Acta Haematologica Polonica 51(4) • December 2020 • 203–211 • DOI: 10.2478/ahp-2020-0036

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REVIEW ARTICLE

journal homepage: https://content.sciendo.com/ahp

Medical dilemmas – erythrocytosis

Article history:
Received: 03.07.2020
Accepted: 23.08.2020

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Abstract
Erythrocytosis is defined not only by an increase in the erythrocyte count, hemoglobin concentration, and hematocrit value, but also by the occurrence of specific symptoms, the intensity and frequency of which depend on the character of the initial genetic lesion. Ischemic episodes and thrombotic complications caused by increased blood viscosity are frequently the first clinical manifestation of the disease. This paper represents the current level of knowledge about the pathogenesis of erythrocytosis and the diagnostic algorithms used to precisely define the type of the disease.

Keywords:
polycythemia, erythrocytosis, thrombosis, molecular defects, inflammation, therapy

Introduction

The term polycythemia was introduced to medical terminology in 1857 by Denis Jourdanet, on the basis of observation of a consistent increase in the viscosity of human blood at high altitudes [1]. Recently, the term polycythemia is sometimes replaced by the term erythrocytosis, but both terms are not synonymous. The term polycythemia refers to any increase in red blood cells, whereas erythrocytosis only refers to a documented increase of the red cell mass. An increase in the red cell mass is usually associated with the appearance of unspecific symptoms like fatigue, headache, dizziness, blurred vision, confusion, arthralgia, and skin itching after bathing and exposition to warm water. Arterial hypertension and severe burning pain in the hands or feet that is usually accompanied by a reddish or bluish coloration of the skin and mucosae membranes are also frequently present. Thromboembolic disease (TED) episodes are frequently observed in subjects with high hematocrit values and erythrocyte count. Recurrent nasal bleeding episodes are thus characteristic. The diagnosis of erythrocytosis is based on a complete blood count result analysis. The absolute erythrocytosis is defined by an increase of the red cell mass above 125% of normal value, with hemoglobin concentration and hematocrit value >185 and 165 g/L in men and women, respectively. The corresponding hematocrit values are 0.52 and 0.48. Abnormal hemoglobin and hematocrit values should be confirmed in two consecutive measurements to diagnose erythrocytosis [2].

The classification of erythrocytosis includes its primary and secondary forms, as well as its inherited and acquired forms (Tab. I).

Despite significant progress in the understanding of the genetic background of erythrocytosis, there are still cases with unknown origin. In such situations, the term idiopathic erythrocytosis should be used [3].

Hematopoiesis and erythropoiesis regulators

The bone marrow is the primary site of hematopoiesis. In normal conditions, hematopoietic stem cells (HSCs) localized in microanatomical organizations, termed “niches”, actively interact with the bone marrow microenvironment. Recent data suggest that hematopoietic progenitor cells differentiation uses different mechanisms under steady-state and stress conditions. The proposed mechanism of such interaction includes HSCs and progenitor cells interplay with growth factors, cytokines, and regulatory factors.

Hematopoiesis is the adaptive process by which mature and functional blood cells are continuously replaced over the entire lifetime of an individual. As a consequence, the daily production of red blood cells, platelets, and neutrophils in homeostatic conditions amounts to more than 300 billion cells. The intensity of this process is even better illustrated by the observation that an average adult produces approximately 2.4 million new red blood cells every second [2].

Erythropoietin (EPO) is the main regulator of red cell production, acting on the late erythroid progenitor cells through specific homodimer receptor (EPOR) and triggering Janus tyrosine kinase 2 (JAK2) activity, with subsequent signal transducer and activator of transcription 5 (STAT5) phosphorylation and STAT5 dimer formation (JAK2-STAT5 pathway). Src-homology 2 domain-containing protein tyrosine kinase 1 (SHP-1) down-regulates the JAK2-STAT5 pathway downstream of the EPOR.

It has been postulated that EPO also ensures EPO progenitors survival, allowing them to continue the differentiation program initiated mainly by GATA-binding factor 1 (GATA-1). The crucial role of EPO in this process is evident, because when the EPO level is low, erythroid progenitor cells undergo apoptosis upon caspase activation [3]. EPO production

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Table I. Absolute erythrocytosis classification based on all known causes [according 2]

<table>
<tr><th colspan="2">Primary erythrocytosis
(erythroid progenitor defects and hypersentitivity to EPO)</th><th colspan="2">Secondary erythrocytosis
(high EPO level and EPO pathway activation)</th></tr>
<tr><th>Acquired</th><th>Inherited</th><th>Inherited</th><th>Acquired</th></tr>
<tr><td rowspan="4">Polycythaemia vera
- JAK2 V617F mutation
- ex 12 JAK2 gene mutation
- others mutation</td><td rowspan="2">Familial erythrocytosis type 1
- EPOR mutations</td><td>High oxygen affinity hemoglobin
- HBB, HBA gene mutation
- BPGM gene mutations</td><td>High EPO blood concentration in relation to pulmonary disease, cardiac failure, kidney disease, liver dysfunction</td></tr>
<tr><td>Familial erythrocytosis type 3
- EGLN1 gene mutations</td><td rowspan="3">Autonomic EPO production (neuroblastoma, renal cancer, hepatoma, liver cancer, uterine tumors, hemangioblastoma, endocrine tumors</td></tr>
<tr><td colspan="2">Familial erythrocytosis type 2
- VHL gene mutations</td></tr>
<tr><td colspan="2">Familial erythrocytosis type 4
- EPAS1 mutations</td></tr>
</table>

EPO – erythropoietin; EPOR – erythropoietin receptor; HBB – beta globin chain gene; HBA – alpha globin chain gene; VHL – von Hippel-Lindau tumor suppressor gene; EGLN1 – prolyl hydroxylase domain 2 gene (previously PDH2); EPAS1 – endothelial PAS domain-containing protein 1 gene (previously HIF2α); BPGM – bisphosphoglycerate mutase gene

in peritubular cells of the kidney is regulated by the oxygen-sensing mechanism [4].

The oxygen-sensing pathway

A sensitive mechanism for sensing oxygen and responding to hypoxia was identified in humans. It involves a number of proteins. Briefly, in normoxic conditions, prolyl hydroxylase domain protein 2 (PHD2) hydroxylates one or both elements of a pair of highly conserved prolines in hypoxia inducible factor alpha (HIF-2α) in an oxygen-dependent manner. This hydroxylation enables specific binding of HIF-2α by a ubiquitin ligase complex containing von Hippel–Lindau (VHL) tumor suppressor, elongin B, and elongin C, which leads to its ubiquitination and subsequent proteasomal degradation. A little amount of HIF-2α remains to bind the 5′ hypoxia responsible element (HRE) of the EPO gene, so the EPO gene transcriptional activation is modest. Under hypoxia, the hydroxylation of HIF-2α is attenuated, HIF-2α is stabilized, binds to its stable partner – aryl hydrocarbon receptor nuclear translocator (ARNT; also known as HIF-β), and mediates the transcriptional activation of the EPO gene by binding to its HRE and EPO synthesis [4]. The detailed mechanism of interaction and activation of target genes in the normoxia and hypoxia conditions in the oxygen-sensing pathway is presented in detail in figure 1.

Genetic variants responsible for erythrocytosis

The primary erythrocytosis is caused by an intrinsic defect in the bone marrow progenitor cells driving the red cell production, and is usually characterized by reduced EPO blood concentration. In the secondary erythrocytosis, a factor extrinsic to the bone marrow is responsible for an increase in the erythroid cells and erythrocyte count. It is associated with elevated or normal EPO blood concentration.

Inherited forms of primary erythrocytosis are caused by EPO receptor (*EPOR*) gene mutations. Rarely, the mutations of others genes, like SH2B adapter protein 3 (*SH2B3*, also known as lymphocyte adapter protein – *LNK*), negatively regulating cytokine initiated cell signaling by the stem cell factor receptor, thrombopoietin receptor, EPOR, platelet-derived growth factor receptor, or the mutations of Janus tyrosine kinase 2 (*JAK2*) gene are responsible for an increase in the erythrocyte count [5, 6, 7].

In the secondary erythrocytosis mutations of oxygen-sensing pathways, genes [*VHL*, *PHD2* (*EGLN1*), *HIF2A* (*EPAS1*), *HBB, HBA1, HBA2,* and *BPGM*] play a causative role in the inherited forms of the disease.

Congenital primary erythrocytosis

EPOR gene mutations

At least 11 mutations of *EPOR* gene leading to premature stop codons and the loss of SHP-1 docking site are reported. The mutations resulted in an abnormal down-regulation of the JAK2-STAT5 pathway downstream of the EPOR by SHP-1 and continuous stimulation of the red cell production without more EPO [4, 8]. Identified mutations are mainly localized in exon 8 of the *EPOR* gene and concern European families only (Tab. II).

Mutations of oxygen-sensing pathways genes

Genetic studies performed in patients' familial erythrocytosis identified a large spectrum of causative mutations in eight candidate genes (Tab. II). Their presence can be confirmed in 30%–40% of the studied subjects with clinical disease diagnosis [2]. In most of the cases, positive family history of erythrocytosis, elevated hemoglobin concentration, and/or hematocrit value is found. A detailed analysis of laboratory data obtained in patients with *PDH2*, *HIF2α,* and *EPO* gene mutation confirms elevated Epo concentration in the blood. Only in persons with *VHL* gene mutations normal Epo blood concentration may be found.

In patients carrying the *HIF2α* mutation, erythrocytosis is observed in the late disease phase, and may proceed by symptoms of neuroendocrine tumor (glioma, paraglioma). In some patients carrying *HIF2α* gene mutations, only neuroendocrine tumor symptoms are present with normal values of hemoglobin concentration and erythrocyte count [9]. Hemoglobin oxygen dissociation curve with a half-saturation of hemoglobin with oxygen (P_{50}) analysis is an important part of the laboratory evaluation of patients with

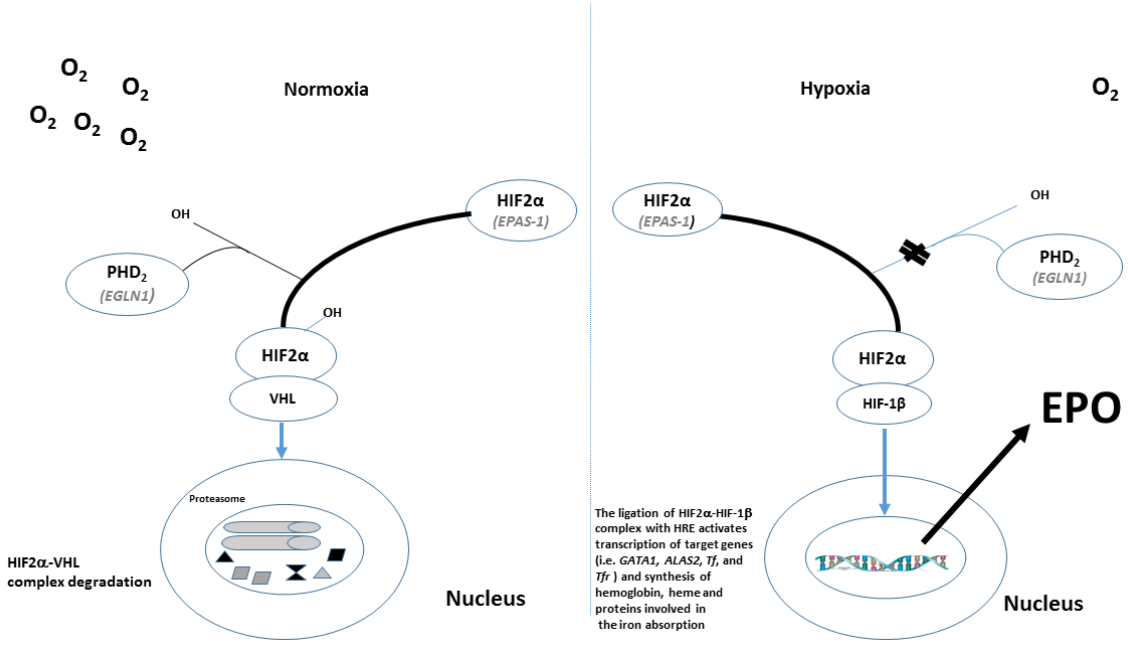

Fig. 1. The oxygen-sensing pathway [4, modified]

HIF2α – hypoxia inducible factor 2α; VHL – von Hippel Lindau tumor suppressor protein; HIF-1β – hypoxia inducible factor 1β; EPO – erythropoietin; PHD2 – prolyl hydroxylase domain 2; OH – hydroxyl group; HRE – hypoxia response element localized in 5' flanking region of target genes (i.e. GATA1, ALAS2, Tf and Tfr). HIF binding to HRE resulted in activations of transcription of proteins involved in the synthesis of hemoglobin, hem and protein participating in iron absorption from the gastrointestinal tract

Table II. Gene mutations responsible for inherited erythrocytosis (according to [2, modified]

Gene	Type of sequence change	Coding protein	Number of described mutations	Mutation consequences	Number of reported cases	Inheritance pattern
HBB *HBA1* and*HBA2*	Missense Missense	HBB HBA	>100 <30	Hemoglobin with high oxygen affinity	- -	Autosomal dominant
BPGM	Missense	2,3-BPG	4	Decreased synthesis of 2,3-BPG	6 (3 families)	-
EPOR	Nonsense/ missense/ small deletions	EPOR	28	C-terminal regulatory domain loss	116 (24 families)	Autosomal dominant
VHL	Missense/ nonsense	VHL	20	Loss of function	55 (30 families, 73 Chuvash people)	Autosomal dominant (recessive in Chuvash polycythemia - *VHL* C598T
EGLN1	Missense/ nonsense/ small deletions and small insertions	PHD2	30	Loss of function	32 (20 families)	Autosomal dominant
EPAS1	Missense	HIF2α	12	Gain of function	24 (14 families)	Autosomal dominant

HBB – beta globin chain gene; HBA – alfa globin chain gene; EPOR – erythropoietin receptor gene;VHL – von Hippel-Lindau tumour suppressor gene; EGLN1 – prolyl hydroxylase domain 2 gene; PDH2 – prolyl hydroxylase 2; EPAS1 – endothelial PAS domain-containing protein 1 gene; HIF2α – hypoxia inducible factor 2 alpha; BPGM – bisphosphoglycerate mutase gene

erythrocytosis. Low P_{50} value suggests the diagnosis of low oxygen affinity hemoglobin variant (LOA HGB), bisphosphoglycerate mutase gene (*BPGM*) mutation, or methemoglobinemia (in some P_{50} value is normal). In the case of a normal P_{50} value, the *EGLN1 (PHD2)*, *EPAS1 (HIF2a),* and *VHL* gene sequencing study should be performed. It should be kept in mind that in some patients with *EGLN1* (rarely *EPOR*) mutation, the P_{50} value might be slightly decreased. Similarly, in less than 5% of α and β hemoglobin variants with high oxygen affinity (HOA HGB), normal or slightly decreased P_{50} may be observed. P_{50} value is normal in patients with erythrocytosis and *BPGM* mutation, as well [9–12].

Congenital erythrocytosis type 1 (ECYT 1; OMIM 133100)

The ECYT1 is primary congenital erythrocytosis and has an autosomal dominant inheritance pattern. However, the mutation of exon 8 of the

EPOR gene (chromosome 19p1) may also be found in patients with no positive family history of erythrocytosis. The mutations, resulting in a loss of the negative regulatory C-terminal domain of the receptor, lead to hyperresponsiveness to EPO due to the lack of a negative regulation of signal transmission by SHP-1 and suppressor of cytokine signaling 3 (SOCS-3). Its presence was described for the first time in 1993 in Finnish family members [11, 13] (Tab. III). The degree of polycythemia may differ between family members [14]. In the majority of cases, the disease is oligosymptomatic. Arterial hypertension and history of arterial and venous thrombosis is rarely positive. In the symptomatic cases, phlebotomy treatment with the normalization of the hematocrit value is needed.

Table III. Indications for erythrocytophoresis in patients with erythrocytosis [46]

Erythrocytosis type	Type of apheresis	Level of evidence[1]	Indication category[2]
Polycythaemia vera	Erythrocytapheresis	1B	I
Secondary erythrocytosis	Erythrocytapheresis	1C	III

[1]Level of evidence: 1A – strong recommendation, high-quality evidence; 1B – strong recommendation, moderate-quality evidence; 1C – strong recommendation, low or very low-quality evidence; 2A – weak recommendation, high-quality evidence; 2B – weak recommendation, moderate-quality evidence; 2C – weak recommendation, low or very low-quality evidence. [2]Indication category: I – disorders for which apheresis is accepted as first-line therapy, either as a primary standalone treatment or in conjunction with other modes of treatment; II – disorders for which apheresis is accepted as second-line therapy, either as a standalone treatment or in conjunction with other modes of treatment; III – optimum role of apheresis therapy is not established. Decision making should be individualized

Congenital erythrocytosis type 2 (ECYT 2; OMIM 263400)

The disease was described for the first time in 2002 in Chuvash people, inhabitants of the mid-Volga River region (the Chuvash polycythemia), and has an autosomal recessive inheritance pattern [12]. Chuvash polycythemia was identified in Poland (Acta Haematol. Pol. 2020, in press). The VHL gene locus associated with Chuvash polycythemia was mapped on chromosome 3p25. In the majority of patients, homozygous missense mutation of VHL 598C>T (Arg200Trp;R200W) is present. Rarely, the disease is caused by combined heterozygous defects. Symptomatic heterozygotes of ECYT 2 were also described. Mutation presence resulted in reduced degradation of HIF2α and increased expression of downstream target genes, including *EPO*, solute carrier family 2 member 1, facilitated glucose transport (*SLC2A1*), transferrin (*TF*), transferrin receptor – CD71 (*TFRC*), and vascular endothelial growth factor (*VEGF*) [12]. The median age of disease diagnosis is 10–19 years [15]. The disease is marked by symptoms of increased blood volume such as headache, vertigo, or dizziness. Frequent physical findings include plethora and a tendency to lower systolic blood pressure. Peptic ulcers, angiomas of the spine, and increased systolic pulmonary artery pressure are also typical. The Chuvash polycythemia carriers are at risk of venous and arterial thrombosis, including cerebral vessels, independently from the hematocrit value [9, 15]. The propensity to thrombosis is higher than in polycythemia vera (PV) [16].

Congenital erythrocytosis type 3 (ECYT 3; OMIM 609820)

Erythrocytosis type 3 causative mutations are identified in Egl nine homolog 1 gene (*EGLN1)*. *EGLN1* is localized on chromosome 1q42 and the coding sequence of prolyl hydroxylase 2 (PDH2). To date, about 30 mutations have been described in the *EGLN1* gene [17]. The majority of *EGLN1* mutations are localized in the catalytic domain and impairing binding of HIF-2a/EPAS1. The c.950C>G(pro317Arg;P317R) mutation was identified as the first one [18].

Congenital erythrocytosis type 4 (ECYT 4; OMIM 611783)

HIF senses and coordinates cellular responses to hypoxia. HIF is a heterodimer consisting of one of three alpha (α) subunits (expression induced by hypoxia) and a constitutively expressed HIFβ subunit (also known as aryl hydrocarbon nuclear translocator; ARNT). In humans, three paralogs of alpha subunit are known: HIF1α (a transcriptional activator and a basic regulator for the expression of genes involved in the response to hypoxia), EPAS1 (endothelial PAS domain-containing protein 1, also known as HIF2α), and HIF3α [19]. Mutations of the *EPAS1* gene (chromosome 2p21) resulted in the synthesis of gain of function variants of HIF2α. Until today, a lot of missense mutations have been identified in exons 2, 9, 12, and 16 of the *EPAS1* gene [20, 21]. A majority of them are associated with amino acid substitution in the proline coding regions of HIF2α(i.e., Pro531). An abnormal proline hydroxylation profile resulted in impaired recognition of HIF2α by VHL. The final ECYT 4 diagnosis should include the EPO blood concentration assessment, which is elevated (Fig. 2), and P_{50} value assessment, which is within the normal range. The other two HIFα subunits, HIF1α and HIF3α, have been studied as potential factors playing a role in the development of erythrocytosis [21].

Congenital erythrocytosis type 5 (ECYT 5; OMIM 617907)

ECYT 5 is associated with a mutation of the EPO gene (chromosome 7q22). Until today, only seven of the *EPO* gene variants associated with erythrocytosis and an increase in the hematocrit value have been identified. They include C306A (upstream), G772T (3′UTR enhancer), G136A (5′UTR), exon 2 sequence mutations – C32del and c.19delC, p7fs, and mutations of the exon 4 sequence – G84R and E99G [13]. Family studies revealed autosomal dominant trait of inheritance. In 10 members of a four-generation Norwegian family, a single nucleotide deletion in exon 2 of the *EPO* gene within the signal peptide sequence was found (c.32delG; OMIM 133170.0002) [22–25].

Congenital erythrocytosis types 6–8 (ECYT 6–8)

Beta chain hemoglobin gene mutations (*HBB*) are responsible for the congenital erythrocytosis type ECYT 6 (OMIM 617980) and

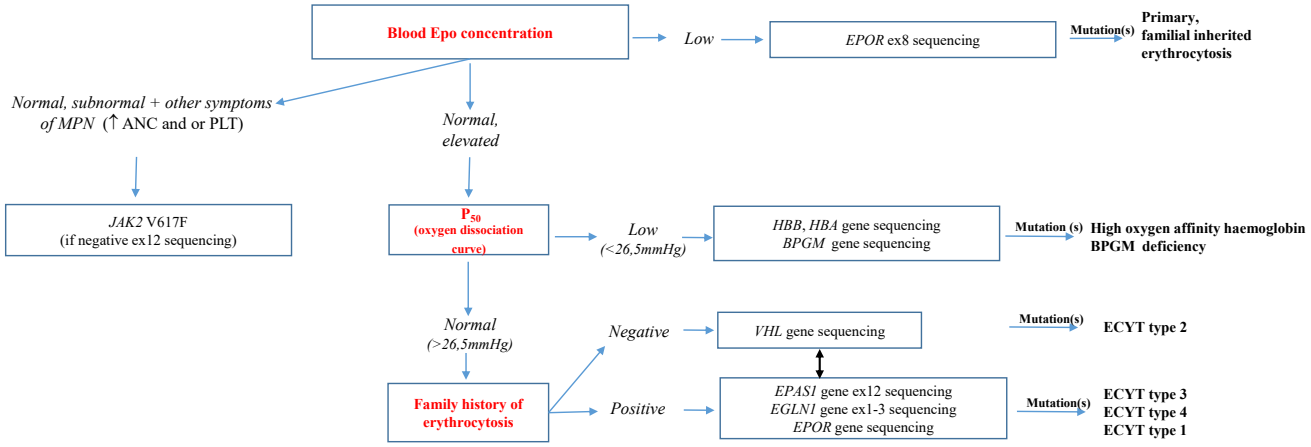

Fig. 2. Algorithm for the laboratory evaluation of erythrocytosis [2, 3; modified]

ECYT – erythrocytosis; HBB – hemoglobin beta chain gene; HBA – hemoglobin alpha chain gene; EPOR – erythropoietin receptor gene; VHL – von Hippel-Lindau tumor suppressor gene; EGLN1 – prolyl hydroxylase domain 2 gene; EPAS1 – endothelial PAS domain-containing protein 1 gene; BPGM – bisphosphoglycerate mutase gene; P_{50} – half saturation of hemoglobin with oxygen; MPN – myeloproliferative neoplasm; JAK2 – Janus kinase 2 gene; ANC – absolute neutrophil count; PLT – platelets; ex – exon

result in the HOA HGB variant. Disease diagnosis is possible on the basis of the P_{50} evaluation and a sequencing study of the *HBB* gene localized on chr11q15. A similar laboratory characteristic is typical for ECYT 7 (OMIM 617981), caused by alpha chain hemoglobin gene mutations (*HBA*), and for ECYT 8 (OMIM 222800), associated with the bisphosphoglycerate mutase deficiency due to *BPGM* gene mutations. The above-mentioned gene aberrations are localized on chromosomal regions 16p13 and 7q33, respectively.

The diagnostic algorithm of congenital absolute erythrocytosis is presented in figure 2. The laboratory consequences of mutations of the genes of the oxygen-sensing pathway (*PHD2-HIF-vHL-EPO*) are presented in table II.

Erythrocytosis and thrombotic risk

The increase of hemoglobin, hematocrit value, and erythrocyte count may lead to the rise of venous and arterial thrombotic risk. The results of a study performed in Sweden among 1.5 million blood donors revealed a 2.4 times higher risk of an ischemic cerebral stroke in both males and females with the hemoglobin value above 17.5 and 16.0 g/dL, respectively. The risk evaluation included the country of origin, age, season of the year, the time from the last blood donation, and the existence of other comorbidities, including pulmonary obstructive disease, cancer, diabetes, atrial fibrillation, nocturnal dyspnea, and coronary artery disease. A similar relationship was documented in terms of the increase of the relative risk of TED in men with elevated hemoglobin levels above 17.5 g/dL (lower limbs thrombosis, pulmonary embolism – 1.5x) [26].

The risk of TED was also studied in familial erythrocytosis caused by genetic variants responsible for erythrocytosis. In the analysis performed by Gordeuk et al. [27] in a six-generation family carrying c.1603A>G (M535V), mutation of the *EPAS1* (*HIF2*α) gene showed that thrombotic episodes were observed even in the young family members treated with phlebotomy in whom the hematocrit value was kept below 45%.

PV (Vaquez disease) is a clonal disorder of the HSC. In 95% of patients, the V617F mutation (exon 14) of the *JAK2* gene localized on chr 9p24.1 is present in the coding sequence of the *JAK2* pseudokinase domain (JH2). The amino acid substitution resulted in an abnormal JH2 inhibitory effect on the adjacent JH1 kinase domain, thus keeping JAK2 in an inactive conformation [28]. In another 3% of patients, mutations of the *JAK2* gene are present in exon 12 (until now, 37 molecular aberrations different in nature were described – synonymous substitutions, deletion variants, duplications) [29]. PV is a rare disease with an annual incidence of 1.5/100,000 people from the general population [26]. Morbidity is estimated at 0.49–46.88/100,000 people/year. The 5-year overall survival is estimated at 88.4% [30]. The diagnostic criteria proposed in 2016 by WHO include an increase in the hemoglobin level above the value of 16 g/dL in females and 16.5 g/dL in males; an increase in the hematocrit value above 48% and 49%, respectively, and total erythrocytes mass. The diagnostic criteria also include bone marrow hyperplasia, the presence of a *JAK2* clonal aberration in the hematopoietic cells (*JAK2* V617F or exon 12 mutation) and subnormal EPO blood concentration. EPO blood concentration is closely associated with the presence of a *JAK2* gene mutation (normal EPO concentration is present only in 32% of *JAK2* mutation carriers). The relationship of the EPO level and the driving defect presence is especially evident in the case of exon 12 *JAK2* mutations (subnormal EPPO level is present in 91% of patients) [31, 32].

The symptoms of PV are associated with an increased blood volume and hyperviscosity and include vertigo, transient ischemic attacks, cardiac ischemia, and TED episodes. A laboratory evaluation of PV patients revealed high hemoglobin and hematocrit values, leukocytosis, and thrombocythosis. The fibrosis of the bone marrow and symptoms of metaplasia with the presence of erythroblasts and unmaturated neutrophils in peripheral blood film are also observed in the late disease phase. In some cases, thrombocytosis is a dominant laboratory abnormality, which may suggest the diagnosis of essential thrombocythemia (masked PV) [33]. A disease evolution

is typical with a possible transformation to secondary acute myeloid leukemia with the frequency of 2.3%–14.4% at 10 years and 5.5%–18.7% at 15 years from the initial diagnosis [34]. The *JAK2* variant allele frequency (VAF) increases during this time, as well. The latter phenomenon is closely associated with an increase of the thrombotic risk [35]. The study by Barbui et al. [36] performed in patients with PV confirmed that 15 years after the diagnosis, 32% of cases experienced arterial and venous thromboembolic complications. Despite the progress in the thrombotic risk stratification and modification of the prophylactic algorithms used, the thrombotic events remain the main cause of mortality and morbidity in patients with PV [37–40].

In Chuvash erythrocytosis the risk of thrombotic episodes occurrence is relatively high. On the basis of data by Sergueeva et al. [16], the propensity to thrombosis of people with Chuvash erythrocytosis is even higher than in PV patients. The thrombotic risk assessment in other types of erythrocytosis is difficult to calculate due to the relatively low disease incidence and lack of cumulative data. A detailed analysis of the available data indicates that thrombosis accounts for the morbidity and mortality of Chuvash erythrocytosis patients, independently from body mass index, systemic blood pressure, serum glucose and glycosylated hemoglobin level, white cell, and platelet count [41, 42]. The frequency of thrombotic complications is high even in children and adolescents with Chuvash polycythemia and high hematocrit value, independently of age, which is not a predictor of thrombotic risk [15]. Moreover, therapeutic phlebothomy seems to increase the thrombotic risk in Chuvash erythrocytosis [16]. This is probably the result of hypoxia-induced prothrombotic state associated with a reduced tissue pathway inhibitor (TFPI) mRNA and protein level, increased tissue factor (TF) mRNA expression [43], the upregulation of tissue factor expression [44], and the expression of thrombospondin-1 (*THBS-1* gene) [16]. It cannot be excluded that the thrombotic risk is also affected by hypoxia-induced reduction of protein S expression [45].

Phlebotomy and cytoreductive treatment of erythrocytosis

Phlebotomy (erythrocytopheresis) is the standard of care of patients with an elevated hemoglobin level, red cell mass, and hematocrit value. Phlebotomy is efficient in the treatment of symptoms associated with high hematocrit value. Its efficacy in the reduction of thrombotic risk in patients with erythrocytosis remains to be clarified [46]. Two prospective randomized studies (PVSG 01 and PVSG 05) demonstrated that phlebotomy used to control hematocrit was associated with a higher thrombotic risk compared to chemotherapy [47, 48]. The higher rate of thrombosis related to bloodletting was also evident when phlebotomy was performed in patients receiving aspirin and dipyridamole treatment (PVSG 05 comparing the clinical efficacy of phlebotomy in combination with aspirin and dipyridamole vs. ^{32}P). In the opinion of the study investigators, the increased thrombotic risk in the phlebotomy treated patients in comparison to the subjects obtaining myelosuppressive therapy (chlorambucil, ^{32}P) seems to be limited to the first 3 years of the treatment [47, 49]. An advantage of cytoreductive therapy with hydroxyurea (HU) over phlebotomy in terms of fatal/non-fatal cardiovascular events incidence was documented by the European Collaboration on Low-Dose Aspirin in the Polycythemia Vera (ECLAP) study (3.0 *vs*. 5.8 per 100 person-years, respectively) on the basis of a clinical evaluation of 1,042 patients with PV [50]. The impact of hematocrit value on the thrombosis risk in PV patients was studied by the Cytoreductive Therapy in Polycythemia Vera (CYTO-PV) Collaborative Group. The study confirmed high thrombotic risk in patients with a high hematocrit value, regardless of whether the patient was treated with chemotherapy and whether the white blood cell count was elevated [51].

The other problem studied was the relationship between the frequency of therapeutic phlebotomies and the thrombotic risk. An observational study performed by Alvarez-Larran et al. [52] confirmed a correlation between the number of phlebotomies and an increased incidence of thrombosis in the HU-treated patients. The existence of such association was not confirmed by Barbui et al. [53].

Recently, the factors influencing the risk of thrombosis in PV patients have been re-evaluated and new scoring systems for more adequate risk stratification have been prepared. One of them is the scale for thrombosis risk assessment, including only two separate categories: high risk (either thrombosis history or advanced age >60 years) and low risk (the absence of both risk factors) with the consideration of arterial hypertension and leukocytosis as additional risk factors in certain circumstances [54]

Janus kinase 2 inhibitors in the treatment of PV

A randomized study result published in 2015 by Vannucchi et al. [55], comparing the best available therapy versus ruxolitinib (RUX) in hydroxyurea-resistant or intolerant PV with splenomegaly, showed higher rates of hematocrit control in RUX arm (60% vs. 20%), ≥35% reduction in the spleen volume (38% *vs.* 1%), and symptom control (49% *vs*. 5%). Unfortunately, the study was not designed to address clinically relevant endpoints in PV, such as the thrombosis rate and thrombosis-free survival. The thrombotic risk was re-analyzed recently, due to the introduction of JAK2 inhibitors to the treatment of PV patients. The data originating from four randomized clinical studies (RESPONSE, RESPONSE-2, RELIEF, MAJIC-PV) including 1,216 patients showed the annual frequency of thrombotic episodes at 4.3%. The thrombotic risk was higher in patients treated with BAT than with RUX (5.51% *vs*. 3.09%) [56].

The thrombosis risk reduction is probably associated with the anti-cytokine effect of JAK2 inhibitors. Chronic inflammatory state as a result of paracrine secretion of inflammatory cytokines by neoplastic cells is present in patients with myeloproliferative neoplasms [57, 58].

Interferon in the treatment of PV

Interferon alfa has the capacity to selectively reduce the malignant JAK2 mutated stem cell clone content [59, 60, 61]. Recently, Gisslinger et al., on the basis of phase 3, randomized, controlled open-label studies (PROUD-PV and its extension study, CONTINUATION-PV), documented that in patients with early PV phase without splenomegaly, pegylated interferon alfa 2b (PEG-interferon alfa 2b) was as effective as hydroxyurea after 12 months of treatment in terms

of complete hematological responses (non-inferior vs. HU). However, in contrast to HU, the PEG-interferon 2b treatment continuation up 36 months was associated with higher response rates and with a higher proportion of patients with molecular response (a steady decrease in the mean absolute *JAK2* V617F allele burden level by month 36 from 42.8% at baseline to 19.7%). The corresponding ratios in the HU treated group was 42.9% at baseline to 39.3% at month 36. Due to good tolerability of PEG interferon 2b treatment and relatively high hematological and molecular response ratios, the study investigators recommend starting the treatment with PEG-interferon 2b in the early disease phase to prevent disease clonal evolution [62].

Conclusions

Recently, a British Society for Hematology guideline for the management of specific situations in PV and secondary erythrocytosis was published [63]. Unfortunately, only the recommendations concerning the treatment of clinical complications in PV are strong and are supported by high-quality evidences. The recommendations concerning the management of secondary erythrocytosis are still a matter of debate and have weak recommendations based on the moderate or even low- or very low-quality evidences. It is a consequence of the lack of the data concerning the patients with a specific molecularly proven diagnosis of secondary erythrocytosis. Therefore, there is a need to collect available data about the clinical outcome and treatments results in patients with inherited, specific forms of erythrocytosis in order to prepare the standards of care.

Authors' contributions

KL – the only author.

Conflict of interest

None.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; uniform requirements for manuscripts submitted to biomedical journals.

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