BASIC SCIENCE HEMATOLOGY

THALASSEMIA

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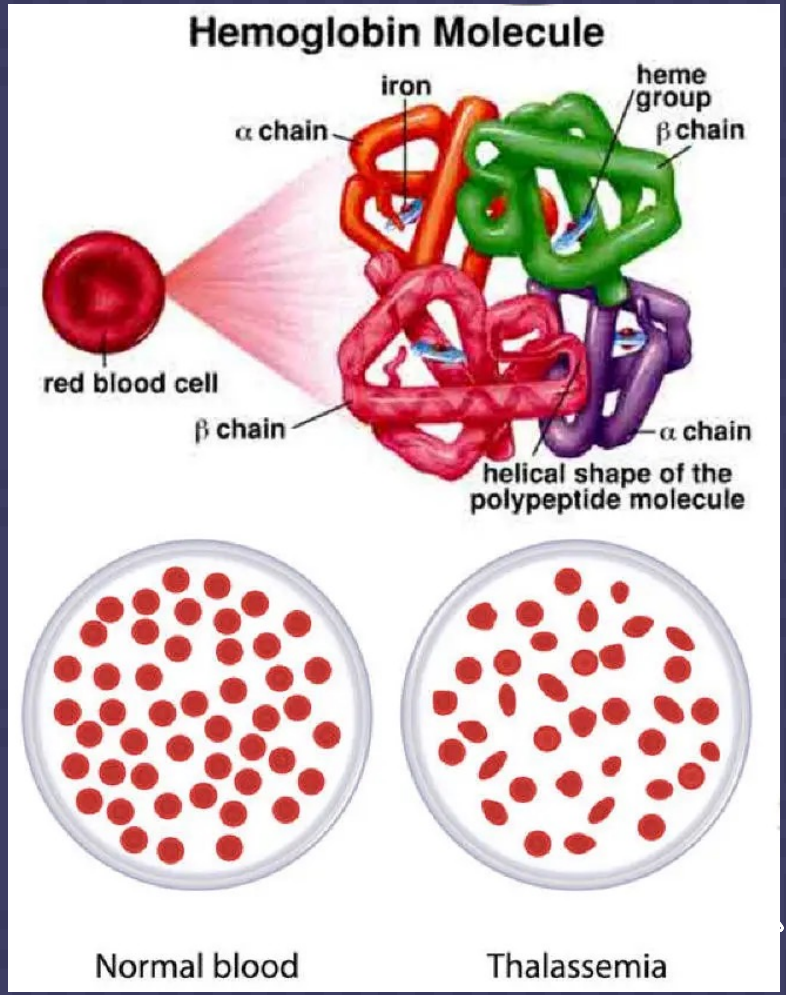
THALASSEMIA

- Inherited hemoglobin disorders, autosomal recessive
- Defective α- or β – globin chain synthesis
- Impaired erythropoiesis, anemia and hypoxia
- Various of clinical phenotypes with marked difference in symptom severity and treatment requirements



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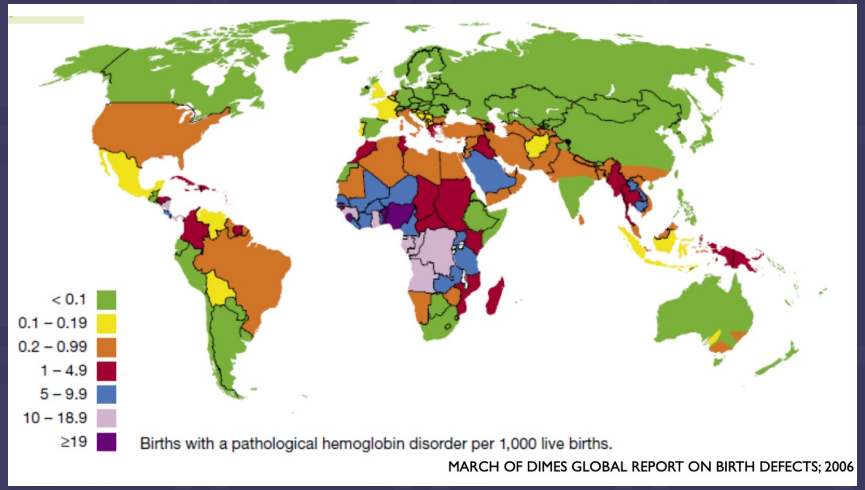
EPIDERMIOLOGY



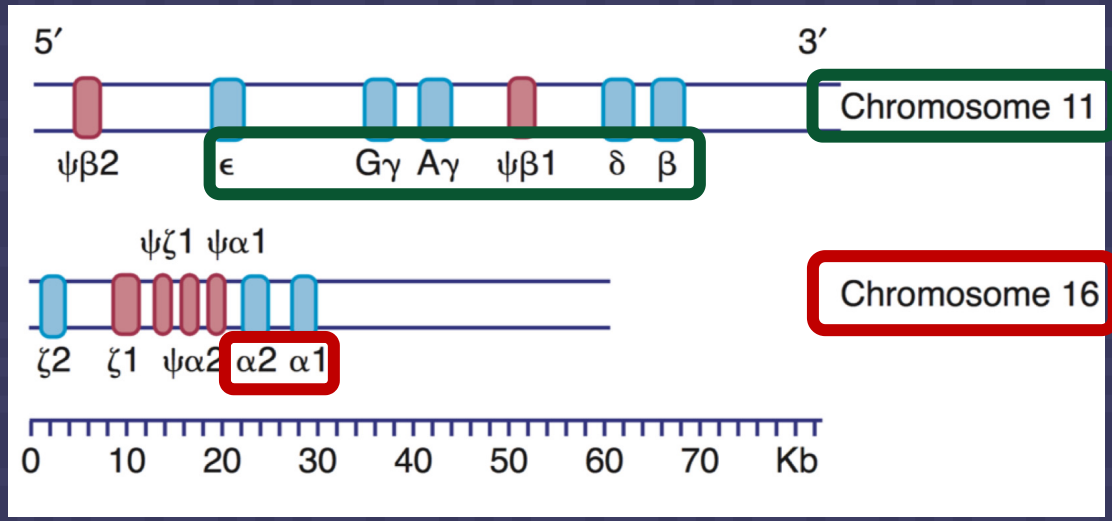
- High incidence in Asia, Southeast Asia, Africa, Mediterranean
- In Thai, Thalassemia disease 1 % of population[1]
- Thalassemia carrier[2] :

➔ α- Thalassemia carrier 20 -30

➔ β – thalassemia carrier 3-9%

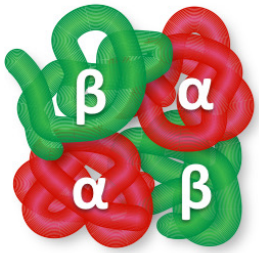
- Hemoglobinopathy : HbE 13% (South east 30 -50%), Hb constant spring (HbCS) 1-8%

1.PanichV,PornpatkulM,SriroongruengW.TheproblemofthalassemiainThailand.Southeast Asian J Trop Med Public Health. 1992; 23Suppl2: 1-6.
2. FucharoenS,WinichagoonP,ThonglairuamV.Beta-thalassemiaassociatedwithalpha-thalassemia in Thailand. Hemoglobin. 1988; 12(5-6): 581-92.



β - gene cluster

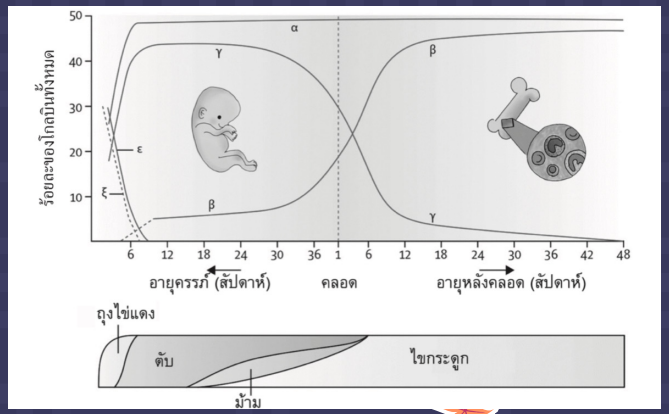
α - gene cluster



$\alpha_2\beta_2$ = HbA

$\alpha_2\delta_2$ = HbA_2

$\alpha_2\gamma_2$ = HbF



Embryonic period	Fetal period	Adult period
Hb Gower-1 $\zeta_2\varepsilon_2$	Hb F $\alpha_2\gamma_2$	Hb A $\alpha_2\beta_2$ 97%
Hb Gower-2 $\alpha_2\varepsilon_2$	Hb A $\alpha_2\beta_2$	Hb A2 $\alpha_2\delta_2$ 2.5%
Hb Portland $\zeta_2\gamma_2$		Hb F <1%

Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2018;391(10116):155-67.
Higgs DR, Engel JD, Stamatoyannopoulos G. Thalassaemia. Lancet. 2012;379(9813):373-83.

TYPE OF THALASSEMIA

α Thalassemia

- 2 alpha globin genes encode on globin chain located on chromosome 16
- Deletion and non –deletion
- Type : 1. carrier (αα/α-)

2.Thalassemia trait
- α thal-2 trait (-α/αα)
- α thal-1 trait (--/αα)

3. Disease
- HbH disease (-- /-α)
- Bart's hydrop fetalis syndrome (- - / - -)

β Thalassemia

- Beta globin gene located on chromosome 11
- Point mutation
- 2 type : 1.Beta thalassemia carrier (β^+/β, β^0/β^0, β^+/β^+)

2.Beta thalassemia disease (β^0/β^0, β^+/β^0, β^0/β^E)

Cao A, Galanello R. Beta-thalassemia. Genet Med. 2010;12(2):61-76.
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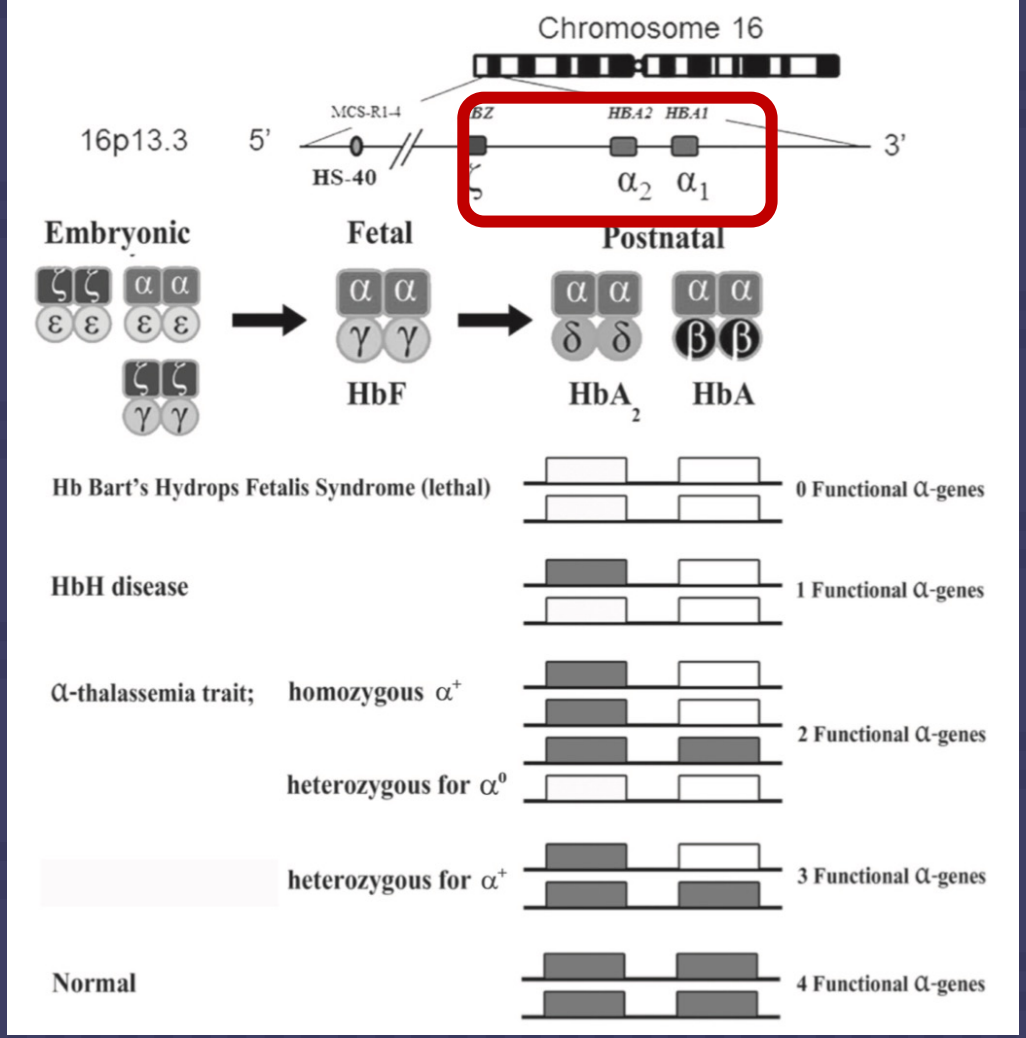


Alpha thalassemia

After GA 6 -8 week : Zetra globin not produce amino acid

***** Large deletion *****

- Defect all α_1 and α_2 – globin from parental : Hb Bart's hydrop fetalis
- Defect α_1 and α_2 – globin from parental : HbH disease (--/- α)
- Homozygous α+ - thalassemia (-α/-α)
- Defect α_1 – globin only : Heterozygous α^0 – thalassemia or α thalassemia 1 trait (--/ αα)
- Defect α_2 – globin : α+ - thalassemia or **silent** α thalassemia or α thalassemia 2 trait or (-α/ αα)

Alpha thalassemia

is commonly caused by

large deletion

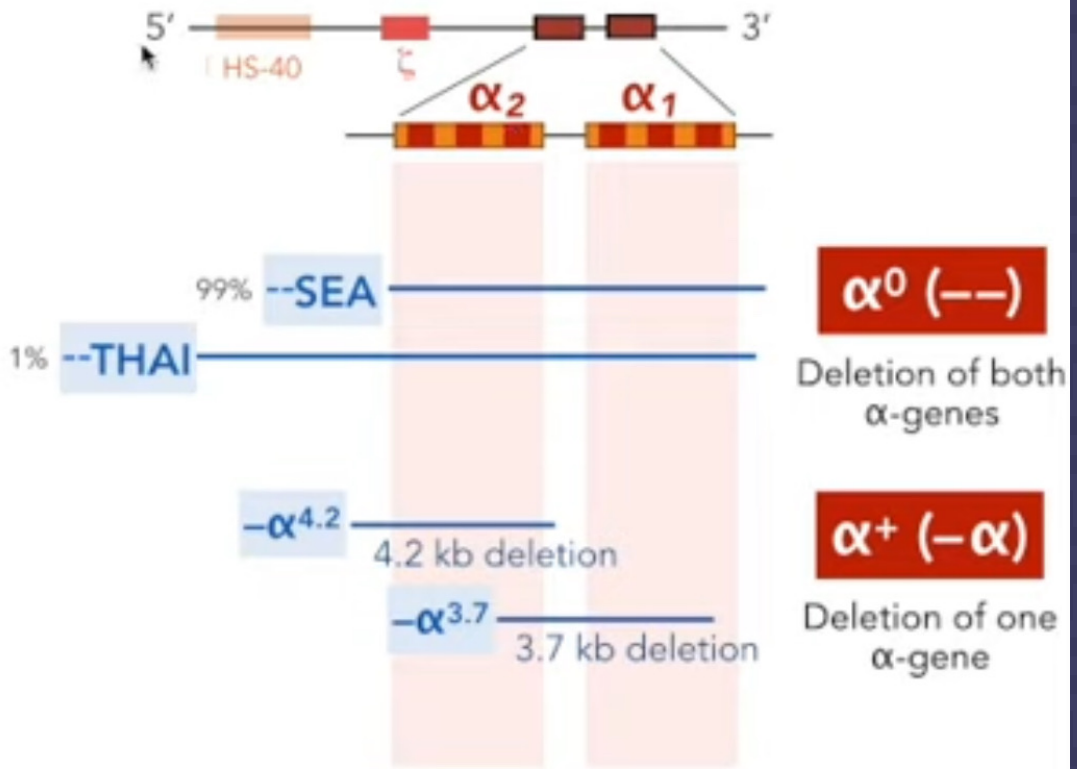
“ Thalassemia effect ”

(–α/αα) **α-thal 2 trait**

(––/αα) **α-thal 1 trait**

(––/–α) **HbH disease**

(––/––) **HbBart's hydrop fetalis**



Warit. .Thalassemia lecture[Video]. 2017 December 17 [cited from 17 Dec 2017] . Available from : https://www.youtube.com/watch?v=Veh9nDPbihU

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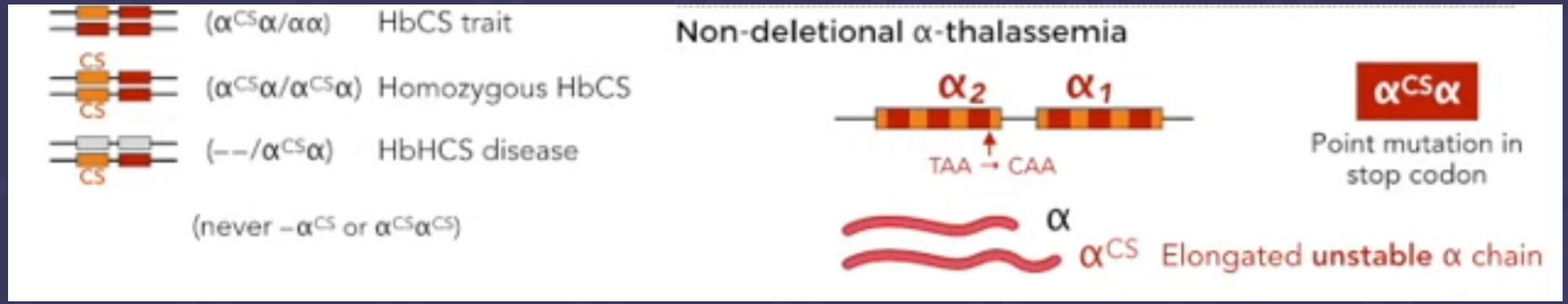
“ Hb variant ”



Abnormal mRNA translocation of termination codon mutation : Stop codon (at location 142 of α_2 - globin) :
1.Hb constant spring (Hb CS) : TAA -> CAA (produce glutamine)
2.Hb Pakse (Hb PS) : TAA -> TAT (produce tyrosine)

-> Hb Quong ze (Hb QS) : abnormal α – globin stability of codon 125 (in exon2) : CTG -> CCG (Leucine -> proline)

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Beta thalassemia

β^0 = ไม่สร้าง β – globin เลย

β+ = สร้าง β- globin ได้แต่ปริมาณลดลง

*** common point mutation ***

(β^0/β) β-thalassemia trait

(β^0/β^0) Homozygous β-thalassemia

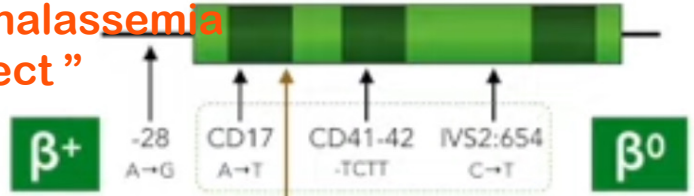
(β^E/β) HbE trait

(β^E/β^E) Homozygous HbE

(β^0/β^E) β-thalassemia / HbE disease

Chromosome 11p 15.4

" Thalassemia effect "



" Hb variant"

HbE

Hb Tak → mutation in stop codon of β-gene
→ elongated β-globin causing **high oxygen affinity**
→ can cause 2° erythrocytosis

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Beta thalassemia

ลักษณะและตำแหน่งของการกลายพันธ์	ความรุนแรง	ตัวอย่างที่พบได้
Point mutations		
Transcription		
Promoter region	β^+	nt-28(A>G), nt-87(C>A)
Cap site	β^+	nt+1 (A>C)
mRNA processing		
Splice junction	β^0	IVS-I-1 (G>T)
Splice site	β^0 or β^+	IVS-I-5 (G>C)
mRNA translation		
Initiation codon	β^0	ATG>ATA
Nonsense codon	β^0	codon 35 (C>A), codon 17 (A>T)
Frameshift	β^0	codon 41/42 (-TCTT)
Deletions		
Large deletion	β^0	619 bp deletion,3.4 kb deletion

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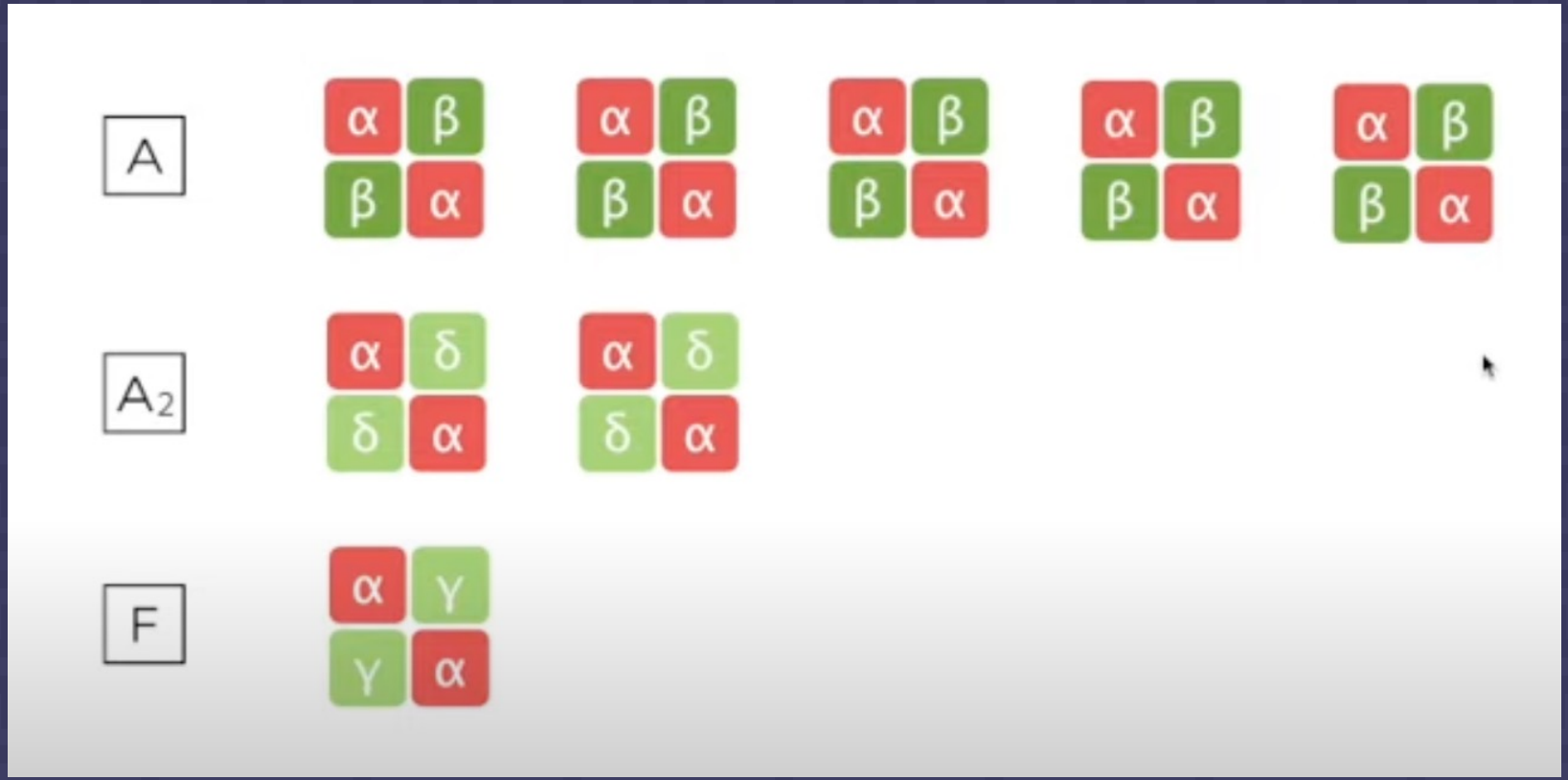
Hb typing : normal



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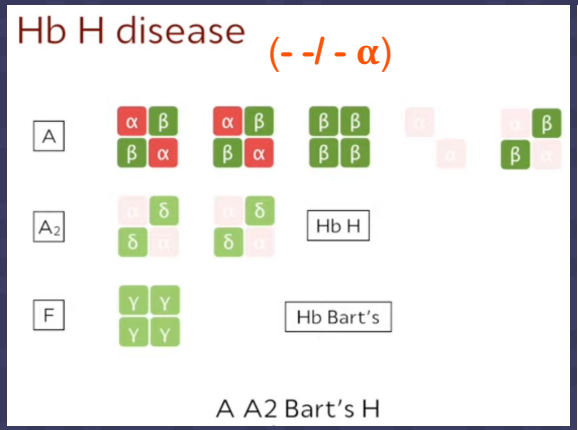
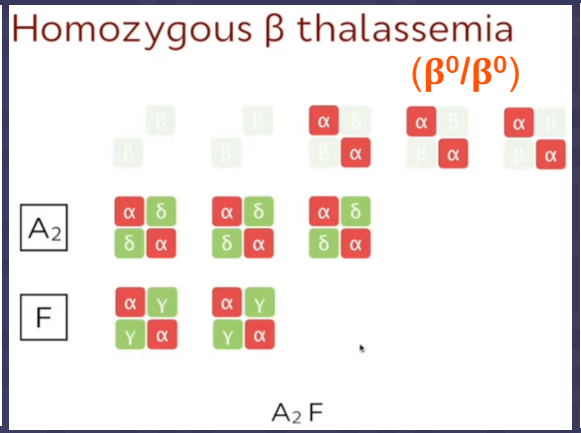
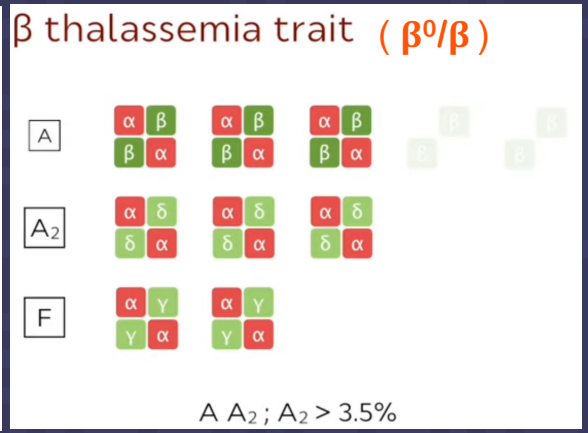
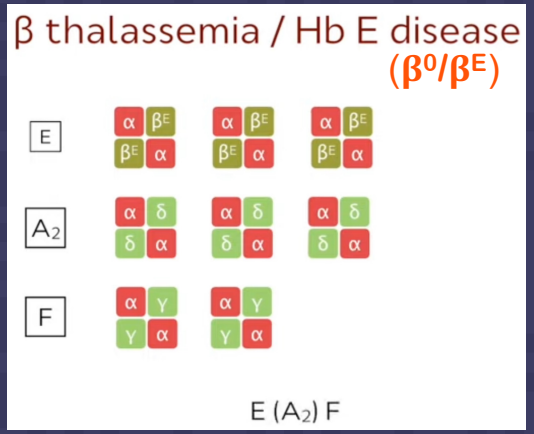
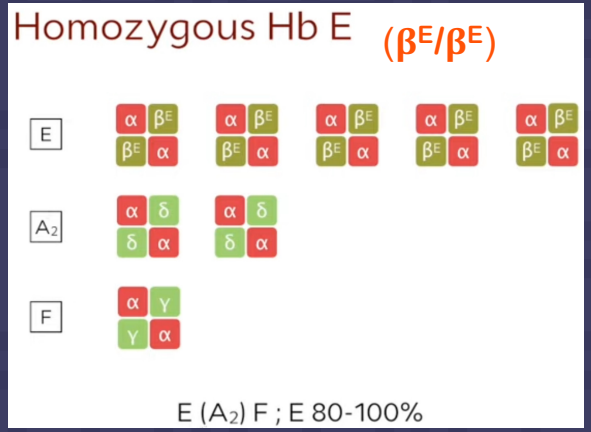
Hb typing

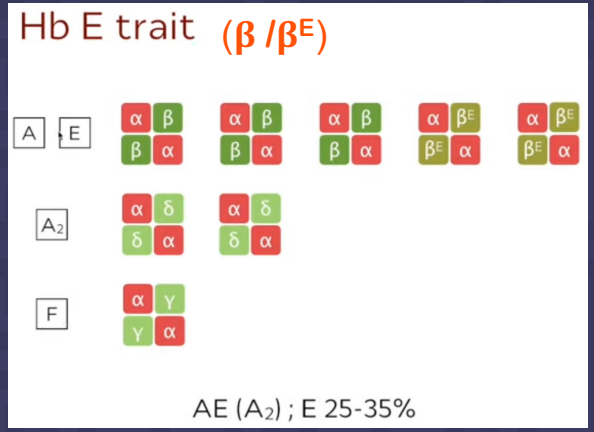












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Alpha thalassemia

	Genotype	Phenotype	Hbtyping
Hb Bart's hydrops	(--/--)	Dead	Hb Bart's > 80%
HbH disease	(--/-α)	Moderate anemia MCV 67±7 fL	A_2A Bart's H, AA_2H H 5 -50%, Bart's 3-4%
HbH c CS disease	(--/$\alpha^{cs}\alpha$)	Moderate anemia MCV 77±5 fL	A_2ABart'sHCS CS 2-5%, H 5-50%, Bart's 3-4%
Homozygous HbCS	($\alpha^{cs}\alpha/\alpha^{cs}\alpha$)	Mild-moderate anemia MCV 86±6 fL	A_2ABart's/A_2ACS CS ~ 5%
Hb CS trait	($\alpha^{cs}\alpha/\alpha\alpha$)	± decrease MCV	A_2ACS
α – thal 1 trait	(--/αα)	± mild anemia MCV 70± 8 fL	$A_2A : A_2 < 3.5$ %
α – thal 2 trait	(-α/αα)	Normal MCV	$A_2A : A_2 < 3.5$ %

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Beta thalassemia

	Genotype	Phenotype	Hbtyping
Homozygous β-thalassemia disease	(β^0/β^0)	Severe anemia MCV 57 -75 fL	A_2F
β- thal/ HbE disease	(β^0/β^E) or (β^+/β^E)	Moderate to severe anemia MCV 67±7 fL	EF (E40-60%)
Homozygous HbE	(β^E/β^E)	Mild anemia MCV 73±6 fL	E; E 80-100%
β- thal trait	(β^0/β) or (β^+/β)	± mild anemia MCV 68± 7 fL	A_2A ; A2 > 3.5%
Hb E trait	(β^E/β^0)	No anemia MCV 68± 7 fL	AE; E 25-35%

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Alpha and Beta thalassemia

	Genotype	Phenotype	Hbtyping
HbAEBart's disease	$(--/-\alpha)(\beta^E/\beta^0)$	Hb 7 -8.5 g/dL MCV 66 $\pm$6 fL	AEBart's E 13-17%
HbAEBart's with CS	$(--/\alpha^{CS}\alpha)(\beta^E/\beta)$	Moderate anemia Decrease MCV	AEBart's CS
HbEFBart's disease	$(--/-\alpha)(\beta^E/\beta^E)$ $(--/-\alpha)(\beta^0/\beta^E)$	moderate anemia Decrease MCV	EFBart's
HbE trait with α-thal trait	$(--/\alpha\alpha)(\beta^E/\beta)$ $(-\alpha/\alpha\alpha)(\beta^E/\beta)$	no anemia Decrease MCV	AE : E < 25%

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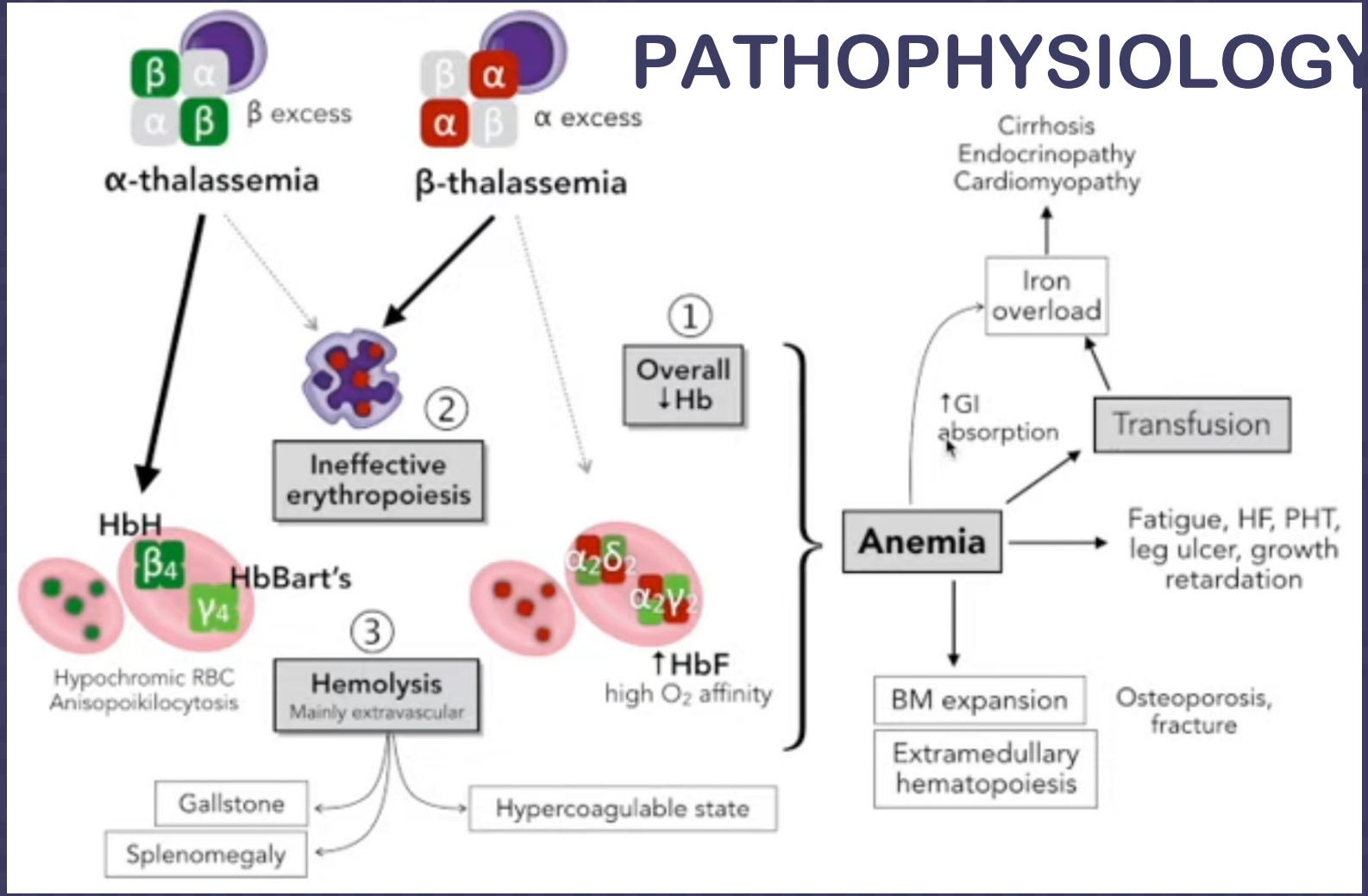
PATHOPHYSIOLOGY



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Clinical presentation

General features

- Pallor
- Fatigue
- Dyspnea on exertion
- Poor appetite
- Palpitation
- Poor growth

Feature of hemolysis

- Jaundice
- Gallstones
- Hyperuricemia (Gout)

** Beta thalassemia major symptoms occur when infant at age 6 -24 months

Excessive erythropoiesis

- Bone marrow expansion and bone disease : Thalassemia facies (prominent maxilla, Frontal bossing, malocclusion of teeth)
- Hepatomegaly
- Splenomegaly/hypersplenism
- Other extramedullary hematopoiesis :
  - Vertebral expansion
  - Medullary expansion
- Hypercoagulable state and vascular disease
  - Deep vein thrombosis
  - Pulmonary embolism
- Iron overload

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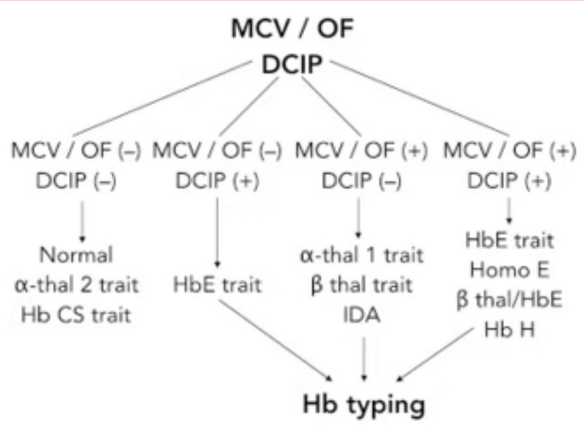
Laboratory

Screening test

- CBC : MCV (< 80 fL), MCH (<27 pg)
- PBS – hypochromia, microcytic , target cell
- One tube osmotic fragility test (OFT) : 0.36% NaCl (hypotonic)
- DCIP precipitation test
- Hb E screen (microcolumn chromatography)

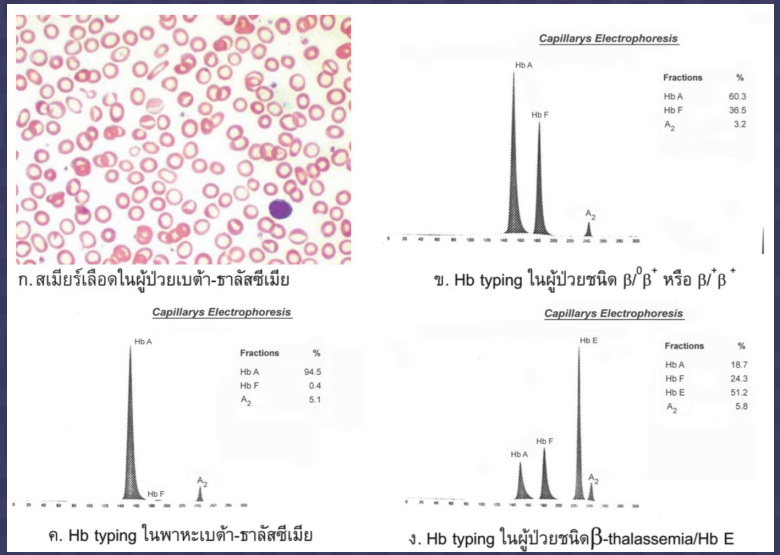


**CONFIRMATION TEST **

Hb typing

- Cellulose acetate
- Isoelectric focusing electrophoresis (IEF)
- High performance liquid chromatography (HPLC)

DNA analysis or PCR ((polymerase chain reaction)



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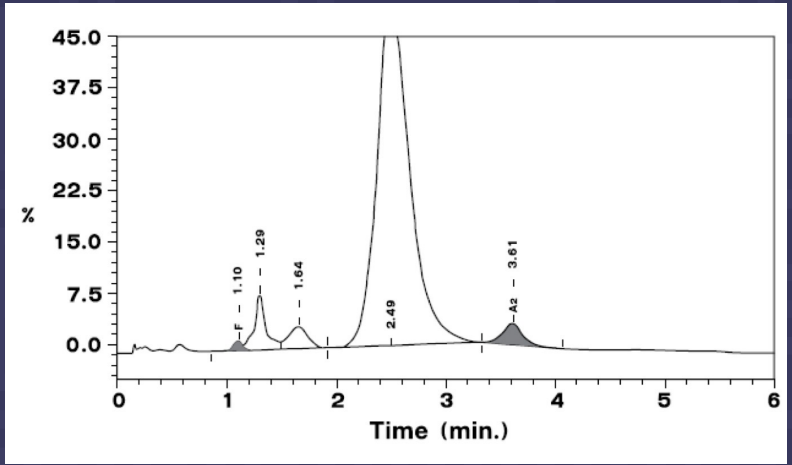
Hb typing

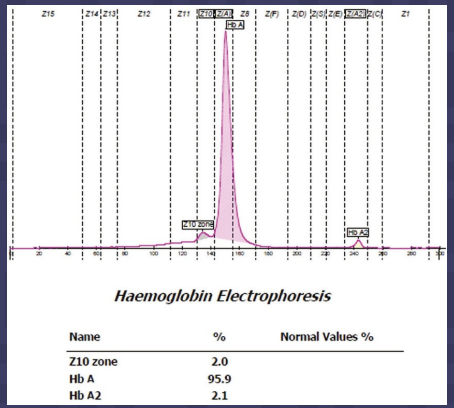


1. High performance liquid chromatography (HPLC)

- Standard procedure for the initial evaluation of hemoglobin variants and thalassemia
- Rapid, automated
- Reliable quantitative measurements of hemoglobin A2 and F
- The chromatograms is analyzed by a microcomputer

2.Capillary electrophoresis (CE) detect

- β-thalassemia trait : HbA2 >3.5%
- Hemoglobinopathies : Hb E trait, homozygous Hb E, Hb CS trait, homozygous Hb CS etc.
- Thalassemia disease : Hb H disease, β-thalassemia /Hb E



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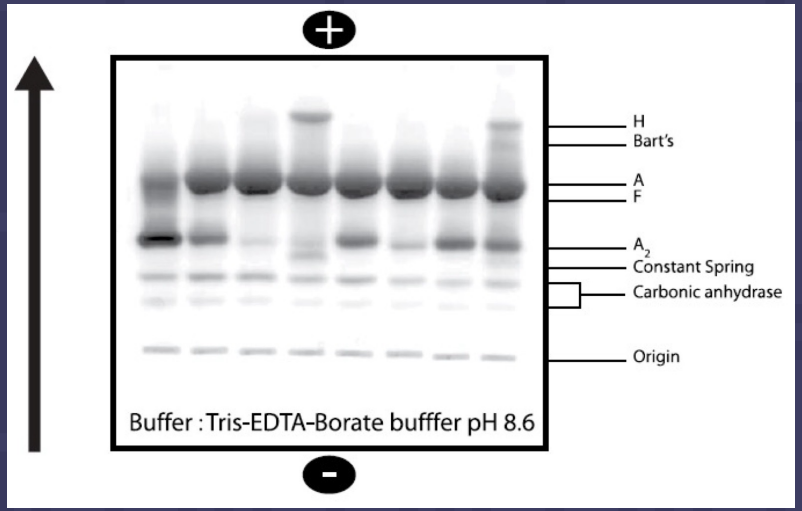
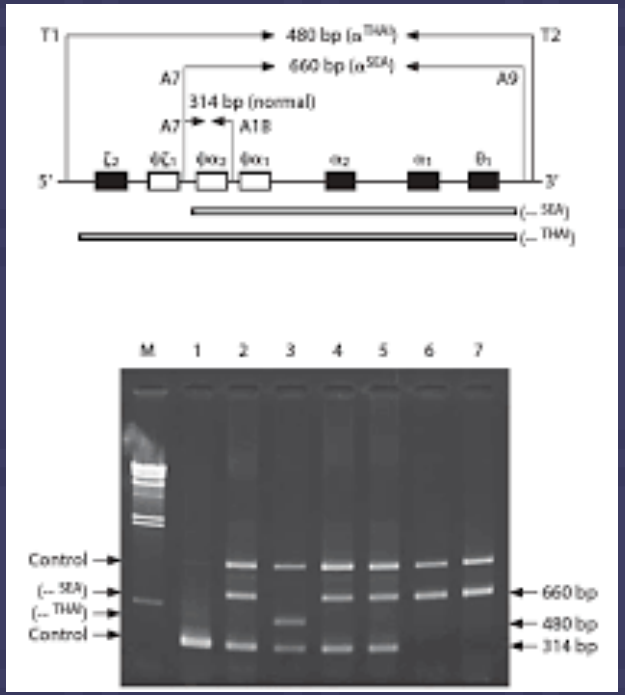
Hb typing





3. Isoelectric focusing electrophoresis (IEF) : separation of amphoteric analytes according to their isoelectric point by the application of an electric field along a pH gradient formed in a capillary

Gap PCR for

- **α – thal 1 SEA (-- ^{SEA}) and THAI (--^{THAI}) deletion**
- **α – thal 2 3.7 kb (- $\alpha^{3.7}$) and 4.2 kb (- $\alpha^{4.2}$) deletion**

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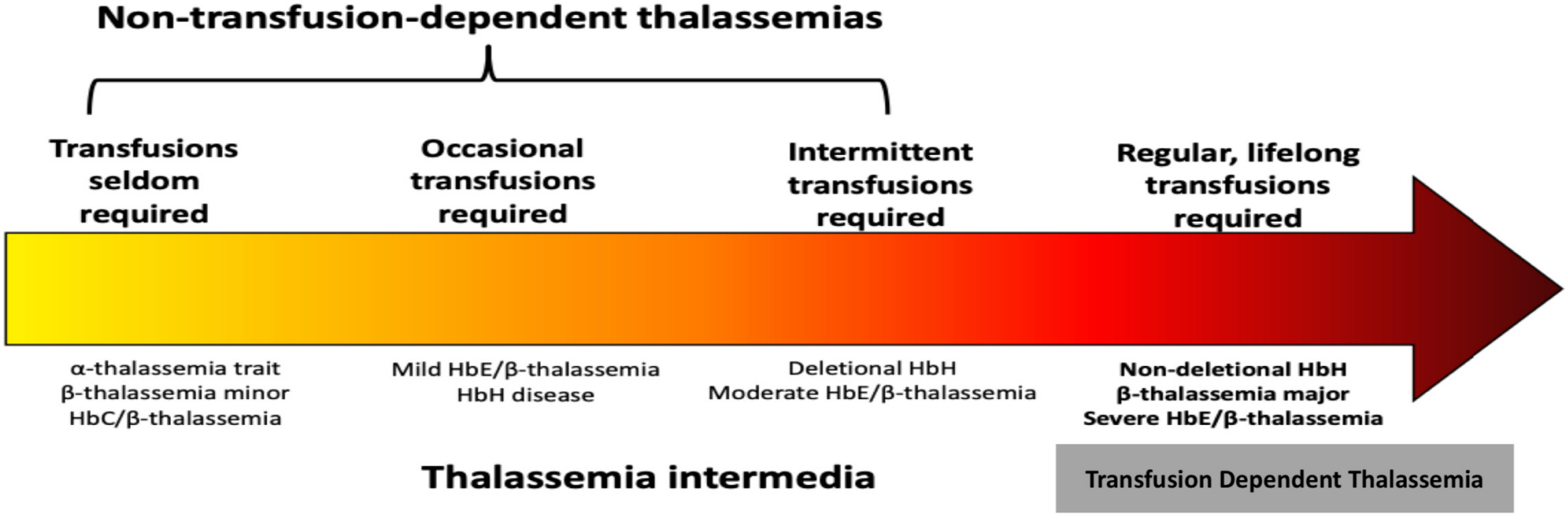
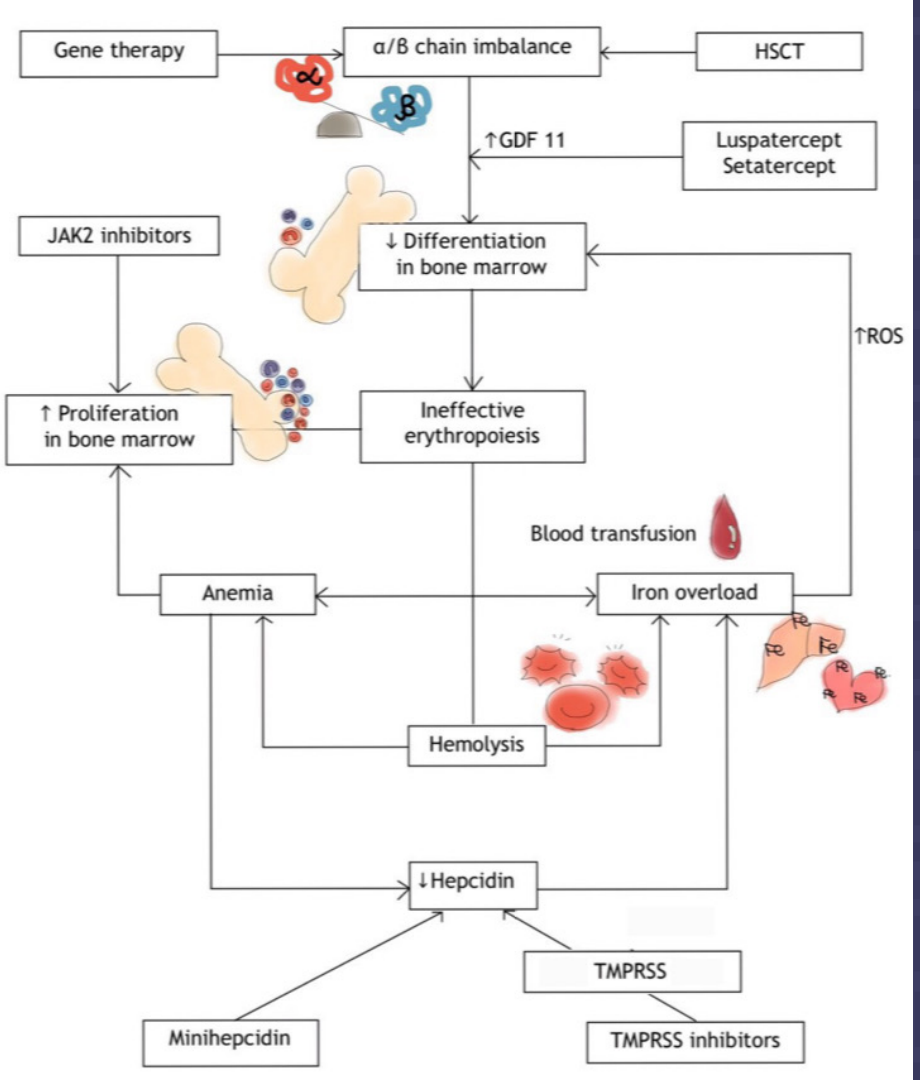
Spectrum of disease



[1]Muncie HL & Campbell JS. *Am Fam Physician* 2009;80:339–344; [2]Galanello & Origa. *Orphanet Journal of Rare Diseases* 2010, 5:11; [3]Harteveld & Higgs. *Orphanet Journal of Rare Diseases* 2010, 5:13; [4]Cohen AR *et al. Hematology Am Soc Hematol Educ Program* 2004;14–34

Management

- Hematopoietic stem cell transplant
  Indication : transfusion dependent thalassemia
- Transfusion
  - occasional transfusion
  - regular transfusion – transfusion dependent (2-6 wks interval), keep pretransfusion Hb 9.0 -10.5 g/dL (but < 14 g/dL)
- Splenectomy
- Treatment of complication
  - Iron – mediated
  - Non iron – mediated
- Manipulation of HbF
- Gene therapy : use of autologous modified stem cells for engraftment



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Transfusion

- To improve the anemia and increase quality of life
- To suppress the ineffective erythropoiesis

Indication for regular transfusion (Age < 18 years)

- Hb < 7 g/dL, 2 consecutive time, without current infection
- Hb > 7 g/dL with
  - facial bone change, Growth retardation (< P10), bone fracture, Extramedullary hematopoiesis : marked hepatosplenomegaly (> 5cm)

Transfusion- dependent

- Severe anemia (Hb < 7 g/dL)
- Hepatosplenomegaly
- Extramedullary hematopoiesis
- Growth failure
- Genotype :
  - β^0/β^0, β^0/β^E, $--^{SEA}/--^{SEA}$
  - some β^0/β^+, $--^{SEA}/\alpha^{cs}\alpha$
- Regular transfusion and iron chelation
- Hematopoietic stem cell transplantation (HSCT)
- Experimental gene therapy

Non- Transfusion dependent

- Mild to moderate anemia (Hb 7-10 g/dL)
- Mild hepatosplenomegaly
- Genotype :
  - β^+/β^+, β^+/β^E, $--^{SEA}/-\alpha$
  - some β^0/β^+, β^0/β^E, $--^{SEA}/\alpha^{cs}\alpha$
- Occasional transfusion (infection or fever, pregnancy)

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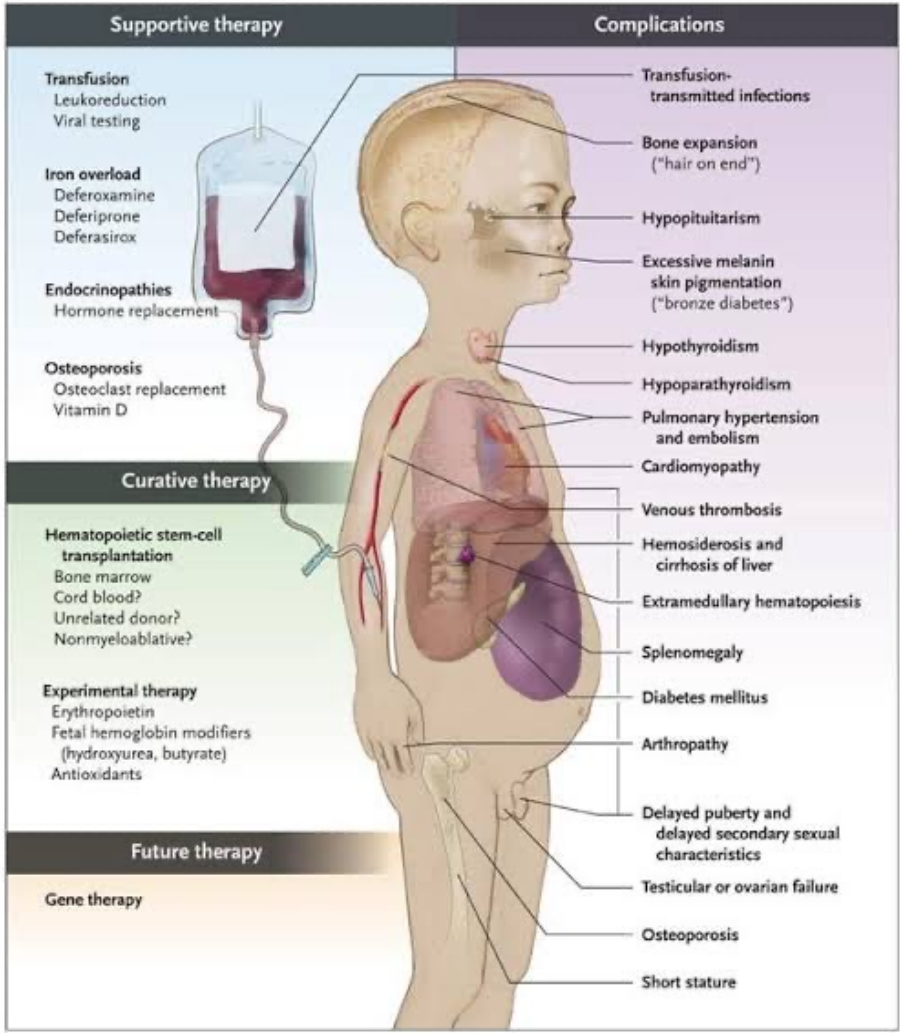

Complication

- Iron overload : cardiac (myocardial dysfunction, arrhythmia), Hepatic (cirrhosis), Endocrine (DM)
- Cardiac complication : CHF, Arrhythmia, pericarditis
- Infection
- Endocrine abnormalities : DM, growth deficiency, Delayed puberty & hypogonadism, hypothyroidism, hypoparathyroidism
- Hypersplenism
- Thrombotic complication : Pulmonary thromboembolism, Pulmonary hypertension, CVD
- Gall stone
- Chronic leg ulcer
- Autoimmune/alloimmune hemolytic anemia

Chelation therapy

- Monitor : CBC, BUN/Cr, serumferritin, UA (proteinuria), LFT
- ** ear and eye examination : Before and the q 1 year

Indication in Non - transfusion dependent
- Age > 10 yr in NTDT with
- serum ferritin > 800 ng/ml, 2 consecutive times within 1 -3 mo
- LIC in MRI T2* > 5 mg/kg (dry weight liver)

Indication in transfusion dependent
1. serum ferritin > 1000 mg/ml, 2 consecutive times within 1 -3 months
2. Regular transfusion > 1 year
3. Blood transfusion > 10 times
4. LIC in MRI T2* >7 mg/kg (dry weight liver)
5. Cardiac MRI T2* < 20 milliseconds

Chelation therapy

Property	DFO (Desferal)	Deferipone (GPO-L-ONE)	Deferasirox (Exjade)
Age	≥ 2 years old	≥ 6 years old	≥ 2 years old
Chelator: iron	1:1	3:1	2:1
Route of administration	Subcutaneous or intravenous	Oral tablet/ solution	Tablet oral suspension
Schedule	Administered over 8-24 hours, 5-7 days/wk	3 times a day	Daily + water 8 oz AC 30 min.
Usual dose	20-40 mg/kg/day	75-100 mg/kg/day	20-40 mg/kg/day
Half-life	20-30 minutes	3-4 hours	12-16 hours
Excretion	Urinary, fecal	Urinary	Fecal
Disadvantages	Local reaction Ear, eye toxicity Growth retardation and skeletal changes	GI disturbances, Transaminitis, Agranulocytosis 1%, Arthralgia, Low plasma zinc	GI disturbances, Transaminitis, GI bleeding, Rise in serum creatinine, Proteinuria, rash

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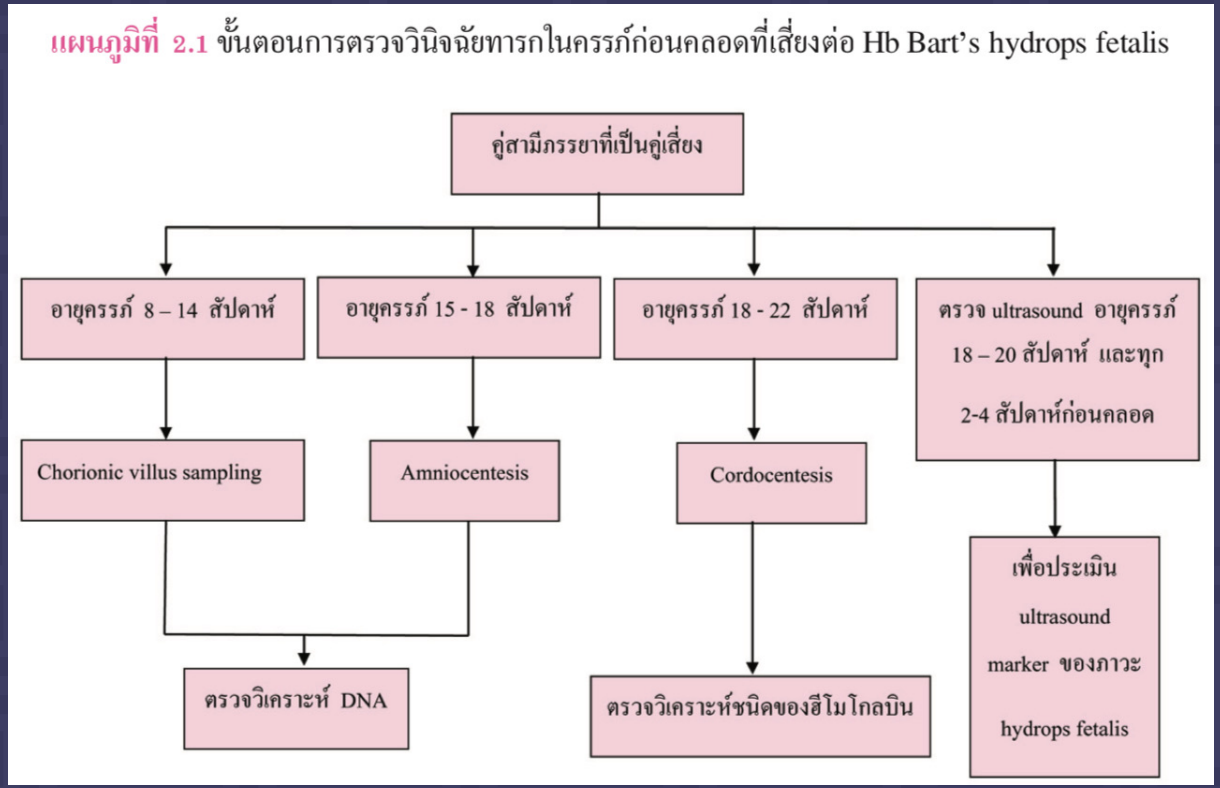
Prevention of thalassemia



Screening for couples at risk and genetic counselling

- Hb Bart's hydrop fetalis (--/--)
- Homozygous β-thalassemia (β^0/β^0)
- β- thalassemia/ Hb E disease (β^0/β^E)

Prenatal diagnosis

- Preimplantation genetic diagnosis (in vitro fertilization)
- Amniocentesis
- Chorionic villi sampling
- Umbilical cord blood sampling

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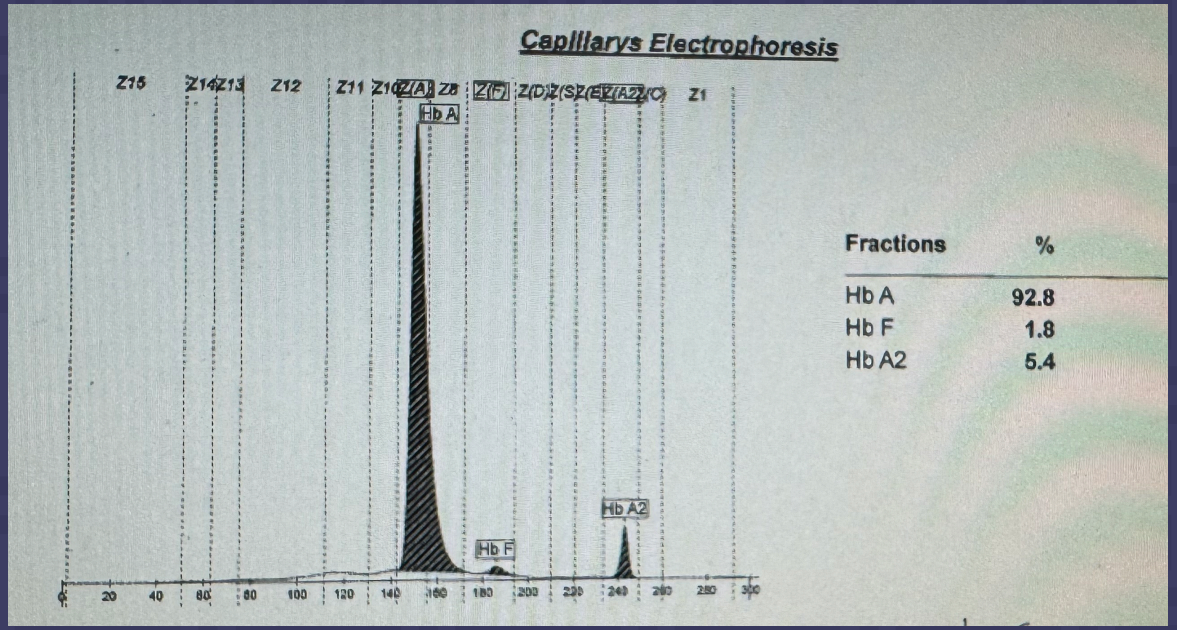
QUIZ

Hb typing report form

- Hb 9.4%
- Hct 31.2 %
- MCV 52.3 fl
- MCH 15.7 pg
- RDW 22.5 %



Interpretation : β- thalassemia trait with or without α thalassemia

TAKE HOME MESSAGES

- α thalassemia : Large deletion VS β thalassemia : Point mutation
- α thalassemia : - carrier (αα/α-)

-> trait : α thal-2 trait (-α/αα) and α thal-1 trait (--/αα)

➔ Disease : HbH disease (-- /-α), Bart's hydrop fetalis syndrome (- - / - -)

- Beta thalassemia - carrier (β^+/β, β^0/β^0, β^+/β^+)

-> Disease (β^0/β^0, β^+/β^0, β^0/β^E)

- **Confirmation test : Hb typing (CE, IEF, HPLC), DNA analysis/ PCR**
- regular transfusion – transfusion dependent [2-6 wks interval], keep pretransfusion Hb 9.0 -10.5 g/dL [but < 14 g/dL]
- complication especially Iron overload
- Indication for chelation : NTDT, TDT
- Prenatal screening : Hb Bart's hydrop fetalis [--/--], Homozygous β-thalassemia [β^0/β^0], β- thalassemia/ Hb E disease [β^0/β^E]

THANK YOU