age at manifestation of 60 years [7]. In childhood and adolescence PV is extremely rare and thus, exact epidemiological data on PV in this age group are not available or at least very limited. Early reports estimated that only 0.1% of the patients present at less than 20 years [8]. This was confirmed by later epidemiological studies reporting an age specific incidence of PV in all age groups at less than 20 years of 0.00–0.02 per 10^5 patient years [9]. To date, only about 50 pediatric patients with PV have been described in the scientific literature [10]. Therefore, any specific diagnostic approach to childhood erythrocytosis should primarily consider congenital erythrocytosis rather than PV.

Characteristics of Erythrocytosis Subtypes in Pediatric Patients and Adults

Data on the clinical and biochemical presentation of patients with congenital erythrocytosis with a defined underlying etiology are very sparse. Systematic evaluations are completely missing. Usually such data are reported in the context of new findings concerning the etiology and pathophysiology of these disorders. The inclusion of pediatric patients depends on the approach used in these studies. They can be focused on patient populations including both adults and children or on only one of these two groups, or these studies are based on the investigation of families affected by a single disorder. As far as it can be concluded from existing reports on congenital erythrocytosis based on these different approaches, the clinical and biochemical characteristics in affected children do not differ from those observed in adult patients, and molecular findings are of course identical. Possible clinical differences are within the range of that caused by heterogeneous phenotypic expression due to varying penetrance or co-inherited factors observed in the general affected patient population.

Since PV in childhood and adolescence is very rare, few data on diagnostic criteria and on the clinical presentation of pediatric patients are available. Overall, the clinical and biochemical presentation in children does not differ significantly from that in young adult patients [10]. Leukocytosis and thrombocytosis appear to be less frequent and less marked than in adult patients. However, more than half of the patients have leukocyte counts above 10 g/L and up to two-thirds of the patients present with platelet counts above 400 g/L in addition to erythrocytosis. [10,11]. Apart from two recent studies, very few data on the examination of erythropoietin-independent erythroid colony (EEC) growth and molecular genetics in pediatric PV patients are available. One cohort included eight sporadic and five familial pediatric PV cases [11,12]. In this series, only three patients had a JAK2 p.V617F mutation and none a *JAK2* exon 12 mutation, and only four patients displayed EEC growth. In contrast, in a second study including eight patients, the JAK2 p. V617F mutation was found in six patients and a JAK2 exon 12 mutation in two patients, and all patients examined for EECs were positive [13].

Specific Aspects of Diagnostic Procedures in Pediatrics

In general, all diagnostic procedures which are sequentially applied in adult patients with erythrocytosis according to the above recommendation can be performed in children and adolescents as well. However, in pediatric patients some investigations have specific requirements. Even blood sampling may be difficult in children. Frequent painful blood sampling is refused not only by the patient but also by the parents. Thus, if any of a pediatric patient's relatives is affected by erythrocytosis, all investigations requiring repeated sampling and/or large volumes of blood should be performed in the relative first. Bone marrow aspiration and trephine biopsy which in children are performed only with deep analgesic sedation or general anesthesia may also be difficult particularly in small children. Technical problems may compromise the intervention itself with an increased risk for injury and bleeding. In addition, small sample size can lead to diagnostic uncertainties. Magnetic resonance imaging which may be necessary to search for causes of acquired secondary erythrocytosis also has to be performed with sedation or even general anesthesia in young children up to the age of 8 years.

Diagnostic Approach to Erythrocytosis in Children and Adolescents

A specific diagnostic algorithm for the diagnosis of erythrocytosis in children and adolescents is regarded as useful because of the different prevalence of PV in addition to the specific requirements for diagnostic procedures in children and adolescents. We propose such an algorithm based on the current state of knowledge which is limited by the few available, reliable clinical and epidemiological data due to the extreme rarity of both congenital erythrocytosis and PV in pediatric patients (Fig. 2).

Diagnostic procedures aimed to identify possible causes of an absolute erythrocytosis in children and adolescents should be initiated if erythrocytosis is suspected based upon increased hemoglobin and hematocrit values in at least two separate blood counts performed at different time points and if relative erythrocytosis is excluded or at least unlikely (values >99th age-adjusted percentile or hemoglobin increase >2 g/dl from baseline).

If an absolute erythrocytosis is diagnosed, possible underlying disorders leading to a hypoxia-induced physiologic Epo-response and acquired secondary erythrocytosis have to be excluded first. An arterial oxygen saturation level (SaO_2) of less than 92% is regarded

Fig. 2. Proposed algorithm for the diagnosis of absolute erythrocytosis in children and adolescents. SpO_2, oxygen saturation measured by pulse oximetry; Epo, erythropoietin, p50, oxygen tension at which hemoglobin is 50% saturated, EEC, endogenous erythroid colonies formation, 2,3 BPG, 2,3 bisphosphoglycerate; [1]Epo may be in the lower normal range in PV. In the case of any typical PV symptom (splenomegaly, thrombocytosis, leukocytosis) diagnostic procedures should focus on PV exclusion first; [2]In the case of suspected PV but negativity for typical JAK2 mutation additional *EPOR* analysis should be considered particularly in cases with only mild PV-like symptoms (e.g., mild splenomegaly). If diagnosis of PV is probable, attempts to confirm this diagnosis can be made by analyzing the formation of endogenous erythroid colonies (EEC) and/or bone marrow (BM) biopsy including cytogenetics; [3]In the case of affected relatives the order of analyses will depend on the presumed inheritance pattern (VHL—recessive, EGLN1, EPAS1—dominant). A list of laboratories performing specific genetic analyses for congenital erythrocytosis is available at http://www.mpneuronet.eu/.

TABLE I. Inheritance and Serum Erythropoietin (Epo) in Congenital and Acquired Erythrocytosis

	Gene	Inheritance	Serum Epo
Familial erythrocytosis type 1 (OMIM #133100) = Primary familial and congenital polycythemia (PFCP)	*EPOR*	Dominant	↓-(↓)
Familial erythrocytosis type 2 (OMIM #263400) = Chuvash polycythemia, VHL-dependent polycythemia	*VHL*	Recessive	↑
Familial erythrocytosis type 3 (OMIM #609820)	*EGLN1* (PHD2)	Dominant	(↑)-↑
Familial erythrocytosis type 4 (OMIM #611783)	*EPAS1* (HIF2α)	Dominant	(↑)-↑
Hemoglobin with increased O$_2$-affinity	*HBB, HBA1*	Dominant	(↑)–↑
2,3 BPG deficiency *BPGM* deficency hereditary ATP increase (*PK hyperactivity*)	*BPGM PKLR*	Dominant	(↑)–↑
Polycythemia vera	*JAK2* (somatic)	(Dominant with variable penetrance)[a]	↓–(↓)
Acquired secondary erythrocytosis	—	—	(↑)–↑

[a]Dominant inheritance with variable penetrance is assumed for familial myeloproliferative neoplasms including PV.

as indicating a causal relationship to absolute erythrocytosis and should therefore lead to further cardiological and pulmonary assessment [14]. It has to be considered that even intermittent reductions of SaO$_2$ as example in the presence of a sleep-apnea syndrome may also lead to the development of erythrocytosis.

A careful physical examination and non-invasive procedures, abdominal ultrasound, pulse oximetry, and pulmonary function tests should be performed before or in parallel to the first blood analysis. At this stage, splenomegaly, and/or additional changes of the complete blood count as thrombocytosis or leukocytosis may indicate PV necessitating prompt targeted further investigation. The initial blood tests should include a blood gas analysis to exclude hypoxia and to calculate the p50 (oxygen tension at which hemoglobin is 50% saturated), liver and renal parameters to exclude severe disorders of these organs which can be associated with erythrocytosis, parameters reflecting iron storage and erythropoietic activity (i.e., serum ferritin, soluble transferrin receptor), and serum Epo.

Significantly increased serum Epo indicates secondary erythrocytosis (Table I) [1,2,15]. It may be caused by a physiologic response to hypoxia due to cardiac, pulmonary, renal, or hepatic disorders (i.e., acquired secondary erythrocytosis) or in congenital secondary erythrocytosis with high oxygen-affinity hemoglobin variants. Increased Epo is found in most cases with congenital erythrocytosis due to genetic defects leading to a dysregulation of oxygen-dependent Epo synthesis, that is, familial erythrocytosis types ECYT2 to ECYT4 (VHL-, PHD2 (*EGLN1*)-, HIF2α(*EPAS1*)-related erythrocytosis) [2,15]. Some patients with one of these disorders may present Epo levels within the normal range which nevertheless are inappropriately high for raised hemoglobin and hematocrit levels [16]. Increased Epo may also indicate an abnormal autonomous Epo production which is found in a variety of neoplasms. Thus, in the absence of an obvious cause for acquired erythrocytosis based on the initial examinations and after exclusion of the known genetic causes for secondary erythrocytosis a careful re-assessment of the possible presence of rare renal and hepatic disorders (e.g., vascular abnormalities) and of Epo-producing tumors (e.g., hemangioblastoma, pheochromocytoma, hepatic adenoma) by MRI and other imaging techniques is mandatory.

Epo levels below the normal range suggest the presence of either *primary familial and congenital polycythemia* (i.e., ECYT1, EPOR-related) or PV [2,15]. However, as in adult patients normal Epo in

children with erythrocytosis does not exclude PV [13]. Also single cases with an *EPOR* mutation presenting with Epo within the lower normal range have been reported.

If diagnosis of PV is probable despite the absence of a *JAK2* mutation, attempts to confirm this diagnosis can be made by analyzing the formation of endogenous erythroid colonies (EEC) or by bone marrow biopsy. The presence of EEC formation would strongly support the diagnosis of PV. However, it has to be considered that the EEC test is not standardized and should be performed only in experienced and specialized laboratories. Bone marrow histology will be useful in only a small number of patients. However, if in an individual patient with suspected PV but *JAK2* negativity there is no other obvious explanation for his erythrocytosis bone marrow histology may be helpful. For example, bone marrow changes affecting non-erythroid cell lines may be present and indicate PV even without corresponding changes in the peripheral blood. In addition, any cytogenetic abnormality will substantiate the presence of clonal hematopoiesis rather than polyclonal congenital erythrocytosis.

An important question to be solved before any further examination is whether other relatives are possibly affected. This is important for several reasons. First, to support evidence for inheritance of the disorder, second, if inheritance is probable to see whether it is dominant or recessive, and third, for the opportunity to examine an adult relative first before further diagnostic work-up of the affected child.

CONCLUSION

The proposed diagnostic approach to absolute erythrocytosis in children and adolescents considers the different prevalence of underlying disorders as well as the particularities in the performance of diagnostic procedures in this age group. Currently, it is expected that the diagnosis of the underlying cause of erythrocytosis is possible in about thirty percent of patients. Future findings of additional genetic causes of inherited erythrocytosis must be integrated into the proposed algorithm.

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