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Review Article

The clinical and laboratory evaluation of the patient with erythrocytosis

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ABSTRACT

Erythrocytosis is frequently encountered as an incidental abnormality on laboratory testing that reveals persistent elevation of the hematocrit level (>52% in men and >48% in women). In many cases, erythrocytosis is the manifestation of an underlying cardiopulmonary process, drug-induced due to androgens, or secondary to smoking, rather than a primary bone marrow disorder such as polycythemia vera. A systematic approach to the clinical and laboratory evaluation of each patient is indicated to consider diverse differential diagnosis possibilities and to identify the underlying etiology of erythrocytosis in order to formulate appropriate subspecialist referral and management plans. A thorough medical history and meticulous physical examination supplemented by a focused initial laboratory evaluation will enable the general practitioner to ascertain the etiology of erythrocytosis in the majority of cases. Patients with clinical and laboratory features suggestive of polycythemia vera and those patients without an apparent underlying condition known to cause erythrocytosis benefit from early referral to a hematologist for further specialized diagnostic evaluation and therapy considerations.

1. Introduction

Erythropoiesis is a tightly regulated process by which the bone marrow produces and maintains a normal red blood cell mass (RCM) required to provide adequate tissue oxygenation. Erythrocytosis refers to an expansion of RCM, frequently manifested as a persistent increase of venous hematocrit levels for >2 months. Hematocrit elevation may occur as a result of either an absolute or an apparent increase in RCM. Individuals with absolute erythrocytosis have raised RCM >25% of the predicted volume adjusted for sex and body mass [1]. On the other hand, individuals with apparent erythrocytosis (or relative erythrocytosis) exhibit elevated hematocrit levels, but have normal RCM associated with contracted plasma volume [2]. A venous hematocrit level >60% in men and >56% in women is nearly always associated with absolute erythrocytosis, as assessed by measurement of RCM [3,4]. This procedure involves radioisotope labeling of an aliquot of the patient's red blood cells with ^{51}Cr and the use of ^{125}I-labeled albumin to simultaneously calculate RCM and plasma volume, respectively [1]. RCM assays, whenever available, are helpful in selected cases, particularly if a patient's hematocrit level is modestly elevated, to confirm the presence of an absolute erythrocytosis that could impact differential diagnosis, further evaluation decision, and management.

2. Classification of erythrocytosis

The terms erythrocytosis and polycythemia are frequently used interchangeably. Erythrocytosis is classified either as a primary or secondary condition. Primary erythrocytosis (or primary polycythemia) refers to the accumulation of red cells associated with autonomous proliferation of red cell progenitor and precursor cells in the bone marrow, caused by inherited or acquired mutations in genes that regulate erythropoiesis. Secondary erythrocytosis occurs when a factor outside the bone marrow – such as inappropriately increased serum erythropoietin (Epo) – stimulates erythropoiesis. Primary and secondary erythrocytoses are further sub-classified into acquired erythrocytosis or familial and congenital erythrocytosis based on the apparent inheritance pattern within a family, the age of onset, and the underlying etiology (Table 1).

3. Initial clinical evaluation of erythrocytosis

3.1. Patient history

The evaluation of an individual with erythrocytosis begins with a thorough history focusing on symptoms, co-morbid medical conditions, medications taken, habits, and family history. Many patients with erythrocytosis are asymptomatic, or they may complain of non-specific symptoms such as fatigue, dizziness, or headache associated with hyperviscosity. Patients should be questioned carefully regarding cardiac and respiratory symptoms and manifestations of chronic hypoxia that may be associated with underlying cardio-pulmonary disease. The presence of excessive daytime sleepiness, loud nocturnal snoring,

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Table 1
Classification of erythrocytosis.

Primary erythrocytosis
Familial and congenital
EPOR gene mutation
Acquired
Polycythemia vera
SH2B3 gene mutation (encodes LNK)
Secondary erythrocytosis
Familial and congenital
High affinity hemoglobin
2,3-biphosphoglycerate (BPG) deficiency
Oxygen-sensing pathway defects
VHL gene mutations (Chuvash polycythemia)
EPAS1 gene mutations (encodes HIF2A)
EGLN1 gene mutations (encodes PHD2)
Acquired
Specific causes listed in Table 2
Idiopathic erythrocytosis

or other sleep-related disturbances may be associated with obstructive sleep apnea. Excessive weight gain and body mass index >30 kg/m2 may be encountered in sleep apnea or obesity–hypoventilation syndromes. Itching may be a non-specific symptom of polycythemia vera (PV), particularly if exacerbated by exposure of the skin to water (aquagenic pruritus). It is important to take note of a prior diagnosis of structural heart disease that may be associated with right-to-left shunting, or pulmonary conditions potentially leading to chronic hypoxemia. A prior episode of arterial or venous thrombosis –particularly in an unusual site such as abdominal visceral veins –may represent a feature of PV, a pre-thrombotic disorder. An occasional patient with renal transplantation may develop significant erythrocytosis. Medications, including parenteral forms, should be reviewed in detail, as they could contribute to apparent erythrocytosis (as in the case of diuretic use) or cause secondary erythrocytosis, as in the case of androgens [5] or tyrosine kinase inhibitors such as sunitinib and sorafinib [6]. In male hypogonadism cases, androgen dose adjustment and sometimes discontinuation is indicated. In addition, all patients should be questioned about exogenous recombinant Epo or anabolic steroid injections. Harmful habits should be reviewed in detail, including abuse of any tobacco products such as cigarettes and cigars, with attention to daily number smoked. A complete and extended family history must be obtained, as erythrocytosis may occur as a familial and congenital disorder, inherited either in an autosomal dominant or recessive manner. The presence of PV or other myeloproliferative neoplasms (MPNs) diagnosed in extended family members may be relevant, given emerging data demonstrating increased prevalence in family members of individuals with apparently sporadic MPNs [7].

3.2. Physical examination

Many patients with erythrocytosis exhibit plethora. A ruddy complexion is frequently seen in patients with PV. The presence of cyanosis or clubbing of the digits may be an indicator of hypoxia associated with cardiopulmonary disease. Erythromelalgia (erythema or pallor of the hands and feet accompanied by burning pain) is often noted in association with PV. Hypertension, if present, may have been associated with difficult control in a patient with newly diagnosed PV or a manifestation of pheochromocytoma. Marked increase in the body mass index should raise suspicion for obesity–hypoventilation syndrome. A low-set soft palate may be a sign of upper airway obstruction causing sleep apnea. Careful cardiac auscultation may reveal a murmur or abnormal heart sounds, such as loud pulmonic component of S2, suggesting an underlying intra-cardiac right-to-left shunt that may, for instance, occur in association with longstanding atrial septal defect. Barrel chest and decreased breath sounds suggest emphysema. Splenomegaly is observed in about 60% of patients presenting with PV and occurs as a result of massive expansion of RCM and varying degrees of extramedullary hematopoiesis. The abdomen should be carefully palpated for the presence of an underlying mass, as a variety of benign and malignant tumors may give rise to secondary erythrocytosis as a paraneoplastic manifestation.

Pulse oximetry can be performed in all patients at the time of the physical examination as a simple assessment of tissue oxygenation. Arterial oxygen saturation <92% has been shown to be associated with compensatory erythrocytosis [8]. In patients with suspected underlying pulmonary disease, continuous pulse oximetry during a brief walk in the clinic may reveal arterial oxygen desaturation during or following exertion. It is important to remember that pulse oximetry may be deceptively normal in patients with a secondary erythrocytosis caused by underlying chronic carbon monoxide poisoning or in patients with obstructive sleep apnea who experience intermittent episodes of nocturnal hypoxia.

4. Practical approach to laboratory evaluation of the patient with erythrocytosis

Evaluation is indicated for patients with high venous hematocrit level >52% in men and >48% in women, persisting for longer than 2 months [4]. Ferritin and transferrin saturation measurements are indicated, especially if hypochromia and microcytosis are noted on red cell indices, because iron deficiency may mask more severe elevation of hematocrit and RCM. The main initial focus of differential diagnosis is to identify, if present, an underlying condition causing secondary erythrocytosis, and to eliminate (or confirm) a diagnosis of PV. Although RCM measurement may be helpful to confirm absolute erythrocytosis in cases with less severe elevation of hematocrit (<56% in women and <60% in men), the test is not available at many medical centers [3,9]. In a series of 102 consecutive patients with isolated erythrocytosis evaluated at a referral center in Europe, 7% of patients were diagnosed with apparent (relative) erythrocytosis as determined by RCM measurement, 49% with secondary erythrocytosis, 13% with PV and 31% had idiopathic erythrocytosis [10].

4.1. JAK2 gene mutation testing and diagnosis of PV

The clinical evaluation of the patient with erythrocytosis has evolved significantly following the discovery of activating, somatic JAK2 gene mutations in PV and other MPNs [11–14]. JAK2 is a cytoplasmic tyrosine kinase involved in the regulation of hematopoietic cytokine receptor-mediated intracellular signal transduction. The gain-of-function JAK2-V617F mutation in exon 14 was detected in 95% of patients with PV. Other JAK2 gene mutations in exon 12 have been identified in 3% of patients with PV [15–17]. Mutation testing is performed in DNA isolated from peripheral blood cells. JAK2 mutation testing is indicated in patients without a readily identifiable erythrocytosis etiology after initial evaluation (such as hypoxia due to cardiopulmonary disease, smoking, androgen use) as illustrated in Fig. 1. JAK2 mutation testing should also be performed in patients with persistent erythrocytosis despite effective treatment of hypoxemia, discontinuation of androgens, or cessation of smoking, and in any patient with clinical features of PV even if a concomitant cardio-pulmonary condition and hypoxia are present.

Detection of a JAK2 gene mutation in a patient with erythrocytosis establishes the presence of a clonal myeloproliferative neoplasm but it is not specific for PV. In the patient with persistently elevated hematocrit level, the presence of a JAK2 mutation is considered diagnostic of PV according to the amended criteria of the British Committee for Standards in Hematology [18]. The elevated hemoglobin value threshold (>18.5 g/dL in men and >16.5 g/dL in women) specified in the 2008 World Health Organization criteria for the diagnosis of PV [19] fails to diagnose PV patients with lower hemoglobin values [3,20,21].

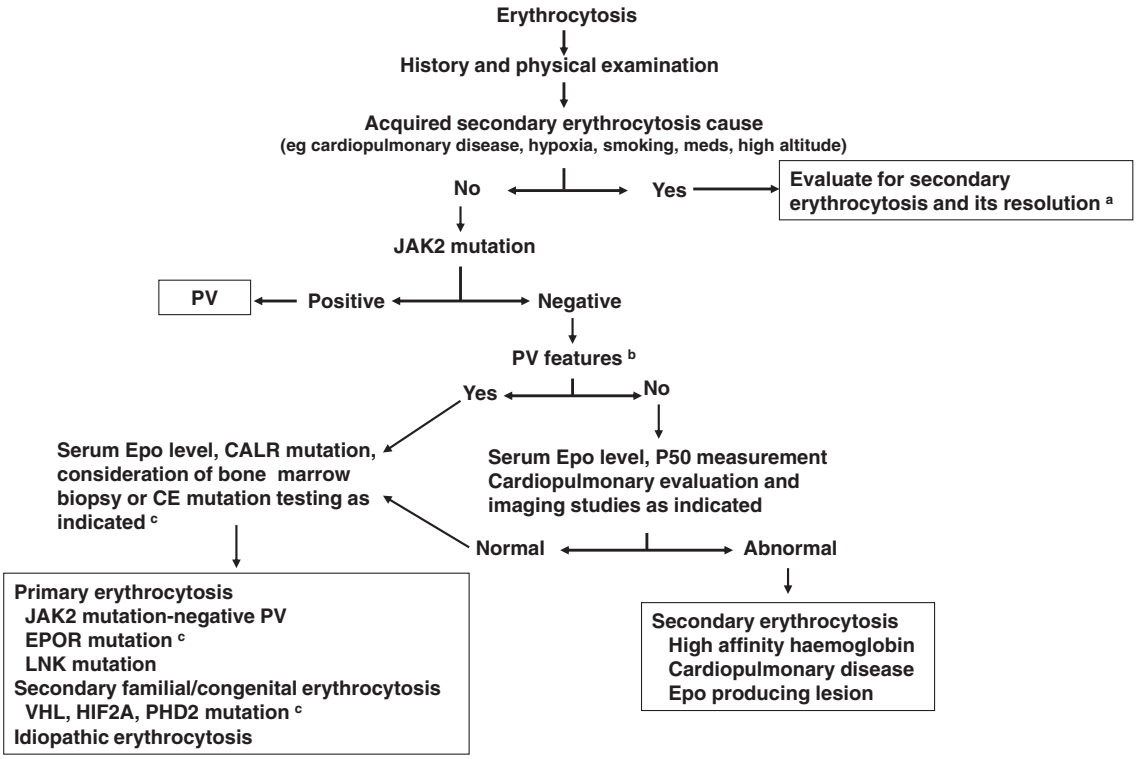

Fig. 1. Clinical and laboratory evaluation of the patient with erythrocytosis. [a] Patients should also be tested for JAK2 mutation if erythrocytosis does not return to normal after resolution of underlying secondary etiology (eg hypoxia, smoking) or if any clinical features of PV are present. [b] PV features include thrombosis, unusual bruising, acral ischemia or erythromelalgia, aquagenic pruritus, splenomegaly, leukocytosis, thrombocytosis, and basophilia. [c] Calreticulin (CALR) mutations were reported in two JAK2-negative PV patients [26]. Mutation testing for genes involved in rare familial and congenital erythrocytosis (CE) syndromes may be available in reference laboratories for research purposes [37].

RCM measurement and bone marrow biopsy are helpful in differentiating PV from a different MPN, such as essential thrombocytosis [9].

For the treatment of patients diagnosed with PV, therapeutic phlebotomy is indicated to reduce red cell mass to target hematocrit <45% as demonstrated in a randomized trial [22]. Another randomized trial showed that many PV patients benefit from low dose aspirin to prevent cardiovascular events [23]. Cytoreductive therapy, with agents such as hydroxyurea or interferon-alfa, is typically indicated in patients at high risk for thrombosis due to a prior thrombosis episode, those patients with persistent PV related symptoms not responsive to therapeutic phlebotomy and low dose aspirin, and older individuals with cardiovascular risk factors and disorders [24]. The JAK inhibitor ruxolitinib has emerged as a new treatment option for patients who are resistant to or intolerant of hydroxyurea [25]. Patients with PV are at risk for developing post-PV myelofibrosis and acute myeloid leukemia. In a cohort of 1545 patients, 9% developed myelofibrosis and 3% evolved to leukemia at a median duration of 10.8 years from PV diagnosis [17].

4.2. The patient with clinical PV features but without JAK2 gene mutation

Patients with erythrocytosis who do not harbor detectable JAK2-V617F or exon 12 mutations and who exhibit any clinical or laboratory features suggestive of PV such as unexplained thrombosis or bruising, acral ischemia or erythromelalgia, aquagenic pruritus, splenomegaly, unexplained leukocytosis or thrombocytosis, or those patients with basophilia, may have JAK2-negative PV constituting about 2% of PV cases, as reported in a large patient cohort [17]. A recent study reported two JAK2-negative PV patients with mutations in the calreticulin (CALR) gene, an abnormality that is much more prevalent in essential thrombocytosis and primary myelofibrosis [26]. In patients without mutations in JAK2 or CALR genes, measurement of serum Epo level may prove helpful if below the reference range, a non-specific finding that is typically associated with PV or other primary erythrocytosis. It is important to remember, however, that normal serum Epo level does not rule out PV because 10–20% of patients with PV exhibit normal or even elevated serum Epo level [17,27].

Patients with any clinical features suggestive of PV but without JAK2 mutation need further hematologic evaluation. In such patients with suspected PV who have relatively less severe elevation of hematocrit values (<56% in women and <60% in men), measurement of RCM, if available, to confirm an absolute erythrocytosis is helpful [18]. A bone marrow aspiration and biopsy is indicated to investigate for the presence of panmyelosis, the characteristic hypercellularity with increased proliferation of all three marrow cell lineages [9,28] and to investigate for clonal cytogenetic abnormalities encountered in 10–20% of patients with PV [29]. A rare patient may have a mutation in the gene encoding LNK, an adaptor protein that normally inhibits JAK2 phosphorylation [30].

4.3. The patient without clinical features of PV and no JAK2 mutation

In this clinical setting, secondary erythrocytosis etiologies are considered and investigated as detailed in Table 2. An elevated serum Epo level above the reference range is generally supportive of a secondary etiology, although in many cases of secondary erythrocytosis, including hypoxia-mediated erythrocytosis, Epo level is normal [27,31]. Acquired secondary erythrocytosis is most commonly associated with hypoxia-driven processes such as underlying pulmonary disease, hypoventilation syndromes such as obesity or obstructive sleep apnea, intra-cardiac shunt, active tobacco use, or chronic carbon monoxide exposure. Chest radiography, pulmonary function testing with diffusion capacity, carboxy-hemoglobin level measurement, and polysomnography may be helpful in selected cases. Treatment of the underlying disorder is expected to lead to the resolution of erythrocytosis. In selected patients with chronic hypoxia-driven erythrocytosis associated with

Table 2
Acquired causes of secondary erythrocytosis.

Physiologic response to chronic or intermittent hypoxemia
Residence at high altitude
Chronic lung disease
Intracardiac right-to-left shunt
Tobacco abuse (smoker's polycythemia)
Chronic carbon monoxide exposure
Hypoventilation syndromes
Obesity–hypoventilation
Neurologic disorders
Sleep apnea syndromes/nocturnal hypoxemia
Non-physiologic stimulation of erythropoiesis by Epo
Drug-induced
Exogenous androgens
Recombinant Epo use
Epo producing tumors
Renal tumors
Hepatocellular carcinoma
Uterine leiomyomas
Cerebellar hemangioblastomas/meningioma
Pheochromocytoma/paraganglioma
Renal disorders
Renal artery stenosis
Renal cysts
Post-renal transplant
Adrenal disorders (Cushing's)
Other rare conditions associated with erythrocytosis
POEMS syndrome
TEMPI syndrome

hyperviscosity-related symptoms or extreme elevation of hematocrit level, limited use of therapeutic phlebotomy may be beneficial [4].

Several malignant and benign tumors are known to be associated with non-physiologic, increased Epo production. Renal conditions such as renal artery stenosis and polycystic kidney disease may be associated with erythrocytosis. Imaging studies such as abdominal ultrasound, and in selected cases, computerized tomography can be helpful, especially if serum Epo level is elevated, to rule out a tumor. An acquired secondary erythrocytosis can be encountered in 10–15% of renal transplant recipients, typically within 8–24 months after transplant, resulting from increased Epo production and possibly from abnormal erythroid precursor sensitivity to Epo or angiotensin II. About 25% of patients with post-renal transplant erythrocytosis will have spontaneous resolution with no intervention. In the remainder of affected individuals, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers have been effective in normalizing Epo levels with resolution of erythrocytosis [32]. TEMPI syndrome (telangiectasias, elevated Epo level and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting) is a recently described syndrome associated with markedly increased serum Epo levels [33].

In searching for secondary erythrocytosis causes, it is helpful to obtain co-oximetry and P50 measurements. Co-oximetry can be performed either on a venous or on arterial blood sample to measure carboxy-hemoglobin and methemoglobin levels. P50 is the partial pressure of oxygen in blood at which 50% of hemoglobin is saturated with oxygen. The P50 value can be directly measured or calculated on a venous sample, with a normal value between 25 and 29 mm Hg [10]. A low P50 value <20 mm Hg reflects a shift in the oxygen dissociation curve to the left, indicating increased affinity of the hemoglobin molecule to oxygen associated with reduced oxygen release to tissues. The most common cause of a low P50 value is elevated carboxy-hemoglobin, frequently encountered in heavy smokers [34]. Rare familial and congenital disorders that lead to secondary erythrocytosis associated with low P50 include high affinity hemoglobins [35], 2,3-biphosphoglycerate deficiency [10], and methemoglobinemia [36]. High affinity hemoglobins are identified on routine hemoglobin electrophoresis in only 20–25% of cases, therefore high-performance liquid chromatography or globin gene sequencing is often necessary if one is suspected. Routine therapeutic phlebotomy or cytoreductive therapy is not indicated in individuals with high affinity hemoglobins.

4.4. The patient with isolated erythrocytosis, no PV features, no JAK2 mutation, and no acquired secondary etiology identified by routine laboratory evaluation

Primary or secondary familial and congenital erythrocytosis syndromes are primarily considered in this cohort of patients, particularly if JAK2-negative PV is not suspected or has been ruled out (Fig. 1). Clues for an inherited or congenital disorder include a family history of erythrocytosis or diagnosis of erythrocytosis at a relatively young age. Testing for specific gene mutations known to be associated with congenital erythrocytosis is encouraged for both diagnostic and research purposes. The European Congenital Erythrocytosis Consortium has cataloged all reported mutations to date [37]. In up to 70% of cases of congenital erythrocytosis, the molecular basis remains unknown and these patients are designated as having idiopathic erythrocytosis.

Several germline Epo receptor (EPOR) gene mutations have been described in patients with primary familial and congenital erythrocytoses [37]. As a result of these gain-of-function mutations, the cytoplasmic portion of EPOR is truncated resulting in a loss of negative regulatory function of this receptor domain on JAK2 down-regulation. Patients heterozygous for one of these mutations are hypersensitive to circulating Epo and exhibit persistent proliferation of erythroid progenitors despite low-normal serum Epo levels. Several germline mutations have been described, inherited in an autosomal dominant fashion, resulting in erythrocytosis that is frequently asymptomatic, although cardiovascular complications have been reported in some patients [37].

Once the presence of a low P50 and high affinity hemoglobins are eliminated, secondary familial and congenital erythrocytosis syndromes are typically associated with a defect in hypoxia inducible factor (HIF) and oxygen sensing pathway that leads to the upregulation of HIF and Epo production. Mutations in HIF pathway genes encoding von Hippel Lindau (VHL), prolyl hydroxylase-2 (PHD2), and hypoxia inducible factor-2α (HIF2A) have been identified [37]. Chuvash polycythemia, an autosomal recessive condition due to VHL gene mutations, was the first recognized congenital defect in the HIF pathway [38]. As a result of the mutation, the abnormal VHL protein can no longer effectively bind HIF. Therefore, HIF is not targeted for ubiquitin-mediated degradation and continues to promote Epo production and accumulation of red cells (Fig. 2). Affected patients have erythrocytosis with an inappropriately normal or elevated serum Epo level, associated with potential risk for thrombotic events [39].

A heterozygous mutation in the EGLN1 gene that encodes PHD2 [40], another major negative regulator of HIF, reduces enzymatic activity of PHD2 and is associated with familial erythrocytosis. Interestingly, a polymorphism in the gene encoding for PHD2 is associated with genetic adaptation to high altitude in Tibetan population who can maintain hematocrit levels comparable to individuals residing at sea level [41]. Gain-of-function mutations in the EPAS1 gene that encodes HIF2A have been reported in families with erythrocytosis, inherited in an autosomal dominant fashion [37,42]. The risk of tumor development in patients with HIF pathway gene mutations is not completely characterized. Unlike inherited cancer mutations recognized in the VHL gene, heterozygosity for the common erythrocytosis-associated VHL mutation does not appear to confer increased risk [37]. Both germline and somatic mutations in PHD1, PHD2, and HIF2A, however, have been discovered in the pheochromocytoma–paraganglioma–polycythemia syndrome [43]. Close follow-up of congenital erythrocytosis patients with HIF pathway defects for tumor development is advisable. There are anecdotal reports that erythrocytosis in patients with PHD2 and HIF2 mutations may be associated with thrombotic complications

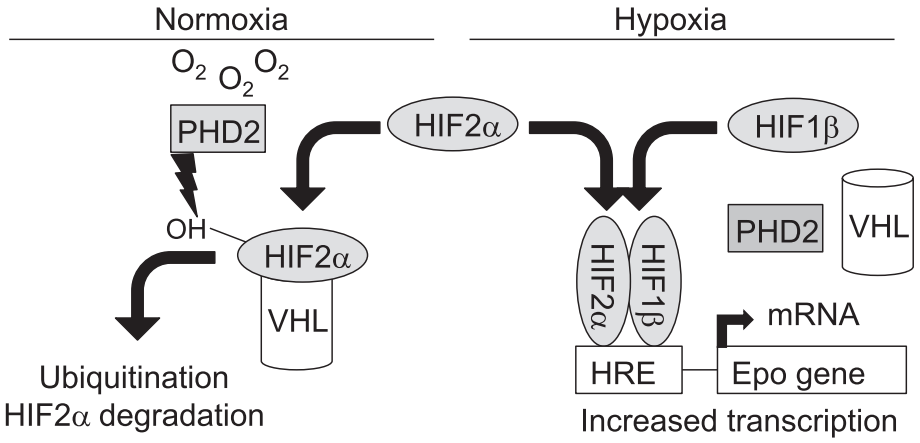

Fig. 2. Oxygen sensing pathway defects and genetic mechanism of erythrocytosis. Under normoxic conditions increased PHD2 activation leads to HIF2α hydroxylation required for VHL protein binding followed by ubiquitination and proteasomal degradation of HIF2α. Under hypoxic conditions, hydroxylation reactions are inhibited and HIF2α is stabilized, dimerizing with HIF1β to bind to hypoxia-responsive elements (HRE) flanking its target genes (including Epo) to upregulate mRNA transcription. Loss of function mutations in negative HIF regulators PHD2 and VHL, or gain-of function mutations in HIF2α are associated with elevated Epo protein levels and erythrocytosis.

[44]. In the management of patients with congenital erythrocytosis syndromes associated with rare HIF pathway mutations and thrombosis, the role and potential benefit of therapeutic phlebotomy requires further study. In a retrospective review of a cohort of patients with Chuvash polycythemia due to VHL mutation, history of phlebotomy was associated with a statistically non-significant 5.6-fold decrease in the risk of thrombosis and 1.7-fold decrease in mortality [39].

Learning points

- Nearly one-half of the patients with erythrocytosis have an underlying non-hematologic etiology such as cardio-pulmonary disorders that can be diagnosed and managed by the practicing internist.
- Evaluation of the patient with erythrocytosis requires a complete clinical assessment beginning with a thorough medical and family history, physical examination, and the identification of symptoms and signs of secondary causes of erythrocytosis as well as PV, a myeloproliferative neoplasm.
- Scientific advances have elucidated the pathogenesis of PV, leading to the development of novel therapies.
- Laboratory evaluation incorporating JAK2 gene mutation testing and further investigation for secondary erythrocytosis etiologies can help guide the evaluation.
- Recognition by the practicing internist of clinical features suggestive of PV and the key role of JAK2 molecular testing facilitates prompt diagnosis, subspecialist referral, therapy initiation, and complication prevention.

Conflict of interest statement

The authors do not have any conflicts of interest in relation to this manuscript.

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