# Diagnosis and management of congenital and idiopathic erythrocytosis

Ther Adv Hematol
(2012) 3(6) 391–398
DOI: 10.1177/2040620712458947



**Mary Frances McMullin**

***Abstract:*** An erythrocytosis occurs when there is an increased red-cell mass. The causes of erythrocytosis are divided into primary, when there is an intrinsic defect in the erythroid cell, and secondary, when the cause is extrinsic to the erythroid cell. An idiopathic erythrocytosis occurs when the increased red-cell mass has no identifiable cause. Primary and secondary defects can be further classified as either congenital or acquired causes. The diagnostic pathway starts with a careful history and examination followed by measurement of the erythropoietin (EPO) levels. This allows a division of those patients with a low EPO level, who can then be investigated for primary causes of erythrocytosis, and those with a normal or high EPO level, where the oxygen-sensing pathway needs to be explored further. Physiological studies in those with congenital defects in the oxygen-sensing pathway show many changes in the downstream metabolism adapting to the defect, which has a bearing on the management of the disorders. Low-dose aspirin and venesection to an achievable target are the main therapeutic options that can be considered in the management of erythrocytosis. Specific guidance on venesection options should be considered with certain causes such as high oxygen-affinity hemoglobins.

***Keywords:*** erythrocytosis, erythropoietin, erythropoietin receptor, oxygen-sensing pathway

## Definition of an erythrocytosis

An erythrocytosis occurs when there is an increase in the red-cell mass to above 125% of the predicted value for the body mass of the patient [Pearson *et al.* 1995]. This is usually manifested by hemoglobin (Hb) levels above 185 g/L or hematocrit (HCT) above 0.52 in males; the equivalent figures in females are Hb 165 g/L and HCT of 0.48. These figures are a guide only and it may be necessary to formally measure the red-cell mass to demonstrate the presence of an absolute or true erythrocytosis, and to distinguish it from an apparent or relative erythrocytosis [Johannsson *et al.* 2005].

## Differential diagnosis of an erythrocytosis

The causes of an erythrocytosis are myriad and frequently unknown as can be noted when reflecting further on their classification. There is no reliable information on incidence and prevalence because this is a group with multiple aetiologies. However, overall these are rare disorders in clinical practice. The initial issue, once the presence of an erythrocytosis has been established, is to determine the cause. An erythrocytosis can be primary where there is an intrinsic defect in the bone marrow resulting in increased red-cell production. In contrast, a secondary erythrocytosis arises when something else drives the production of red cells. This is usually erythropoietin (EPO), the hormone that drives red-cell production. Those with a primary cause have a low EPO level, whereas those with a secondary cause are expected to have either inappropriately normal EPO levels for the raised Hb, or raised EPO levels. The causes of erythrocytosis can also be classified into congenital and acquired depending on the age of onset and family history. Using these definitions an extensive list of the causes of erythrocytosis results (see Table 1).

Primary causes consist mainly of the classical acquired polycythemia vera, but there are also rare congenital primary causes where there is an

Correspondence to:
**Mary Frances McMullin, MD, FRCPath**
Department of Haematology, 'C' Floor, Belfast City Hospital, Queen's University Belfast, Lisburn Road, Belfast, BT9 7AB, Northern Ireland, UK
**m.mcmullin@qub.ac.uk**

**Table 1.** Causes of erythrocytosis

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|---|
| **Primary erythrocytosis** |
| Congenital |
| EPO receptor mutations |
| Acquired |
| Polycythemia vera |
| **Secondary erythrocytosis** |
| Congenital |
| Oxygen-sensing pathway mutations |
| High oxygen-affinity hemoglobin |
| Bisphosphoglycerate mutase deficiency |
| Acquired |
| EPO mediated |
| Hypoxia driven |
| Central hypoxic process |
| Local renal hypoxia |
| Pathologic EPO production |
| Tumours |
| Exogenous EPO |
| Drug associated |
| **Idiopathic erythrocytosis** |

EPO, erythropoietin.

intrinsic defect in the erythropoietin receptor on the red cell resulting in a truncated receptor that signals for red-cell production without the presence of EPO. A number of these congenital anomalies have been described (as reviewed by Percy [Percy, 2007]).

Secondary erythrocytosis can result for a wide variety of reasons. Some rare congenital causes have been described. The oxygen-sensing pathway has a number of genes that are involved in the production of proteins which are linked and then degraded in the presence of oxygen. In hypoxia these proteins are not degraded, but survive and translocate to the nucleus where they facilitate the transcription of a number of genes, including the *EPO* gene, resulting in increased EPO production, and ultimately increased red-cell production. Mutated genes in the oxygen-sensing pathway result in proteins that do not degrade, survive and allow increased EPO production. To date, mutations have been described that result in erythrocytosis in those with homozygous or compound heterozygous mutations in the *VHL* gene [Ang *et al.* 2002], heterozygote mutations in the *PHD2* gene [Percy *et al.* 2006], and heterozygote mutations in the *HIF2A* gene [Percy *et al.* 2008; McMullin, 2010].

There are also other congenital defects that can result in a secondary erythrocytosis. High oxygen-affinity Hbs bind oxygen more tightly than normal and do not release it easily to tissues. A compensatory erythrocytosis results [Percy *et al.* 2009]. Rare enzyme deficiencies, such as bisphosphoglycerate mutase deficiency, can result in decreased 2,3 bisphosphoglycerate, a shifted oxygen dissociation curve, tissue hypoxia, and erythrocytosis [Rosa *et al.* 1978].

Acquired secondary erythrocytosis can result from several causes. A hypoxic process results in increased EPO production, which then results in erythrocytosis. The hypoxic process can be central as in chronic lung disease or a high altitude habitat. It can also be local to the kidney, the site of EPO production. An example of this is renal artery stenosis, which would produce an area of hypoxia. EPO can also be produced in a pathological setting. A number of different tumours that produce EPO have been described, such as some cerebellar haemangioblastomas. There are some situations where exogenous EPO is present, such as when EPO is administered deliberately to gain the benefits of increased Hb [Jelkmann and Lindby, 2011]. The administration of androgens can also have the effect of increasing Hb production [Dickerman *et al.* 1999].

## Idiopathic erythrocytosis

Once all the causes of primary and secondary erythrocytosis have been considered, there remains a group for which no cause for the erythrocytosis can be identified. This group is in a category termed 'idiopathic erythrocytosis'. The group consists of those with a low EPO level, which would suggest that there is an unidentified primary cause, and those with inappropriately normal or raised EPO level, which would suggest a secondary unidentified cause. The number of patients classified as having idiopathic erythrocytosis is decreasing as specific primary and secondary causes are identified, however, currently there remain a considerable number of individuals who despite extensive investigation fall into this category.

## Diagnostic pathway

Having made a diagnosis of erythrocytosis, a careful history and examination must be undertaken. This will allow exploration of the possible reasons for the erythrocytosis and any obvious causes can

then be investigated further. In those for whom there is no obvious explanation, further investigation can proceed with measurement of the EPO level. The results will fall into two categories. The first category consists of those with an EPO level below the normal range. This suggests a primary abnormality intrinsic to the erythroid cell and further investigation should be directed to the exploration of primary causes.

The second category is those with an inappropriately normal or raised EPO level, which suggests a secondary cause for the erythrocytosis, and further investigations should be directed toward a search for secondary causes, including defects in the oxygen-sensing pathway.

## Management of an erythrocytosis

Management of the specific disorder polycythemia vera has been discussed [McMullin *et al.* 2005], and will change as newer treatments, such as JAK2 inhibitors, come into use. However, the management of a congenital or idiopathic erythrocytosis is much more problematic as there is little evidence available for guidance. There have been a number of physiological investigations in some patients with congenital defects of the oxygen-sensing pathway, the results of which should be considered in deciding management options.

## Chuvash polycythemia

After the discovery of the homozygote arg200Trp mutation in the Chuvash cohort, studies were carried out retrospectively and cross-sectionally on a cohort of these patients compared with two control groups, spouses and community members (some of whom were heterozygotes for the mutation). Treatments in these patients included venesection, aspirin and, in a few early cases, busulfan. Venesection was associated with a nonsignificant decreased risk of thrombotic events and aspirin with a nonsignificant increase. Survival was decreased in patients compared with controls due to cerebrovascular and other thrombotic events. There was no evidence of increased deaths from malignancy in the Chuvash patients. Cross-sectional investigation in the living cohorts showed that homozygotes had higher Hb, a history of more frequent thromboses, lower systolic and diastolic blood pressure, and more venous varicosities compared with controls. It was of note that in the small number of heterozygote controls blood pressures were lower than in normal controls. Serum levels of EPO, transferrin receptor, vascular endothelial growth factor (VEGF), and total plasminogen activator inhibitor-1 were significantly higher in patients than any control group. In imaging studies significantly more vertebral body hemangiomas were identified in the Chuvash homozygotes [Gordeuk *et al.* 2004]. These retrospective studies did not show any benefit of venesection or aspirin use on events and mortality in the Chuvash patients, but they were small studies and therefore limited [Gordeuk and Prchal, 2006]. These studies show increased thrombolic events of relevance in Chuvash polycythemia patients, and therefore management should consider methods to prevent these events. However, nothing shown to be of therapeutic benefit has emerged from these studies.

## Physiological studies of Chuvash polycythemia

Careful and extensive physiological studies have been carried out in small numbers of Chuvash patients with parallel studies in controls. Patients with Chuvash polycythemia were treated with venesection. The controls were unaffected people and other polycythemia vera and erythrocytosis patients. Subjects underwent a series of experiments where they were subjected to degrees of hypoxia and their responses measured. Exposure to mild hypoxia provoked a greater increase in ventilation compared with all controls. Moderate hypoxia was poorly tolerated, but Chuvash patients also had abnormally high set points around which pulmonary artery pressure was regulated. Hypoxia produced much larger changes in pulmonary artery pressure. Parallel studies investigating the lymphocytes from patients and controls showed increased expression of aldolase C and VEGF on exposure to hypoxia. These studies suggest that the Chuvash patients demonstrated features that are characteristic of acclimatization to the hypoxia of high altitudes [Smith *et al.* 2006].

Further studies in this group of patients examined their response to the metabolic stress of exercise and physiological responses to a standardized meal. Exercise capacity was measured using an exercise test on a cycle ergometer. Magnetic resonance spectroscopy was used to measure calf muscle energy metabolites during exercise, and muscle biopsy samples were taken at rest after exercise. In patients with Chuvash polycythemia ventilation rates rose more rapidly than controls,

blood lactate concentrations rose early, and the maximum work rates achieved were lower. During exercise calf muscle phosphocreatine was depleted and inorganic phosphate increased markedly accompanied by a fall in pH. After a standard meal increases in plasma pyruvate and blood lactate levels were significantly greater in the Chuvash patients.

Muscle biopsies showed significant increases in mRNA expression of muscle phosphofructokinase, pyruvate dehydrogenase kinase isoforms 1, 2 and 4. Other mRNAs, protein expression levels, and enzyme activities studied did not differ from controls. These physiological studies show that although the Chuvash polycythemia is a relatively subtle defect, it has a major effect on the overall metabolism as soon as the metabolism is stressed [Smith *et al.* 2008; Formenti *et al.* 2010].

## Physiological studies of other oxygen-sensing pathway defects

Physiological studies were also carried out on a small number of those patients with HIF2A mutations, and these were compared with controls and the Chuvash patients. Compared with controls, HIF2A patients had significantly higher pulmonary arterial systolic pressures at baseline and a significant increase in response to moderate hypoxia. At baseline they had higher heart rates and greater cardiac output than controls, but did not differ from controls in response to mild or moderate hypoxia. In response to incremental exercise testing, there was overlap and therefore no significant differences between HIF2A patients and controls. The hyperventilation and hypocapnia at rest in the HIF2A patients persisted throughout the period of exercise.

In summary, HIF2A gain-of-function mutations are associated with pulmonary hypertension, increased cardiac output, increased heart rate, and increased pulmonary ventilation relative to metabolism. However, compared with the Chuvash patients, other aspects seen in the Chuvash phenotype are less obvious or absent. A further experiment in this series examined the physiological effect of venesection on an HIF2A patient by studying the patient prevenesection and postvenesection. This procedure reduced the haematological parameters as expected. However, there was no effect on ventilation and pulmonary arterial systolic pressure, heart rate, blood pressure, or cardiac output at baseline and with hypoxia. It also did not affect the accumulation of lactate, ventilation, maximum work rate, and maximum venous blood lactate during exercise to exhaustion. All parameters remained within the normal range. The benefit of venesection is not demonstrated in this study despite a reduction of HCT [Formenti *et al.* 2011].

Overall, physiological studies in patients with congenital erythrocytosis and defects in the oxygen-sensing pathway show considerable variations from normal, and these differences may be part of the body's adaptation to the defect. This suggests that venesection to a normal HCT may not be beneficial but in fact would disrupt the adapted physiology.

## Therapeutic options for the management of an erythrocytosis

### *JAK2 and Chuvash polycythemia*

Chuvash polycythemia patients have raised EPO levels suggesting a secondary erythrocytosis. However, they also have features seen in primary erythrocytosis, such as hypersensitivity to EPO. Recently, studies have been carried out to investigate further the downstream signalling of the VHL mutants. The VHL protein is a component of the E3 ubiquitin ligase, elongins BC/Cul2/VHL (ECV), which polyubiquitylates α subunits of HIF. Chuvash VHL mutants have a reduced capacity to form the ECV complex. JAK2 through STAT5 signalling is a critical mediator of proliferation and survival of erythroid progenitor cells. VHL is shown to bind the suppressor of cytokine signalling 1 (SOCS1) to form the heterodimeric E3 ligase, which targets the phosphorylated JAK2 for ubiquitin-mediated destruction. Chuvash VHL mutants have a reduced capacity to promote proteasome-dependent degradation of JAK2 and have an altered affinity for SOCS1, and therefore fail to engage and degrade phosphorylated JAK2. This would account for the hypersensitivity to EPO seen in Chuvash polycythemia. It also raises the possibility of JAK2 inhibition as a means of interrupting the disease process. The JAK2 inhibitor TG101209 was administered to $vhl^{R200W/R200W}$ knock-in mice and this reversed the disease phenotype. Therefore, JAK2 inhibition may be considered as a possible therapeutic manoeuvre in human disease in the future [Ryan *et al.* 2011].

### *Aspirin*

Aspirin suppresses the production of thromboxane by platelets and, therefore, is thought to be of benefit as an antithrombotic agent. This has been tested in patients with polycythemia vera in a randomized trial, the European Collaboration on Low-dose Aspirin in Polycythemia Vera (ECLAP), where treatment with low-dose aspirin compared with placebo resulted in a significant reduced risk of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes [Landolfi *et al.* 2004]. The use of aspirin has not been tested in any formal way in patients with erythrocytosis from other causes. However, it would seem reasonable, given the proven efficacy in other situations such as polycythemia vera, that it should be given at low dose to those with no specific contraindication. Asprin may reduce the incidence of thromboembolic events in these disorders.

### *Venesection*

In those with a raised Hb and HCT, reduction of the HCT by venesection is a possible therapeutic manoeuvre. It reduces the viscosity and therefore may be of therapeutic benefit. It has been used in the management of polycythemia vera for many years. The efficacy and best therapeutic target for the HCT is not certain. In a retrospective study, the relationship between the HCT and platelet count in 69 patients with polycythemia vera and the incidence of vascular occlusive events was investigated [Pearson and Wetherley-Main, 1978]. Patients were treated with venesection with or without chemotherapy. The incidence of vascular occlusive episodes correlated positively with the HCT level. The risk of vascular occlusive episodes was increased when the HCT was increased from a low normal to a high level within the normal range. From this data the optimum therapeutic HCT level to reduce the risk of events was below 0.45. Another study from the same institution studied cerebral blood flow in 16 patients with polycythemia prevenesection and postvenesection compared with a control group. Cerebral blood flow was decreased in those with polycythemia, and reduction of the HCT to a mean of 0.45 was associated with a 73% increase in cerebral blood flow [Thomas *et al.* 1977a]. An accompanying study found that cerebral blood flow was significantly lower in 38 patients with HCTs in the range 0.47–0.52 compared with another group with HCTs in the range 0.36–0.46. Furthermore, cerebral blood flow improved by 50% when remeasured after the HCT was reduced by venesection. [Thomas *et al.* 1977b]. These studies provide evidence for the use of a venesection to a target of 0.45 in polycythemia vera.

The ECLAP study recommended that the HCT was maintained below 0.45. However, entrants to the study had a range of HCTs, which varied with time. Throughout the study 10% of patients had HCTs above 0.50. In this study, there was no difference in the risk of death or thrombosis in those with HCTs below 0.45 than in those with HCTs above this value [Di Nisio *et al.* 2006]. However, it is noted that the number with much higher HCTs was small and decreased during the period of the study, but it does not support reduction of the HCT to the low value of 0.45. In polycythemia vera the most efficacious HCT is currently under investigation in a randomized trial.

In other causes of erythrocytosis reduction of the HCT by venesection may be of therapeutic benefit. However, as noted from physiological studies, this is not necessarily the case as the system may require the higher HCT to maintain physiological processes. Some reduction of the HCT may be beneficial in reducing the thromboembolic risk and for the treatment of specific symptoms. A therapeutic target is unknown but reduction to 0.45, as has been used in polycythemia vera and has been demonstrated from evidence to be the target in that disorder, is unlikely to be possible in those with a very high HCT, and may not be the best management given the adapted physiology that has been demonstrated in some of the congenital erythrocytoses. Judicious venesection with a higher HCT as target may be considered a therapy.

## Management of idiopathic erythrocytosis

The group termed ‘idiopathic erythrocytosis’ is that where no cause for the erythrocytosis has been identified. This group has been studied in the past and referred to as ‘benign erythrocytosis’ [Modan and Modan, 1968], but it has not always been shown to have a benign course. Other authors have referred to it as ‘pure erythrocytosis’ [Najean *et al.* 1981]. In a published series [Modan and Modan, 1968; Pearson and Wetherley-Mein, 1979], the incidence of vascular complications is as high as 46% at presentation, and 17% died of cerebrovascular accidents [Pearson and Wetherley-Mein, 1979], or comparable events rates to polycythemia

vera [Modan and Modan, 1968]. The management plan must consider these facts, but also note that these series are very old and do not have the benefit of more recent diagnostic investigation. With this in mind experts have reviewed the evidence and suggested a management plan. This recommends the reduction of the HCT to less than 0.45 if it is greater than 0.54. Reduction of the HCT to less than 0.45 if it is less than 0.54 should be undertaken if there is an increased risk of thrombosis as suggested by evidence of ischemia, previous history of thrombosis, peripheral vascular disease, diabetes, or hypertension. In these individuals with no identified cause of erythrocytosis cytoreductive therapy is contraindicated [McMullin *et al.* 2005].

## Management of high oxygen-affinity Hbs

A high-affinity Hb leads to a high Hb by physiological adaptation. When considering venesection in these patients, it is of note that no randomized trials of venesection to prove efficacy have been undertaken. A number of observations when considering venesection should be noted. Most individuals with high-affinity Hb are asymptomatic but hyperviscosity symptoms and thromboembolic events have been reported [Fairbanks *et al.* 1971; Weatherall *et al.* 1977]. Experiments using isovolemic venesection that reduced the HCT from 0.55 to 0.41 can reduce exercise performance [Butler *et al.* 1982; Winslow *et al.* 1983]. In some families thrombotic episodes have only occurred in compound heterozygotes for high-affinity Hb and a thrombophilic defect [Berruyer *et al*, 1994; Hanss *et al.* 2002]. However, individuals with dizziness, dyspnoea, or angina may obtain clinical benefit from venesection [Fairbanks *et al.* 1971; Grace *et al.* 1992].

With evaluation of all these factors it is suggested that consideration should be given to venesection to reduce the HCT in those with symptoms such as dizziness, dyspnoea, or angina where the HCT may be a contributory factor. Venesection should also be considered in those with one or more previous thrombotic episodes and in affected asymptomatic individuals where a family member with a high oxygen-affinity Hb has developed thrombotic problems. Partial exchange should be considered if the HCT is greater than 0.60 and major surgery is required [Larson *et al.* 1997]. Venesection to reduce the HCT to less than 0.60 is recommended [Weatherall *et al.* 1977], but when thrombosis or symptoms compatible with hyperviscosity have developed at a lower HCT then a target of 0.52 is suggested [McMullin *et al.* 2005].

## Management options: conclusion

The therapeutic options for a patient with congenital and erythrocytosis are limited and largely of unproven efficacy. Low-dose aspirin in those without a specific contraindication and venesection to an achievable target HCT are the main treatments to be considered. Specific measures can be considered in situations such as high-affinity Hb. In the future JAK2 inhibitors may have a role in the treatment of Chuvash polycythemia.

## Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## Conflict of interest statement

The author declares no conflicts of interest in preparing this article.

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