



Case report

Haemoglobinopathy Ypsilanti – A rare, but important differential diagnosis to polycythaemia vera [☆]



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ABSTRACT

We present a case of a mother and daughter who were initially diagnosed with polycythaemia vera and treated with venesection. As JAK2 V6217F/exon 12 mutation analyses became available, these were performed and turned out negative. Haemoglobin electrophoresis was performed and the patients were found to have high oxygen affinity haemoglobin Ypsilanti. It is important and relevant to look for high oxygen affinity variants of haemoglobin when there is a family history of erythrocytosis, in young persons and when there is no apparent reason or clonal marker present.

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1. Introduction

Haemoglobin Ypsilanti is a rare high oxygen affinity haemoglobin variant. The haemoglobinopathy is inherited autosomally dominant and most affected individuals are heterozygote. Due to the high oxygen affinity of the haemoglobin (Hb) molecule, hypoxia arises in the tissues, and secondary erythrocytosis develops [1].

More than 900 structural haemoglobin variants have been described. Of these more than 100 show high oxygen affinity [2]. Erythrocytosis caused by Hb Ypsilanti was first described in 1967 [3], and the mutation results in substitution of amino acid (β 99 Asp→Tyr) involved in the stability of the quaternary structure of the haemoglobin tetramer [2,4]. Hb Ypsilanti is unusual, because the tetramer of the liganded form of Hb Ypsilanti is more stable than the deoxy tetramer. Hb Ypsilanti also exhibits large quaternary enhancement effect, where the binding of all 4 ligands is greater than for the isolated $\alpha_1\beta_1$ dimers [4].

Since 2008, diagnostic make-up of patients presenting with polycythaemia includes examination for the JAK2 V6217F/exon 12 mutations [5]. The JAK2 V617F mutation is present in more than 95% of patients with polycythaemia vera (PV) and in the small group of patients without this clonal marker a variant mutation in exon 12 is almost always found [6]. Furthermore endogenous erythroid colony (EEC) formation *in vitro* may be a criterion of PV [5].

We report two patients with high oxygen affinity Hb and highlight the importance of differential diagnoses in erythrocytosis.

2. Cases

In 2003 a 51-year-old woman (patient 1) was referred due to a B-Hb up to 21 g/dl and a haematocrit of 0.67. The patient presented with symptoms of a (transient) cerebral ischaemic attack. Magnetic resonance imaging of the cerebrum showed no apoplexy but hydrocephalus. The patient was later treated with a ventriculo-peritoneal shunt.

The patient suffered from hypertension and had been smoking about 20 cigarettes daily for her entire adult life. She had experienced a pressing sensation in the head, had difficulty concentrating and was chronically tired. As long as she could remember, she had had elevated B-Hb.

In 2007 her daughter, a 24-year-old woman, (patient 2), was referred for evaluation due to headaches for 3 weeks and an elevated B-Hb (16 g/dl).

Both patients were considered as atypical PV (Table 1). From the time of diagnosis the patients have been treated with venesection, when the haematocrit was above 0.42, or the patient experienced symptoms such as itching and sweating – and almost always followed by a subjective relief and benefit. Patient 1 also received acetylsalicylate 75 mg daily and later marcoumar after multiple pulmonary embolisms due to immobilisation after surgery. Patient 2 had no thromboembolic complications. She has received acetylsalicylate prophylaxis, and had two uneventful pregnancies and deliveries.

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Table 1
Manifestations and analysis at diagnosis and later in the course (year).

Parameter (normal range)	Patient 1	Patient 2
Age at diagnose	51 years	24 years
Comorbidity	Hypertension, hydrocephalus	None
Symptoms at diagnose	TCl, fatigue, pressing sensation in the head, difficulty concentrating	Headache for 3 weeks
Haemoglobin (11.3–16.1 g/dL)	21 (2003)	16 (2007)
Haematocrit	0.67 (2003)	0.56 (2007)
MCV (80–100 fL)	n.a.	81 (2007)
MCHC (19–22 mmol/L)	n.a.	20.1 (2007)
White blood cells (3–9 billions/L)	7.0 (2003)	7.0 (2007)
Platelets (150–400 billions/L)	235 (2003)	179 (2007)
Iron (10–35 µmol/L)	4 (2007)	7 (2007)
Ferritin (12–300 µg/L)	6 (2007)	5 (2007)
Reticulocytes (25–99 billions/L)	113 (2006)	56 (2007)
Cobalamin (145–640 pmol/L)	569 (2007)	349 (2007)
Homocystein (4–15 µmol/L)	11.0 (2007)	11.3 (2007)
Methylmalonat (< 0.28 µmol/L)	0.17 (2007)	0.20 (2007)
Erythropoietin (5–30 IU/L)	23 (2003) 118 (2010)	8.9 (2007)
Bone marrow biopsy	Found in concordance with a chronically myeloproliferative disorder (hypercellularity, domination of erythropoiesis, but also numerous partly hyperlobular megakaryocytes scattered in the reticulum, mild eosinophilia, a myelopoiesis that was well represented and with normal maturation. No iron deposits)	Showed hypercellularity with slight left shift, no sign of malignancy. Iron deposits scarce. Normal cytogenetics, normal HUMARA test X-inactivation pattern (25:75)
Peripheral blood smear	Normal (2003)	Normal (2009)
JAK 2 V617F exon 12 mutation	Negative (JAK 2 V617F – 2008 and exon 12 – 2010)	Negative (JAK 2 V617F – 2007 and exon 12 – 2010)
Abdominal ultrasound	Normal in 2003, in 2009 splenomegaly with longest diameter 13.7 cm.	Normal (2007)
Chest X-ray	Normal (2003)	Normal (2007)
PCR for BCR-abl transcript	n.a.	Negative (2007)
Endogenous Erythroid Colony formation <i>in vitro</i>	Normal (2012)	Normal (2009)
CFU-GM colony growth	n.a.	Normal (2009)

In 2008 and 2010 the JAK2 V617F and exon 12 mutation analyses were performed, and showed a *wild type* status in both patients. EEC was found normal by *in vitro* analysis (Table 1).

Since patient 1 was young, there was a family history, and no clonality was found, other causes for erythrocytosis than PV were considered. In 2012 separation of haemoglobins was performed revealing the haemoglobin variant Hb Ypsilanti.

Three other family members have been tested for erythrocytosis, which was not observed (Fig. 1). Further follow-up is not possible due to lack of contact, death or very young age. The two children of patient 2 have not had haemoglobin measured at birth. Besides, for patient 1 thrombosis have occurred in at least 2 other family members and one has been treated with anticoagulants (cause unknown) (Fig. 1). In both patients reported here we have reduced the venesection frequency, allowing a B-Hb higher in the normal range.

3. Methods

Haemoglobin fractions were obtained by liquid chromatography on a Waters UPLC (Waters, Milford, MA, USA) by using a Cation-exchange PolyCat column (35 × 4.6 mm, 3 µm, 1500 Å) (PolyLC Inc., Columbia, MD, USA). Genomic DNA was purified from leucocytes using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Part of the β-globin gene was amplified using the forward primer A (5'-ATA TCT TAG AGG GAG GGC-3') and the reverse primer B (5'-CCC ATT CTA AAC TGT ACC-3'). Platinum®Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) was used according to the manufacturer's

instructions for polymerase chain reaction (PCR) amplification. The following conditions were used: denaturation for 120 s at 95 °C, 32 cycles of 30 s at 9 °C, 40 s at 60 °C and 60 s at 72 °C followed by a final elongation of 7 min at 72 °C. The PCR product was purified using QIAquick (Qiagen) PCR purification kit according to the manufacturer's instructions. Sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) and subsequently purified using the BigDye® XTerminator purification kit according to the manufacturer's instructions. Primers for sequencing were primers A and B. Sequencing was performed on an Applied Biosystems 3500 Genetic Analyser (Life Technologies, Carlsbad, CA, USA).

In vitro test for EEC, JAK2 mutations and paraclinical parameters were performed by routine analysis.

4. Discussion

It is always important to make the correct diagnosis – in particular when molecular biological analyses are available. The pursuit of the correct diagnosis was intensified in these patients when signs of clonality were not demonstrated. Since there was a family history with erythrocytosis, and furthermore, because patient 2 was young to develop PV, other reasons for polycythaemia were considered. The inheritance in myeloproliferative disorders is complex and difficult to evaluate in a single family (Fig. 1) [7]. Hereditary erythrocytosis can be associated with mutations in the gene encoding the erythropoietin receptor, defects in the oxygen sensing pathways and abnormal oxygen delivery [8]. Calculation of P50 may be indicative of abnormal oxygen delivery

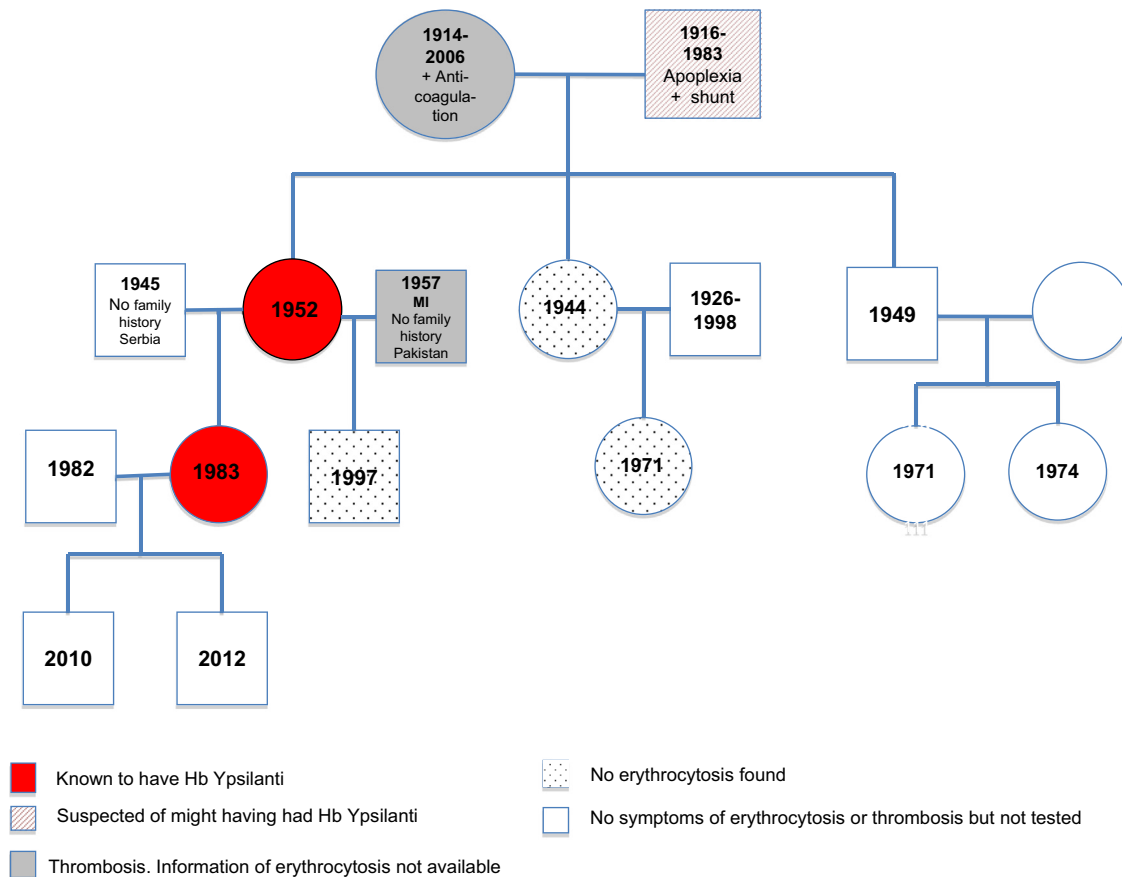


Fig. 1. Family pedigree in haemoglobinopathy Ypsilanti.

in patients with erythrocytosis [8]. If a low value is found the next step would be sequencing of the globin genes. In the patients reported here P50 was not calculated, specifically due to marcumar anticoagulation therapy in patient 1. Alternatively, the specific diagnostic analyses were performed.

The therapeutic strategy in PV and high affinity oxygen states is very different. The strict venesection activity and reduction of haematocrit to 0.45 or below is not rational in a patient with a high affinity haemoglobinopathy. Furthermore no cytoreductive therapy is necessary in haemoglobinopathy. The introduction of hydroxyurea or interferon-alpha therapy may induce side effects and in principle a risk for long-term complications. The risk of transformation to leukaemia in clonal erythrocytosis is not present in a haemoglobinopathy, and this fact has a significant positive psychological impact. However, the risk to transfer a hereditary disease may be just as difficult to acknowledge and handle.

A review of the literature and reported cases show that erythrocytosis due to high oxygen affinity haemoglobin is usually well tolerated in younger patients, but in the elderly patients the risk of thrombosis is increased [1]. The development of pulmonary embolism in patient 1 seems to be secondary to other causes. Treatment strategy in high affinity hemoglobinopathy should be decided individually [1,2].

It is important and relevant to look for high oxygen affinity variants of haemoglobin when there is a family history of erythrocytosis, in

young persons and when there is no apparent reason or clonal marker present.

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