Anaemia due to a red blood cell membrane defect
Red blood cell membrane defect

- Pathologies
- Clinical signs
- Diagnostic criteria
- Treatment (HS)
The pathologies

**Structural organisation**
- Hereditary spherocytosis
- Hereditary elliptocytosis

**Transport function**
- “Stomatocytosis”

**The other hereditary forms:**
- CDA II

**The other acquired forms:**
- PNH
- Zieve, ...

Mohandas, 2008 Br J Haematol
Hereditary spherocytosis (HS)
Hereditary elliptocytosis (HE)

Pyropoikilocytosis (PPE)

Junction complex

> 90%

α-spectrine  β-spectrine

Auto-association sites
Hereditary « Stomatocytosis » (HSt)

- Dehydrated form (DHSt) ou xerocytosis
- Overhydrated (OHSt) ou hydrocytosis
- Intermediate forms:
  - Cryohydrocytosis
  - Familial pseudohyperkaliemia
Congenital dyserythropoïèsis (CDA)

CDA II

Deglycosylation B3

Mutation SEC23B Gene: protein involved in maturation, proliferation and division of erythroblasts
The pathologies

Caucasians ++

Hereditary spherocytosis

Sub-Saharan Africa (caucasians)

Hereditary elliptocytosis

“Stomatocytosis”

CDA
The pathologies

Caucasians ++

Hereditary spherocytosis

1/2000 - 1/5000

Sub-Saharan Africa (caucasians)

Hereditary elliptocytosis

1/100 West Afr.

“Stomatocytosis”

1/50,000 (DHSt)

(Cryohydrocytosis, OHSt: a few families)

CDA

< 1/100,000
The pathologies

Caucasians ++
Hereditary spherocytosis
65-75% dominant

Sub-Saharan Africa (caucasians)
Hereditary elliptocytosis
70% dominant

“Stomatocytosis”
Dominant
OHSt de novo

CDA
Recessive
Red blood cell membrane defect

- Pathologies
- Clinical signs
  - Paediatric/adult
  - Splenomegaly/biliary lithiasis
- Diagnostic criteria
Paediatric/adult
First visit at what age?

Depends on the degree of decompensation of anaemia

- Variable age at diagnosis, but
  - HS ± 65% with neonatal icterus
  - Each “stress” on the RBCs = decompensated anaemia
    - Birth
    - Infection (i.e. Parvovirus B19)
    - Pregnancy
Red blood cell membrane defect

- Pathologies
- **Clinical signs**
  - Paediatric/adult
  - Splenomegaly/biliary lithiasis
- Diagnostic criteria
Splenomegaly/ biliary lithiasis
Haemolysis ± compensated

- Differential diagnosis (*MCV, AutoImmune Haemolytic Anaemia*)
  - Measurement RBC enzymes (G6PD, PK, GPI, ...)
  - *Hb* fractions
  - Screen for *Hb* H
  - Heat and isopropanol tests
  - Screen for Heinz bodies

Enzyme deficiency

Haemoglobinopathy
*HbCC, HbSC, ...*

Unstable *Hb*
Red blood cell membrane defect

- Pathologies
- Clinical signs
  - Paediatric/adult
  - Splenomegaly/biliary lithiasis
- Diagnostic criteria
## Diagnostic criteria

RBC membrane pathology

<table>
<thead>
<tr>
<th></th>
<th>MCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>N or ↓</td>
</tr>
<tr>
<td>EH</td>
<td>N or ↓</td>
</tr>
<tr>
<td>DHSt</td>
<td>↑</td>
</tr>
<tr>
<td>OHSt</td>
<td>↑</td>
</tr>
<tr>
<td>CDA I</td>
<td>↑</td>
</tr>
<tr>
<td>CDA II</td>
<td>N</td>
</tr>
</tbody>
</table>
Diagnostic criteria
RBC membrane pathology

Membrane defect = regenerative

Except CDAs
Diagnostic criteria
CDA II

- Aniso-poikilocytosis
- Fex spherocytes

Binuclearity of bone marrow erythroblasts
Diagnostic criteria
RBC membrane pathology
Electrophoresis (SDS-PAGE)

CDA II

Band 3
# Diagnostic criteria CDAs

Table 2. General definition criteria of the CDAs

1. Evidence of congenital jaundice/jaundice or a positive family history
2. Evidence of ineffective erythropoiesis
3. Typical morphological appearance of bone marrow erythroblasts, and
4. Exclusion of congenital anemias that fulfill criteria 1 and 2, but were classified according to the underlying defect, such as the thalassemia syndromes, Unclear?) or hereditary sideroblastic anemias

*Heimpel H, ENERCA white book*

[www.enerva.org](http://www.enerva.org)
Diagnostic criteria
HE and HPP

Study of the parents
Diagnostic criteria
Hereditary spherocytosis

β-spectrin

Bande 3
Diagnostic criteria
Screening tests for HS

- **Cryohaemolysis test**
  - Osmotic fragility

- **Eosin-5-maleimide binding test:** decrease fluorescence
  - Membrane protein defect
Diagnostic criteria
Osmotic gradient ektacytometry

Osmoscan curve

Control

HS

Osmolality (mOsm/kg)

0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
70 120 170 220 270 320 370 420 470

control

HS
Confirmation of diagnosis (± 80%)
Hereditary spherocytosis

Clinical
Individual and family history

First line tests
Confirm haemolysis and erythropoietic answer
Exclude AIHA, enzymopathy, other
Search for HS characteristics

Second line tests
Search for osmotic fragility and RBC membrane deficiency
(Cryohaemolysis, EMA binding test)

In any doubt, diagnostic tests
(Ektacytometry, SDS-PAGE)
Hereditary spherocytosis

• **Follow-up**
  – Childhood: annual visit unless new symptoms, acute event...
  – *Chronic haemolysis: look for co-inheritance of a haemochromatosis gene*

• **Treatment**
  – Folate therapy in severe and moderate HS

  *Splenic conditioning:*
  *Further membrane loss*

  – Splenectomy (HS confirmed)
    • Indications depends on symptoms and complications (individual tolerance)
Hereditary spherocytosis

• **Treatment**
  – Splenectomy (HS confirmed)
    • Lifelong small risk of overwhelming sepsis (*grade 1 recommendation, grade B evidence*)
    • National guidelines for immunization; reimmunization when and how? Duration of AB prophylaxis? (*grade 2 recommendation, grade C evidence*)
    • No indication for extended thrombosis prophylaxis after splenectomy. Adults should receive perioperative thromboprophylaxis in the usual way;
    • To be avoided in patients with some forms of hereditary stomatocytosis (*grade 1 recommendation, grade B evidence*).
Red blood cell membrane defect
Home message

- The most frequent in Belgium: hereditary spherocytosis
- Haemolysis ± compensated except for CDAs= dyserythropoiesis
- Differential diagnosis with other causes of haemolysis
- Clue: clinical features, screening tests
- Final diagnosis
  - Simple (family history)
  - Complex: diagnostic tests available un expert centres
- Treatment
  - Symptoms, complications
  - Eliminate the amplifier (spleen)
## RBC membrane pathology

### Summary: diagnostic criteria

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>Cryohemolysis or EMA, <em>ektacytometry</em>, SDS-PAGE</td>
</tr>
<tr>
<td>EH</td>
<td>Morphology (<em>Parents</em>), EMA, <em>ektacytometry</em></td>
</tr>
<tr>
<td>DHSt</td>
<td>Ektacytometry</td>
</tr>
<tr>
<td>OHSt</td>
<td>SDS-PAGE (absence of stomatin)</td>
</tr>
<tr>
<td>Cryohydro.</td>
<td>MCV, MCHC and $K^+$ after 2H on ice</td>
</tr>
<tr>
<td>CDA II</td>
<td>SDS-PAGE (no glycosylation of Band 3)</td>
</tr>
</tbody>
</table>
Red blood cells enzyme deficiencies
Red blood cell enzyme disorder

• If persistent haemolytic anaemia and
  – Normocytic
  – Regenerative
  – Haemoglobinopathies excluded
  – Negative Coombs test

• Acute haemolytic anaemia
Enzymatic equipment of the RBC

Other deficiencies
- Very rare
- Sometimes other symptoms (TPI)
- Inheritance: Autosomal recessive
  Consanguinity?

Pyruvate Kinase
- < 1/20,000
- Inheritance: Autosomal recessive
  Consanguinity?
  Chronic haemolytic anaemia
Enzymatic equipment of the RBC

First step of the pentoses pathway: production of NADPH which gives protection against oxydatives agents.
# G6PD Deficiency

## Epidemiology

### Classification II et III

#### Table IIa: World Distribution of G6PD Deficiency

<table>
<thead>
<tr>
<th>Region</th>
<th>Country</th>
<th>Estimated Population (x1000) 1966</th>
<th>Frequency (in males) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>Ghana</td>
<td>7,300</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Nigeria</td>
<td>9,104</td>
<td>2-25</td>
</tr>
<tr>
<td></td>
<td>Angola</td>
<td>5,084</td>
<td>11-27</td>
</tr>
<tr>
<td></td>
<td>Congo</td>
<td>15,300</td>
<td>6-23</td>
</tr>
<tr>
<td></td>
<td>Kenya</td>
<td>9,104</td>
<td>2-25</td>
</tr>
<tr>
<td></td>
<td>Tanzania</td>
<td>9,900</td>
<td>2-28</td>
</tr>
<tr>
<td></td>
<td>South Africa</td>
<td>17,474</td>
<td>3-9</td>
</tr>
<tr>
<td></td>
<td>Ethiopia</td>
<td>22,200</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Algeria</td>
<td>11,600</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Americas</td>
<td>USA</td>
<td>192,119</td>
<td>11 (in Blacks)</td>
</tr>
<tr>
<td></td>
<td>Venezuela</td>
<td>8,427</td>
<td>2-12</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>78,809</td>
<td>0</td>
</tr>
<tr>
<td>Asia</td>
<td>China</td>
<td>686,400</td>
<td>2-5</td>
</tr>
<tr>
<td></td>
<td>Hong Kong</td>
<td>3,692</td>
<td>3-7-5.5</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td>471,627</td>
<td>4-19</td>
</tr>
<tr>
<td></td>
<td>Japan</td>
<td>96,906</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Europe</td>
<td>Greece</td>
<td>8,480</td>
<td>1-32</td>
</tr>
<tr>
<td></td>
<td>Italy</td>
<td>50,762</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>Sardinia</td>
<td></td>
<td>3-35</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td></td>
<td>rare</td>
</tr>
</tbody>
</table>

Adapted from - WHO report

![Map of G6PD deficiency distribution](image)
• Mechanisms of R against malarial infection
  – RBC infection equal in RBC with and without the deficiency
  – RBC with G6PD deficiency:
    • Alterations of the parasites and the RBCs
      – Phagocytosis++ and earlier: lowered parasitaemia
    • Probably not a decrease of the parasite growth (discordant literature)
Question

- Do you think that women are affected by G6PD deficiency?
G6PD deficiency

- X-linked recessive inheritance
  - Men affected: ±1/20
  - Women affected (homozygosity/compound heterozygosity/X inactivation): ±1/400
G6PD deficiency

- **Class I (very rare)**
  - Neonatal icterus
  - Chronic haemolytic anaemia

- **Class II (Asia, Med. Bassin)**
  - Neonatal icterus
  - Severe haemolytic crisis when exposed to any oxydant (drugs, infection, ...)

- **Class III (Sub-Saharan. Africa)**
  - Neonatal icterus if preterm newborn
  - Haemolytic crisis when exposed to any oxydant (drugs, infection, ...)

*jaundice +++ if Gilbert’s disease*
Precautionary measures in case of G6PD deficiency (fayism)

What is G6PD deficiency?
G6PD (glucose-6-phosphate dehydrogenase) catalyzes an oxidation/reduction reaction and is responsible for maintaining adequate levels of NADPH inside the red blood cell. It is thus essential for ensuring a normal life span. G6PD deficiency is an X-linked inherited disease. It means that boys are more often affected than girls. The deficiency is most prevalent in the Mediterranean basin (Italy, Greece, Cyprus, ...) in Africa, and Southeast Asia.

Risk?
Most often, individuals who have G6PD deficiency have a perfectly normal life but this enzyme deficiency may provoke the sudden destruction of red blood cells and lead to haemolytic anaemia with jaundice following the intake of fava beans (Vicia fava – see photograph) and of various drugs (see the list below).

Symptoms?
Within hours after the sudden destruction of red blood cells, symptoms are: palor, fatigue, general demotivation of physical condition, dark yellow-orange urine, yellow colouring of skin and mucous membrane, ...

At birth, the yellow colouring of skin and mucous membrane might be the unique symptom.

Measures?
Warn your physician or pediatrician. Avoid the following drugs and food.

<table>
<thead>
<tr>
<th>TO PROSCRIBE (proved risk)</th>
<th>TO AVOID (same class)</th>
<th>TO AVOID (same class)</th>
<th>RISK AT HIGH DOSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Deoxyx - Metamizole / Noramidopyrine (Aspirine, Norsol, Bucopin Compositum) - Nitrofurantoin (Furadantine) - Rasburicase (Fasurec) - Sulfranidine - Sulfamethoxazole (Bactrim, Eusaprim) - Sulfamethizide (Sulfasedemol) - Sulfasalazine (Salazopyrine) - Trimetoprine (Bactrim, Eusaprim) - Valadinic acid - Sulfadiazine (oral) - Sulfasulfrol</td>
<td>- Chloropyruvate (Nixaquene) - Ciprofloxacin (Ciproline, Ciprobel, Ciprotoxem) - Dimercaproxy - Glibenclamide (Revorea, Diabron, Glibenclamide) - Levoflaxacin (Tavanic) - Norfloxacin (Cenovis) - Phenytoin (convulsions) (Keskin, Vitamon K) - Spiramycine (Roventin) - Sulfadiazine (Taxane)</td>
<td>- Glutamazide (Diamicron) - Glimiprid (Amaryl) - Glipizide (Jibensene, Minipil) - Glucophage (Glytenorm) - Hydroxychloroquine (Plaquenil) - Lomefloxacin (Oxacein) - Norfloxacin (Avelox, Prodox) - Ofloxacin (Oloxacin, Tarivid) - Phenazone (local) (Hemoholin, Oflozin) - Prilocaine (Esha, Citrast) - Quinone - Sulfamethizide (Aminoside, Ancobon, Sulfa 19)</td>
<td>- Acetylsalicylic acid (Aspirin) - Acetaminophen (Paracetamol) - Paracetamol (Dafalgan, Dolprof, Perdatin) - Benzonate - Carbasalate</td>
</tr>
<tr>
<td>FOOD TO AVOID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fava beans (Vicia fava) - Drinks containing quinine (Schweppes, Gini ...) - Drinks with high content of vitamin C</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VARIOUS TO AVOID

- Some medicinal plants (Acalypha indica, Capsis chinensis) - Naparbrane - Henné

These information are dedicated to health professionals. If you believe that you, or a family member, are concerned don't take any decision (for example stopping drug intake) before you have taken your physician advice.

Adapted from Medicaments et déficit en Glucose-6-Phosphate Dehydrogenase (G6PD) - Février 2008 - Agence française de sécurité sanitaire et de prévention des risques - www.sante.gouv.fr (C June 2008)

40
G6PD deficiency
Biological diagnosis

- Regenerative haemolytic anaemia
- Measuring the enzyme activity

\[
\begin{align*}
glucose-6-P + NADP^+ & \xrightarrow{G6PD} 6-P-gluconate + NADPH + H^+ \\
6-P-gluconate + NADP^+ & \xrightarrow{6PGD} ribulose-5-P + CO_2 + NADPH + H^+
\end{align*}
\]

Suivi à 340 nm

<table>
<thead>
<tr>
<th>G6PD activity/Reticulocytes</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD (7.0-17.0)</td>
<td>7,5</td>
<td>9,6</td>
<td>&lt; 0,5</td>
</tr>
<tr>
<td>HK (1.0-3.5)</td>
<td>2,0</td>
<td><strong>7,2</strong></td>
<td>2,9</td>
</tr>
</tbody>
</table>
G6PD deficiency
Treatment

• Prevention of crisis
  - To avoid the trigger

• Transfusion if necessary (+ Folic acid)

• Life expectancy similar to the general population
G6PD deficiency
Home message

- X-linked recessive inheritance (BUT not only boys)
- Resistance against malarial infection (BUT sometimes in individuals from North Europe)
- Haemolytic anaemia (trigger)
  - Intravascular++
  - Regenerative, normocytic
- Final diagnosis
  - Measurement of the enzyme activity
- Treatment
  - Removal of the trigger and its avoidance
- Class I = chronic haemolytic anaemia (rare)
  - No relationship with endemic zones for malarial infection