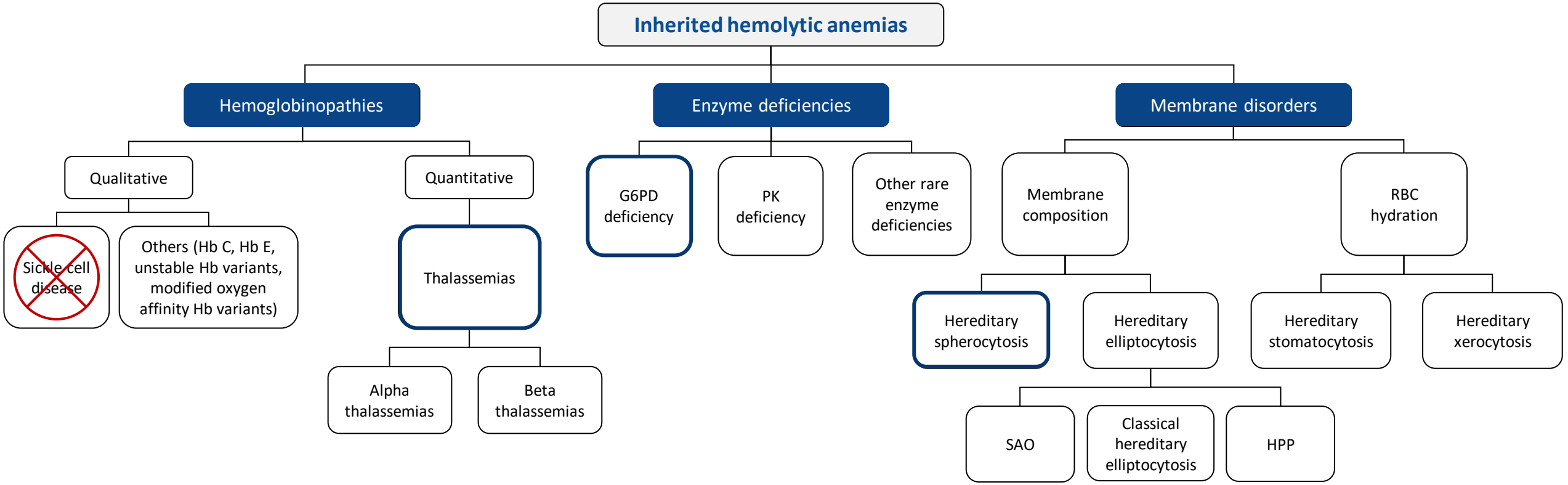
LABORATOIRE HOSPITALIER UNIVERSITAIRE DE BRUXELLES
LHUB-ULB
UNIVERSITAIR LABORATORIUM BRUSSEL

Tips and tricks to diagnose congenital red blood cell disorders

Anne-Sophie Adam

Inherited Hemolytic (Anemias)



HEMOGLOBINOPATHIES

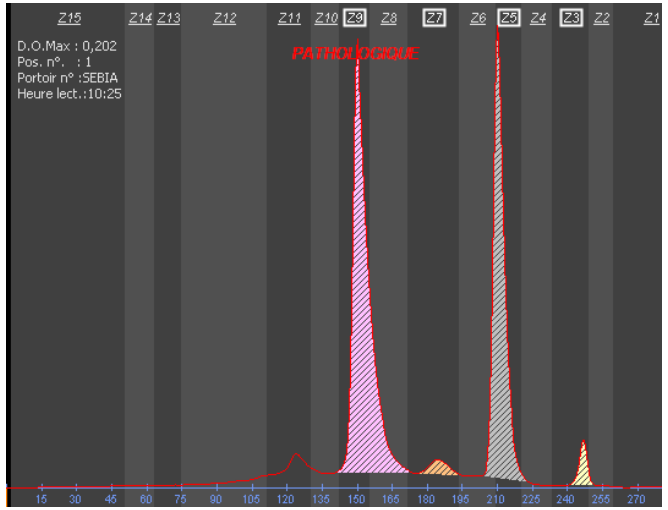
Laboratory techniques

1. Separation and quantification of Hb fractions
2. If presence of Hb variant
   - Use of a second method based on a different separation principle
3. Give a conclusion
   - Interpretation of the different techniques

- Eurobloodnet recommandations: http://www.eurobloodnet.com/best-practices/guidelines-repository/16/prevention-and-diagnosis-of-haemoglobinopathies-a-short-guide-for-health-professionals-and-laboratory-scientists
- Traeger-Synodinos, J., et al. (2015). "EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies." Eur J Hum Genet 23(4): 426-437.

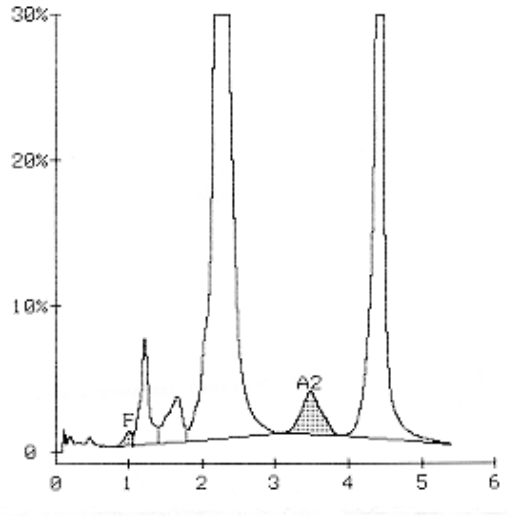
Laboratory techniques

- **Capillary electrophoresis**



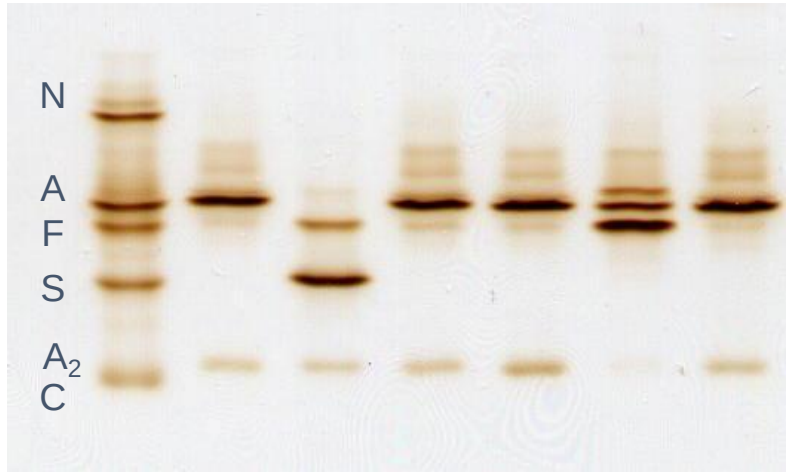
Quantitative

- **HPLC**



Quantitative

- **Isoelectric focusing (IEF)**



Qualitative

- Each technique is based on a **different principle of separation of Hb fractions**
  - ➢ Variable sensitivity and specificity

Laboratory techniques

- **Important to use a quantitative method** to determine:
  - **Hb variant** level
  - **Hb A2** level
  - **Hb F** level

Table 1. Conditions in which Hb F is raised

Physiological
Neonates
Pregnancy
Hereditary
δβ thalassaemia
β thalassaemia major and intermedia
β thalassaemia trait (sometimes)
Hereditary persistence of fetal haemoglobin
Sickle cell anaemia ± treatment with hydroxycarbamide (hydroxyurea)
Unstable β chain variants
Acquired (Hb F sometimes raised)
Recovery from bone marrow hypoplasia
Leukaemia
Myelodysplasia
Thyrotoxicosis
Hepatoma

Table 2. Main pre-analytical subject-related factors that may ***increase*** Hb A_2 levels

Thalassaemic syndromes	Heterozygous β-thalassaemia
Other haemoglobinopathies	Artefact in the presence of Hb S
	Some haemoglobin variants with thalassaemic phenotype
Acquired conditions	Hypertrophic osteoarthropathy
	Megaloblastic anaemia
	Pseudoxanthoma elasticum
	Hyperthyroidism
Treatment-related situations	Antiretroviral therapy in patients with HIV

Table 3. Main pre-analytical subject-related factors that may normalize or ***decrease*** Hb A_2 levels

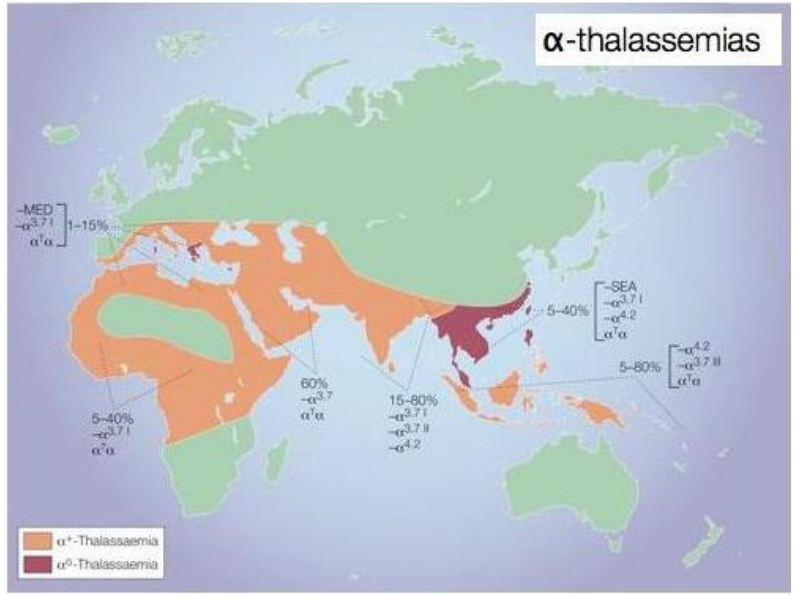
Thalassaemic syndromes	'Silent' β-thalassaemia alleles
	Interaction between δ- and β-thalassaemia (δ+β-thalassaemia)
	δβ-thalassaemia
	Hb H disease and other α-thalassaemias
Other haemoglobinopathies	α-chain variants
	δ-chain variants
Acquired conditions	Erythroleukaemia
	Severe iron-deficiency anaemia
	Sideroblastic anaemia

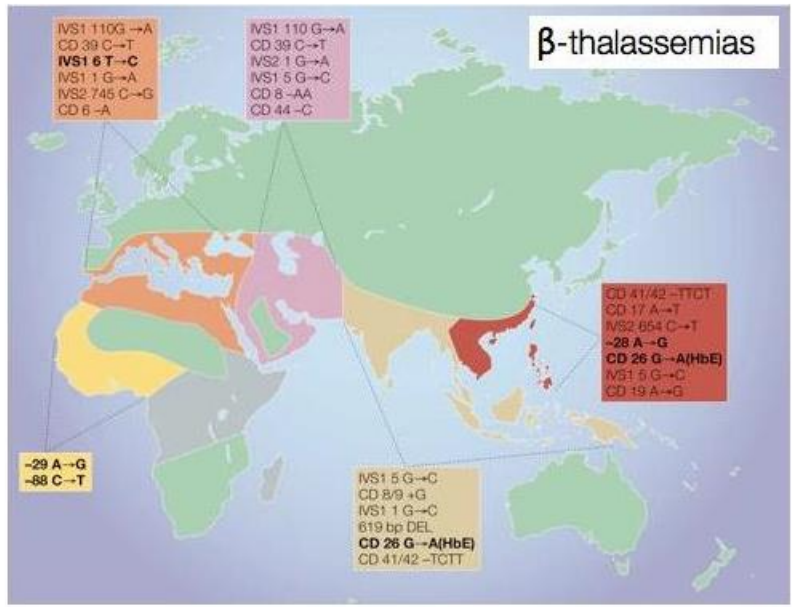
Int. Jnl. Lab. Hem. 2012, 34, 1-13
Int. Jnl. Lab. Hem. 2012, 34, 14-20

Thalassemias

- Among the **most common genetic disorders in the world:**
  - **± 5% of the world population** has at least one thalassemia variant allele
  - Approx. **56,000 infants** born annually with major thalassemia

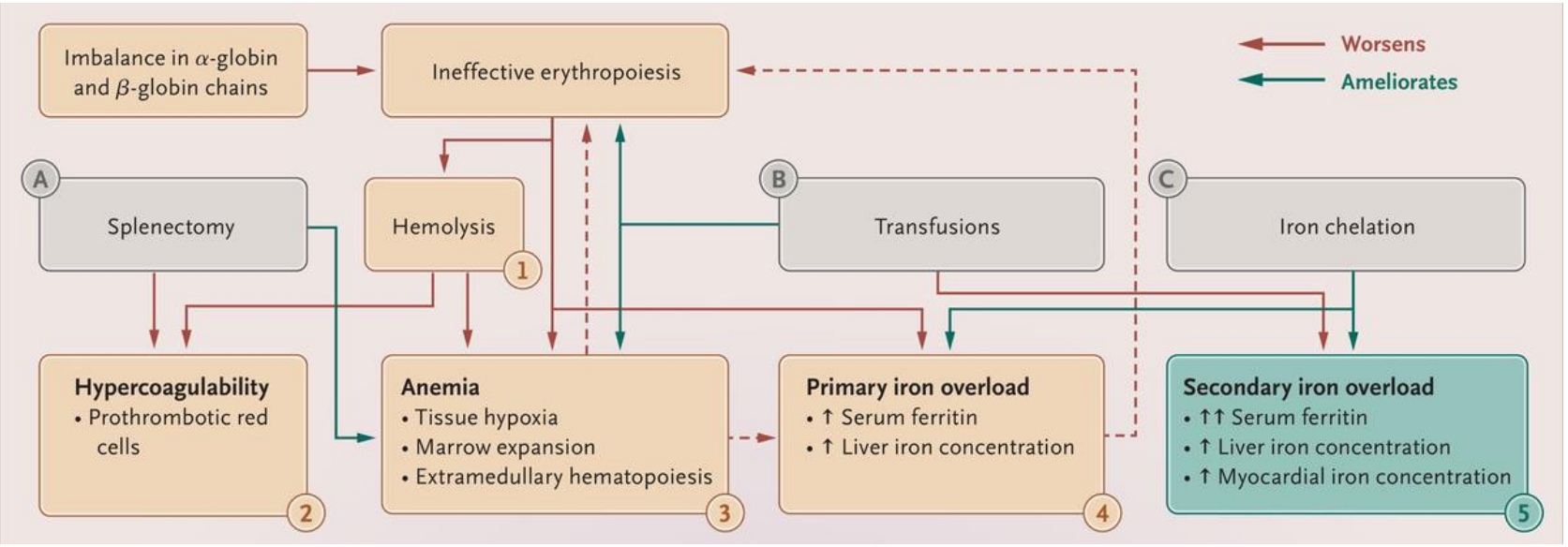
- **Epidemiology:**
  - Prevalent from sub-Saharan Africa, through the Mediterranean region and Middle East, to the Indian subcontinent and East / South-East Asia
  - Increased incidence in our country due to migration of groups with a high frequency of thalassemic mutations





Thalassemias

- **Physiopathology:**
  - Due to genetic defects in the α- or β-globin genes
  - Precipitation of the unpaired chains → Destruction of RBC precursors:
    - In the bone marrow = Ineffective erythropoiesis
    - In the circulation = Hemolysis



Thalassemias

- **Thalassemia syndromes:** Highly complex

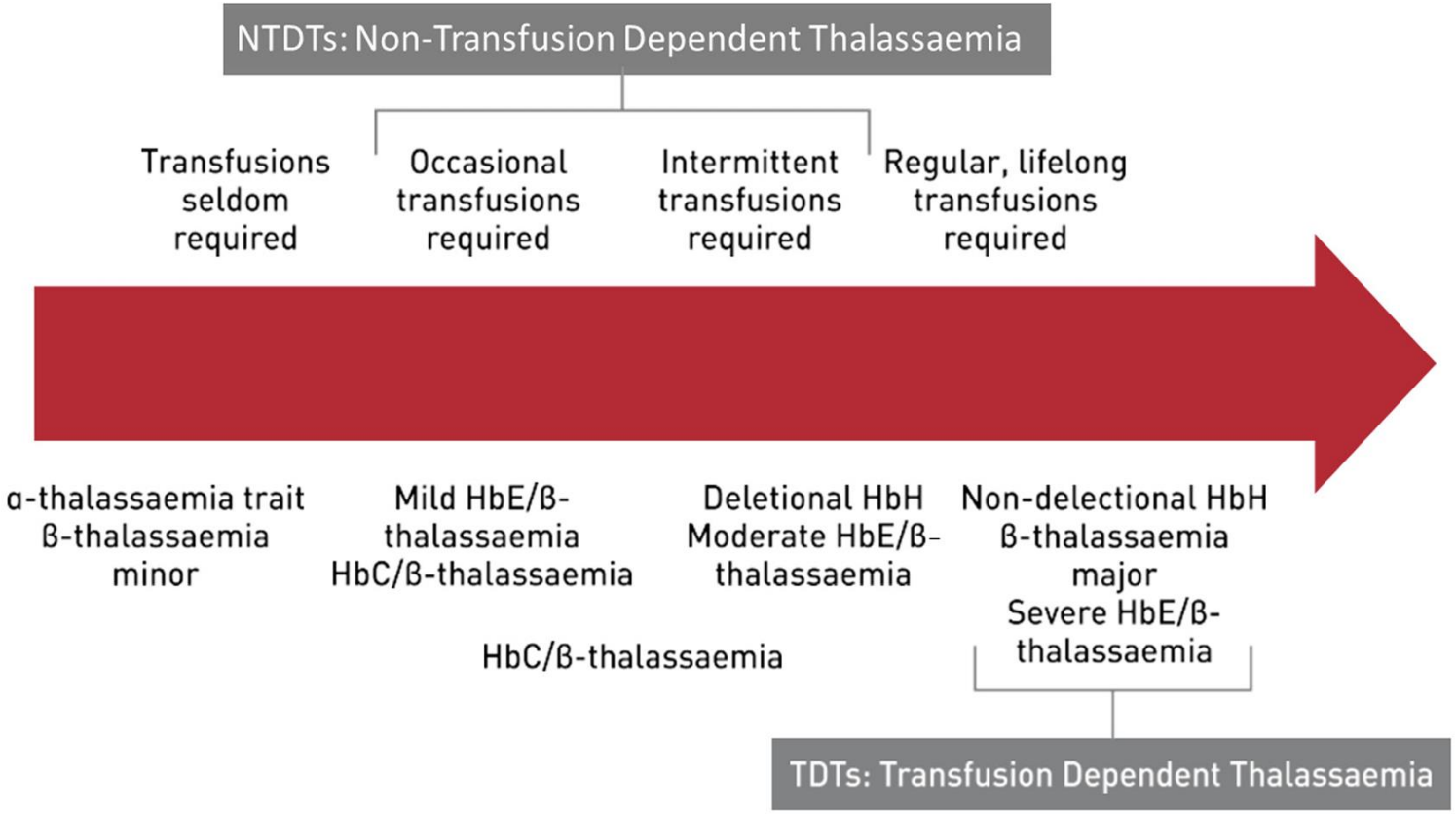
Disorder	Genotype	MCV	Anemia	
Alpha thalassemia				
silent carrier	α α / α -	nl	none	NTDT
minor	α α / - - or α - / α -	low	mild	
HbH disease	α - / - -	low	moderate	NTDT or TDT
Bart's syndrome	- - / - -	low	fatal	
Beta thalassemia				
minor	β / β° or β / β^+	low	mild	NTDT
intermedia	β^+ / β^+ or β° / β^+	low	moderate	NTDT or TDT
major	β° / β° or β° / β^+	low	severe	TDT

Genotype interaction	*Disorder expected*
Homozygous	
β° or severe β^+-thalassaemia	Thalassaemia major
Mild β^+-thalassaemia	Thalassaemia intermedia
Mild β^{++}-thalassaemia (silent)	Very mild thalassaemia intermedia
$\delta\beta^\circ$-thalassaemia	Thalassaemia intermedia
Hb Lepore	Thalassaemia intermedia to major (variable)
HPFH	Not clinically relevant
Hb C	Not clinically relevant
Hb D-Punjab	Not clinically relevant
Hb E	Not clinically relevant
Hb O-Arab	Not clinically relevant
Compound heterozygous	
β°/severe β^+-thalassaemia	Thalassaemia major
Mild β^+/β° or severe β^+-thalassaemia	Thalassaemia intermedia to major (variable)
Mild β^{++}/β° or severe β^+-thalassaemia	Mild thalassaemia intermedia (variable)
$\delta\beta^\circ/\beta^\circ$ or severe β^+-thalassaemia	Thalassaemia intermedia to major (variable)
$\delta\beta^\circ$/mild β^+-thalassaemia	Mild thalassaemia intermedia
$\delta\beta^\circ$/Hb Lepore	Thalassaemia intermedia
Hb Lepore/β° or severe β^+-thalassaemia	Thalassaemia major
Hb C/β° or severe β^+-thalassaemia	β-thalassaemia trait to intermedia (variable)
Hb C/mild β^+-thalassaemia	Not clinically relevant
Hb D-Punjab/β° or severe β^+-thalassaemia	Not clinically relevant
Hb E/β° or severe β^+-thalassaemia	Thalassaemia intermedia to major (variable)
Hb O-Arab/β°-thalassaemia	Severe thalassaemia intermedia
$\alpha\alpha\alpha/\beta^\circ$ or severe β^+-thalassaemia	Mild thalassaemia intermedia
$\alpha\alpha\alpha\alpha/\beta^\circ$ and $\alpha\alpha\alpha\alpha\alpha\alpha/\beta^\circ$-thalassaemia	Mild to severe thalassaemia intermedia (variable)

Thalassemias

- **Classification:** based on clinical severity and transfusion requirement

2021 Thalassaemia International Federation Guidelines for the Management of Transfusion dependent Thalassemia. HemaSphere6(8):e732, August 2022.



Thalassemias

- **Clinical manifestations:**

  - Range from **asymptomatic carrier status** to **profound abnormalities** including
    - Severe anemia
    - Extramedullary hematopoiesis
    - Skeletal and growth deficits
    - Iron overload

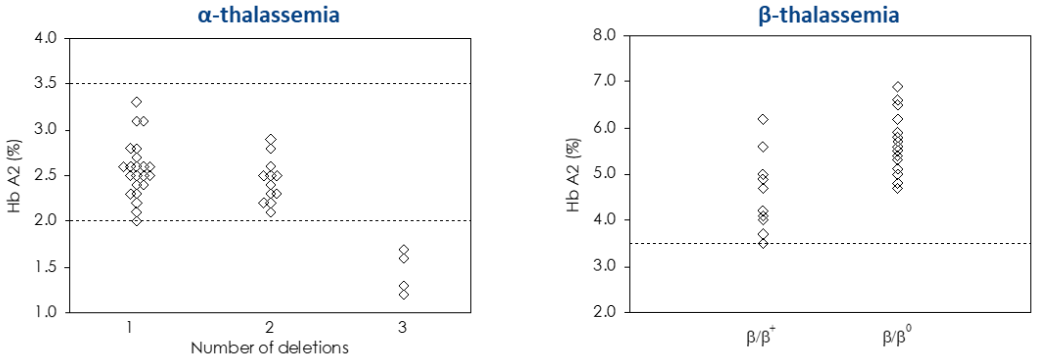
    ⇨ Dramatically shortened life expectancy in the absence of aggressive treatment

  - **Severity** correlates with:
    - The number of functioning globin chains that are lost
    - The ratio of alpha to beta chains

Thalassemias

Thalassemia should be consider in all those who have hypochromic microcytic anemia after exclusion of IDA

- **Diagnostic:**
  - **Hematological parameters:** RBC indices and morphology
  - **Phenotype**: Quantitative separation of Hb fractions
    - **β-thalassemia:** Hb A2 > reference values
    - **α-thalassemia:** Hb A2 ± reference values
      - (except in Hb H disease)

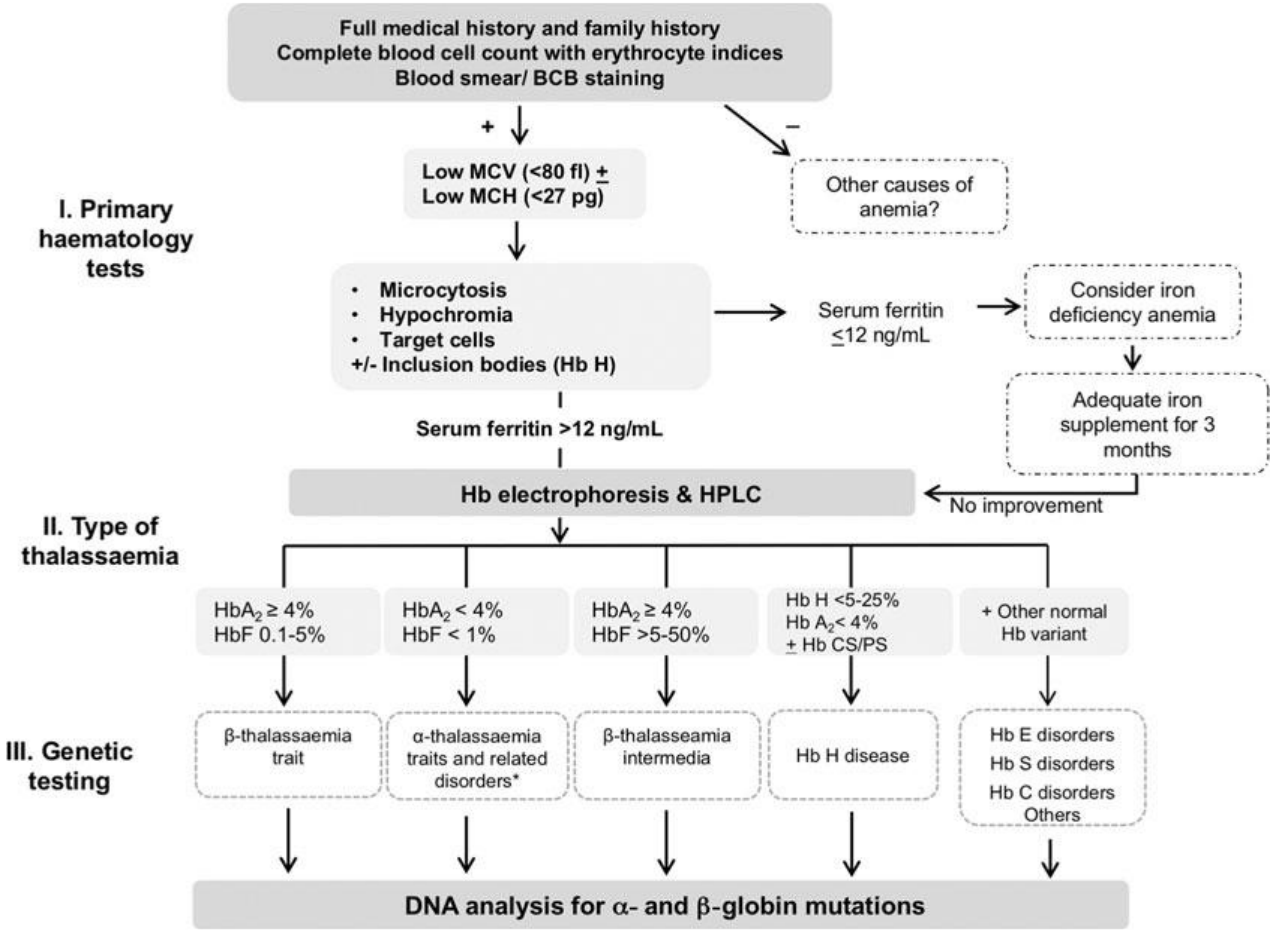


		β-TM	β-TI	HβE / βThal		HbH
Hb levels		<5g/dL	~7 – 10 g/dL	Mild	9 – 12 g/dL	2.6 – 13.3 g/dL
				Moderately/ Severe	6 – 7 g/dL	
				Severe	4 – 5 g/dL	
BLOODSMEAR	Low Hb Production	Red cell hypochromia microcytosis, Target cells				
	Haemolysis	Irregularly crenated RBC, increased reticulocytes [5 – 10%]				
	Ineffective erythropoiesis	Nucleated RBC, Basophilic stripling				
	Special Features	+Numerous F-cells/ Acid elution	+F-cells/ Acid Elution	+DCIP staining [HbE] +F-cells/ Acid Elution		HbH inclusion bodies
Haemoglobin study		HbF up to 100% HbA2↑	HbF 10 – 50% [up to 100%] HbA2 > 4%	HbE [40 – 60%] HbF [60 – 40%] ±HbA [with β-thal] HbA2↑		Variable HbH [0,8 – 40%] HbA2↓ the presence of α-variants e.g. Hb CS, Hb PS etc.
DNA analysis		• Common known mutations of both β0 and β – thal mutations in population specific set can be done by PCR based methods. • For rare or unusual mutations, direct sequencing or array analysis is required • Other analysis for β-TI included α- and β- globin rearrangements, Xmn I polymorphism and other QTLs for γ-globin expression				Gap-PCR developed for 7 common α-thal deletions and RDB for non-deletional mutations. For unknown mutations, MLPA analysis and sequencing required

Summary of diagnostic methods for thalassemia and hemoglobinopathies. DCIP = dichlorophenolindophenol; Hb = hemoglobin; MLPA = multiplex ligation-dependent probe amplification. QTL = quantitative locus; PRC = polymerase chain reaction; RBC = red blood cells; RDB = reverse dot blot; TI = thalassemia intermedia; TM = thalassemia major.

2021 Thalassaemia International Federation Guidelines for the Management of Transfusion dependent Thalassemia. HemaSphere6(8):e732, August 2022.

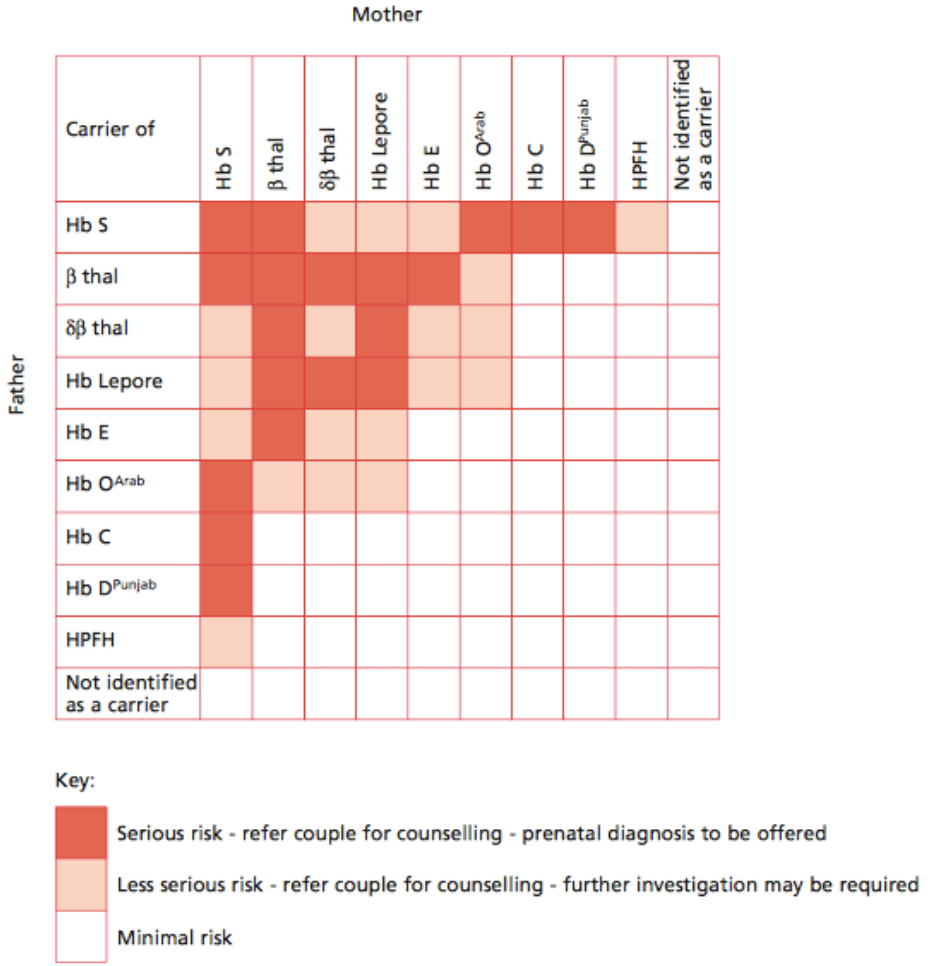
Thalassemias



IJLH 2016, 38, 32-40

Thalassemias

- **Diagnostic:**
  - **Genotype:**
    - **Not required** to confirm the diagnosis of **β-carrier**
    - But **ALWAYS** in **prenatal diagnosis**
    - Necessary to confirm the **α-thalassemia carrier status** (genetic counselling)
    - Other situations: to be discussed



Thalassemias

- **Treatment:**

  - **Blood transfusions:** decided upon the following criteria
    - Confirmed diagnosis of thalassemia
    - **Laboratory criteria:** **Hb < 7 g/dL** on 2 occasions, > 2 weeks appart (excluding all other contributory causes such as infections)

      Or
    - **Clinical criteria irrespective of Hb level:** **Hb > 7 g/dL** with any of the following
      - Significant symptoms of anemia
      - Poor growth/failure to thrive
      - Complications from excessive intramedullary hematopoiesis such as pathological fractures and facial changes
      - Clincially significant extramedullary hematopoiesis

  - **Iron chelation**

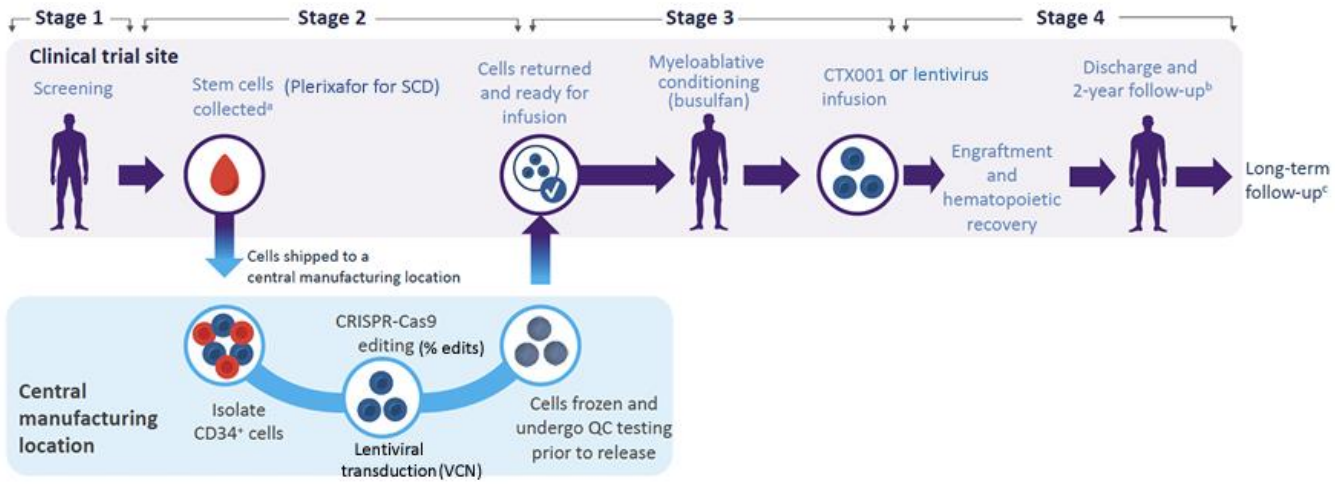
Thalassemias

- **Treatment:**

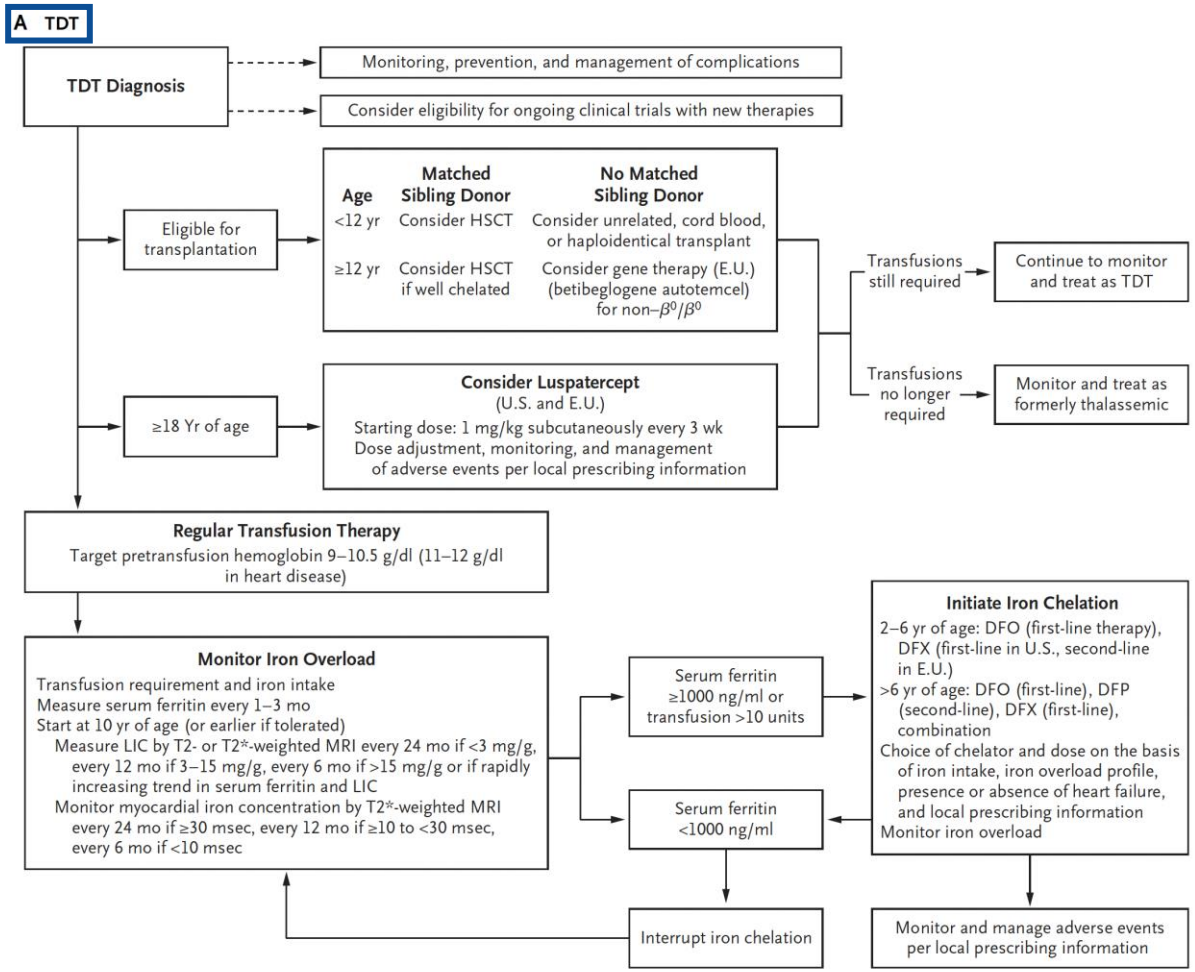
  - **Allogenic hematopoietic stem cell transplantation:** only curative treatment

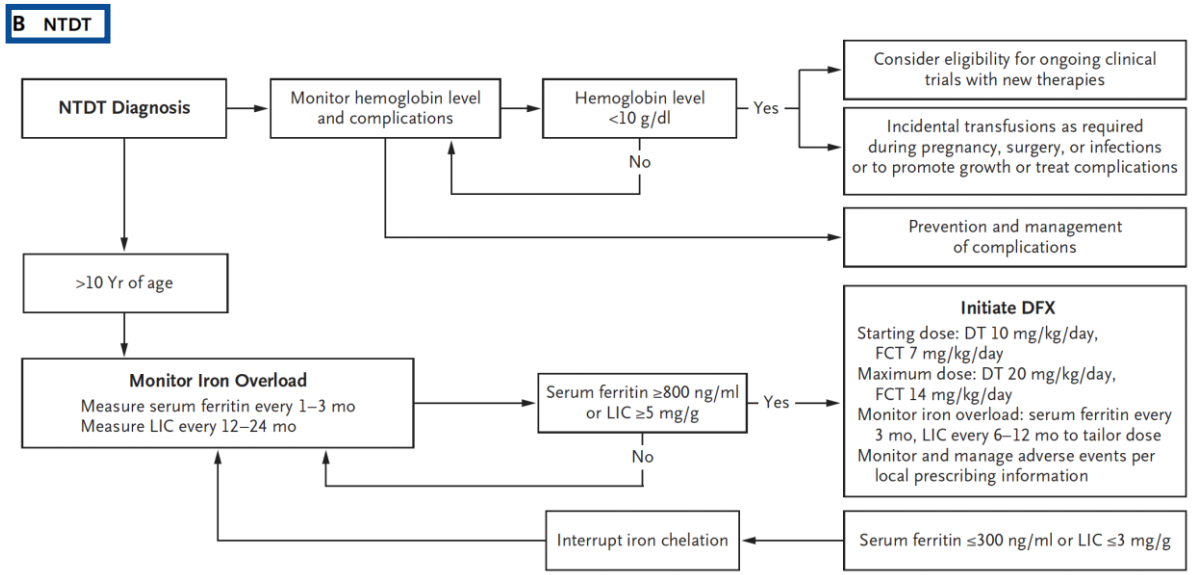
  - **Lustapercept:**
    - Enhances erythroid maturation → Reduction in transfusion burden
    - Only for patients > 18 y.o.

  - **Gene therapy:** gene addition or gene editing



Thalassemias





The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

β-Thalassemias

Ali T. Taher, M.D., Ph.D., Khaled M. Musallam, M.D., Ph.D., and M. Domenica Cappellini, M.D.

N ENGL J MED 384;8 NEJM.ORG FEBRUARY 25, 2021

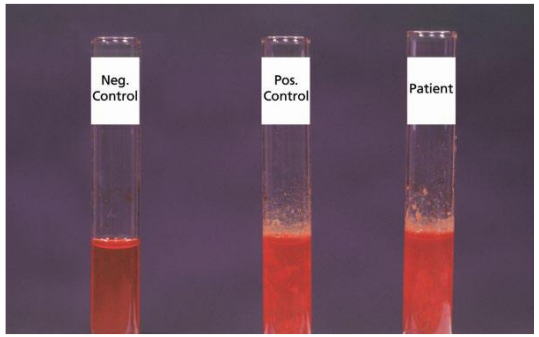
Unstable Hemogloin Variants

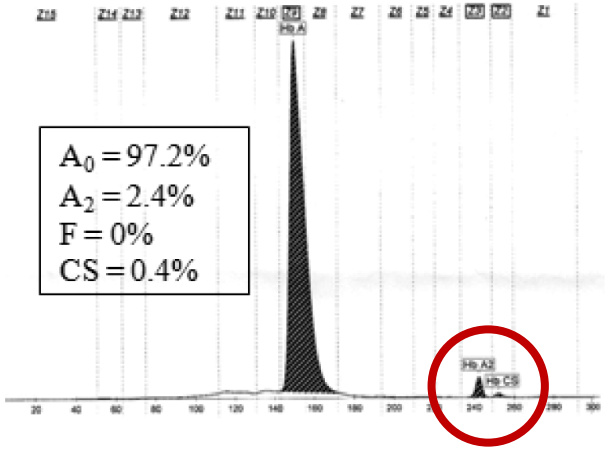
- Group of disorders characterized by **clinical, laboratorial and genetic heterogeneity**:
  - Approx. 150 Hb variants
  - Chronic or episodic hemolysis
  - Mild, moderate or severe hemolytic anemia (depending on the severity of the molecular defect)

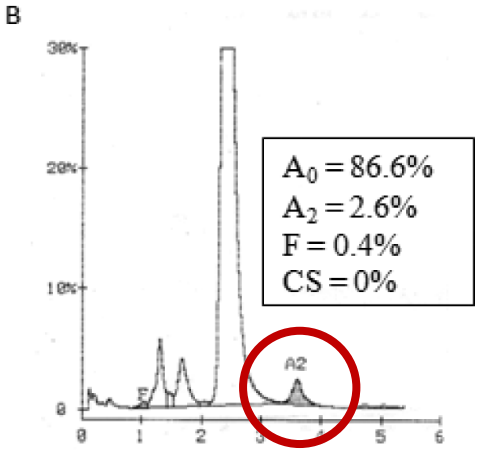
No Hb variant
≠
No Hbpathies

- They undergo **rapid denaturation, precipitation and degradation within the RBC**
  - **Misleading** electrophoretic results

- **Diagnosis of unstable Hb variants:**
  - **Blood smear**: Heinz bodies
  - **Laboratory tests**:
    - Heat test
    - Isopropanol test
  - **Genetic analysis**







Hemoglobinopathies

Public Health England

NHS

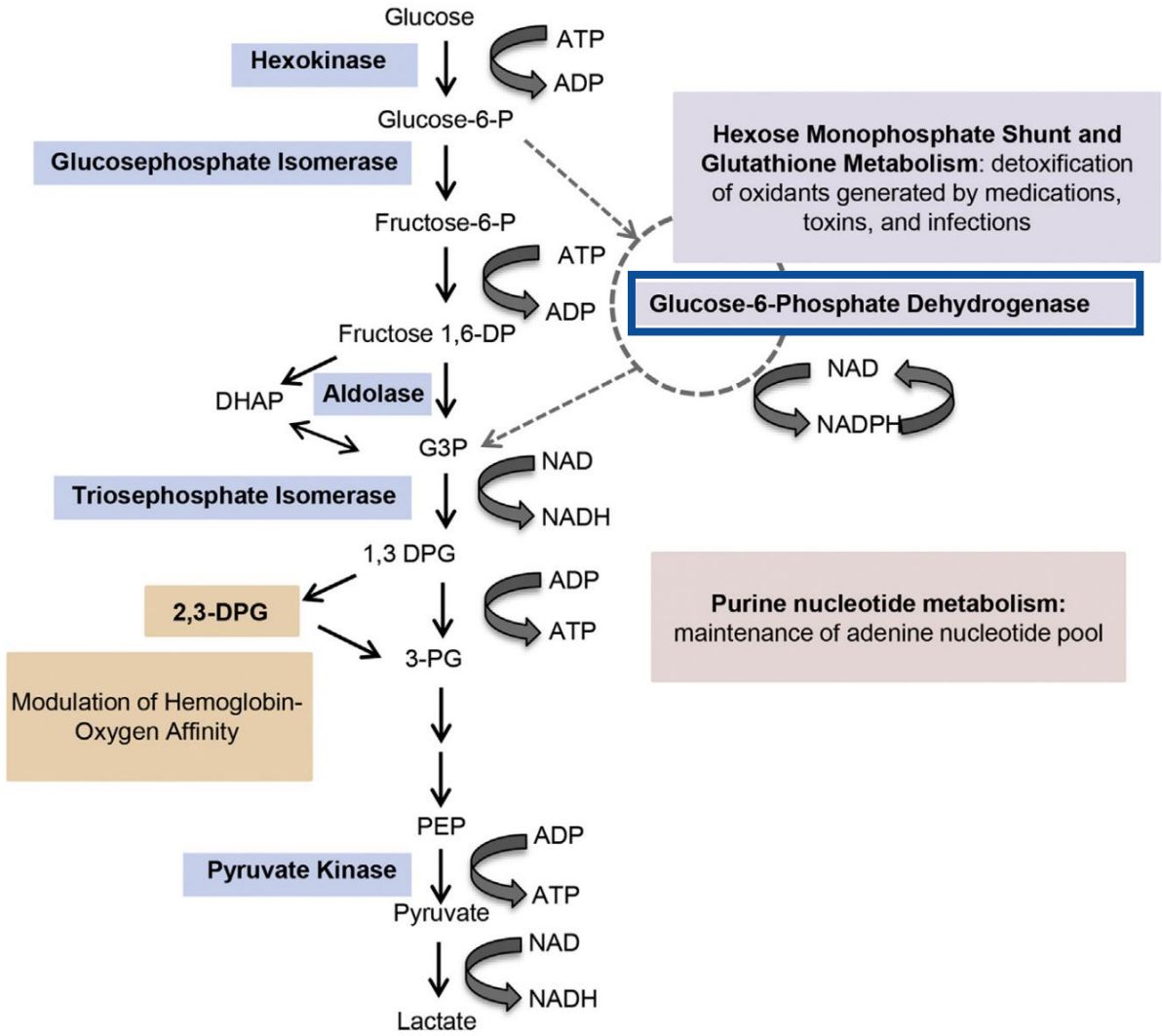
NHS Sickle Cell and Thalassaemia Screening Programme

Handbook for antenatal laboratories

https://www.gov.uk/government/publications/sct-screening-handbook-for-antenatal-laboratories

RBC ENZYME DEFICIENCIES

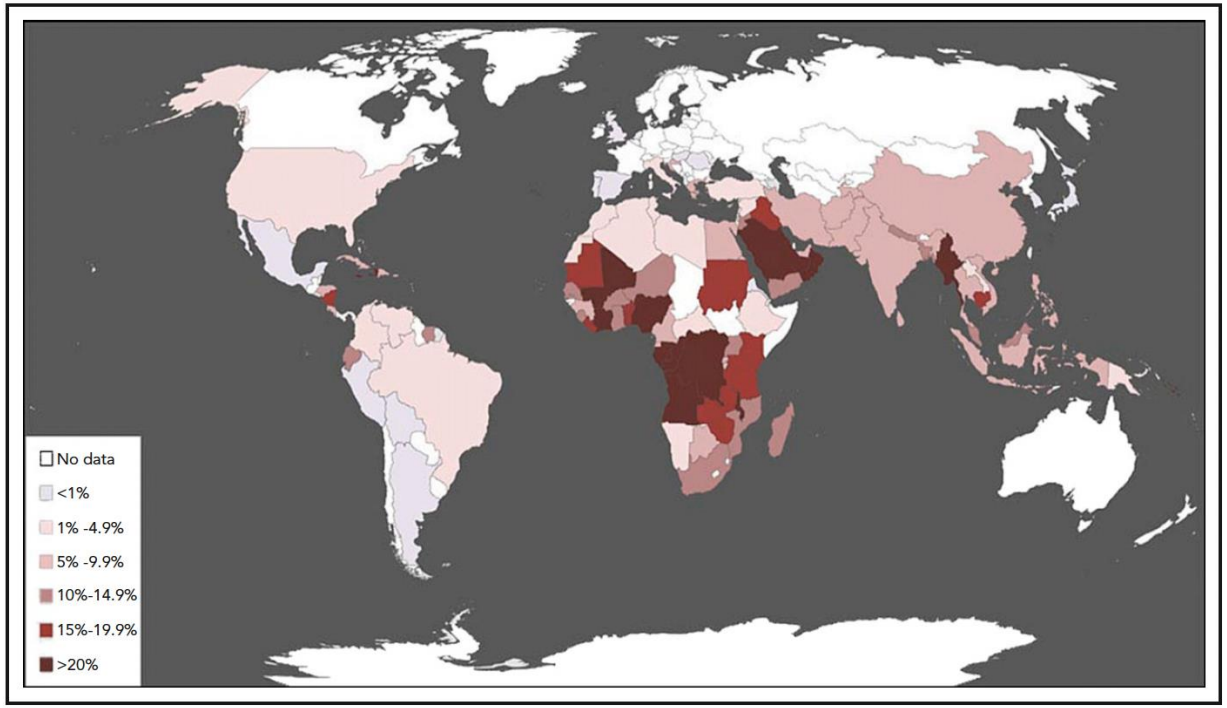
RBC Enzymes


Glucose
Hexokinase
ATP
ADP
Glucose-6-P
Glucosephosphate Isomerase
Fructose-6-P
ATP
ADP
Fructose 1,6-DP
DHAP
Aldolase
G3P
Triosephosphate Isomerase
NAD
NADH
1,3 DPG
2,3-DPG
ADP
ATP
3-PG
Modulation of Hemoglobin-Oxygen Affinity
PEP
Pyruvate Kinase
ADP
ATP
Pyruvate
NAD
NADH
Lactate
Hexose Monophosphate Shunt and Glutathione Metabolism: detoxification of oxidants generated by medications, toxins, and infections
Glucose-6-Phosphate Dehydrogenase
NAD
NADPH
Purine nucleotide metabolism: maintenance of adenine nucleotide pool

G6PD Deficiency

- **Most common enzymatic disorder of RBCs:**
  - Affecting **400 to 500 million** people worldwide
  - **Global distribution** but more common in areas in which malaria is endemic
    - Selective advantage against infection by *P. falciparum*

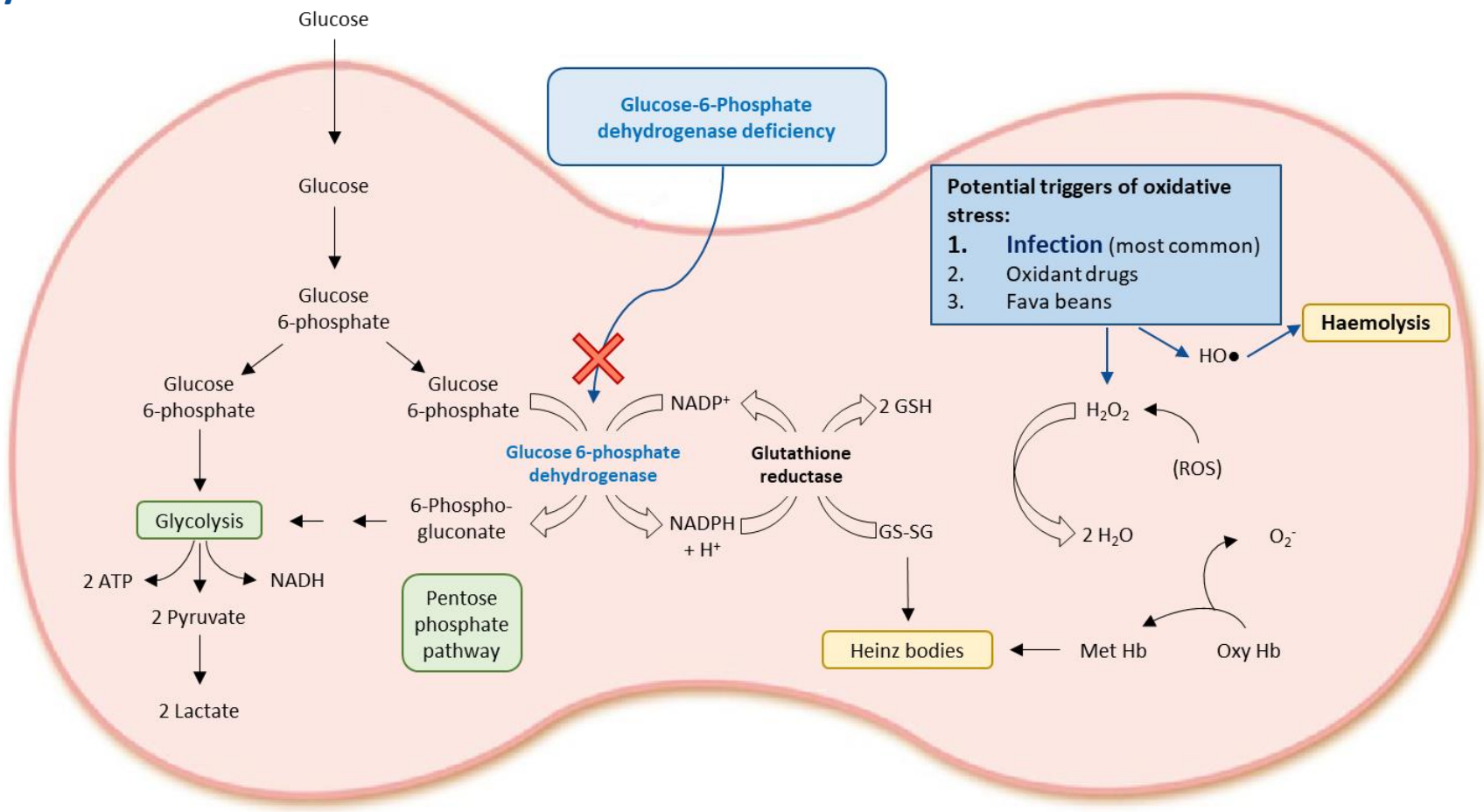
- **Mode of inheritance: X-linked** disorder
  - Men are more commonly affected than women



Blood. 2020, 136(11):1225-1240

G6PD Deficiency

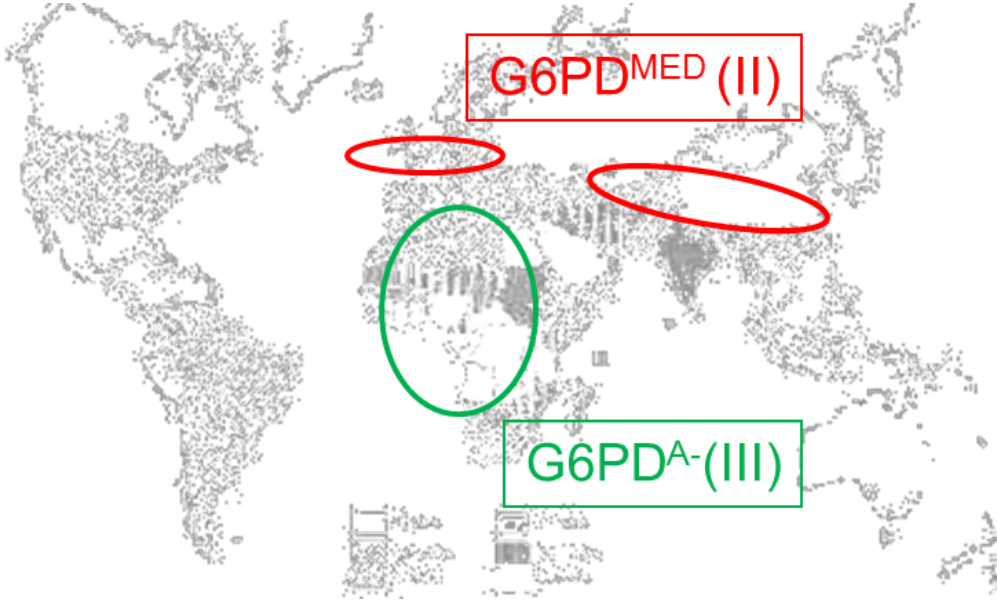
- **Physiopathology:**



G6PD Deficiency

- **Clinical expression:** from **asymptomatic** to **episodic** to **chronic hemolysis**
  - Depends on the degree of the enzyme deficiency
  - Which is determined by the characteristics of the G6PD variant

- **3 clinical entities:**
  - Neonatal hyperbilirubinemia
  - Acute hemolytic anemia (drugs, infections, fava beans)
  - Chronic non-spherocytic hemolytic anemia (class I variants)



G6PD Deficiency

- **Classification of G6PD variants:**

Malaria Policy Advisory Group Meeting
23—24 March 2022, Geneva, Switzerland
Background document for Session 2

World Health Organization

LHUB-ULB

Technical consultation to review the classification of glucose-6-phosphate dehydrogenase (G6PD)

25 & 27 January 2022, virtual meeting

Current World Health Organization (WHO) classification and guidance

The first international WHO meeting on G6PD was convened in December 1966, when just 20 G6PD variants had been described according to their biochemical characteristics, such as percent activity (measured by gold standard spectrophotometric assay), electrophoretic mobility (K_m) value, activity on substrate analogues, pH optimum, and thermostability *(2)*. This meeting proposed that an indication be given for each variant in terms of the enzyme activity in males. This led to a proposed classification published by Yoshida et al. *(3)*. WHO convened a Working Group on G6PD in 1985, which made some minor modifications to the Yoshida classification *(4)*. This modified classification remains in use today.

G6PD classification	Level of residual enzyme activity (% of normal)
Class I (Severe enzyme deficiency with CNSHA)	<10% with CNSHA
Class II (Severe)	<10%
Class III (Moderate to mild)	10–60%
Class IV (Very mild or no enzyme deficiency)	60–150%
Class V (Increased enzyme activity)	more than twice normal

Revised classification

In future, G6PD variants should be classified based on the median residual enzyme activity in male hemizygous individuals for each variant expressed as percentage of normal activity as follows:

WHO classification of G6PD variants in homozygous and hemizygous individuals		
Class	**Median of G6PD Activity**	**Haemolysis**
A	<20%	Chronic (CNSHA)
B	<45%	Acute, triggered
C	60–150%	No haemolysis
U	Any	Uncertain clinical significance

G6PD Deficiency

- **Severity** and **course** of **acute hemolytic episode** depend on:
  - G6PD variant
  - Type and duration of oxidative stress

  + Age & Coexisting disease conditions

- Only a **few drugs** with well-documented causal relationship
  - No test available: *in vitro ≠ in vivo*
  - Individual drug metabolization

- Hemolysis most frequently related to the **infection** than to the drug
  - Treat the infection
  - Change the treatment

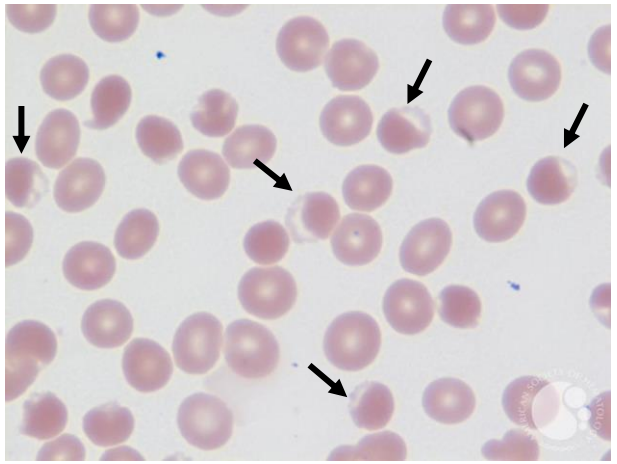
Category of drug	Predictable hemolysis	Possible hemolysis
Antimalarials	Dapsone Primaquine Pamaquin Tafenoquine Methylene blue	Chloroquine Quinine
Analgesics/Antipyretic	Phenazopyridine	Aspirin (high dose) Paracetamol (Acetaminophen)
Antibacterials	Cotrimoxazole Sulfadiazine Quinolones Nitrofurantoin	Sulfasalazine
Other	Rasburicase Toluidine blue Niridazole Pegloticase	Chloramphenicol Isoniazid Ascorbic acid Glibenclamide Vitamin K (Menadione) Isosorbide Dinitrate

BJH 2020, 189, 24-38

G6PD Deficiency

- **Diagnosis:**

  - **During acute hemolytic episode: blood smear** (Bite cells, Heinz bodies) and **biological parameters of hemolysis**



  - **G6PD activity:**
    - **Qualitative: screening tests**
      - Not reliable for female patients
      - Suitable for emergency screening for G6PD deficiency
        - (eg. administration of rasburicase prior to initiation of chemotherapy)
    - **Quantitative : spectrophotometric assay**
      - Should always follow an abnormal/borderline qualitative test
      - Normal if > 60% (steady-state)

G6PD Deficiency

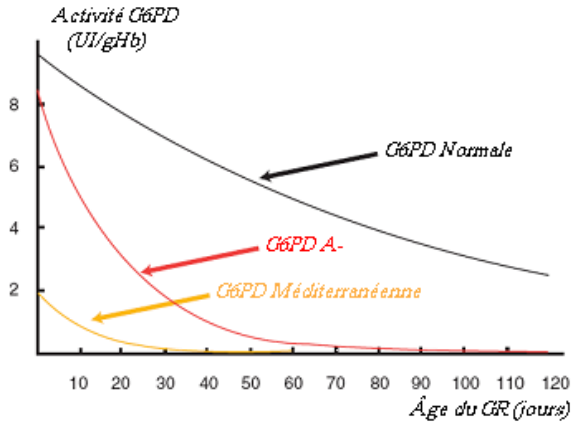
- **Diagnosis:**

  - **Pitfalls of quantitative methods:**

    - Final G6PD activity should be **interpreted in light of the reticulocyte count and/or other RBCs enzyme levels** (HK, PK, 6PGD) measured <u>on the same sample</u>
      - Young RBCs/Reticulocytes have much higher G6PD activity than mature RBCs

    - **Acute hemolytic episode:** RBCs with the most severely reduced G6PD activity will have hemolyzed
      - May result in N or ↑ enzyme levels and a false-normal result
      - Test should be repeated 2-3 months after resolution

    - **Samples with very low MCH (< 25 pg):** may give G6PD activity above the reference range → Caution with values at the lower end of the range



https://doi.org/10.1016/j.jpp.2021.04.001

/!
Sample conservation

<u>Half-life</u>:
Normal: 62 days
A-: 13 days
Med: 8 days

G6PD Deficiency

- **Diagnosis:**

  - **Molecular analysis :** if
    - Results of initial diagnostic procedures are **equivocal or borderline**
      - (Heterozygous) woman
      - Male individuals with Klinefelter syndrome (XXY)
    - To confirm the condition in a **recently transfused patient**
    - **Type I** G6PD deficiency
    - **Sickle cell patients**

G6PD Deficiency

- **Management:**

➢ **Neonatal hyperbilirubinemia :**

- **Moderate:** phototherapy
- **Severe:** may require exchange transfusion

➢ **Acute hemolytic episodes: Make the diagnosis** and

- **Remove any inciting agent(s)**
- If severe anemia: **Blood transfusion**
- Recommendations cut-off: Hb 7 g/dL
  - If rapid decrease in Hb and hemoglobinuria: cut-off Hb 9 g/dL
  - If acute renal failure: Hemodialysis might be required

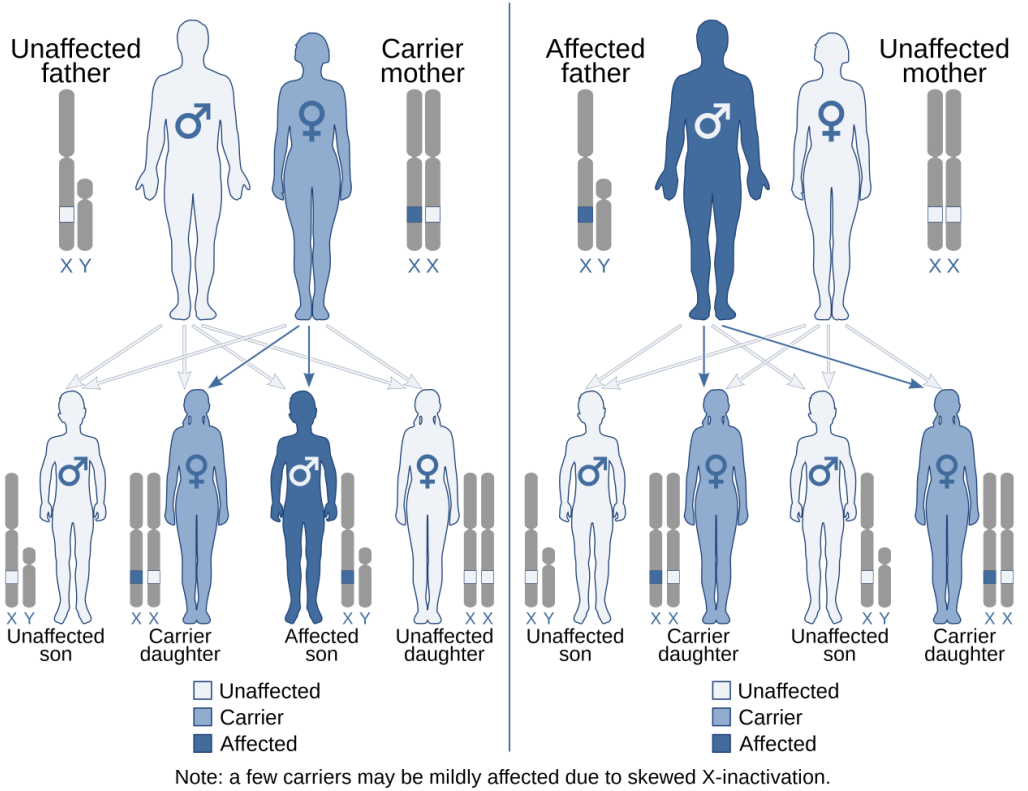
➢ **Chronic hemolysis:**

- **Chronic transfusions**
- **Folic acid**
- **Symptomatic treatments** (cholecystectomy, iron chelation)

G6PD Deficiency

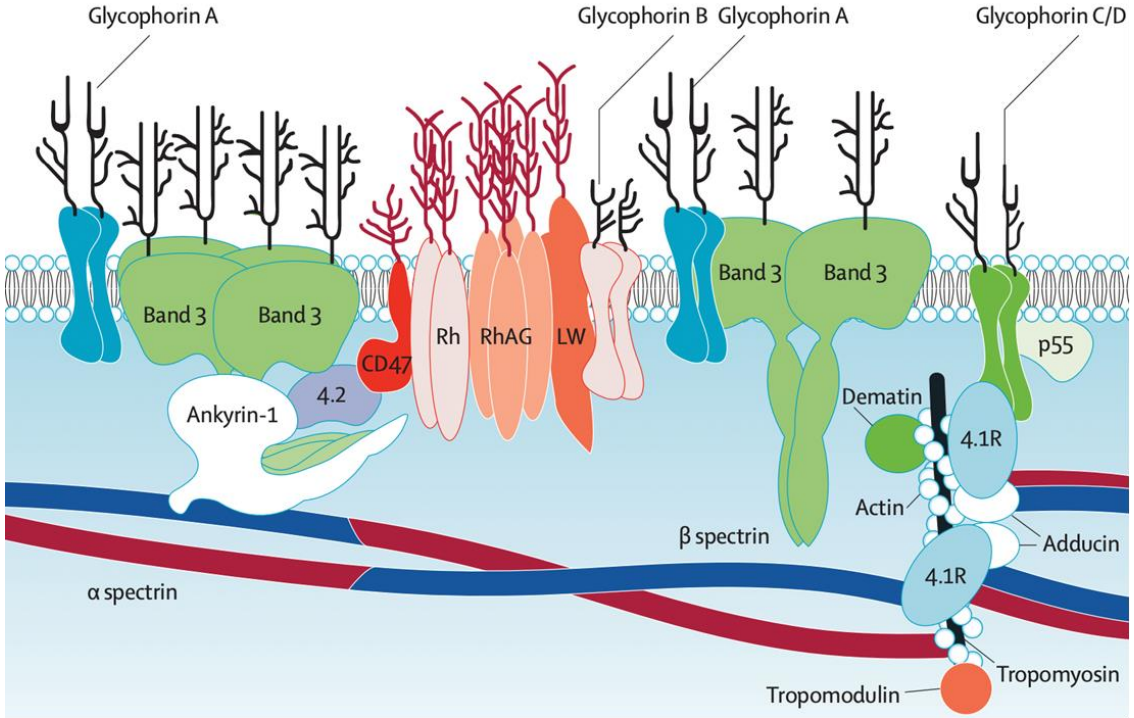
- **Prevention:**
  - ➢ Avoidance of **unsafe drugs and chemicals**
  - ➢ **Dietary restrictions:** Fava beans
    - Variable sensitivity: G6PD MED & Canton +++

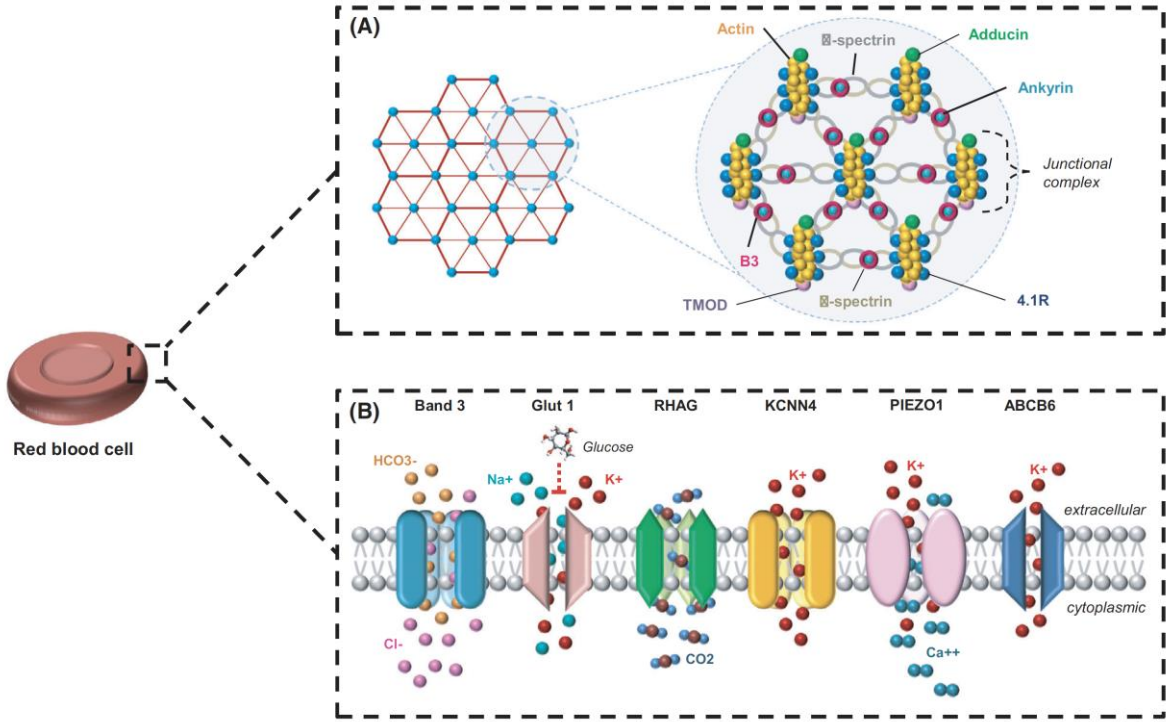
- **Genetic counseling:** X-linked disorder



MEMBRANE DISORDERS

Red Cell Membrane





Hereditary Red Cell Membrane Disorders

Structural Disorders

Hereditary Spherocytosis (HS)

Hereditary Elliptocytosis (HE)

Hereditary Pyropoikilocysosis (HPP)

Membrane Transport Disorders

Overhydrated Hereditary Stomatocytosis (OHSt)

Dehydrated Hereditary Stomatocytosis (DHSt)*

Familial Pseudohyperkalaemia (FP)

Cryohydrocytosis (CHC)

Southeast Asian Ovalocytosis (SAO)

*Hereditary Xerocytosis (HX)

Hereditary Spherocytosis

- First described in **1871** as **microcythemia** in a case history by 2 Belgian physicians

- **Most common inherited hemolytic anemia**:
  - **Prevalence**: 1/2.000 – 1/5.000 in Nothern Europe
  - Probably **higher** (undiagnosed mild cases)

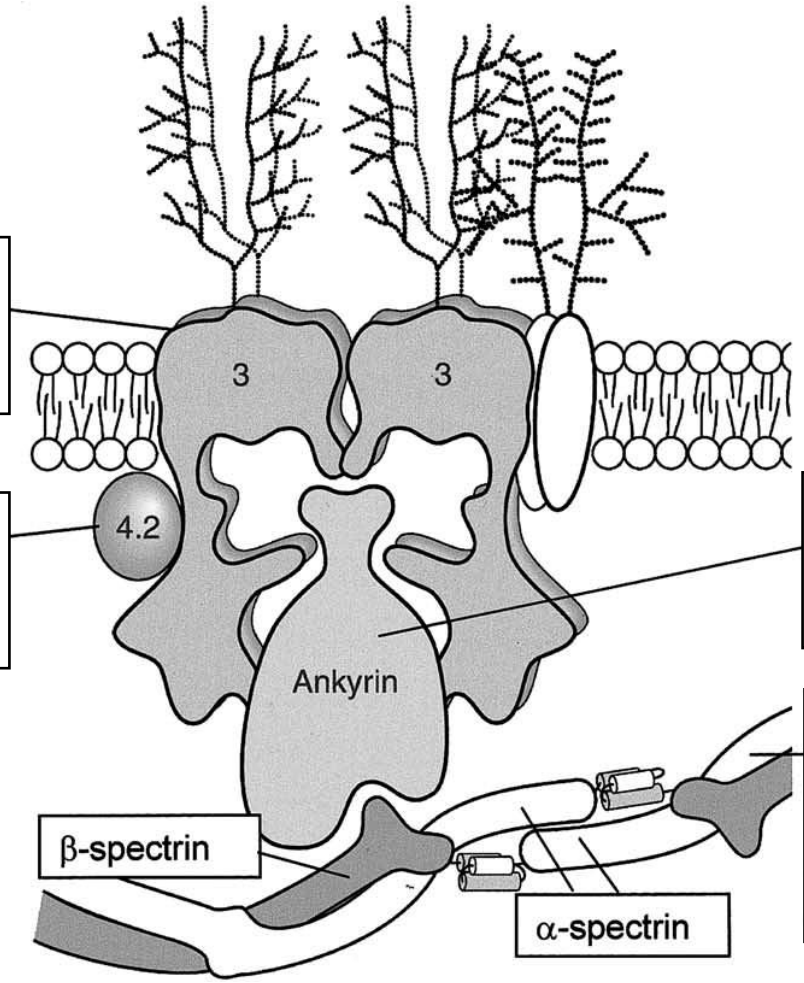
- **Highly heterogeneous group of disorders:**
  - **Clinical severity**: fully compensated hemolysis to transfusion-dependant anemia
  - **Protein defect**: α- and β-spectrins, ankyrin, band 3 and protein 4.2
  - **Mode of inheritance**: 75% dominant ; 25% recessive/de novo
  - **Age of diagnosis**

Hereditary Spherocytosis

Protein	Gene	Chromosome localisation
α-spectrin	SPTA1	1q22-q23
β-spectrin	SPTB	14q23-q24.2
Ankyrin	ANK1	8p11.2
Band 3	EPB3	17q12-q21
4.2 protein	ELP42	15q15-q21

Mutations of **Band 3** in **20-35%** of HS – mostly **dominant**

Mutations of **Protein 4.2** in **recessive** HS (**<5%** of HS)



Mutations of **Ankyrin** in **40-65%** of HS – both **dominant** and **recessive**

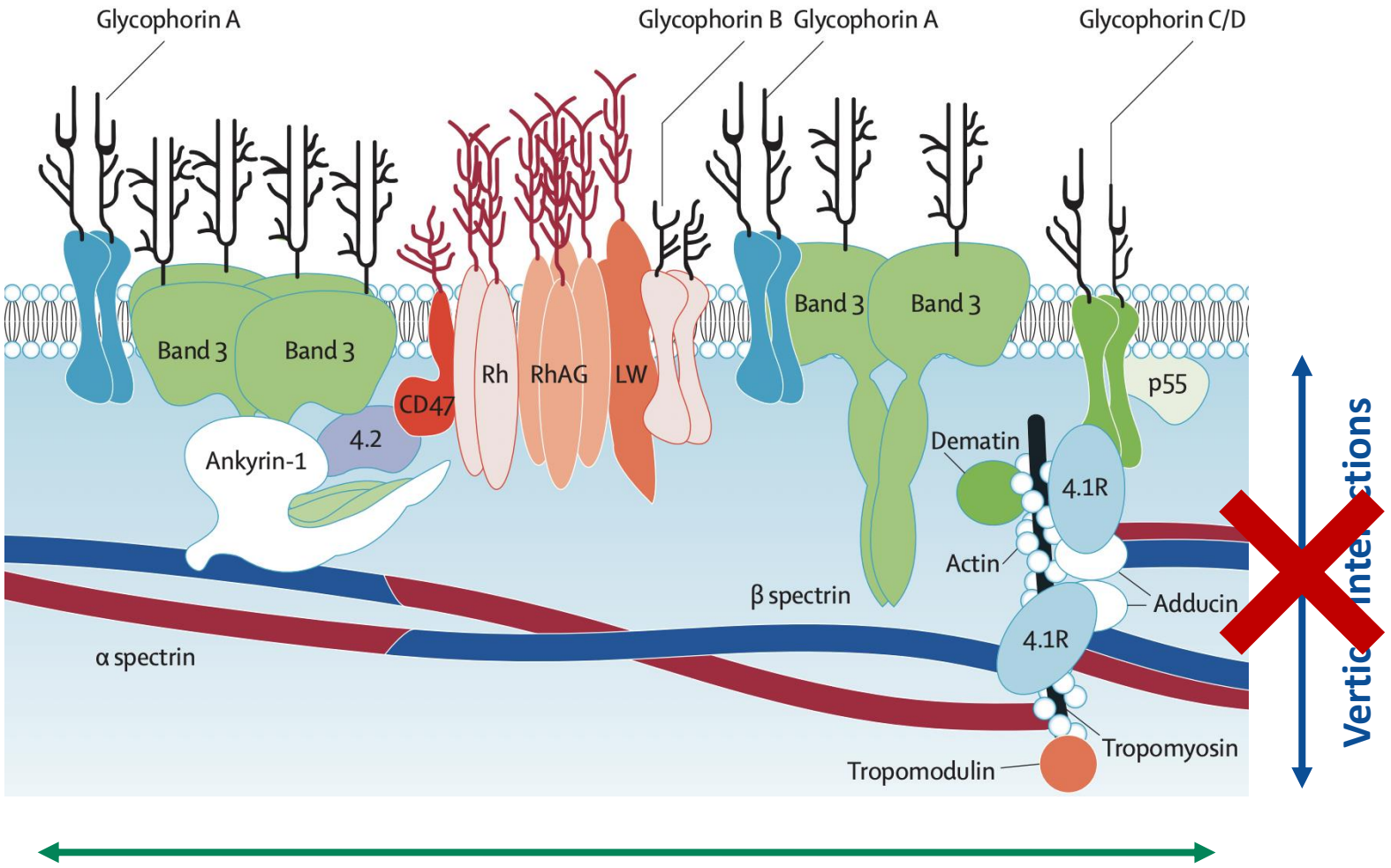
Mutations of **α-spectrin** in **recessive** HS (**<5%** of HS)

Mutations of **β-spectrin** in **dominant** HS (**15-30%** of HS)

Adapted from Eber et al. 2004 and Perrotta S, A et al. 2018

Hereditary Spherocytosis

- **Physiopathology:**

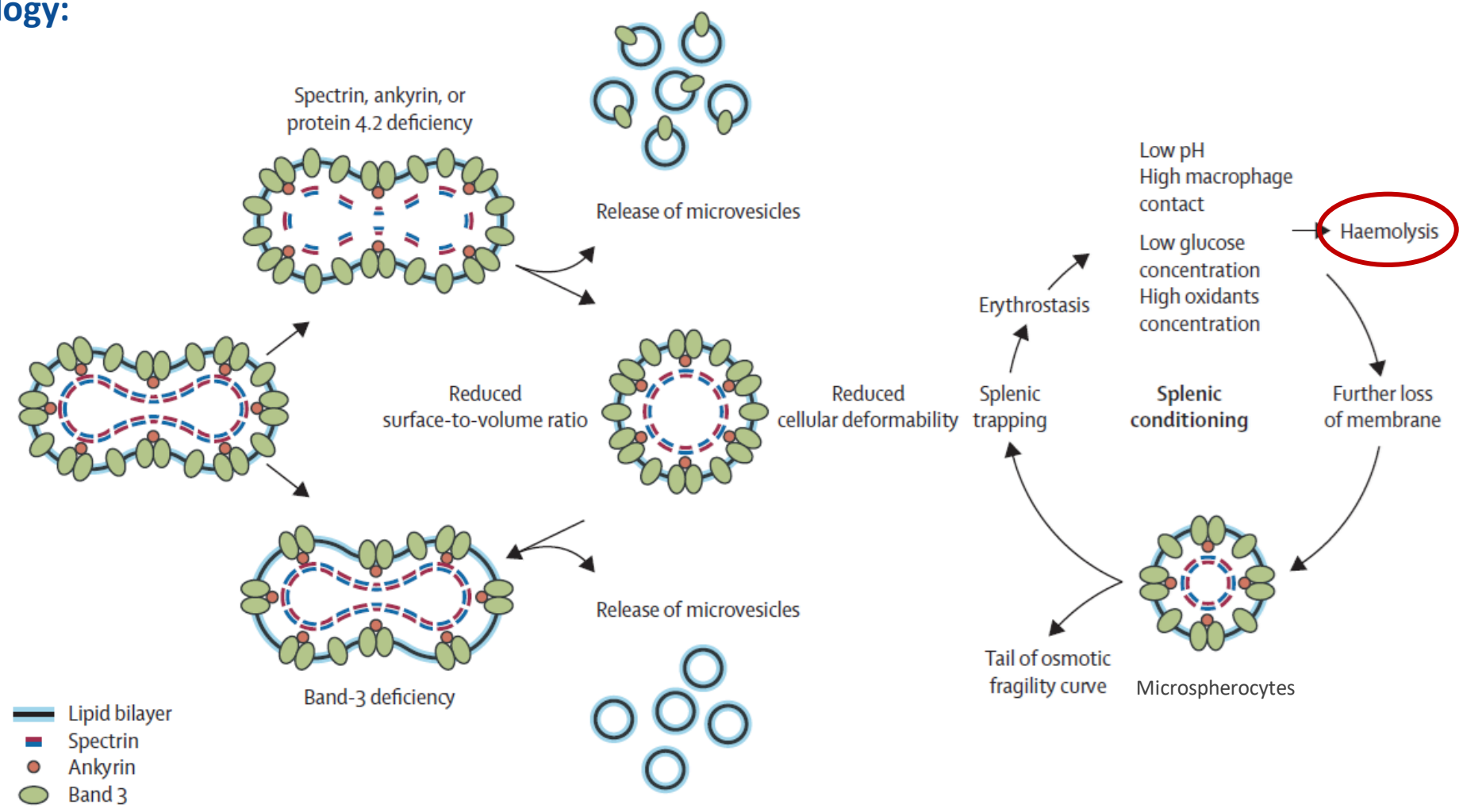


⇨ **Membrane loss (µvesicles)**

Perrotta S, A et al. 2018

Hereditary Spherocytosis

- **Physiopathology:**



Hereditary Spherocytosis

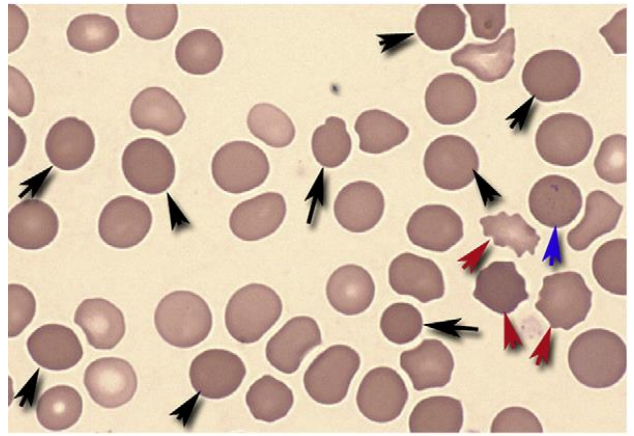
- **Clinical presentation:**
  - ➢ **Neonatal period/Infancy:**
    - Neonatal jaundice
    - Hb level:
      - Normal at birth
      - Rapid fall within the 1st month after birth
    - Anemia: mostly improves during the 1st year of life
  - ➢ **Childhood/Adulthood:**
    - Persistent jaundice, anemia, splenomegaly, gallstones
    - Hemolysis: can be compensated in adults
- **Positive familial history** in 75% of cases
- **Classification:**

	Trait	Mild	Moderate	Severe
Haemoglobin (g/dL)	Normal	11 – 15	8 – 12	6 – 8
Reticulocytes count (%)	Normal (< 3%)	3 – 6	> 6	> 10
Bilirubin (µmol/L)	< 17	17 – 34	> 34	> 51
Splenectomy	Not required	Usually not necessary during childhood and adolescence	Necessary during school age before puberty	Necessary – delay until 6 years if possible

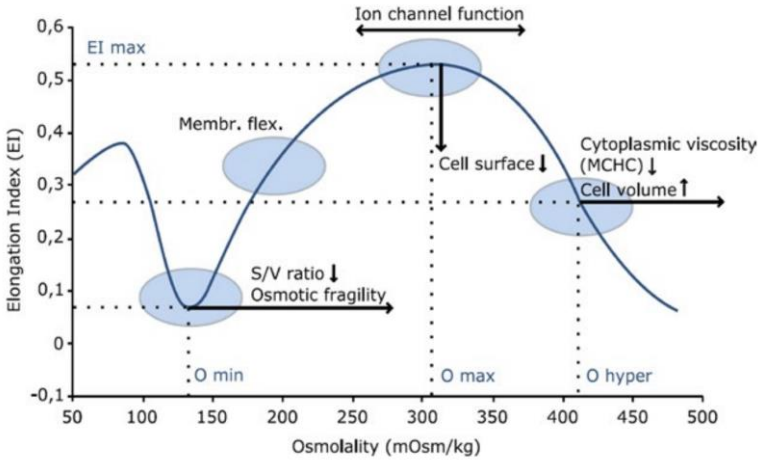
Hereditary Spherocytosis

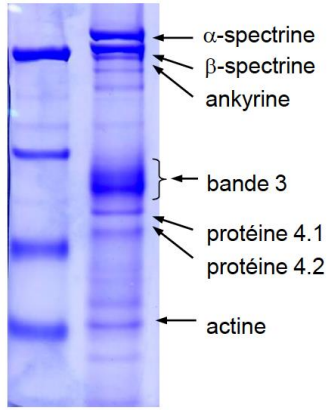
Hemoglobin level can be normal

Da Costa L, 2013



- **Diagnosis:**
  - **Family and Clinical Histories**
  - **Laboratory investigations:**
    - **First tier screening tests**
      - RBC morphology: spherocytes, mushroom cells, etc.
      - Biological parameters of hemolysis
      - New RBC and reticulocyte parameters
    - **Second tier screening tests**
      - Eosine-5-maleimide (EMA) binding test
      - Cryohemolysis test / OF tests
    - **Diagnostic tests**
      - Ektacytometry
      - SDS-PAGE





LHUB-ULB: National Reference Center since 2019

Hereditary Spherocytosis

- **Abnormal RBC indices have been proposed but their sensitivities are low**
  - ↑ MCHC
  - ↑ RDW
  - ↑ Hyperdense cells (Advia analyzer)

- Development of additional parameters based on **complete blood count** (CBC) and **reticulocyte parameters** on last generation hematology analyzers:
  - Many publications about their effectiveness as **HS first tier screening tool**

Hereditary Spherocytosis

Parameters not standardized and method-dependent

Analyzers	Parameters of interest	Definition	Usefulness in HS	Explanation
Siemens (Advia 2120)	% Hyper	% Hyperdense RBCs (MCHC > 41 g/dL)	% Hyper ↑	Membrane loss associated with increased permeability to monovalent cations ➢ Sodium pump hyperactivity ➢ Na^+ loss > K^+ entry ➢ Dehydration ➢ ↑ MCHC
Abbot Diagnostics (CELL-DYN Sapphire)	% HPR	% Hyperchrome RBCs (MCHC > 41 g/dL)	% HPR ↑	
Beckman-Coulter (DxH800, LH755, LH780)	MSCV	Mean Sphered Cell Volume	MSCV < MCV	Reduced deformability of the spherocytes ➢ Incapacity to increase their volume in hypo-osmotique solution
	MRV	Mean Reticulocyte Volume	MRV ↓	Membrane loss and decreased surface area in HS ➢ Already occur during erythropoiesis
Sysmex (XNs, XT-4000i, XE-5000)	IRF	Immature Reticulocyte Fraction	Rét/IRF ↑	High reticulocyte count without an equally elevated IRF ➢ Hypothesis: abnormal/decreased coloration of the reticulocytes
	MicroR	% Microcytic RBCs (MCV < 60 fL)	Micro R ↑ & MicroR/Hypo-He ↑	Spherocytes = small hyperdense cells ➢ ↑ microcytic RBCs % ➢ ↑ Hyper-haemoglobinized % = ↓ hypo-haemoglobinized %
	Hypo-He	% Hypo-haemoglobinized (MCH < 17 pg)		

Hereditary Spherocytosis

- **Management/Treatment:**

  - **Monitoring:** neonates +++

  - **Supportive measures:**
    - Treatment of hyperbilirubinemia
    - Folic acid
    - EPO

  - **Transfusions** (+ iron chelation)

  - **Splenectomy:** based on the severity of hemolysis, age of the patient and potential perioperative and post-splenectomy long-term complications
    - Total or partial
    - > 5 y.o
    - !! Immunizations for encapsulated organisms !!
    - Simultaneous cholecystectomy: only if symptomatic gallstones

Important to confirm the diagnosis prior splenectomy

Table 1. Summary of splenectomy recommendations for hemolytic disorders. Haematologica 2017 Volume 102(8):1304-1313

Disease	When splenectomy recommended? *
Hereditary spherocytosis	Patient is transfusion-dependent or suffers severe anemia. Patient has moderate disease: decision based on spleen size and quality of life parameters. No need to perform cholecystectomy.
Pyruvate kinase deficiency	Consider if patient is transfusion-dependent or severely anemic. Cholecystectomy should be performed at time of splenectomy.
Splenectomy in congenital non-spherocytic hemolytic anemia due to G6PD deficiency	Consider if patient is transfusion-dependent and/or has massive splenomegaly and/or has symptomatic splenomegaly.
Hereditary stomatocytosis	Contraindicated.
Congenital dyserythropoietic anemia type II	Consider if patient is transfusion-dependent and/or has symptomatic splenomegaly.
Sickle cell disease	Patient has had two acute splenic sequestration crises and/or has massive splenomegaly and/or suffers symptomatic hypersplenism.
Unstable hemoglobin	Consider only if patient has transfusion-dependent anemia and/or symptomatic splenomegaly.

*For all indications splenectomy should be performed after 6 years of age. G6PD: glucose-6-phosphate dehydrogenase.

ALGORITHM

Testing Algorithm for HHA

+ Family History & Clinical exam

1. INITIAL ROUTINE TESTS

Initial laboratory tests to confirm HA

CBC
Reticulocyte count
Chemistry panel: LDH, bilirubin, haptoglobin, etc.

Always interpret reticulocyte count based on the Hb level

Haptoglobin levels can be low in neonates without hemolysis

N or ↑ haptoglobin does not eliminate the possibility of hemolysis

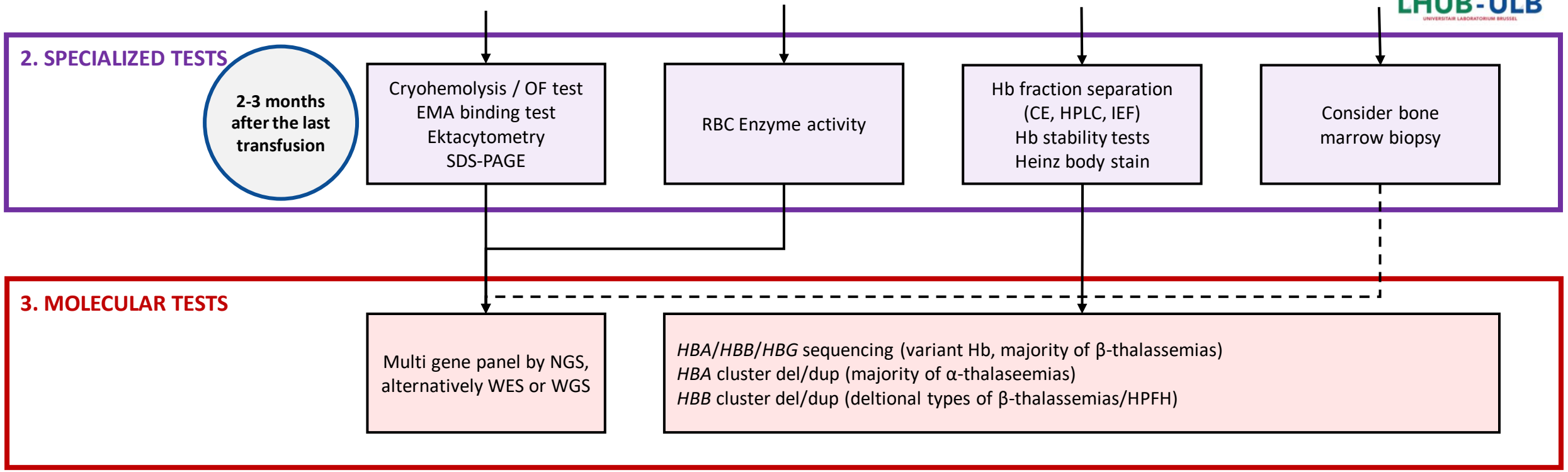
Rule out acquired HA

AIHA: DAT
MAHA workup: D-dimer
ADAMTS13, *E. coli* Shiga-like toxin, etc.

Peripheral blood smear review

Schistocytes	Spherocytes Ovalocytes Stomatocytes	Bite cells Echinocytes Normal morphology	Microcytic RBCs Bite cells	Macrocytic or normocytic RBCs ↓ Reticulocytopenia
Consider **MAHA**	Consider **Membrane defect**	Consider **Enzyme deficiency**	Consider **Hb disorder**	Consider **CDA**

Testing Algorithm for HHA



Molecular analysis: Gene panel

- **« In house » panel of 4427 genes (mendeliome)**
  a. Ataxia (524 genes)
  b. Congenital malformation syndromes (853 genes)
  c. Early onset epileptic encephalopathy (836 genes)

  d. Hereditary Hemolytic Anaemias due to unknown or doubtful origin (56 genes)

  e. Hereditary spastic paraplegia (160 genes)
  f. Neurodevelopmental disorders (1376 genes)
  g. Neuromuscular disorders (535 genes)
  h. Dermatogenetic panel, severe, rare and hereditary genodermatoses (374 genes)

CONCLUSION

Take home messages

- **Hemoglobinopathies:**
  - **Thalassemia syndromes:** hemolysis and inneffective erythropoiesis
  - Don’t forget about unstable Hb variants

- **Enzyme deficiencies:**
  - **G6PD deficiency:** make the diagnosis to be able to prevent hemolysis as much as possible

- **Membrane disorders:**
  - **Hereditary spherocytosis:** guidance by clinical picture

- **Consider NGS in selected patients**

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Thank you !

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