A Rare Hemoglobinopathy as a Cause of Erythrocytosis in a Patient with Suspected JAK2V617F Negative Polycythemia Vera: A Case Report

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Abstract

Introduction: Differential diagnosis of polycythemia vera (a clonal myeloproliferative neoplasm) because of erythrocytosis. One year before, he suffered from an ischemic central retinal vein occlusion of unknown cause. Aspirin treatment was initiated. His mother was diagnosed with a JAK2V617F negative polycythemia vera years earlier and treated with phlebotomies as well as aspirin. Apart from erythrocytosis, laboratory analysis showed a normal white blood cell and platelet counts. The differential blood picture and lactate dehydrogenase were within the normal ranges. Molecular testing for BCR-ABL was initiated. His mother was diagnosed with a JAK2V617F negative polycythemia vera years earlier and treated with phlebotomies as well as aspirin. Apart from erythrocytosis, laboratory analysis showed a normal white blood cell and platelet counts. The differential blood picture and lactate dehydrogenase were within the normal ranges. Molecular testing for BCR-ABL, JAK2V617F and Calreticulin gene mutations by PCR was negative. Bone marrow biopsy was normal without signs for a myeloproliferative neoplasm. As almost all patients with polycythemia vera carry the phenotype-driver mutation JAK2V617F, further genetic testing for congenital causes of erythrocytosis was conducted. Mutations in the EPO-receptor gene were not found, but a very rare heterozygous point mutation in the beta-globin-chain [exon 2 (c.119A>C) leading to a change in codon 40 (CAG>CCG)] was detected by next generation sequencing. This rare variant belongs to the high oxygen affinity hemoglobinopathies leading to a reduction of oxygen supply in tissues and an increase in red cell production.

Conclusion: The diagnosis of a JAK2 mutation negative polycythemia vera must always be questioned and congenital causes of erythrocytosis excluded as the therapeutic and prognostic consequences are immense. Rare variants of hemoglobinopathies, particularly those with high oxygen affinity need to be excluded by molecular testing.

Keywords: Polycythemia vera • Polyglobulia • High oxygen affinity variant haemoglobins

Abbreviations: BCR-ABL: Breakpoint Cluster Region-Abelson • Hb: Haemoglobin • Hct: Hematocrit • EPO: Erythropoietin • JAK2: Janus Kinase 2 • MPN: Myeloproliferative Neoplasms • O2: Oxygen • PV: Polycythemia vera

Introduction

By definition, an absolute erythrocytosis is present when the red cell mass is greater than 125% of the predicted value for sex and body mass [1]. Nevertheless, an erythrocytosis is suspected when the hemoglobin (Hb) and/or hematocrit (Hct) is above the normal range, although a direct correlation between Hb and Hct measurement and red cell mass cannot be assumed [2].

A distinction is made between primary and secondary causes as well as acquired and congenital causes. An erythrocytosis is classified as primary when an intrinsic defect in the erythroid compartment of the bone marrow is present, for example in patients with congenital erythropoietin (EPO) receptor gene mutations and aquired polycythemia vera (PV). The secondary form is characterized by an overproduction of red cells in the bone marrow driven by endogenous and exogenous EPO or other reasons (e.g. Methemoglobinemia or hypoxia). Measuring the EPO level helps to distinguish between primary and secondary causes [3].

According to the WHO-classification, the diagnosis of PV can be done by the presence of an elevated Hb level above 16.5 g/dl in men and 16.0 g/dl in women or a Hct above 49% in males or greater than 48% in females plus hypercellularity and panmyelosis in a bone marrow biopsy and a phenotype-driver mutation in JAK2, which can be detected in more than 95% of patients with PV [4,5]. The most frequent JAK2 mutation is JAK2V617F (exon 14) which is responsible for 97% of JAK2 mutations seen in PV. The remainder are spread across exons 12, 13, and 14 [6].

A correct work-up of erythrocytosis is sometimes a diagnostic challenge. Yet, a precise diagnosis is mandatory for accurate counselling of patients and initiating the optimal treatment.

Case Report

We present a case of a 31-year-old man referred to our center in September 2019 with a suspected PV. One year earlier, he suffered from an ischemic central retinal vein occlusion of unknown cause and was treated with intravitreal Bevacizumab and aspirin 100 mg QD. Clinically, he complained of headache and visual impairment in both eyes. Social history was significant for smoking until 2015. The use of testosterone and steroids was denied. Cardiac-ultrasound showed normal results. At presentation, he was in a good...
general condition with a normal body mass index. Blood pressure and pulse were within normal ranges. Interestingly, his mother was diagnosed with a JAK2V617F negative PV some years before and was treated in an outpatient clinic with phlebotomies and aspirin. Furthermore, the patient stated that 13 years earlier, an elevated Hct was present in a routine blood examination.

Laboratory analysis showed normal white blood cell and platelet counts with 7.7 and 204 \( \times 10^9/\text{L} \) respectively. Hb was 18.4 gm/dL with a Hct of 54% and an erythrocyte count of 6.25 million cells/\text{mL}. The differential blood picture, lactate dehydrogenase, creatinine, albumin, C-reactive protein, as well as liver enzymes were within the normal ranges. Hepatosplenomegaly and lymphadenopathy were not detectable by physical examination and ultrasound. The EPO level was within the normal range (11.1 U/l) and the hemoglobin-electrophoresis showed no abnormalities (97.2% HbA and 2.8% HbA2).

Molecular testing for BCR-ABL, JAK2V617F and Coereticulin gene mutations by PCR from the peripheral blood was negative. Furthermore, the histopathological examination of the bone marrow biopsy presented a mature hematopoiesis with predominant granulopoiesis and regular cellularity (Figure 1a,b). There was no apparent fibrosis and no atypia in the megakaryopoiesis (Figure 1c,d). Blast count in bone marrow smears was below 5%. Cytogenetic analyses showed a normal karyotype. In summary, there were no signs for myeloproliferative neoplasia (MPN).

Because of the young age of the patient, the long history of polyglobulia, and the unusual JAK2 mutation negative polycythemia vera of his mother, we initiated genetic testing for an EPO-receptor mutation which yielded no pathological finding. A subsequent molecular testing by next generation sequencing for mutations, deletions, and duplications in the alpha-globin-genes BPGM, EGLN, EPAS1 and VHL was negative. Yet, a very rare heterozygous variant in the beta-globin-chain was detected. This variant was a point mutation in exon 2 (c.119A>C) leading to a change in codon 40 (CAG>CCG) resulting in a glutamine to proline replacement in the amino acid sequence of the beta globin chain. This variant is found in the \( \alpha_1 \beta_2 \) interface and belongs to the group of high oxygen (\( O_2 \)) affinity hemoglobinopathies.

The symptoms of hyperviscosity (headache and visual impairment) disappeared under aspirin and phlebotomies. The target Hct is kept around 50%. The patient and his family received an extensive genetic counselling because of the possible autosomal dominant inheritance of the disease. The subsequent molecular testing revealed the presence of the same mutation in his mother.

**Discussion**

Clarifying the cause of polyglobulia is sometimes a diagnostic challenge. A distinction must be made between primary and secondary as well as acquired and congenital causes [3]. First clues to the cause could often be found in the medical history of patients.

An increase in Hb levels, Hct, and the absolute number of red blood cells may be evidence of PV. However, more often erythrocytosis is caused by an underlying non-hematological disease. Reasons for an acquired secondary cause of polyglobulia such as pulmonary, renal, and cardiac causes have to be excluded first. Measuring the EPO-level could help differentiate between primary and secondary forms.

If there is no evidence for an acquired secondary cause or if there are additional features suggesting MPN such as abnormal white blood and/or platelet counts or splenomegaly, a hematological diagnostic work-up must be initiated including molecular testing for BCR-ABL and the typical phenotype-driver mutations for BCR-ABL negative MPN as well as a bone marrow examination to confirm the diagnosis.

According to the WHO-classification of 2016, PV belongs to the classical BCR-ABL negative MPN [4]. MPN are characterized by a persistent hyperactivation of the Janus kinase 2 (JAK2)-signal transducer and activator of transcription (STAT) signaling pathway which is driven by various somatic mutations.

In patients with a positive family history of polyglobulia, congenital causes should always be included in the differential diagnosis. Although most MPN cases are sporadic and occur in elderly adults, familial cases are described and there is a growing evidence for a germline predisposing factor in the MPN pathogenesis [7-9]. Therefore, the exclusion of a familial MPN should be part of the diagnostic work-up.

Yet, in the majority of patients with PV, a JAK2 mutation could be detected [7]. Thus, the diagnosis of a JAK2 mutation negative PV is very unusual and must always be questioned and congenital causes for erythrocytosis such as mutations in the EPO-receptor gene as well as mutations in the \( \alpha \)- and \( \beta \)-globin-chains investigated [10]. Today and due to the improvement in molecular testing, congenital causes of erythrocytosis could be identified more easily [11]. One molecularly characterized form of congenital primary erythrocytosis is the autosomal dominant primary familial and congenital polycythemia caused by gain-of-function mutations in the EPOR gene which is found in around 12% of cases with erythrocytosis [12].

In 1986, Charache et al first described a molecular defect in the alpha-chain associated with an increased \( O_2 \) affinity called Hb-Chesapeake [13]. At present, more than 100 Hb-variants with high \( O_2 \) affinity are described. Most of these Hb variants have substitutions at one of three regions that are crucial

![Figure 1](image-url)
for Hb function: 1. the α1β2 interface; 2. the C-terminal end of the β-chain; and 3. the 2,3-bisphosphoglycerate mutase binding site [14]. The variant found in our patient is located at the α1β2 interface. To our knowledge, data to this variant are very limited.

Clinical data on patients suffering from congenital erythrocytosis are sparse. Patients are mostly asymptomatic and may have facial and mucosal erythrosis. Nevertheless, hyperviscosity symptoms and thromboembolic episodes have been reported and seem to be related to the high Hct. The higher affinity of O2 to Hb leads to a lower oxygenation of tissues and consecutively to an erythrocytosis [10].

The primary goal of treatment is the prevention of thrombembolic complications and reduction of symptoms caused by hyperviscosity. Patients with thrombembolic events should be treated with antithrombotic agents as is the case in our patient. In patients with hyperviscosity, therapeutic phlebotomy could be indicated, targeting a Hct at which hyperviscosity symptoms are minimized, while ensuring that patients are not experiencing symptoms associated with reduced oxygen-carrying capacity. Basic measures include good hydration and the avoidance of activities that potentially increase blood viscosity (e.g., mountain climbing, smoking, dehydration) [15].

Guidelines on the investigation and management of erythrocytosis published in 2005 suggest that the target Hct with phlebotomy for patients with high affinity Hb should be < 60%. If thrombosis or symptoms of hyperviscosity occur at lower Hct, then a target Hct of 52% is suggested [18]. It is important to note that the target Hct proposed here is higher than the 45% currently employed in patients with PV.

Conclusion

In conclusion, targeted NGS is cost-efficient, provides rapid and accurate mutation analysis, and could be applied directly to erythrocytosis cases where a genetic cause is suspected.

Compliance

Compliance with Ethical Standards.

Conflict of Interest

The authors declare that they have no conflict of interest.

Informed Consent

The patient has given his informed consent for each diagnostic and therapeutic step presented in this case report as part of the routine clinical work up.

References
