Diagnosis and Treatment of Erythrocytosis

Mary Frances McMullin

Professor of Clinical Haematology, Queens University, Belfast

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Abstract

An erythrocytosis arises when the red cell mass is increased. This can be due to a primary intrinsic defect in the erythroid progenitor cells or secondary to erythropoietin production from some source. Primary and secondary causes can be congenital or acquired. Rare, primary congenital defects are due to mutations leading to truncation of the erythropoietin receptor. The main acquired, primary erythrocytosis is polycythaemia vera. Among the congenital secondary causes, a number of defects in the genes in the oxygen-sensing pathway have recently been described, which lead to a secondary erythrocytosis. An extensive list of acquired secondary causes needs to be considered. A number of patients do not have an identifiable cause of erythrocytosis and are therefore described as having idiopathic erythrocytosis. Investigation should commence with careful clinical evaluation. Determination of the erythropoietin level is then a first step to guide the further direction of investigation. In those with congenital defects, a number of serious thromboembolic events have been described, but there is little information available about outcomes in these individuals and, therefore, no evidence to guide management. In this group, consideration should be given to the use of venesection to attain an achievable haematocrit level, and also low-dose aspirin therapy.

Keywords

Erythrocytosis, erythropoietin, prolyl hydroxylases, von Hippel-Lindau protein, hypoxia-inducible factor, idiopathic erythrocytosis

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

Red cells constitute the vast majority of the cells in circulation in the human body. The amount of red cells has an influence on the viscosity of the blood. Red cell production is under exquisitely sensitive control. Any disruption of this system can lead to an increase in red cell production – erythrocytosis – leading to increased viscosity, with clinical consequences.

Erythrocytosis

An absolute erythrocytosis is present when there is an increase in the red cell mass over 125% of that predicted for the individual's body mass.[1] This can be measured. If the haematocrit (Hct) level is over 0.60 in a male or 0.56 in a female, the red cell mass has been shown to be increased, and it can be assumed in these cases that there is an absolute erythrocytosis. Haemoglobin (Hb) above 18.5g/dl or Hct above 0.52 in a male are judged to be elevated and warrant further investigation. The equivalent figures for females are 16.5g/dl and 0.48. These numbers do not always translate to an absolute erythrocytosis, as has been shown in a comparative study,[2] and it may be necessary to formally measure the red cell mass to establish the presence of an absolute erythrocytosis. Red cells contain haemoglobin, which supplies oxygen to the tissues. The oxygen supply to the tissues is under fine control and the hormone produced in response to hypoxia is erythropoietin (EPO). EPO is mainly produced by the kidneys. Any fall in oxygen levels in the tissues in the kidneys will result in increased EPO production and, consequently, an erythrocytosis.

Classification of Erythrocytoses

When an erythrocytosis is established, it is necessary to look for a cause. An absolute erythrocytosis can be classified depending on its aetiology. Erythrocytoses are classified as primary if there is an intrinsic defect in the erythroid progenitor cells in the bone marrow, or secondary if the increased red cell mass results from factors external to the erythroid progenitor cell (i.e. increased EPO production from any cause, driving red cell production).

Primary and secondary erythrocytoses can be subdivided into congenital and acquired groups (see *Table 1*). Primary erythrocytoses will have an EPO level below normal, as the intrinsic defect in the progenitor is responsible for the red cell production, and the physiological response to this is depression of EPO levels. In secondary erythrocytoses, EPO levels will be normal (inappropriate for a raised Hb) or elevated as the EPO is driving the erythrocytosis.

Primary Erythrocytoses

Primary Congenital

A congenital erythrocytosis can arise due to mutations of the EPO receptor. Under normal physiological circumstances, EPO docks with its receptor and a series of events then occur leading to gene transcription and increased red cell production. This process is switched off after about 30 minutes when SHP-1 binds to the receptor and further red cell production is halted. The EPO receptor mutations lead to truncation of the receptor above the binding site for SHP-1,

Table 1: Classification of an Erythrocytosis

Primary Erythrocytosis
Congenital
Erythropoietin receptor mutations
Acquired
Polycythaemia vera
Secondary Erythrocytosis
Congenital
Oxygen-sensing pathway gene mutations (*VHL*, *PHD2*, *HIF2A*)
High oxygen-affinity haemoglobins
Bisphosphoglycerate mutase deficiency
Acquired
Erythropoietin-mediated
Central hypoxia
Chronic lung disease
Right-to-left cardiopulmonary vascular shunts
Carbon monoxide poisoning
Smoker's erythrocytosis
Hypoventilation syndromes including obstructive sleep apnoea
High-altitude
Local hypoxia
Renal artery stenosis
End-stage renal disease
Hydronephrosis
Renal cysts (polycystic kidney disease)
Post-renal transplant erythrocytosis
Pathological erythropoietin production
Cerebellar haemangioblastoma
Meningioma
Parathyroid carcinoma/adenomas
Hepatocellular carcinoma
Renal cell cancer
Pheochromocytoma
Uterine leiomyomas
Iatrogenic
Erythropoietin
Androgens
Idiopathic Erythrocytosis

Table 2: Diagnostic Criteria for Polycythaemia Vera[13]

World Health Organization – Revision 2007	
Major	Minor
Hb >18.5g/dl (men) or >16.5g/dl (women), or other evidence of increased red cell mass	Bone marrow biopsy showing panmyelosis
JAK2 mutation	Serum EPO below the normal reference range
	EPO-independent erythroid colonies

thus binding of SHP-1 cannot occur, the receptor remains in the 'on' state and red cell production continues. At least 14 such mutations have now been described (reviewed by Percy).[3] The original mutation[4] has arisen on more than one occasion independently.[5]

Primary Acquired

The main acquired primary erythrocytosis is polycythaemia vera (PV). This arises in the bone marrow from a clone of progenitor cells, which show an enhanced response to cytokines, and results in increased production of red cells and, frequently, also white cells and platelets. The clone has a 'gain-of-function' mutation in exon-14 of *JAK2*, Val617Phe, which results in a constitutively activated *JAK2*, enhanced downstream signalling and increased cell proliferation.[6–9] A small minority of cases do not have the exon-14 mutation but have a variety of mutations in a region of exon-12 of *JAK2*.[10] These patients tend to have a predominantly erythroid phenotype, and before the discovery of the mutations many would have been described as having an idiopathic erythrocytosis.[11] Diagnostic criteria have been defined for PV, and these have been simplified with the discovery of the clonal marker.[12,13] *Table 2* outlines current diagnostic criteria for PV.

Secondary Erythrocytoses

Secondary Congenital

There is a carefully balanced pathway that senses and responds to oxygen levels in the human body. This pathway consists of a number of proteins:

- prolyl hydroxylases (PHDs), which exist in a number of isoforms – PHD1, PHD2 and PHD3;
- hypoxia-inducible factor (HIF), consisting of an unstable alpha subunit and a stable beta subunit; and
- the von Hippel Lindau tumour suppressor protein (VHL).

HIF-α also has three isoforms: HIF-1α, HIF-2α and HIF-3α. In conditions of normal oxygen tension, oxygen activates the PHDs, which in turn hydroxlate HIF-α. This binds to VHL, and HIF is then ubiquinated and destroyed in the proteasome. In hypoxic conditions hydroxylation is reduced, HIF-a escapes degradation, associates with the beta subunit and binds to the transcriptional enhancer element 3' to the *EPO* gene. Regulation of EPO transcription and a number of other proteins then occurs, with resultant increased EPO production. Mutations in the genes for any of these proteins would result in an abnormal protein, which would not be destroyed in normoxic conditions; this leads to an increase in levels of HIF-α, which then travels down the hypoxic pathway, ultimately resulting in increased EPO production.

A number of mutations resulting in abnormal proteins have been described in individuals with erythrocytosis. These proteins have all been shown to have abnormal function. A mutation in the *PHD2* gene, a heterozygous C950G change, was identified in a family with erythrocytosis.[14] A number of other mutations of the gene associated with erythrocytosis have since been identified (reviewed in McMullin).[15] The original defect in the oxygen-sensing pathway was discovered in the VHL protein when an extensive kindred with erythrocytosis were discovered in the Chuvash area of Russia. These individuals were all homozygotes for a single mutation, C598T.[16] This mutation, and a number of other compound heterozygote mutations of VHL, have now been described (reviewed in McMullin).[15] A gain of function of the *HIF2A* gene, G1609T, has been described in three generations of the same family with erythrocytosis.[17] To date, four further amino acid changes in the protein associated with erythrocytosis have been described.[15]

A high-affinity Hb will bind oxygen tightly and release it less readily to the tissues. This will result in tissue hypoxia and a compensatory erythrocytosis. Over 90 such Hb variants have been identified, and this is a cause of secondary erythrocytosis.[18] Another rare cause of

congenital secondary erythrocytosis is bisphosphoglycerate mutase deficiency, where a mutation in the bisphosphoglycerate mutase gene leads to decreased levels of the enzyme, resulting in low 2,3-bisphosphoglycerate levels and increased binding of oxygen by Hb, tissue hypoxia and erythrocytosis.[19]

Secondary Acquired

Anything leading to the increased production of EPO can produce an erythrocytosis. This can happen when there is central hypoxia from any cardiac or lung disease. Local hypoxia of the kidneys will also lead to increased EPO production and a resulting erythrocytosis. EPO can also be produced pathologically. This has been described in a variety of tumours where EPO RNA is identified in tumour tissues in combination with increased circulating EPO levels. Iatrogenic drug administration can also lead to a secondary erythrocytosis. This may be done deliberately with EPO to boost Hb levels and, thus, performance in sports. It is also observed with androgen administration. The causes of secondary acquired erythrocytosis are listed in *Table 1*.

Idiopathic Erythrocytosis

There remains a small group of patients who do not have an identified cause for their erythrocytosis and are consequently diagnosed with idiopathic erythrocytosis. Likely pathological processes for further investigation can be identified on the basis of EPO levels, but in many no cause is identifiable.

Pathway for Investigation

Once an erythrocytosis has been established by repeat testing and red cell mass measurement if necessary, it is essential to take a thorough history and make a full physical examination. Any possible diagnoses should be explored, for instance respiratory investigations in an individual with a history of respiratory disease. In those where the cause of the erythrocytosis is not clear, a first step in further investigation would be to measure the EPO level. This divides individuals into two groups: those with low or absent levels, who may have a defect in the EPO signalling pathway, and those with inappropriately normal or elevated levels, in whom a source of the EPO must be sought. This would include investigation of the oxygen-sensing pathway (see *Figure 1*).

Management

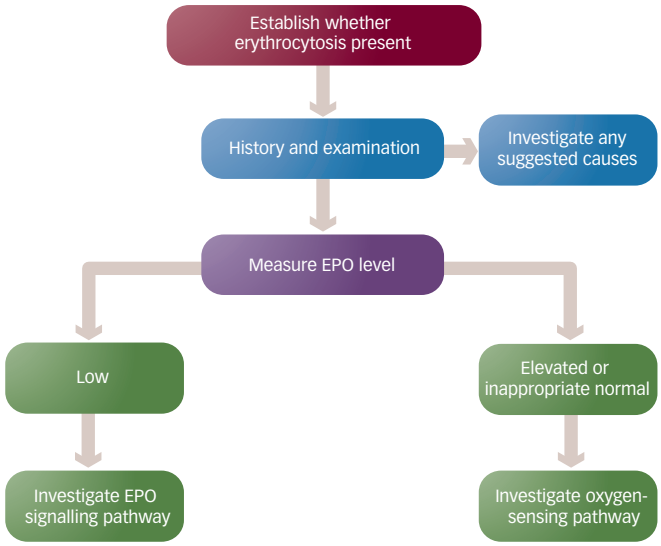
For patients with PV there are guidelines used in practice for their management.[20] These include aspirin for all who can tolerate it, venesection to an Hct level of 0.45 and agents to reduce cell counts, directed by age, complications and symptoms. The JAK2 mutant protein provides a specific target for therapy, and a number of agents are in development. Clinical trials directed at this protein are likely to add to the therapeutic armamentarium for PV in the future.[21]

In many of the other secondary erythrocytoses, such as those from cardiac or respiratory disease, there is limited evidence to guide management and consider when venesection may be appropriate, and to what specific Hct level. Close liaison with specific specialists, such as paediatric cardiologists, is required.[20]

Clinical Events

In many of the more recently described congenital erythrocytoses, and in idiopathic erythrocytosis, there is a paucity of evidence around the clinical consequences and effective management. The largest group in whom the clinical consequences of erythrocytoses have been studied is those with the *VHL* mutation C598T in Chuvashia, Russia. A group of homozygotes with this mutation had a lower median survival than unaffected controls, and the increased mortality was due to thromboembolic events.[22] In the other reported defects of the oxygen-sensing pathway, there are a small number of reports in families of individuals presenting with serious thrombotic events at young ages.[15] This suggests, but does not prove, that the increased Hct levels with erythrocytosis causing an increase in viscosity may lead to an increase in morbidity and mortality; as such, reduction of Hct levels by venesection could be of benefit.

Figure 1: Diagnostic Pathway



EPO = erythropoietin.

Venesection

Venesection may be a therapeutic measure for those with congenital or idiopathic erythrocytosis. It must be considered on a patient-by-patient basis if it is likely to be both a necessary and a beneficial therapeutic measure. Consideration of any symptoms the patient is experiencing, previous history and, if available, history in similarly affected family members is needed. If venesection is undertaken, a target therapeutic Hct level is also needed. The only evidence-based target for Hct is 0.45, the advised target for those with PV on the basis of a retrospective study that showed that the incidence of thromboembolic events increased if Hct was above this cut-off.[23] This target would be very difficult to achieve in those with congenital erythrocytosis and may not be of any benefit. A target of 0.50 is more achievable in those in whom a programme of venesection is undertaken.

Aspirin

In PV the administration of low-dose aspirin has been shown to be beneficial, with a significantly reduced incidence of thromboembolic events in those on therapy in the European Collaboration on Low Dose Aspirin study (ECLAP).[24] Except in those with a specific contraindication, this is a relatively safe therapy and it should be considered in those with erythrocytosis, as it is a low-risk way of potentially reducing the possibility of thromboembolic events.

Conclusion

An erythrocytosis can arise from primary and secondary causes. Investigation should be directed by the EPO level, and may then explore EPO signalling in those with low EPO levels or investigation of oxygen sensing in those with normal or elevated levels. Management is directed by the primary cause of the erythrocytosis. In congenital erythrocytoses, consideration needs to be given to venesection (to an achievable Hct level) and low-dose aspirin administration. There is little evidence available to support management decisions in this group. ■

Mary Frances McMullin is a Professor of Clinical Haematology at Queens University Belfast and a Consultant Haematologist at Belfast City Hospital. She has an extensive specialist practice in myeloproliferative neoplasms, acute myeloid leukaemia and allogeneic bone marrow transplantation. Her research interests include the investigation of the causes of erythrocytosis and pathogenesis of polycythaemia vera and myeloid leukaemia. She is President of the Haematology Association of Ireland.

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