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JAK2 unmutated erythrocytosis: 2023 update on diagnosis and management

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Abstract

Disease Overview: *JAK2* unmutated or non-polycythemia vera (PV) erythrocytosis encompasses a heterogenous spectrum of hereditary and acquired entities.

Diagnosis: Foremost in the evaluation of erythrocytosis is the exclusion of PV through *JAK2* (inclusive of exons 12-15) mutation screening. Initial assessment should also include gathering of previous records on hematocrit (Hct) and hemoglobin (Hgb) levels, in order to streamline the diagnostic process by first distinguishing longstanding from acquired erythrocytosis; subsequent subcategorization is facilitated by serum erythropoietin (Epo) measurement, germline mutation screening, and review of historical data, including comorbid conditions and medication list.

Hereditary erythrocytosis constitutes the main culprit in the context of longstanding erythrocytosis, especially when associated with a positive family history. In this regard, a subnormal serum Epo level suggests *EPO* receptor *(EPOR)* mutation. Otherwise, considerations include those associated with decreased (high oxygen affinity hemoglobin variants, 2, 3 bisphosphoglycerate deficiency, *PIEZO1* mutations, methemoglobinemia) or normal oxygen tension at 50% hemoglobin saturation (p50). The latter include germline oxygen sensing pathway (*HIF2A-PHD2-VHL*) and other rare mutations.

Acquired erythrocytosis commonly results from central (e.g., cardiopulmonary disease, high-altitude habitat) or peripheral (e.g., renal artery stenosis) hypoxia. Other noteworthy conditions associated with acquired erythrocytosis include Epo-producing tumors (e.g., renal cell carcinoma, cerebral hemangioblastoma) and drugs (e.g., testosterone, erythropoiesis stimulating agents, sodium glucose co-transporter-2 inhibitors).

Idiopathic erythrocytosis is an ill-defined terminology that presumes the existence of an increased Hgb/Hct level without an identifiable etiology. Such classification often lacks accounting for normal outliers and is marred by truncated diagnostic evaluation.

Management: Current consensus treatment guidelines are not supported by hard evidence and their value is further undermined by limited phenotypic characterization and unfounded concerns for thrombosis. We are of the opinion that cytoreductive therapy and indiscriminate use of phlebotomy should be avoided in the treatment of non-clonal erythrocytosis. However, it is reasonable to consider therapeutic phlebotomy if one were to demonstrate value in symptom control, with frequency determined by symptoms rather than Hct level. In addition, cardiovascular risk optimization and low dose aspirin is often advised.

Future directions: Advances in molecular hematology might result in better characterization of "idiopathic erythrocytosis" and expansion of the repertoire for germline mutations in hereditary erythrocytosis. Prospective controlled studies are needed to clarify potential pathology from *JAK2* unmutated erythrocytosis, as well as to document the therapeutic value of phlebotomy.

Disease Overview

The term "Erythrocytosis" or "Polycythemia" refers to an absolute or relative increase in hemoglobin (Hgb)/hematocrit (Hct) levels from baseline sex- race- and altitude-adjusted normal values.[1,2] In 2016, the World Health organization (WHO) lowered the proposed Hgb and Hct diagnostic thresholds for polycythemia vera (PV) to 16.5 g/dL/49% and 16 g/dL/48% for Caucasian males and females, respectively.[3,4,5,6] The lower Hgb/Hct thresholds for PV were introduced to facilitate diagnosis of masked PV and remain unchanged in the contemporary 2022 International Consensus Classification (ICC) of myeloid neoplasms.[7] Unsurprisingly, there has been an unprecedented rise in erythrocytosis referrals to hematology which are frequently triggered by Hgb/Hct levels that exceed thresholds for PV. Although *JAK2* unmutated or non-polycythemia vera (PV) erythrocytosis has a higher prevalence than PV, accurate epidemiology has been difficult to ascertain as it represents a spectrum of heterogeneous diseases ranging from hereditary to acquired medical conditions. In a large population-based cohort (n=147 167), the incidence of erythrocytosis ranged from 0.3% to 3.4% based on application of the WHO 2008 (Hgb/Hct > 18.5g/dl/52%, males and >16.5 g/dl/48%, females) and 2016 (Hgb/Hct > 16.5 g/dl/49%, males and >16 g/dl/48%, females) criteria, respectively; predisposing factors included older age, higher body mass index, history of smoking, hypertension and use of androgens.[8] Similarly, in a Canadian study, erythrocytosis was noted in 4.1%/0.35% males, and 0.35%/0.13% females, according to WHO 2016 and 2008 thresholds, respectively.[9] On the other hand, in a National Health and Nutrition Examination Survey (NHANES) analysis, the overall prevalence of unexplained erythrocytosis was 35.1 per 100,000 population and was significantly higher in older patients aged 50-59 years and 60-69 years, at 128.7 per 100,000 and 102.5 per 100,000, respectively.[10]

In clinical practice, the diagnostic evaluation of *JAK2* unmutated erythrocytosis remains a challenge, and patients are frequently subjected to non-uniform testing.[11,12] In a recent single center study on non-clonal erythrocytosis, additional investigations beyond *JAK2* testing were not pursued by the treating hematologist in 58% of patients.[13] On the other hand, in another study, nearly as many secondary erythrocytosis (31%) as PV (39%) patients underwent bone marrow biopsies.[14] Similarly, a marked heterogeneity in treatment patterns stems from an unsubstantiated concern for thrombosis, and whether to initiate phlebotomy, and desired Hct target, continue to be unresolved issues.[15] In the current review, we provide our contemporary diagnostic and therapeutic approach to known hereditary and acquired entities associated with *JAK2* unmutated erythrocytosis.

Pathogenesis

The hypoxia-inducible factor (HIF)-prolyl hydroxylase domain (PHD)-Von Hippel-Lindau (VHL) pathway regulates erythropoiesis and Epo production within renal peritubular cells, in an oxygen-dependent manner.[16-18] HIF transcription factor is a heterodimer with alpha and beta subunits, the latter - is constitutively expressed, while hypoxia affects function of the former. HIFA has three known isoforms (HIF1A, HIF2A, HIF3A), amongst which HIF2A is mainly involved in regulation of erythropoiesis; *HIF2A* knockout mice demonstrate a hypocellular marrow and anemia as a result of inadequate Epo production. [19] Under normoxic conditions, HIF2A undergoes hydroxylation at two critical proline residues (Pro405 and Pro531) through prolyl hydroxylase (PHD2),[20,21] followed by VHL (a recognition subunit of E3 ubiquitin ligase) mediated degradation via the ubiquitin proteasomal pathway.[22] In addition, a 2-oxoglutarate dependent oxygenase, factor inhibiting HIF (FIH), catalyzes hydroxylation of a specific arginine residue within HIF and inhibits HIF binding to p300, a transcriptional co-activator. [23] The end result is reduced transcriptional activation of *EPO*.

Conversely, under hypoxic conditions, PHD2 enzymatic activity is reduced, resulting in diminished hydroxylation and degradation of HIF2A, in other words stabilization of HIF2A occurs,

following which the HIF complex binds to hypoxia responsive elements within the *EPO* gene and turns on transcription.[24-26] This constitutes the pathogenetic basis for hypoxia-induced acquired erythrocytosis associated with chronic obstructive pulmonary disease (COPD), cyanotic heart disease with right to left shunt, and high-altitude habitat. Amelioration of tissue hypoxia reverses the process resulting in compensated normal Epo in such conditions, consistent with the assertion that regulation of erythropoiesis by HIF is exquisitely sensitive to oxygen levels.[27,28] Additionally, HIF2A expression is also modulated by iron regulatory proteins 1 and 2 (IRP) through its iron responsive elements, and deletion of *Irp1* in murine models has been shown to increase HIF2A expression, which in turn stimulates Epo, resulting in erythrocytosis.[29]

Diagnosis

Hematology referrals for erythrocytosis are often triggered by Hgb/Hct levels that exceed 16.5 g/dl/49% and 16 g/dl/48% in Caucasian males and females, respectively, and should be confirmed by at least two separate blood counts evaluated at different time-points. An understanding of sex-, race-and altitude-adjusted normal values, and extreme high normal values which exceed the 95th percentile is required for interpretation of test results. It is important to highlight the ill-defined nature of the term "erythrocytosis" and the arbitrary measures of its definition which are extrapolated from diagnostic thresholds used for PV. In the evaluation of erythrocytosis, inapparent (masked) erythrocytosis which may result when an increase in red cell mass is accompanied by a concomitant increase in plasma volume and therefore masked by a normal Hgb/Hct should be considered. Similarly, in situations with normal Hgb/Hct and concomitant iron deficiency, PV is often under-recognized. Historically, red cell mass (RCM) measurement was recommended to confirm absolute erythrocytosis, but is no longer widely available, including at the Mayo Clinic. Presently, increased Hgb/Hct levels are acceptable surrogates for an elevated RCM. Overall, RCM measurements have been found to be suboptimal in distinguishing PV from other causes of erythrocytosis; in a study of 105 patients with erythrocytosis, RCM was elevated in 76%, 20%, 21% and 57% with PV, secondary erythrocytosis, apparent erythrocytosis, and essential

thrombocythemia, respectively. [30] Importantly, in the particular study, RCM showed significant correlation with Hgb/Hct levels; elevated RCM was not observed with Hgb/Hct < 16/48% in males and 13/39% in females, in contrast, Hgb/Hct > 19.5/58% in males and 17.5/53% in females were almost always associated with increased RCM.[30] Furthermore, obesity is a confounding factor that hinders accurate interpretation of RCM. [31]

The discovery of *JAK2* exons 14 and 12 mutations in 2005 and 2007, respectively, and their almost invariable association with PV has greatly simplified our current diagnostic approach to erythrocytosis. [32,33] This is because the most treatment-relevant step in addressing the differential diagnosis of erythrocytosis is the exclusion of PV, because of its specific association with increased risk of thrombosis and fibrotic/leukemic transformation.[5] Therefore, foremost in the evaluation of erythrocytosis, PV should be excluded by absence of peripheral blood *JAK2* mutation (*V617F* exon 14 and exon 12).[33,34] Serum Epo measurement may also be helpful, especially in cases of *JAK2* exon 12 mutated PV, where serum Epo levels are often subnormal. [35] If PV is suspected clinically or Epo is subnormal with absence of *JAK2* mutations, a bone marrow examination is recommended in order to identify histological features associated with myeloproliferative neoplasm (MPN). Additional testing for MPN associated driver mutations; *CALR* and *MPL*, albeit rarely found in PV, should be performed. [36-40] Furthermore, in patients with unexplained erythrocytosis, subnormal serum Epo levels, and absence of *JAK2, MPL* and *EPOR* mutations*, SH2B3/LNK* exon 2 mutations or polymorphisms may be present.[41,42] In one study, *LNK* mutations were detected in 6 of 112 (5.3%) patients with *JAK2* negative (idiopathic) erythrocytosis.[43] An additional test, bone marrow erythroid progenitor cultures, although not uniformly performed at all centers, may serve as a useful adjunct to rule-out endogenous erythroid proliferation.[44,45] According to a seminal study on the subject, endogenous erythroid colonies (EEC) were noted in the vast majority of PV (43 of 46) and in 0 of 17 secondary erythrocytosis cases.[46]

As a next step in the evaluation of erythrocytosis, a comprehensive medical assessment including careful review of prior blood counts, medications, and family history is recommended. The clinical

phenotype of secondary erythrocytosis differs from PV; patients with secondary erythrocytosis are more likely to be males, display isolated erythrocytosis with lower white and platelet counts, higher Epo levels, lower lactate dehydrogenase levels, without palpable splenomegaly.[14,47,48] Most studies have observed a lower incidence of thrombosis with secondary erythrocytosis in comparison to PV,[47-49] while two retrospective studies have suggested a similar thrombotic risk as in PV.[14,50] It is to be noted that the latter studies did not account for the causal diversity of secondary erythrocytosis.

The historical overview guides further investigations focused on conditions associated with hereditary or acquired erythrocytosis. Figure 1 is an illustration of our diagnostic algorithm for *JAK2* unmutated erythrocytosis which centers upon duration of erythrocytosis and family history (if known), and serum Epo levels.

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Diagnostic approach to hereditary erythrocytosis

Hereditary erythrocytosis is suspected in children and young adults with long-standing erythrocytosis, particularly with a positive family history.[51,52] A subnormal serum Epo level is suspicious for presence of an *EPOR* mutation. On the other hand, if serum Epo is normal or elevated, high oxygen affinity hemoglobin variants (HOAV) should be considered foremost. Historically, venous P50 measurement (oxygen tension at which hemoglobin is 50% saturated) was recommended which can also be calculated from venous blood gas using the following mathematical formula https://www.medsci.org/v04/p0232/ijmsv04p0232s1.xls.[53] A left shift of the oxygen dissociation curve, that is venous P50 <24 mmHg is a good screening test for HOAV, although a small proportion of cases could be missed if arterial instead of venous blood gas is analyzed and if analytical conditions are compromised with the presence of air bubbles in the syringe.[54] Therefore, it is best to confirm that P50 was obtained on venous blood through venous oxygen saturation levels between 30-55%. At the Mayo Clinic, P50 testing is no longer available due to assay issues and detection of HOAV is accomplished

through cation exchange high performance liquid chromatography (HPLC), capillary electrophoresis, and mass spectrometry. Sequencing of *HBB*, *HBA1, HBA2* is not required; in our experience, all HOAV were identified through either capillary electrophoresis, HPLC or mass spectrometry, moreover, sequencing may miss a rare polymorphism that is located under the amplification primers. Therefore, in situations when P50 testing is unavailable or inaccurate, HOAV is investigated through capillary electrophoresis, HPLC and mass spectrometry with or without sequencing of *HBB*, *HBA1, HBA2*. If HOAV is absent and P50 <24 mmHg, the possibility of defective 2, 3 bisphosphoglycerate (2,3- BPG) mutase causing 2, 3-BPG deficiency, methemoglobinemia or *PIEZO1* mutations is investigated.[55] Pathogenic *PIEZO1*/*FAM38A* mutations were recently described in up to 4% of patients with idiopathic erythrocytosis, in association with clinical or biological manifestations of hereditary xerocytosis (HX) (iron overload, splenomegaly, hemolysis, decreased venous P50).[56] In a large series of patients with HX and *PIEZO1* mutations, 68% were not anemic; moreover, seven patients had Hgb > 16 g/dl, with two patients known to have erythrocytosis.[57] Functionally, Piezo1-HX impacts red cell energy metabolism and glycolysis, resulting in reduced BPG levels, conferring a high oxygen affinity state, which explains the erythrocytosis associated with this disorder.[58,59]

Germline mutations in the oxygen sensing pathway (*VHL, PHD2, HIF2A*) do not affect the venous P50 measurement and Epo is either elevated or inappropriately normal; in this regard, it is important to note that serum Epo might be impacted by phlebotomy. If oxygen sensing pathway mutations are absent and P50 is normal, the presence of germline mutations in the *EPO* gene is considered, whereby a single nucleotide deletion in exon 2 causes a frameshift that truncates translation of the main *EPO* messenger RNA (mRNA) transcript, and typically a noncoding mRNA, is transcribed from an alternative promoter within intron 1, and produces excess functional Epo mainly through the liver.[60] In addition, a novel heterozygous 5`UTR *EPO* variant was discovered in a five generation kindred with erythrocytosis; the mutated 5`UTR of *EPO* augments interaction with HIF2A, leading to increased Epo production.[61]

In the situation of a negative work up and high index of clinical suspicion for hereditary erythrocytosis, one should consider the possibility of *HFE* mutations (H63D and C282Y).[62,63] In a study of 132 patients with idiopathic erythrocytosis, *HFE* mutations were identified in 73 (55%) patients, which included C282Y (n=18), H63D (n=56), and C65S (n=3).[63] The frequencies of C282Y (15%) and H63D mutations (40%) were higher than reported frequencies of 3.8-9.2% and 13.6-22% for C282Y and H63D, respectively, in the European population.[63] Similarly, a study of 56 patients (79% males) with idiopathic erythrocytosis from the UK and Italy, demonstrated a higher incidence of *HFE* mutations (45%) compared to the general population.[62] Most patients were either homozygous, or heterozygous for C282Y or H63D, or compound heterozygotes for C282Y/H63D except two patients carried the S56C mutation.[62] Importantly, only 12 of 25 (48%) patients with *HFE* mutations had high ferritin levels.[62] HFE influences iron metabolism through hepcidin, and low hepcidin is a major factor leading to iron overload in hereditary hemochromatosis. The postulated mechanism for erythrocytosis is increased bioavailability of iron, which facilitates erythropoiesis as demonstrated in *HFE* knock out mice.[64] An additional consideration in children with unexplained erythrocytosis includes *SLC30A10* mutations, that have been reported in association with hypermanganesemia, parkinsonism and hepatic cirrhosis.[65,66]

Presently, the Mayo clinic laboratory offers an algorithmic hereditary erythrocytosis evaluation in which all cases are first tested for HOAV through cation exchange HPLC, capillary electrophoresis, and mass spectrometry. Additional testing is pursued in a reflexive manner and may include Sanger sequencing of *HBA1/HABA2, HBB, EPOR* (exon 8), *VHL* (exons 1-3), *EGLN1(PHD2)* (exons 1-5), *EPAS1(HIF2A)* (exons 9 and 12) and *BPGM.* Our laboratory experience lends to the rarity of the above entities; of 1192 cases tested for hereditary erythrocytosis only 143 (12%) cases had identified abnormalities of which 85 were pathogenic or likely pathogenic mutations (inclusive of HOAV) and 58 variants of unknown significance.[55]

Diagnostic approach to acquired erythrocytosis

Acquired erythrocytosis warrants a comprehensive medical evaluation with attention to place of residence, tobacco use and medications both prescription and supplements particularly testosterone, androgens, erythropoietin, diuretics, and sodium-glucose cotransporter 2 inhibitors (gliflozins). Physical examination should focus on identifying hypoxia, cardiopulmonary disease, telangiectasias, cushingoid or virilization features, abdominal masses raising concern for renal or hepatic tumors and abdominal bruit in case of renal artery stenosis. Renal cell carcinoma is the most frequent cause of paraneoplastic erythrocytosis; distinguishing features include the subacute development of erythrocytosis with a significant increase in Hct level.[67] Initial testing should include arterial blood gas, overnight oximetry, and abdominal ultrasound to assess for renal/hepatic tumors/renal artery stenosis. An echocardiogram with shunt study is indicated in the presence of relevant cardiac findings. Computed tomography (CT) or magnetic resonance imaging (MRI) of the brain may be considered in case of neurological symptoms.[68] In females, myomatous erythrocytosis syndrome should be kept in mind, particularly since erythrocytosis is often masked by menorrhagia.[69] Rare acquired disorders to consider include the monoclonal gammopathy driven TEMPI syndrome which is characterized by telangiectasias, elevated serum Epo (which might be >5000 mIU/ml) and erythrocytosis, monoclonal gammopathy, perinephric fluid collections and intrapulmonary shunting; [70,71] erythrocytosis and telangiectasis are often the initial manifestation. Similarly, erythrocytosis along with thrombocytosis may be a part of polyneuropathy, organomegaly, endocrinopathy, M-protein, skin changes (POEMS) syndrome .[72]

Management approach

i) General considerations

In general, management of acquired erythrocytosis warrants addressing the underlying predisposition. Management recommendations are often extrapolated from studies conducted in PV or through consensus

guidelines.[73,74] In order to formulate our recommendations summarized in figure 2, we reviewed the relevant literature pertaining to secondary erythrocytosis associated with common hereditary and acquired conditions (Tables 1 and 2). Overall, data on thrombosis in secondary erythrocytosis has been conflicting; particularly since thrombotic risk assessments did not factor in causal diversity. In a large population-based series, erythrocytosis defined by higher 2008 WHO criteria Hgb/Hct thresholds, was associated with higher cardiovascular morbidity and mortality; [8] in the particular study, etiologies of erythrocytosis were not specified, however, a high incidence of clonal hematopoiesis (38%) and cardiovascular risk factors was reported which accounted for a higher incidence of cardiovascular events. Similarly, in another series of thirty-five patients with secondary erythrocytosis, thrombosis rates at or prior to diagnosis were similar to those reported in PV, which was likely due to clustering of cardiovascular risk factors in patients with secondary erythrocytosis.[14] Accordingly, aspirin 81 mg daily should be instituted in the presence of cardiovascular risk factors, along with cardiovascular risk factor modification. Arterial and venous thromboses are managed with anti-platelet agents and systemic anticoagulation, respectively.

ii) Role of phlebotomy

Current guidelines formulated by the British Society of Hematology lack hard evidence and recommend phlebotomy for patients with recent thrombosis, and those with risk factors for thrombosis or Hct > 52% and > 56% in patients with hereditary erythrocytosis and COPD, respectively.[74] Nonetheless, phlebotomy might be detrimental to most patients with secondary erythrocytosis including those with COPD, cyanotic heart disease or hereditary erythrocytosis. [75-78] In such instances, erythrocytosis is a physiologically appropriate compensatory mechanism in an effort to maintain tissue oxygenation. Therefore, the institution of phlebotomy requires careful assessment of risk-benefit balance and should be reserved only for relief of symptoms with documented response to the particular treatment modality; reported symptoms in patients with non-clonal erythrocytosis include fatigue, generalized weakness, headaches, visual changes, mental fog, tinnitus, chest pain, palpitations, dyspnea, abdominal and bone

pain. Similar symptoms can also be a consequence of phlebotomy-induced dehydration and iron deficiency, which should be concomitantly addressed.

iii) Perioperative management

There is limited guidance regarding perioperative management; a recent study compared perioperative outcomes of 24 patients with secondary erythrocytosis and PV, and highlighted higher rates of thrombosis and conservative perioperative practices with underutilization of phlebotomy and limited antiplatelet therapy in patients with secondary erthrocytosis.[79] On the other hand, in a matched control study of 100 patients with COPD and Hgb > 16 g/dl and controls matched for age, gender, physical status and type of surgery, risk of perioperative thrombotic or hemorrhagic complications was not increased; however details on perioperative management were not provided.[80] Regardless, an individualized approach is recommended, and in patients with physiologically appropriate erythrocytosis, phlebotomy should be performed with caution after consideration of the balance between tissue oxygenation and thrombotic risk.

The next section provides an in-depth review on the incidence, pathogenesis, clinical course, and management specifics for erythrocytosis associated with common hereditary and acquired conditions.

Erythrocytosis associated with high oxygen affinity

i)High oxygen affinity hemoglobin variants

HOAV constitute the majority of cases of hereditary erythrocytosis.[55] Transmission is autosomal dominant, and usually with a documented family history of erythrocytosis. Structurally, hemoglobin is a tetramer, comprised of two alpha and beta globin subunits (an $\alpha_1\beta_1$ dimer and an $\alpha_2\beta_2$ dimer in hemoglobin A) with two conformationally stable states; the relaxed (R), high oxygen affinity state and tense (T), low oxygen affinity state.[81,82] Oxygen binding to hemoglobin subunits demonstrates cooperativity, resulting in

a sigmoidal oxygen dissociation curve which is left-shifted (low p50) with HOA variants.[83] The R-T transition is impacted by mutations in critical regions of the globin chain in HOAV. Oxygen delivery is compromised at the tissue capillary level, resulting in hypoxia which serves as a stimulus for Epo production and subsequent erythrocytosis. To date, 103 HOAV have been described in the HbVar database (https://globin.bx.psu.edu/cgi-bin/hbvar/query_vars3), the majority (80%) are β chain variants. Between 1974-2018, the Mayo Clinic laboratory has identified 80 distinct variants (60 β, 20 α), including 12 novel variants.[55] These mutations were mostly missense impacting the heme pocket, α1β2 contact sites, 2, 3 BPG binding sites and C-terminal conformation stabilization regions, with the most common variants being Hb Tarrant (α chain variant) and Hb Malmo (β chain variant).[55]

Phenotypically, the majority of patients with HOAV are asymptomatic and only one-third demonstrate erythrocytosis, likely due to low-level expression of the variant or concomitant hemolysis.[84,85] The incidence of thrombosis is unknown and management guidelines, arbitrarily recommend phlebotomy for Hct > 52%.[74] Among 41 patients with HOAV-associated erythrocytosis evaluated at the Mayo Clinic over five decades (median follow up; 10 years), 34 (83%) patients harbored β-chain (13 Malmo, 4 Olympia, 3 San Diego, 2 Wood) and 7 (17%) α-chain (4 Dallas and one each Columbia-Missouri, Jackson, and Wayne) variants. Family history was documented in 24 (56%) patients with history of thrombosis in two (5%) patients.[86] Treatment included phlebotomy in 23 (56%) and antiplatelet agents in 21 (51%) patients. 23 (56%) patients reported one or more symptoms likely related to increased Hct while thrombosis was documented in 10 (24%) patients (6 arterial and 4 venous thrombotic events).[86] Neither Hgb/Hct level nor phlebotomy showed a significant correlation with thrombotic complications or symptoms. On the other hand, arterial thrombosis was associated with older age, male gender and cardiovascular risk factors, while half of venous events developed in the context of other predisposing factors. Importantly, 20% of younger patients (median age 28 years) without cardiovascular risk factors, were observed for up to two decades, and did not experience thrombosis.[86] Symptom relief from phlebotomy was noted in 42% of patients; meanwhile 30% of patients suffered

additional adverse symptoms following the procedure.[86] Taken together, phlebotomy has limited therapeutic value in management of HOAV-associated erythrocytosis.

ii) 2,3 bisphosphoglycerate (2, 3 BPG) deficiency

2, 3 BPG deficiency resulting from 2, 3, BPGM (bisphosphoglycerate mutase) variants is an uncommon condition associated with HOA and erythrocytosis with limited number of cases described in the literature.[87-90] 2, 3, BPGM catalyzes conversion of 1, 2 BPG to 2, 3 BPG within red cells, the latter binds deoxy hemoglobin tetramer and allosterically converts it to a low oxygen affinity state, prompting release of oxygen. However, with 2, 3 BPGM deficiency, conversion of 1, 2 BPG to 2, 3 BPG is impaired, which enables the hemoglobin tetramer to assume a HOA state.

Erythrocytosis associated with germline EPAS1(HIF2A), EGLN1(PHD2), VHL, EPOR variants

Germline mutations in the oxygen-sensing pathway (*VHL-HIF2A-PHD2-EPOR*) are relatively rare, however may result in erythrocytosis with normal P50 measurement accompanied by either an elevated or inappropriately normal Epo (*VHL-HIF2A-PHD2*) or subnormal Epo (*EPOR*).[12] Between 2012-2021, the Mayo Clinic laboratory tested 592 patients for *HIF2A/PHD2/VHL/EPOR* alterations and identified a total of 12 pathogenic variants in *HIF2A* (n=6, 1%), *PHD2* (n=3, 0.5%), and *EPOR* (n=2, 0.3%), while one of 446 (0.2%) patients harbored *VHL* variant.[91] It is to be noted that interrogation of this pathway is an evolving area of investigation with recent identification of novel zinc finger domain *PHD2* mutations, splicing mutations in *VHL* and *HIF2A* variants. [92-94]

i)Germline EPAS1(HIF2A) variants

In 2008, a heterozygous missense gain of function *EPAS1(HIF2A)* mutation, located near the primary site of HIF hydroxylation (Pro-531) was discovered upon investigation of a family with erythrocytosis; the particular mutation results in stabilization of HIF2A due to impaired hydroxylation.[95]

HIF2A mutations may be associated with neuroendocrine tumors with a genotype/phenotype study illustrating the clinical spectrum of HIF2A mediated diseases (n=66): class 1a (n=6, sporadic) with pheochromocytoma, paraganglioma, somatostatinoma and erythrocytosis; class 1b (n=12; 1 familial, 11 sporadic) with pheochromocytoma, paraganglioma and erythrocytosis; class 1c (n=20; all sporadic) with pheochromocytoma and paraganglioma, and class 2 (n=28; 9 familial, 6 sporadic) with erythrocytosis alone. Each class was found to be driven by a unique set of non-overlapping mutations with class 1 mutations typically located between amino acid residues 529 and 532, which contain Pro-531, whereas class 2 mutations were found exclusively between residues 533 and 540. These findings highlight the differential impact of *HIF2A* mutations on HIF2A-VHL interaction with higher HIF2A levels implicated in tumorigenesis (class 1 disease) as opposed to slight increases in HIF2A activity that are sufficient to induce erythrocytosis in class 2 disease. [96] In a European collaborative study on 41 patients and 28 relatives with erythrocytosis associated with germline *HIF2A* variants, 11 mutations were classified as pathogenic with identification of four new mutations (D525G, L526F, G527K, A530S).[94] The study also unveiled two young cases with *HIF2A* variants in a mosaic state, suggesting that mosaicism may be present at a very low level that is not necessarily detectable by next generation sequencing (NGS). It is therefore necessary to lower the variant detection thresholds or to manually analyze the NGS results. In the particular study, pulmonary arterial hypertension was described in two patients, and thrombosis was reported in six patients (five families).[94] On the other hand, in a prospective study of eight patients harboring the *HIF2A* p.M535V variant, five experienced thrombotic events *versus* none of seventeen *HIF2A* wild-type patients.[78] Furthermore, thrombotic events occurred despite ongoing phlebotomy with Hct < 45% and in the absence of cardiovascular risks.[78] Similarly, three of six Mayo Clinic patients with *HIF2A* pathogenic variants experienced thrombosis, all had one more cardiovascular risk factors, two were receiving phlebotomy, with dual antiplatelet therapy in one patient.[91] Interestingly, treatment with belzutifan, a small molecule inhibitor of HIF2A, led to rapid and sustained tumor response together with resolution of hypertension, headaches and polycythemia in a patient with polycythemia and multiple paragangliomas (Pacak-Zhuang syndrome).[97]

ii) Germline EGLN1(PHD2) variants

Loss of function *EGLN1*(*PHD2)* mutations (P317R and the P371H variants) affect the catalytic rate and substrate-binding of PHD2, thereby hindering HIF hydroxylation. *PHD2* mutations (P317R), are implicated in hereditary erythrocytosis; Epo levels were inappropriately normal, pointing towards secondary erythrocytosis.[98] These mutations are not typically associated with tumors except for a patient with a recurrent extra-adrenal paraganglioma harboring the *PHD2* H374R mutation.[99]

iii) Germline VHL variants (Chuvash polycythemia)

Chuvash polycythemia (CP) is an autosomal recessive condition resulting from homozygous *VHL*(R200W) mutation. It was first described in Chuvashia (Russia) in 1997, and subsequently noted in the Italian island of Ischia and worldwide [100-103] Functionally, the VHL-HIF2A interaction is disrupted with impaired ability of VHL to target hydroxylated HIF2A for proteasomal degradation, resulting in secondary erythrocytosis from increased HIF2A and Epo levels under normoxic conditions.[100] In addition, mutant VHL exhibits altered affinity for suppressor of cytokine signaling 1 (SOCS1), impeding formation of a heterodimeric E3 ligase involved in targeting phosphorylated JAK2 for ubiquitin-mediated proteasomal degradation.[104] Accordingly, erythroid progenitors in affected patients display hypersensitivity to Epo, akin to PV (primary erythrocytosis). In contrast to the autosomal dominant "VHL syndrome", malignant and benign tumors, including spinocerebellar hemangioblastomas, pheochromocytoma and renal cell carcinoma are not seen in patients with CP, reflecting the differential impact of the mutation on HIF regulation.[105] On the other hand, erythrocytosis seldom accompanies "VHL syndrome" and is typically mediated by ectopic Epo production by tumor.[106,107]

Patients with CP demonstrate an unusual propensity for vascular events leading to early mortality.[102] In a prospective, age, sex-matched controlled study of 155 patients with CP, age and prior thrombotic events but not Hct were independent predictors of thrombosis; moreover, phlebotomy was associated with an increased incidence of thrombosis in CP and aspirin 75 mg daily was not

protective.[102] In a separate comparison of CP and PV patients, mortality was higher in CP (47% vs 18.5%), with a higher proportion of deaths due to cerebrovascular events or peripheral thrombosis (46.1% vs 21.9%), despite younger age distribution of the CP cohort (median age; 16 vs 60 years).[102] In the particular study, 78% of CP patients received phlebotomy without significant benefit in thrombotic risk or mortality reduction, suggesting thrombotic events are not associated with elevated Hct; moreover elevated vascular endothelial growth factor (VEGF), plasminogen activator inhibitor- 1, 2 (PAI-1,2) and homocysteine levels may contribute to thrombosis. [102,108] JAK2 inhibitor therapy has shown efficacy in patients with CP. In murine models, administration of a highly selective JAK2 inhibitor, TG101209 was able to reverse the CP phenotype,[104] moreover, ruxolitinib therapy in three patients led to hematologic and symptomatic improvement, without appreciable impact on thrombotic events or mortality.[109]

In general, we recommend close monitoring with stringent cardiovascular risk modification and antiplatelet therapy for patients with erythrocytosis associated with germline mutations in the oxygen-sensing pathway. In addition, symptoms suggestive of neuroendocrine tumors and pulmonary hypertension in patients with *HIF2A* mutations warrant prompt investigation. The routine use of phlebotomy, cytoreductive therapy, belzutifan (HIF2A inhibitor) or ruxolitinib (JAK2 inhibitor) is not advised.

iv) Germline EPOR (EPO receptor) variants

EPOR mutations are relatively rare with an incidence rate of 1.1% in a series of 270 consecutive unrelated patients with idiopathic erythrocytosis.[110] Transmission is usually autosomal dominant and should be suspected in patients with erythrocytosis accompanied by low serum Epo levels and/or family history. The clinical course of patients harboring EPOR variants is not well-known. EPOR is part of the cytokine class I receptor superfamily and following binding of Epo, it undergoes dimerization and activates JAK2, which in turn leads to tyrosine phosphorylation of its distal region.[111] Epo-induced JAK2

activation leads to intracellular activation of the Ras/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt pathways, and signal transducer and activator of transcription (STAT 1, 3, 5A, 5B) which turns on numerous target genes that promote red cell survival, proliferation and maturation.[111] In essence, Epo in synergy with several growth factors (SCF, GM-CSF, 1L-3, and IGF-1) enhances red cell survival by inhibition of apoptosis together with maturation and proliferation of erythroid progenitor cells. Germline heterozygous nonsense and frameshift mutations in exon 8 of *EPOR* have been described which cause truncation of the C-terminal distal portion of the receptor which contains several tyrosine residues that serve as docking sites for SHP1, SOCS3 that are negative regulators of Epo signaling.[110,112-114] The aforementioned alterations confer hypersensitivity to Epo via excessive activation of EPOR, resulting in primary erythrocytosis.[115-117]

Erythrocytosis associated with cardiopulmonary disease

i) Chronic obstructive pulmonary disease (COPD)

The prevalence of erythrocytosis (Hgb ≥ 17 g/dl in males and ≥ 15 g/dl in females) in patients with COPD, ranges from 5.9-18.1% and is declining with the implementation of long-term oxygen therapy.[118,119] In a contemporary observational cohort of moderate to very severe COPD patients enrolled in the COPDGene study (n=1928), prevalence of WHO 2016 defined erythrocytosis was 6.6%, and was significantly higher in males, non-Hispanic whites, and current smokers.[120] Severe resting hypoxia, impaired diffuse capacity for carbon monoxide (DLCO), were identified as risk factors for erythrocytosis, on the other hand continuous or nocturnal supplemental oxygen were associated with a reduced risk.[120] With respect to thrombosis, a study of 86 patients with COPD associated erythrocytosis, found a similar incidence of venous thrombosis compared to age and sex matched COPD patients without erythrocytosis (19.8%vs 14%; p=0.42).[121] In another study that investigated the impact of phlebotomy on arterial and venous thrombosis in COPD associated erythrocytosis, thrombosis rates were similar with or without

phlebotomy (31% vs 22%; p=0.28).[122] Moreover, more stringent Hct control did not appear to influence the incidence of thrombosis; Hct <52% or ≥ 52% (25% vs 37%; p=0.45).[122] Therefore, phlebotomy should be avoided and restricted to symptom control in patients whose symptoms can clearly be correlated to the increased Hct with documentation of symptom relief from phlebotomy. [123] Furthermore, hypoxia should be corrected with supplemental oxygen and smoking cessation counselling provided when applicable.

ii) Obstructive sleep apnea (OSA)

The prevalence of erythrocytosis in patients with OSA ranges widely from 0.3% to 8%, and it is unclear whether treatment of OSA results in resolution of erythrocytosis.[124,125] In a large study of 1,604 patients with suspected OSA, only 1.6% had erythrocytosis defined by Hct ≥51% and 48% in males and females, respectively.[126] On the other hand, in a recent meta-analysis which included a total of 3,654 patients with OSA, the overall prevalence of erythrocytosis was 2% and 6%, in patients with mild-moderate, and severe OSA, respectively.[127] In the particular study, treatment with continuous positive airway pressure (CPAP) reduced Hgb and Hct levels by 3.76 g/l and 1%, respectively.[127] Management of OSA includes weight loss and referral to a sleep specialist for institution of overnight CPAP. Regarding thrombotic risk and OSA, a recent study evaluating 330 cases of erythrocytosis found thrombotic rate to be significantly lower in patients with OSA versus those with either PV or chronic pulmonary disease (incidence rate of 1.46 vs 4.51/6.24 cases per 100 person-years respectively, p=0.009).[128]

iii) Cyanotic congenital heart disease

Patients with cyanotic congenital heart disease have a predisposition for thrombosis; in a cross-sectional study of 98 adult patients, the prevalence of cerebral and pulmonary thrombosis by imaging was 47% and 31%, respectively, and was not associated with degree of erythrocytosis.[129] In addition, another retrospective study of 162 adults with erythrocytosis associated with cyanotic heart disease, reported a higher incidence of cerebrovascular events in patients despite the implementation of phlebotomy.[76] In the

particular study, patients were stratified into two groups based on absence/presence of prior cerebrovascular events: Group 1 (n=140) and Group 2 (n=22) respectively. Amongst 46/162 (28%) of patients that underwent phlebotomy, cerebral events occurred in 25% of Group 1 (n=35) vs 50% of Group 2 (n=11), (p=0.016). Other noteworthy findings were a strong association of iron deficiency and/or microcytosis with cerebrovascular events; reported in 11/41(27%) with iron deficiency.[76] Hence, phlebotomy is discouraged in patients with congenital heart disease and might be detrimental through exacerbation of iron deficiency and symptoms related to hyperviscosity.[130] Similar symptoms often result from dehydration and iron deficiency which should be corrected. The use of hydroxyurea is not generally recommended; in a series of four patients with symptomatic secondary erythrocytosis and congenital heart disease, symptom relief was achieved with hydroxyurea but without a clear impact on thrombosis and at the cost of myelosuppression requiring dose reduction in three patients. [131]

Erythrocytosis associated with drugs

i) SGLT-2 inhibitor therapy

SGLT-2 inhibitors **(**canagliflozin, empagliflozin, dapagliflozin, and ertugliflozin) also referred to as "gliflozins" are FDA-approved for diabetes mellitus, heart failure, and chronic renal disease.[132] Treatment with empagliflozin in the context of a large, placebo-controlled study demonstrated a dose-dependent increase in Hgb/Hct levels, with a baseline increase in Hct levels of 4.8% ± 5.5% in patients treated with empagliflozin 10 mg and 5.0% ± 5.3% in those receiving empagliflozin 25 mg, vs 0.9% ± 4.7% in the placebo arm.[133] Similarly, Hgb changes were 0.8 ± 1.3 g/dL with empagliflozin vs 0.1 ± 1.2 g/dL with placebo.[133] The putative mechanisms underlying erythrocytosis include increased Epo production via hypoxia-induced activation of HIF2A, modulation of iron metabolism through hepcidin, and hemoconcentration.[134,135] Studies have also implied shared molecular mechanisms for SGLT-2

inhibitor–associated erythrocytosis and cardioprotection, whereby a drug-induced hypoxic and nutrient-deprived state activates sirtuin 1 and HIF2A.[134,136]

In the Mayo Clinic experience with SGLT-2 inhibitor associated erythrocytosis, 30 patients with normal baseline Hgb/Hct developed a peak increase in Hct of 7.4% (2-14.1) following therapy; 8 (27%) patients had an additional predisposition which included OSA alone (n = 5) or in association with smoking (n = 2) and smoking alone (n = 1).[137] Serum Epo measurement was inappropriately normal or increased in all cases, with a median EPO level of 10.3 mIU/mL (range, 6.1-37.3 mIU/mL).[137] Blood donation/phlebotomy was instituted in seven cases, half of the patients received antiplatelet therapy (n = 14) whereas five received systemic anticoagulation.[137] Thrombotic events (unstable angina) occurred in two patients (7%), despite the use of phlebotomy in both cases and aspirin in one patient.[137] Erythrocytosis was found to be self-limiting with complete resolution following discontinuation of therapy.[137] Erythrocytosis observed with SGLT-2 inhibitor therapy serves as a biomarker of a drug-induced hypoxic and nutrient-deprived state, in which activation of sirtuin 1 and HIF2A, promotes Epo production and also ameliorates cardiac dysfunction.[136] Therefore, given the association of erythrocytosis with cardiac benefits, discontinuation of SGLT-2 inhibitor therapy is not generally advised.

ii) Testosterone therapy

Erythrocytosis may develop in cis and trans men receiving testosterone therapy.[138,139] The reported prevalence of erythrocytosis (Hct > 50%) in testosterone treated hypogonadal cis men is variable (5%-66%) with the highest risk associated with injectable compared with trans-dermal testosterone.[138-140] Current guidelines generally advise against androgen replacement when Hct is >50% and in patients with Hct > 54%, therapeutic phlebotomy may be implemented, testosterone withheld until Hct is at an acceptable level < 50%, and therapy resumed at a reduced dose.[141 142] In a large cohort of trans men (n=1073), receiving testosterone and followed for 20 years, the prevalence of erythrocytosis defined by Hct > 50%, 52%, 54%, was 11%, 3.7% and 0.5%, respectively.[143] Although the largest rise in Hct was

seen in the first year, the probability of erythrocytosis increased over time (10% after 1 year and 38% after 10 years), and was associated with smoking, long acting undecanoate injections, older age, higher body mass index and pulmonary disease, underscoring the need for regular Hct monitoring and risk factor modification.[143] In another study of 519 transgender individuals on testosterone therapy, 20% developed Hct > 50%, testosterone dose was reduced in 42%, and 4.8% underwent phlebotomy, however, thrombosis was documented in only a minority (0.9%) of cases. [144] Although this is discrepant with a recent study in which 5,842 men who received testosterone therapy and developed erythrocytosis (Hct ≥52%) were matched and compared to a cohort having received testosterone but who did not develop erythrocytosis.[145] Interestingly, those with erythrocytosis had a higher risk of major adverse cardiovascular events and venous thromboembolic events (number of outcomes: 301, 5.15%) compared to men who had hematocrit within normal range (226, 3.87%) while on testosterone (OR 1.35, 95% CI 1.13-1.61, p <0.001).[145] Accordingly, we recommend close Hct monitoring with target Hct < 50% in patients on androgen replacement therapy.

Erythrocytosis associated with renal transplant

The incidence of post-renal transplant erythrocytosis ranges from 8 to 15% and is declining with widespread use of angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARB).[146,147] It usually develops within the first two years, with spontaneous resolution reported in one fourth of patients within two years of diagnosis.[146] Predisposing factors include male gender, preserved glomerular filtration rate, rejection-free course, retained native kidneys, long pre-transplant dialysis course, absence of anemia pre-transplant, renal artery stenosis, polycystic kidney disease and glomerulonephritis.[146] In earlier studies, it was associated with increased risk of thromboembolic events, which has not been the case in a contemporary series.[148] The mechanism remains obscure but is felt to be multi-factorial with excessive Epo production driven by renin-angiotensin, insulin-like growth factor 1

(IGF-1) and endogenous androgens. [149] Accordingly, both ACE-I and ARB have been used to control post-renal transplant erythrocytosis, obviating the need for intermittent phlebotomy. Enalapril was studied in a randomized trial in post-renal transplant erythrocytosis with administration of either 2.5 mg daily (n=15) or placebo (n=10) for 4 months. Hct decreased from 52.7 to 47.1 at 1 month and to 46.1 after 4 months of therapy with enalapril; no change with placebo without any thrombotic events. [150] Similarly, both enalapril and losartan were also evaluated in a prospective randomized study, and both drugs demonstrated a significant decline in Hgb. [151] Theophylline is another agent, which significantly reduced Hct and EPO levels in renal transplant patients (58 to 46%; 60 to 9 units; $p<0.05$) vs controls (43 to 39%; 6.9 to 4.7 units; $p<0.05$), thereby eliminating phlebotomy needs and may be considered in the second-line setting [152] We recommend ACE-I or ARB as first-line therapy and reserve phlebotomy for patients with suboptimal response to therapy. In addition, low dose aspirin and cardiovascular risk factor modification is advised.

Erythrocytosis not otherwise specified (Idiopathic erythrocytosis)

Erythrocytosis is referred to as "idiopathic" or "not otherwise specified" or "unexplained" when a specific etiology remains elusive. It is an ill-defined terminology that presumes the existence of an increased Hgb/Hct level without an identifiable etiology. Such classification often lacks accounting for normal outliers and is marred by truncated diagnostic evaluation. In such situations, homogeneity in testing for known hereditary and acquired etiologies cannot be overemphasized.

In recent years, NGS testing has improved molecular characterization of idiopathic erythrocytosis cases. [110,153] A noteworthy observation is the association of idiopathic erythrocytosis with *JAK2* GGCC_46/1 haplotype and *CALR* rs1049481_G allele;[154,155] additionally, 22 variants affecting 7 genes (*ASXL1*, *TET2*, *DNMT3A*, *JAK2*, *KIT*, *RUNX1*, *ANKRD26*) were detected in approximately 40% of patients with idiopathic erythrocytosis.[155] Based on these preliminary findings, it is hypothesized that

genomic instability manifested by a high prevalence of mutations in genes involved in clonal hematopoiesis might predispose to development of erythrocytosis in such cases. An additional new observation through a genome wide association study is the identification of loss and gain of function mutations in *ACO1* which encodes cytosolic aconitase 1 (iron-responsive element binding protein 1(IRP1) and is involved in iron sensing.[156] Accordingly, in an effort to unveil novel entities, research testing with expanded and highly sensitive NGS panels and functional assays is encouraged.

Conclusion

JAK2 unmutated erythrocytosis is an evolving subject which remains diagnostically and therapeutically challenging. Impactful discoveries within the last five years, include mutations in the *EPO* gene, novel zinc finger domain *PHD2* mutations, splicing mutations in *VHL,* and *PIEZO* mutations.[56,60,92,93,110] Furthermore, improved molecular characterization of "idiopathic erythrocytosis" cases through expanded and highly sensitive NGS platforms coupled with increased utilization of functional assays is anticipated in the near future.[153,94] In the current document we share a practical and comprehensive diagnostic approach to *JAK2* unmutated erythrocytosis. Importantly, the prompt and accurate distinction between underlying physiological *vs* pathological causes of erythrocytosis is crucial, as management is directly impacted by etiology, and incorrect diagnosis may lead to *under* or *over* treatment. In this regard, an individualized management approach is underscored with phlebotomy reserved for symptom control rather than Hct level. Collectively, there is a critical need for prospective controlled studies in order to define optimal management strategies and new therapies such as HIF2A inhibitors (bezultifan), although mechanistically enticing, require further investigation.

Author contributions

NG, NS and AT co-wrote the paper.

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Figure legends

Figure 1. Diagnostic algorithm for *JAK2* unmutated erythrocytosis.

Abbreviations: Hgb- hemoglobin, Hct- hematocrit, P50- oxygen tension at which hemoglobin is 50% saturated, HPLC- high performance liquid chromatography, HFE- homeostatic iron regulator, CO- carbon monoxide

Figure 2. Treatment algorithm for *JAK2* unmutated erythrocytosis.

Abbreviations: COPD- chronic obstructive pulmonary disease, CPAP- continuous positive airway pressure, ACE – angiotensin converting enzyme, ARB- angiotensin receptor blocker

Table 1. Selected practically relevant studies focusing on management issues in hereditary erythrocytosis.

Condition predisposing to erythrocytosis	Study/Reference	Noteworthy findings
Chuvash polycythemia (CP)	Thrombosis in CP. (Prospective study) *Sergueeva et al, Haematologica (2017)*	- CP pts matched by age, sex and place of residence (n=128). - CP pts with lower blood pressure, body mass index, white count, but more smokers than controls. - New thrombosis in CP pts 0.031 events/pt/year (34 arterial + venous events vs 3 arterial events in controls), history of phlebotomy but not Hct predictor of thrombosis. - 9 (7.0%) deaths in CP pts (median age; 54 years) compared to 2 (1.6%) controls (median age at death; 81 years) (p=0.058). All deaths related to thrombosis.
Congenital erythrocytosis	Thrombotic risk in congenital erythrocytosis. (Prospective study) *Gordeuk, et al, Haematologica (2020)*	- CP pts and matched controls (n=155). - 40 thrombotic events in CP at enrollment (n=27) with 37 new events (n=33) including 9 fatal events during 11-year observation. Thrombosis in 3 controls at enrollment with 5 new events. - In multivariate analysis in CP pts, age and past thrombosis but not Hct were independent predictors of new events. Phlebotomy associated with increased thrombosis (HR 1.9, p=0.028). None were on anticoagulation at second event; aspirin 75 mg/day was not protective. - HIF2A p.M535V variant six-generation pedigree (8 subjects), arterial/venous thrombosis in 5 of 8 *vs* none in 17 HIF2A wild-type pts (p=0.001). - Thrombotic events occurred despite phlebotomy and Hct < 45%.
High oxygen affinity hemoglobin variant (HOAV)	Clinical outcomes and impact of therapy. (Retrospective study) *Gangat et al, Am J Hematol (2021)*	- 41 pts with 34 beta and 7 alpha chain HOAV. - 23 pts with one more symptom related to Hct. - Neither Hct (p=0.32), phlebotomy (p=0.16) or aspirin (p=0.75) influenced symptoms - 10 pts (24%) experienced thrombosis - Neither Hct at time of event (p=0.67) nor phlebotomy (p=0.66) correlated with thrombosis - Cardiovascular risks factors (p=0.002), older age (p=0.04) and male sex (p=0.01) were predictive of arterial thrombosis
Germline *EPAS1(HIF2A), EGLN1(PHD2), VHL, EPOR, BPGM* mutations	Mayo Clinic experience. (Retrospective study) *Gangat et al, Haematologica (2022)*	- 14 pathogenic variants, HIF2A (n=6), PHD2 (n=3), EPOR (n=2), BPGM (n=2) and VHL (n=1). - 22 variants of uncertain significance, EPOR (n=1), HIF2A (n=3), PHD2 (n=10), BPGM (n=2), VHL (n=6).

		- All six patients with HIF2A mutations had one or more cardiovascular risk factors, with three patients (50%) experiencing thrombosis, two of which occurred with ongoing phlebotomy. - None of three pts with pathogenic PHD2 variants experienced thrombosis. - None of two pts with EPOR mutations experienced thrombosis with ongoing phlebotomy. - Two pts with BPGM variants with uneventful course, one patient on phlebotomy and aspirin.

Abbreviations: pts- patients, Hct- hematocrit, *EPAS1* (*HIF2A*)- endothelial PASS domain protein 1(hypoxia-inducible factor 2 α), *EGLN1* (PHD2)- egl-9 family hypoxia inducible factor 1 (prolyl hydroxylase 2), *VHL*- von Hippel Lindau, EPOR- erythropoietin receptor, BPGM- 2,3-Bisphosphoglycerate Mutase.

Table 2. Selected practically relevant studies focusing on management issues in secondary acquired erythrocytosis.

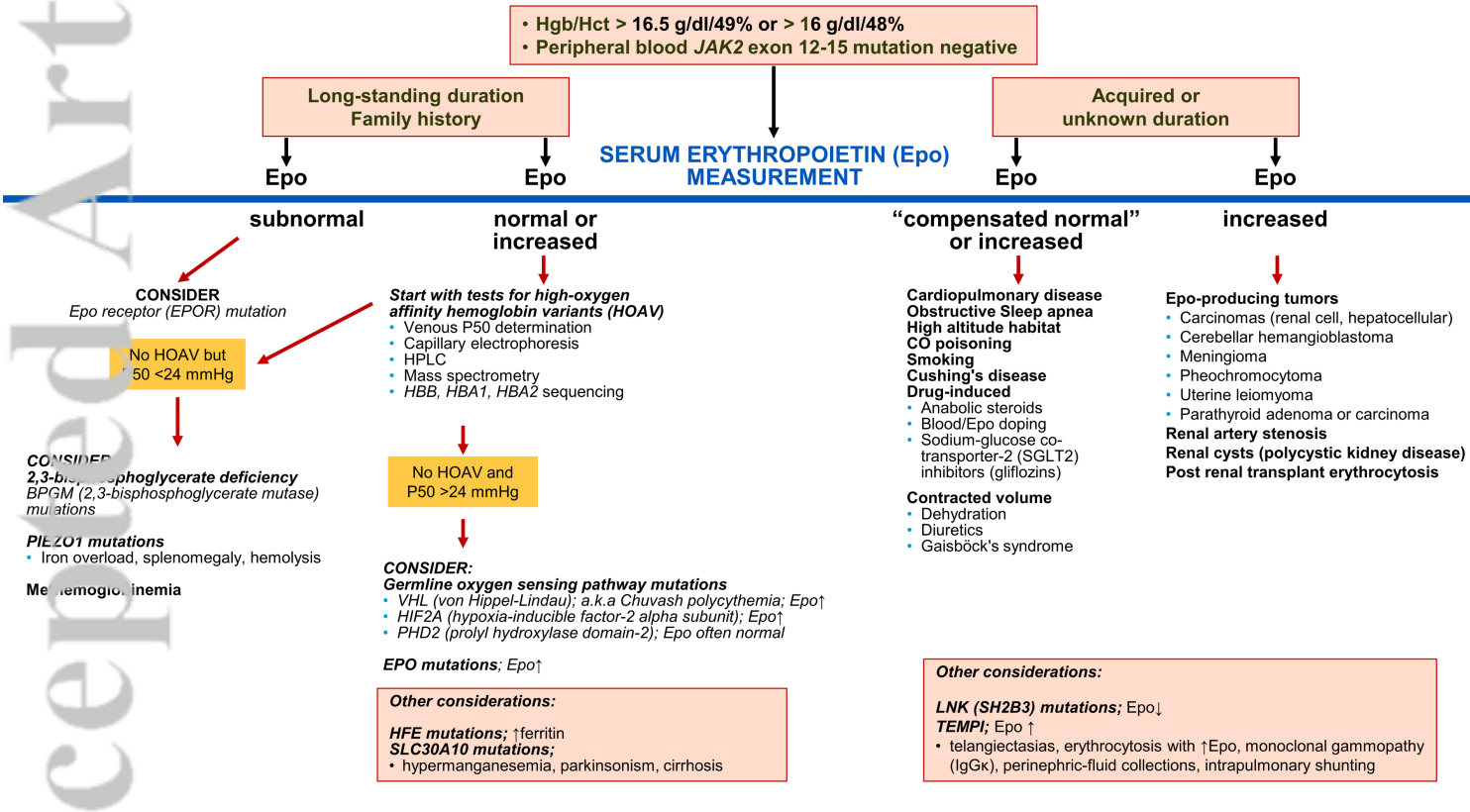
Condition predisposing to erythrocytosis	Study/Reference	Noteworthy findings
COPD (Chronic obstructive pulmonary disease)	Pulmonary embolism in chronic hypoxemic patients. (Prospective study) *Ristic et al, Med Glas (Zenica) (2013)*	- 362 pts with severe COPD exacerbation or respiratory failure, with D-dimer ≥500µg/l referred for Doppler ultrasound and CT. - Group I (100 pts with erythrocytosis) *vs* Group II (262 without erythrocytosis). - Higher pulmonary embolism in Group I vs II (39% vs 11.1%)
	Prevalence of VTE in COPD with erythrocytosis. (Retrospective case-control study) *Nadeem et al, Clinical and Applied Thrombosis/Hemostasis (2012)*	- COPD with erythrocytosis, Hct≥50 (n=86) vs age, sex matched COPD without erythrocytosis, Hct<50 (n=86). - No difference in VTE ;17 (19.8%) cases *vs* 12 (14%) controls, p=0.42 - Trend toward higher incidence of idiopathic VTE in cases (n=10 *vs* 4, p=0.16) - Similar BMI, smoking, cancer, OSA with differences in hematocrit, oxygen use, pulmonary HTN. Phlebotomy data not provided
	Incidence of cardiovascular and thrombotic events in secondary polycythemia. (Retrospective study) *Mao et al, Blood (2019) (Supplement_1)*	- Impact of phlebotomy on prevalence of arterial/venous thrombosis in COPD with erythrocytosis (n=115). - Thrombosis in 11/35 (31.4%) phlebotomized vs 26/116 (22.4%) in non-phlebotomized (p=0.28). - Amongst phlebotomized pts, thrombosis in 4/16(25%) with Hct < 52% vs 7/19(36.8%) in those with higher Hct (p=0.45)
	Secondary polycythemia and perioperative hemorrhage or thrombosis. (Retrospective case-control study) *Lubarsky et al, J Clin Anesth (1991)*	- COPD with erythrocytosis, Hgb > 16g/dl (16.2-20.1 g/dl) (n=100) vs age, sex, surgery, ASA physical status-matched controls without erythrocytosis. - No thrombosis in erythrocytosis group vs 3 events in controls. - Significantly less transfusion requirement in erythrocytosis group vs controls (p<0.025).
	Symptomatic and pulmonary response to acute phlebotomy in secondary polycythemia. (Double blind clinical trial) *Dayton et al, Chest (1975)*	- 11 pts with hypoxic lung disease associated erythrocytosis, Hct >54 underwent phlebotomy *vs* 8 pts as controls (sham procedure). - 8/11 phlebotomized patients with symptom (dyspnea, fatigue, headache) improvement within 24 hrs and lasted > 7 days in 5 pts, particularly if Hct > 60 vs no improvement in controls (p< 0.005). - No objective improvement in airway obstruction, gas exchange or exercise tolerance.
Cyanotic congenital heart disease	Risk of stroke in adults with cyanotic congenital heart disease. (Observational study)	- 112 adults with cyanotic heart disease excluding those with independent risk factors for stroke were observed for 748 pt-years.

	Perloff et al, Circulation (1993)	- 2 groups, (i) compensated (Hct 46-72%, absent/mild symptoms, iron replete with phlebotomy at intervals > 1 yr if symptoms) (n=101) vs (ii) decompensated (Hct 61.5-75%), iron deficiency, marked/severe symptoms with phlebotomy every 3 to 6 months) (n=11). - None of the pts in group (i) had cerebral arterial thrombosis, vs one pt in group (ii) with amaurosis fugax.
	Cerebrovascular events in adults with cyanotic congenital heart disease (Retrospective study) *Ammash et al, J Am Coll Cardiol (1996)*	- 162 adults with cyanotic congenital heart disease; Group I (n=140) no history of cerebrovascular event after age 18 years, Group II (n=22) well documented cerebrovascular event (TIA (n=19), reversible ischemic neurologic deficit n=4, infarct, n=6). - 46/162 (28.4%) underwent phlebotomy, with increased risk of events after phlebotomy (35/140 in Group I vs 11/22 in Group 2, p=0.016). - Strong association of iron deficiency and/or microcytosis with cerebrovascular events (11/41; p=0.004).
	Hydroxyurea for secondary erythrocytosis in cyanotic congenital heart disease. (Case series) *Reiss et al, Am J Hematol (2007)*	- 4 pts with symptomatic secondary erythrocytosis and cyanotic congenital heart disease. - 2 pts with recent TIA or stroke, one with extreme fatigue/dyspnea and one with extreme exhaustion following phlebotomy. - Median hydroxyurea use: 15 months; symptomatic improvement, but myelosuppression in 2 pts and thrombocytopenia in 1 pt which required dose reduction. 1 pt with TIA on hydroxyurea. Minimal increase in HbF.
Post-renal transplant	Enalapril for erythrocytosis after renal transplant. (Randomized double-blind study) *Beckingham et al, Nephrology Dialysis Transplantation (1995)*	- 2.5 mg of enalapril daily (n=15) or placebo (n=10) for 4 months. - Hct decreased from 52.7 to 47.1 at 1 month and 46.1 after 4 months with enalapril, no change with placebo (p = 0.004). No change in erythropoietin level. - No phlebotomy or thrombosis.
	Effects of theophylline on erythrocytosis after renal transplant. (Prospective study) *Bakris et al, N Engl J Med (1990)*	- Pts with post-renal transplant erythrocytosis (n=8) vs normal controls (n=5). - 8 weeks of theophylline, EPO significantly reduced in transplant pts (60 to 9 units; p<0.05) and controls (6.9 to 4.7 units; p<0.05). - Hct reduced in transplant pts (0.58 to 0.46; p<0.05) and controls (0.43 to 0.39; p<0.05). Requirement of weekly phlebotomy eliminated.
	Comparison of enalapril and losartan on post-renal transplant erythrocytosis. (Prospective randomized study) *Yildiz et al, Tranplanation (2001)*	- 27 pts treated with enalapril 10 mg/day (n=15), vs losartan 50 mg/day (n=12) for 8 weeks. - Hgb significantly decreased with both losartan (17.1 to 15.9 g/dl, p=0.01) and enalapril (17.4 to 14.9 g/dl, p=0.001), greater decrease with enalapril (-3.26 vs -1.70, p=0.05). - Among the responders who discontinued treatment, there was a trend for longer time to relapse with losartan (7.38 months) compared with enalapril (2.75 months) (p=0.11).

	Post-transplantation erythrocytosis in kidney transplant recipients- A retrospective cohort study *Hofstetter et al, European Journal of Haematology (2021)*	- 169/1304 (12.9%) with erythrocytosis, associated with male gender, higher glomerular filtration rate and polycystic kidney disease. - 73 patients received treatment for erythrocytosis, phlebotomy (n=51), ACEi/ARB (n=41), phlebotomy +ACEi/ARB (n=19). - 7/138 (5.1%) VTE events in erythrocytosis group vs 2/161 (1.2%) in the control group (p=0.086). - Mean Hgb at event 17.1-18.1 g/dl. - None of the 7 pts received ACEi/ARB prior to event.
Testosterone therapy	Prevalence and management of secondary erythrocytosis in transgenders on testosterone. (Retrospective study) *Oakes et al, Blood (2020) (Supplement_1)*	- 234 pts, mean pre-testosterone Hgb 13.5 g/dL and Hct 40.3%. Mean Hgb peak 15.7 g/dl, Hct 47.2% at an average of 21 months post therapy. - 23.5% pts with Hct > 50%, and 8.5% hgb > 17.5 g/dL. Only one thrombotic event. - Dose reduction in 14.5% with erythrocytosis, no phlebotomy. - 88.9% of patients with erythrocytosis had received testosterone cypionate
	Erythrocytosis and thromboembolic events in transgender individuals receiving gender-affirming testosterone. (Retrospective study) *Oakes et al, Thrombosis Research (2021)*	- 519 pts, with mean peak Hgb/Hct 15.7 g/dl/47% - Mean time to peak Hgb/Hct 31.2 months. - 7.8% developed Hgb > 17.5 g/dl, 20% developed Hct > 50%. - Dose reduction in 42%, and 4.8% underwent phlebotomy. - Thrombosis in 0.9%, of which 80% with erythrocytosis by either Hgb or Hct
	Secondary polycythemia in men receiving testosterone therapy and risk of major adverse cardiovascular events and VTE in the first year of therapy. *Ory et al, Journal of Urology (2022)*	- 5,8442 men who received testosterone and developed erythrocytosis Hct≥52% matched to 5,842 men who did not develop erythrocytosis. - Higher major adverse cardiovascular outcomes/VTE (events: 301, 5.15%) in men with erythrocytosis compared to men without erythrocytosis (226, 3.87%) while on testosterone (OR 1.35, 95% CI 1.13-1.61, P<0.001)

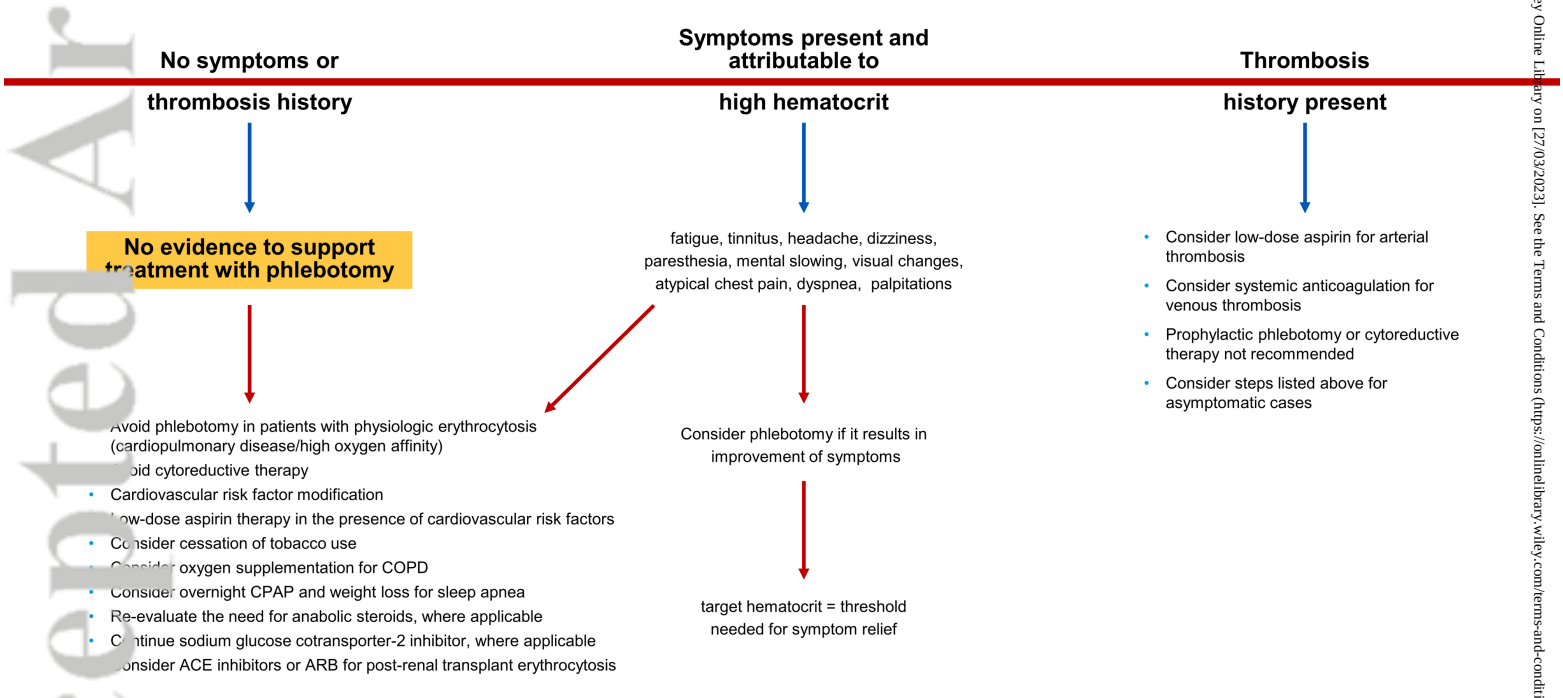
Abbreviations: pts- patients, VTE- venous thromboembolism, CT- computed tomography, BMI- body mass index, OSA- obstructive sleep apnea, HTN- hypertension, Hct- hematocrit, Hgb- hemoglobin, TIA- transient ischemic attack, EPO- erythropoietin, ACEi- angiotensin converting enzyme inhibitor, ARB- angiotensin receptor blocker.

Figure 1



AJH_26920_AJH 2023 erythrocytosis figure 1.tif

Figure 2



AJH_26920_AJH 2023 erythrocytosis figure 2.tif