Laboratory Diagnosis of Rare Anaemias: Hereditary Red Blood Cell Enzyme Disorders

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Red blood cell enzyme disorders cause hereditary nonspherocytic hemolytic anemia (HNSHA)

The diagnosis of HNSHA should be assumed in cases of persistent normocytic hemolytic anemia in which hemoglobin abnormalities and antiglobulin reactions have been excluded, spherocytes are absent, and osmotic fragility is normal.
The red blood cell

- Highly differentiated enucleated cell
- Transport of oxygen
- Mainly one protein: Hb
- Life-span 120 days
Metabolic energy is needed:

- to maintain red cell shape
- to keep the iron of hemoglobin in the divalent ($\text{Fe}^{2+}$) form
- to pump ions against electrochemical gradients (intracellular: high potassium, low sodium and calcium levels)
- to keep sulphydryl groups of red cell enzymes, hemoglobin, and membranes in the active, reduced forms
Metabolic pathways of the erythrocyte
Enzymes of the red blood cell...

- Acetylcholinesterase
- Adenosine deaminase
- Adenylate kinase
- Aldolase
- Bisphosphoglycerate mutase
- Catalase
- NADH-cytochrome b5 reductase
- Enolase
- Galactokinase
- Galactose-4-epimerase
- γ-Glutamylcysteine synthetase
- Glucose phosphate isomerase
- Glucose-6-phosphate dehydrogenase
- Gluthathione peroxidase
- Gluthathione reductase
- Glutathione synthetase
- Glutathione-S-transferase
- Glyceraldehyde 3-phosphate dehydrogenase
- Hexokinase
- Lactate dehydrogenase
- Monophosphoglyceromutase
- Multiple inositol polyphosphate phosphatase
- NADPH diaphorase
- Phosphofructokinase
- Nucleoside phosphorylase
- Phosphoglucomutase
- 6-Phosphogluconate dehydrogenase
- 6-Phosphogluconolactonase
- Phosphoglycerate kinase
- Phosphoglycolate phosphatase
- Phosphomannose isomerase
- Pyrimidine-5'-nucleotidase
- Pyruvate kinase
- Ribosephosphate isomerase
- Superoxide dismutase
- Transaldolase
- Transketolase
- Triosephosphate isomerase
...hereditary deficiencies of which lead to hematologic disease

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- Adenosine deaminase (hyperactivity!)
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Clear cause and effect relationship
...hereditary deficiencies of which lead to hematologic disease

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Associated with erythrocytosis and methemoglobinemia
...hereditary deficiencies of which lead to hematologic disease

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Associated with hemolysis
Lack of energy may shorten the red blood cell’s life span

Lack of energy  Premature removal from the circulation by the spleen and monocyte-macrophage system (Decreased survival: hemolysis)

Extravascular hemolysis
- low hemoglobin levels
- normal MCV (Mean Corpuscular Volume)
- high number of reticulocytes
- high bilirubin levels
- low haptoglobin levels

Hereditary NonSpherocytic Hemolytic Anemia (HNSHA)
Acute or chronic
I. Enzyme deficiencies of the hexose monophosphate shunt and glutathione metabolism

Hereditary red blood cell enzymopathies
I. Enzyme deficiencies of the hexose monophosphate shunt and glutathione metabolism

Inadequate levels of reduced glutathione (GSH): inability to withstand oxidative stress

→ Periodic hemolytic episodes (acute HNSHA) induced by oxidant drugs, food (favism), infection, physiologic stress

Associated deficiencies:
- **Glucose-6-phosphate dehydrogenase (G6PD)**
- Glutathione reductase (GR)
- γ-Glutamylcysteine synthetase (GCS)
- Glutathione synthetase (GSH-S)
II. Enzyme deficiencies of glycolysis and nucleotide metabolism

**ATP** (Adenosine TriPhosphate)
II. Enzyme deficiencies of glycolysis and nucleotide metabolism

Continuously impaired ATP generation: lack of sufficient energy → Chronic HNSHA; exacerbated by infection, physiologic stress

Associated deficiencies*:
- hexokinase (HK)
- glucosephosphate isomerase (GPI)
- phosphofructokinase (PFK)
- aldolase
- triosephosphate isomerase (TPI)
- phosphoglycerate kinase (PGK)
- pyruvate kinase (PK)
- pyrimidine 5’-nucleotidase (P5N)
- adenylate kinase (AK)
- adenosine deaminase (hyperactivity*) (ADA)
• G6PD deficiency may also be associated with chronic HNSHA!
• Mutations located in specific regions of the G6PD dimer (interface)
Clinical features of HNSHA

HNSHA is highly heterogeneous!

- severe hemolytic anemia, diagnosed at birth (hydrops fetalis)
- (fully) compensated mild hemolysis, diagnosed later in life
- (chronic) hemolysis with exacerbation during infection
- icterus (gall stones) and splenomegaly
- there may be non-hematological effects:
  - myopathy: phosphofructokinase deficiency
  - (severe) neurological abnormalities: - triosephosphate isomerase def.
    - phosphoglycerate kinase def.
    - $\gamma$-glutamylcysteine synthetase def.
    - glutathione synthetase def.
    …
Laboratory diagnosis of HNSHA

- No morphological abnormalities, except:
  Pyrimidine 5’-nucleotidase deficiency:
  prominent basophilic stippling

- Other cases: demonstration of the specific enzyme defect by measuring red blood cell enzyme activities
  Little correlation between residual enzyme activity and severity of clinical expression

- Molecular characterization of the defect on the DNA level
• Blood transfusions

• Leucocyte and platelet contamination (α-cellulose pre-treatment)

• Storage and shipment of samples
  – *e.g.* instability of PFK, TPI, ADA

• Enzyme deficiencies may be less pronounced under optimized *in vitro* conditions (compared to *in vivo*) → low [S] assays
Reticulocytosis: most red cell enzymes are red cell age-dependent: enzyme activities decreases with age (HK, PK, G6PD, aldolase, P5N)
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Reticulocytes: 638‰ (5-25‰)
• **Reticulocytosis:** most red cell enzymes are red cell age-dependent: enzyme activities decreases with age (HK, PK, G6PD, aldolase, P5N)

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Reticulocytes: 638‰ (5-25‰)

Patient: PK activity relatively too low, PK deficiency?!
Reticulocytosis: most red cell enzymes are red cell age-dependent: enzyme activities decreases with age (HK, PK, G6PD, aldolase, P5N)

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reticulocytes: 638‰ (5-25‰)

Patient: compound heterozygous for *PKLR* mutations c.1436G>A / c.507+1G>A
Inheritance of red blood cell enzyme disorders

- Most enzyme disorders are transmitted as autosomal recessive disorders
- ADA hyperactivity: autosomal dominant
- G6PD and PGK deficiencies are X linked
  - men: usually affected
  - women: usually unaffected
    (due to random inactivation of one chromosome: lyonisation)
...never trust a woman!

Reference value:
G6PD: 7.1 – 11.5 U/gHb

* Acute hemolysis (favism?)
Reference value:
G6PD: 7.1 – 11.5 U/gHb

* Acute hemolysis (favism?)

heterozygous
G6PD Vanua Lava

(1975) G6PD 6.1 L

(1977) G6PD 4.2 L


(1948) G6PD: 3.6 L

(1980) G6PD 1.6 L
...never trust a woman!

Reference value:
G6PD:  7.1 – 11.5 U/gHb

* Acute hemolysis (favism?)
‘Skewed’ X chromosome inactivation may contribute to the clinical expression of G6PD deficiency!

* Acute hemolysis (favism?)
• Most red blood cell enzyme disorders cause hereditary nonspherocytic hemolytic anemia (HNSHA)
• HNSHA is highly heterogeneous
• Loss of energy leads to extravascular hemolysis (acute or chronic)
• Most common: G6PD deficiency (acute) and PK deficiency (chronic)
• Exacerbation of hemolysis during oxidative stress and/or infection
• Some enzymopathies are expressed throughout the body; myopathy, neurological symptoms
• Except for P5N deficiency, red cell morphology is unremarkable
• No correlation between residual enzymatic activity and clinical severity
• Reticulocytosis may mask the diagnosis of enzymopathies
• Confirmation of the diagnosis by molecular analysis
• Beware of X-linked diseases in women!
Added value of DNA analysis

- Male, 1966
- Admitted with headache, tiredness and abdominal pain
- Spleen enlarged and painful
- Lab:
  - Hb 10 g/dL
  - Reticulocytes 9%
  - Bilirubine 81 μmol/L
  - LDH 790 U/L
  - Haptoglobin 0.2 g/L
  - Directe antiglobine test (Coombs): negative
- Family history: several members with splenomegaly

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Conclusion: congenital hemolysis due to PK-deficiency
PKLR analysis: heterozygous for mutation c.1618+1delG / ?
PK deficiency is an autosomal recessive disease
We would not expect severe hemolysis

SLC4A1 (band 3) analysis

Patient: abnormal osmotic fragility
PKLR analysis: heterozygous for mutation c.1618+1delG / ?
PK deficiency is an autosomal recessive disease
We would not expect severe hemolysis

Patient: hereditary red blood cell membrane disorder!