ERYTHROCYTOSIS

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Definition of an erythrocytosis

It is suspected that a patient has an erythrocytosis when presenting with a hemoglobin (Hb) or hematocrit (Hct) above the normal range. Each laboratory quotes a normal range for the analyzer in use, for each sex. There is considerable variation in the quoted normal range. Reviewing this showed that normal Hb ranges for men in published studies varied from 13.3–16.7g/dl to 13.2–18.0 g/dl and women from 11.8–14.8g/dl to 12.2–16.5g/dl (1-4). Similarly there is a spread of Hct ranges. Therefore, there is considerable variation in what would be considered an Hb or Hct above the upper limit of normal.

However, to demonstrate that there is truly an erythrocytosis, it is necessary to show that the red cell mass (RCM) is increased above a defined level that is greater than 125% of predicted. This is measured and calculated using internationally defined methods and standards and surface area (5). An increased RCM in men is defined as plus 25% of the 98% limits and in women plus 25% of the 99% limits. Thus, while it defines a borderline between normal and abnormal, it is a somewhat arbitrary defining line.

It is often considered that a raised Hb or Hct equates to an elevated RCM. This is not always the case and was demonstrated by a systematic study by Johansson and colleagues (6). They had cohorts of males and females in whom they had data on both the RCM and Hb. In males, only 35% of those with absolute erythrocytosis had an Hb above 18.5g/dl, which is the cut off level currently used by the WHO to define an elevated Hb in Polycythaemia Vera (PV) (7). Similarly, in the female cohort with an upper limit of Hb of 16.5g/dl, only 63% with absolute erythrocytosis were above this level. Thus, relying on an Hb will miss those with absolute erythrocytosis and mislabel some who have an absolute erythrocytosis but do not have an Hb above the normal range. It cannot be advised to rely solely on the Hb and Hct to assume there is an erythrocytosis.

In practical terms, an erythrocytosis is suspected whenever the Hb and Hct are reported above the normal range. RCM measurement may be required to confirm this. However, in a patient in whom the diagnosis is obvious and supported by other tests (e.g., PV with a positive test for a *JAK*2 mutation) or where the Hct is so high that it is always associated with an increased RCM (Hct 0.06 or above in men and 0.56 or above in women), RCM measurement is deemed unnecessary (8) but is used as a confirmatory test in doubtful cases.

Classification of an erythrocytosis

Once it has been established that an absolute or true erythrocytosis is present, the next question that arises is the cause. An erythrocytosis can result from a primary bone marrow problem where the bone marrow has an intrinsic defect resulting in increased red cell production. This is referred to as a primary erythrocytosis. In contrast, if erythropoietin (EPO), the hormone, which drives red cell production, is produced for any reason then this will drive the bone marrow to produce more red cells and a secondary erythrocytosis (secondary to EPO for whatever reason) is present. The remaining group, for which a cause for the erythrocytosis cannot be determined, is then termed idiopathic erythrocytosis.

Primary erythrocytosis

An intrinsic primary erythrocytosis can be congenital or acquired. Congenital causes are those where there is a mutation of the *erythropoietin receptor (EPOR*) gene (9,10). Under normal physiological circumstances, the hormone EPO docks with its receptor on the cell surface, other proteins are then recruited, phosphorylated, and then translocated to the nucleus where a further signal results in red cell production. After a few minutes, this process is turned off by the attachment of the SHP1 protein to the EPOR, which dephosphorylates the receptor (11). A number of mutations have been discovered in the *EPOR* gene that result in truncation of the EPOR above the attachment site for SHP1. This causes a receptor that is activated but does not turn off and therefore continues to signal without the need for EPO. These rare mutations are a cause of congenital primary erythrocytosis in those with increased RCM and an EPO level below the normal range (a full list of EPOR mutations reviewed by Percy (12)). They usually present at a young age and may have a family history in keeping with the congenital causation.

The major cause of acquired primary erythrocytosis is PV. In order to fulfill the criteria for diagnosis of this condition, at least an elevated Hb and the presence of an acquired *JAK*2 clone is required (13). This is at least sufficient to make the diagnosis although white cells and platelets may of course also be elevated (7).

Another protein involved in the EPO signaling pathway is the lymphocyte-specific adaptor protein (LNK). LNK modulates thrombopoietin and EPO signaling by interaction with JAK inhibiting downstream activation (14,15). Aberrant LNK function could interfere with EPO induced signaling resulting in hypersensitivity to EPO and erythrocytosis in the presence of a low EPO level. *LNK* mutations have been described in patients with myeloproliferative neoplasms (16,17), and a few with erythrocytosis and low EPO levels (18,19) would appear to explain the erythrocytosis in some instances.

Secondary erythrocytoses: congenital

A secondary erythrocytosis is indicated in a patient with an increased RCM and a serum EPO level above the normal range or what is termed an inappropriately normal EPO level. The term inappropriately normal is used, as the physiological response to a raised Hb level would be a reduced EPO level. There are a number of congenital causes for this, which should be considered in the differential diagnosis.

The oxygen-sensing pathway

The body has a physiological mechanism for the detection of oxygen levels and maintenance of oxygen homeostasis. Under normal oxygen tensions, proteins are degraded but if there is hypoxia, they survive, are processed, and ultimately signal for more protein production, including the production of EPO. This would result in more red cell production to carry oxygen to the tissues and restoration of the oxygen homeostasis. Some of the main proteins in this pathway are the HIF proteins, the prolyl hydroxylases (PHD), and von Hippel–Lindau protein (VHL). The HIF proteins and the prolyl hydroxylases (PHD) both have three different isoforms (20,21). The process is that in normoxic conditions, PHDs site-specifically hydroxylate the oxygen dependent degradation domain of HIFα. After this hydroxylation has taken place then VHL, a tumor suppressor protein, associates with HIFα (22,23). VHL provides the recognition site for a multi-component ubiquitin ligase complex. HIFα is then targeted for proteasomal proteolysis by the ubiquitin-proteasome pathway and destroyed (24-26).

When hypoxia is detected, prolyl hydroxylation is suppressed and PHD is no longer able to associate with VHL. HIFα then accumulates and associates with the stable HIFβ in the nucleus forming a transcriptionally active HIF complex. This complex binds to promoters and enhancers of a range of genes and leads to the transcription of a large number of genes and thus protein production, including EPO (27). A defect in the genes coding for any of these proteins could result in the production of an abnormal protein, which would behave abnormally and not degrade in the presence of normal oxygen tension. This would lead to protein survival, increasing HIFα levels, and ultimately increased EPO levels, which would cause a secondary erythrocytosis.

There are a number of individuals and families in which such defects have been described. In the *VHL* gene, a cohort of individuals were described with erythrocytosis in an area of Russia, Chuvashia (28,29). These individuals are all homogygotes for a single mutation C598T leading to an Arg200Trp change. These and other homozygote and compound heterozygote *VHL* mutations are described,

which cause congenital erythrocytosis (30-36). In *PHD2*, a family was initially described who were heterozygous for C950G resulting in a Pro317Arg change in *PHD2*. This mutation was shown by *in vitro* studies to cause erythrocytosis (37). This and other *PHD2* mutations have subsequently been seen in those with erythrocytosis (38-42). The final mutations in the oxygen-sensing pathway so far discovered are in *HIF2*A. The initial family had a heterozygous mutation of G1609T, resulting in a Gly537Trp amino acid change present in three generations. This mutation again was demonstrated to cause EPO induction and erythrocytosis (43). A number of other heterozygotes for *HIF2A* mutations have subsequently been described (44-48). Thus, a number of reported congenital mutations in the genes in the oxygen-sensing pathway could result in secondary erythrocytosis.

Other causes of congenital erythrocytosis

There are a number of other rare circumstances where a congenital erythrocytosis can occur. Oxygen release to the tissues depends on Hb and on how it is bound to oxygen. A number of variant hemoglobins have been described that have higher than normal affinity for oxygen. Such hemoglobins give up oxygen less readily than normal to the tissues and thus the patient has an elevated Hb and EPO level. Over 90 such high oxygen affinity variants have been described with defects of both α and β globin genes occurring (49,50).

Oxygen delivery to tissues is dependent upon levels of 2,3 bisphophoglycerate (BPG), which binds to Hb and converts the molecule to a lower oxygen affinity state. 2,3BPG is produced from 1,3BPG, and this reaction is catalyzed by the enzyme bisphosphoglycerate mutase. A deficiency of the enzyme results in reduced 2,3BPG levels and a shift in the hemoglobin-oxygen dissociation curve to the left. This causes decreased oxygen release to the tissues and a compensatory erythrocytosis. Cases of deficiency of 2,3BPG have been described in some families, inherited in both autosomal dominant and autosomal recessive patterns (51).

Congenital methemoglobinaemia is another cause of congenital erythrocytosis. This can occur because of an abnormal M hemoglobin variant or deficiency of a cytochrome reductase. M hemoglobin variants have an amino acid substitution near the hem pocket allowing the formation of methaemoglobinaemia. They are autosomal dominant and α,β,γ globin variants have been reported. α and γ present with cyanosis at birth (with γ variants, the situation reverses in the first few months after birth whereas β globin variants present a few months postpartum when the switch from fetal Hb occurs) (52). The enzyme NADH-cytochrome *b*5 reductase 3 (CYB5R3) (also known as diaphorase-1 and NADH-methemoglobin reductase) catalyses electron transfer from NADH to cytochrome *b*5, which in turn then acts as an electron donor for methemoglobin. Deficiency of this enzyme will lead to an increased methaemoglobin level and erythrocytosis as a compensatory process. A single gene on chromosome 22q13 encodes for two protein variants of the enzyme. The soluble form is present mainly on erythrocytes, and the membrane bound form is localized in the endoplasmic reticulum in a wide range of tissues. The rare autosomal deficiency of CYB5R3 presents as a type I disorder where the enzyme deficiency is restricted to the erythrocytes and leads to a relatively benign cyanosis and a type II disorder, where the deficiency affects all cells resulting in cyanosis and a severe neurological disorder (53). There are also a series of reports of families with erythrocytosis who have an inherited increase in ATP levels associated with low 2,3BPG. These cases, which have an autosomal dominant pattern of inheritance, have been shown to have inherited elevated erythrocyte pyruvate kinase (PK) activity, and the molecular change in the PK gene has been identified in at least one case. The relationship between increased PK activity and resulting hereditary increase in ATP is complex (54).

Secondary erythrocytoses: Acquired

The secondary acquired causes of erythrocytosis are legion. These can be considered as those that are hypoxia driven, resulting in a compensatory production of EPO and thus erythrocytosis and those where there is pathological presence of EPO either endogenously produced or exogenously administered. Hypoxia can result from a central systemic process, either respiratory or cardiac issues, or from a hypoxic environment as in high altitude habitat. Local renal hypoxia, which can be caused by a variety of local renal disease, can result in a secondary acquired erythrocytosis as the local renal hypoxia results in a signal for the kidney to produce more EPO in response. A variety of tumors is described in the literature that has been demonstrated to produce EPO and thus drive erythrocytosis (55-61) as listed in Table 1. Secondary erythrocytosis can also arise because

of EPO administration. This will produce an erythrocytosis, which may be advantageous to certain activities. This can be very difficult to detect (62). Androgen administration is a cause of a secondary erythrocytosis (63).

Idiopathic erythrocytosis

A group of patients remains in whom the cause of the erythrocytosis cannot be identified, and these are termed idiopathic erythrocytosis. This is a mixed group and falls into two categories: those with low EPO levels who must have an undefined primary cause, and those with normal or elevated EPO levels who have an unidentified secondary cause. Those who fall into this group have diminished with time as causes of either primary or secondary erythrocytosis have been discovered.

Management of a congenital erythrocytosis

Unfortunately, there is very little available evidence on management strategies and outcomes in congenital erythrocytosis. Any information comes from the Chuvash polycythaemia cohort where some retrospective studies have been carried out. There is also emerging individual case reports with other oxygen-sensing pathway defects, some of which report increased thromboembolic events of a serious or life threatening nature associated with these mutations.

Low dose aspirin has been shown to be of benefit in prophylaxis of thromboembolic events in PV, where it has been shown in a randomized placebo controlled trial that those on aspirin have reduced incidence of thromboembolic events (64). No relationship to aspirin usage and outcome was shown in retrospective studies in Chuvash polycythaemia (65). Nevertheless, given the widespread known efficacy of aspirin as a prophylactic agent for thromboembolic events, it would seem logical to consider its use in congenital erythrocytosis in those without a specific contraindication. It would also be expedient to undertake vigorous prophylaxis of any other risk factors for thromboembolism.

Reduction of the Hct by venesection will reduce the blood viscosity and could be of benefit in reduction of the risk of thromboembolic events. This has been shown to be of benefit in PV in retrospective studies and is part of the management of the condition (66). The relationship between Hct, venesection, and outcomes are inconclusive in the Chuvash cohort (65). It is also of note that some of the mutations in the oxygen-sensing pathway lead to physiological alterations, which include raised Hct, and may be part of the required functioning in those with the mutation (67-69). However, it would seem logical to consider attempting to reduce the Hct by venesection particularly in those with very high Hcts and in individuals who are symptomatic. It can be very difficult to get the Hct reduced with venesection in these patients and an achievable level must be considered. A hematocrit of 0.50 may be a reasonable target. Symptomatic response should be assessed (Table 3).
It has been shown that the VHL protein binds to the suppressor of cytokine signaling (SOCS1) and then after binding to E3ligase, targets JAK2 for ubiquitin mediated destruction. The Chuvash VHL mutants have altered affinity for SOCS1 and do not degrade JAK2. In mice, a selective JAK2 inhibitor reverses the disease phenotype (70). This raises the possibility that inhibitors of the JAK pathway that are becoming available for therapeutic use (71,72). may be of benefit in the treatment of at least this congenital erythrocytosis to block the JAK pathway and thus block the development of erythrocytosis.

Some guidance for the management of a high affinity Hb based on the sparse evidence is available. 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 effected asymptomatic individuals in whom 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 (73). Venesection to reduce the Hct to less than 0.60 is recommended (74) but when thrombosis or symptoms compatible with hyperviscosity have developed at a lower Hct then a target of 0.52 is suggested (75).

Management of idiopathic erythrocytosis

For those with idiopathic erythrocytosis, there is little evidence to guide management. However, the suggested management plan would recommend reduction of the Hct to less than 0.45 if the Hct is greater than 0.54. Reduction of the Hct to less than 0.45 if the Hct 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 (75).

Conclusions

Causes of a proven erythrocytosis are varied and include rare congenital events. Investigation can follow a logical pathway driven by the clinical presentation. There is little evidence to provide clear management guidance, and there is a need for long-term follow up of outcomes in those with rare causes of erythrocytosis in order to gain better knowledge in the future.

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