Other red blood cells disorders

Béatrice GULBIS, M.D., PhD
Objectives of the training course

- Appreciate the place of red blood cell membrane defects and G6PD deficiency within the haemolytic (anaemias) at the level of:
  - Prevalence / Incidence
  - Clinical diagnostics aspects
  - Useful diagnostic tests
  - Therapeutics
Haemolytic (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 I et II (III, variants)

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

Mohandas, 2008 Br J Haematol
Hereditary spherocytosis (HS)

- RhAG
- B3
- 4.2
- Ankyrin
- Spectrins

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1%</td>
<td></td>
</tr>
<tr>
<td>15-20%</td>
<td></td>
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<tr>
<td>&lt; 5%</td>
<td></td>
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<tr>
<td>50-60%</td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td></td>
</tr>
</tbody>
</table>
Hereditary elliptocytosis (HE)  
*Pyropoikilocytosis (PPE)*  

> 90%  

*α*-spectrine  

*β*-spectrine  

**Auto-association sites**  

**Junction complex**
Hereditary « Stomatocytosis » (HSt)

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

CDA I

Deficiency

CDA II

Deglycosylation B3

B3 B3
The pathologies

Non Sub-Saharan Africa
++
Hereditary spherocytosis

Sub-Saharan Africa
(caucasians)
Hereditary elliptocytosis

“Stomatocytosis”

CDA
The pathologies

Hereditary spherocytosis

Non Sub-Saharan Africa

++

1/2000 - 1/5000

“Stomatocytosis”

1/50.000 (DHSt)
(Cryohydrocytosis, OHSt: few families)

Hereditary elliptocytosis

Sub-Saharan Africa (caucasians)

1/100 West Afr.

CDA

< 1/100.000
(CDA III: few families)
The pathologies

Non Sub-Saharan Africa

++

Hereditary spherocytosis

65-75% dominant

Sub-Saharan Africa (caucasians)

Hereditary elliptocytosis

70% dominant

“Stomatocytosis”

Dominant

OHSt de novo

Recessive

CDA
Red blood cell membrane defect HS

- Pathologies
- Clinical signs
- 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
• Mostly extravascular haemolysis
  – Jaundice
  – Splenomegaly
  – Biliary lithiasis
Red blood cell membrane defect

- Pathologies
- Clinical signs
- Diagnostic criteria
Diagnostic criteria
RBC membrane pathology

Membrane defect = regenerative except CDAs

CDAs
Diagnostic criteria
Screening tests for HS

• **Disease of the “reticulocyte”**
  – Reticulocytosis with or without anaemia
  – Volume of the reticulocytes
    • MRV ou MCVr
  – Immature Reticulocyte Fraction
    • IRF
Diagnostic criteria
Screening tests for HS

- Ret/IRF (Ann Hematol. 2011;90:759-)

Mai 2015
HS and other conditions affecting the level of erythropoiesis

Comparison of screening tests results in hereditary spherocytosis patients (HS, n=48), controls (n=213, 82 healthy subjects and 131 cryohaemolysis negative without anaemia) and in patients with anaemia of different origins: auto-immune haemolytic anaemia (AIHA, n=7), G6PD deficient patients (n=7), Hb SS (n=5), Hb AS (n=9), beta-thalassemia patients (n=6), and iron deficiency (n=4)

Diagnostic criteria
HE and HPP

HE

HPP
Diagnostic criteria
Screening tests for HS

β-spectrin

Bande 3
Diagnostic criteria
CDA I and II

CDA I

CDA II
Jaundice/Splenomegaly/Biliary lithiasis
Haemolysis ± compensated

- Differential diagnosis
  - **Morphology**
  - **Auto Immune Haemolytic Anaemia – Clinical context - Direct Coombs test**

- Measurement RBC enzymes (G6PD, PK, GPI, ...)
- Hb fractions
- Screen for Hb H

- Enzyme deficiency
  - **Haemoglobinopathy**
    - HbCC, HbSC, ...

- Unstable Hb
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)

EI
Diagnostic criteria
Electrophoresis (SDS-PAGE)

Confirmation of diagnosis (± 60%)
Diagnostic criteria
RBC membrane pathology
Electrophoresis (SDS-PAGE)

CDA II
### Diagnostic criteria

**CDAs**

<table>
<thead>
<tr>
<th>Table 2. General definition criteria of the CDAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Evidence of congenital jaundice/jaundice or a positive family history</td>
</tr>
<tr>
<td>2. Evidence of ineffective erythropoiesis</td>
</tr>
<tr>
<td>3. Typical morphological appearance of bone marrow erythroblasts, and</td>
</tr>
<tr>
<td>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</td>
</tr>
</tbody>
</table>

*Heimpel H, ENERCA white book*

*www.enerca.org*
Hereditary spherocytosis

Clinical features
Personal 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)
# RBC membrane pathology

## Summary: diagnostic criteria

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>SH</strong></td>
<td>Cryohemolysis or EMA, ektacytometry, SDS-PAGE</td>
</tr>
<tr>
<td><strong>EH</strong></td>
<td>Morphology <em>(Parents)</em>, EMA, ektacytometry</td>
</tr>
<tr>
<td><strong>DHSt</strong></td>
<td>Ektacytometry</td>
</tr>
<tr>
<td><strong>OHSt</strong></td>
<td>SDS-PAGE (absence of stomatin)</td>
</tr>
<tr>
<td><strong>Cryohydro.</strong></td>
<td>MCV, MCHC and $K^+$ after 2H on ice</td>
</tr>
<tr>
<td><strong>CDA I</strong></td>
<td>Morphology (BM), molecular diagnosis</td>
</tr>
<tr>
<td><strong>CDA II</strong></td>
<td>SDS-PAGE (no glycosylation of Band 3)</td>
</tr>
</tbody>
</table>
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 \((\text{grade 1 recommendation, grade B evidence})\)
    • National guidelines for immunization; reimmunization when and how? Duration of AB prophylaxis? \((\text{grade 2 recommendation, grade C evidence})\)
    • No indication for extended thrombosis prophylaxis after splenectomy. Adults should receive perioperative thromboprophylaxis in the usual way;
    • N.B. To be avoided in patients with some forms of hereditary stomatocytosis \((\text{grade 1 recommendation, grade B evidence})\).
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 in expert centres
- Treatment
  - Symptoms, complications
  - Eliminate the amplifier (spleen)
Red blood cells enzyme deficiencies
Red blood cell enzyme disorder

• If acute or persistent haemolytic anaemia and
  – Normocytic
  – Regenerative
  – Haemoglobinopathies, membranopathies excluded
  – Negative Coombs test
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
G6PD deficiency

- Favism has been first reported as an allergic reaction.
- In the 50s, US troops going to Korea were preventively treated by primaquine. A significant amount of soldiers became jaundiced and anaemic.
G6PD deficiency

- Drop of Hb about 5 g/dL, nadir at day 7
- Hemoglobinuria at the beginning (Days 1-3)
- Reticulocytes count increases
- Jaundice

- More variable picture with favism
Enzymatic equipment of the RBC

G6PD

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>Central</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>East</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>South Africa</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 report54
• **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)
G6PD deficiency

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

- **Class I (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*
G6PD deficiency
Factors that influence severity of haemolysis

• Drug dose
  – The same dose of a drug gives the same level of haemolysis

• RBC ageing
  – The oldest RBC have the least G6PD and are the first to haemolyse
  – If a drug is given at a high dose, almost all RBC will haemolyse (ageing as well as young). If a lower dose is given, only the ageing RBC will be concerned. For the next dose, RBC with high amount of G6PD will not be concerned.
G6PD deficiency
Factors that influence severity of haemolysis

Proc. Nati. Acad. Sc. USA
Vol. 75, No. 4, pp. 1979-1983, April 1978
G6PD deficiency

- Mostly intravascular haemolysis
  - Acute
  - Fever
  - Lower back pain
  - Renal insufficiency
G6PD deficiency
Drugs that trigger haemolysis

<table>
<thead>
<tr>
<th>Category of drug</th>
<th>Predictable haemolysis</th>
<th>Possible haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimalarials</td>
<td>Dapsone Primaquine Methylen blue</td>
<td>Chloroquine Quinine</td>
</tr>
<tr>
<td>Analgesics/Antipyretic</td>
<td>Phenazopyridine</td>
<td>Aspirin (high doses) Paracetamol (Acetaminophen)</td>
</tr>
<tr>
<td>Antibacterials</td>
<td>Cotrimoxazole Sulfadiazine Quinolones (including nalidixic acid, ciprofloxacin, ofloxacin) Nitrofurantoin</td>
<td>Sulfasalazine</td>
</tr>
<tr>
<td>Other</td>
<td>Rasburicase Toluidine blue</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isoniazid Ascorbic acid Glibenclamide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin K Isosorbide Dinitrate</td>
</tr>
</tbody>
</table>

- Possible haemolysis
  - *Dose related*,
  - *Combination of drugs*
  - *Co-morbidity*

- No drug is tested, no test is available
- In many cases haemolysis was probably triggered by the infection and not the drug.

G6PD deficiency
Biological diagnosis

• Regenerative haemolytic anaemia

• Measuring the enzyme activity

\[
\text{glucose-6-P + NADP}^+ \xrightarrow{\text{G6PD}} 6-P\text{-gluconate + NADPH + H}^+ \\
6-P\text{-gluconate + NADP}^+ \xrightarrow{\text{6PGD}} \text{ribulose-5-P + CO}_2 + \text{NADPH + H}^+ \\
\]

Measure 340 nm

G6PD activity/Reticulocytes

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G6PD</strong> (7.0-17.0)</td>
<td>7.5</td>
<td>9.6</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td><strong>HK</strong> (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 or “control”
- Class I = chronic haemolytic anaemia (rare)
  - No relationship with endemic zones for malarial infection
Iron overload

- NTBI
  - Effective Erythropoiesis (-)
  - Liver damage (+)
  - Splenectomy (+)
- Liver damage (+)
- Ineffective erythropoiesis (+)
- Transferrin
- Transfusion rate (+)
- Haemolysis (-)
- Inflammation (-)
- Ascorbate deficiency (-)

Risk of cardiac iron overload:
- Transfusion-dependent blackfan diamond
- Transfusion-dependent CDA
- Thalassaemia major
- Aplastic anaemia
- Red cell aplasia
- PK deficiency
- Sickle cell disease
- Thalassaemia intermedia
- Hereditary haemochromatosis