HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

3 - Hemoglobinopathies and Thalassemias

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1

Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- Grant support - CSL Behring, Global Blood Therapeutics, Imara, Ironwood, Novartis
- Consulting- CSL Behring, Global Blood Therapeutics, Novartis, Forma

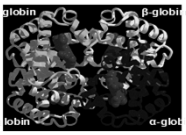
2

Outline

1. General considerations
2. Beta-thalassemias
3. Alpha-thalassemias
4. Hemoglobinopathies other than sickle cell disease

3

Ribbon structure of hemoglobin A



Tetramer

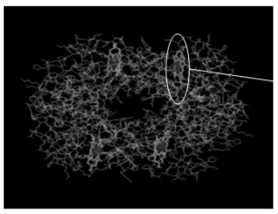
- 2 pairs of globin (polypeptide) chains
- Alpha-beta dimers aggregate to form tetramers

Heme

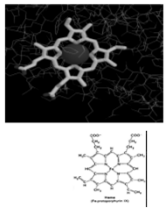
- Complex of Fe^{+2} and protoporphyrin
- Covalently bound to each globin monomer
- Reversibly binds one molecule O_2

4

Stick Structure of Hemoglobin A



Close-up view of heme with Fe at center



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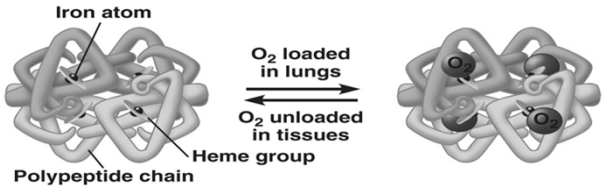


6

Normal Hbs found in Adults

Hb A: $\alpha_2\beta_2$ 97%

Hb A_2: $\alpha_2\delta_2$ 3%

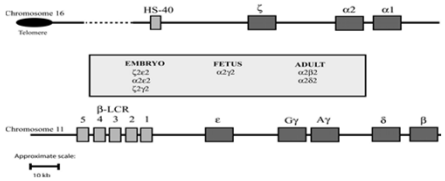
Hb F: $\alpha_2\gamma_2$ <1%

Primary structure

- Alpha globin- 141 amino acids
- Beta globin- 146 amino acids

7

Human Globin Genes



DNA hypersensitive sites:
- β-globin LCR [locus control region]
- α-globin HS-40 [hypersensitive site-40]

(Weatherall and Proven, Lancet 2000;355:1169-1175)

8

Chromosome 16

--------α--------- α---------

--------α--------- α---------

Chromosome 11

-----$^{G}\gamma$-- $^{A}\gamma$ ----- δ-- β----

-----$^{G}\gamma$-- $^{A}\gamma$ ----- δ-- β----

9

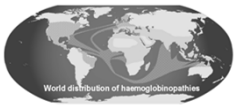
Hemoglobinopathies and Thalassemias

- Mankind's most common single gene, Mendelian diseases
- Disorders of the synthesis or structure of Hb
- Almost 1500 described

10

Geographical overlap in distribution

Distribution of hemoglobinopathies



Distribution of malaria

Evidence that these red cell disorders protect against malarial infection.

11

Hemoglobinopathies and Thalassemias

Thalassemias:

reduced amounts or absence of structurally nl globin chain

- α-thalassemia
- β-thalassemia

Hemoglobinopathies:

amino acid substitutions; structurally abnl globin

- Hb S, Hb C, Hb G-Philadelphia, Hb D, Hb O-Arabia
- Hb E
- Unstable Hbs
- Altered O_2 affinity
- Hb M

12

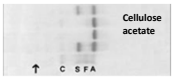
Hemoglobinopathies and Thalassemias

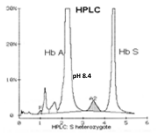
- Interactions among thalassemias and hemoglobinopathies are common
  - Hemoglobin S / beta thalassemia
  - Hb S and alpha thalassemia
  - Hemoglobin E / beta thalassemia

13

Hemoglobinopathies: Laboratory Dx

- Hb electrophoresis
  - Cellulose acetate (alkaline): provisional ID of Hb A, Hb F, Hb S, Hb D, Hb C, Hb E, Hb O, Hb H
  - Citrate agar (acidic): distinguish Hb C from Hb E, and Hb C from Hb O
- HPLC
  - Retention time, peak characterisitic influenced by single aa substitutions
  - Accurately identifies 75% of Hb variants
- Molecular biology
  - PCR; gene sequencing





14

Thalassemia Mutations

α-Thalassemia

- clinically expressed in fetus and at birth
- mostly caused by gene deletion

β-Thalassemia

- expressed after several mos of age because of switching from γ- to β-globin
- mostly caused by point mutations

(Steensma, Blood, 2005)

15

Beta-Thalassemias

- ↓ synthesis of β-globin chains
- Excess of α-globin chains
  - α-globin aggregates to form insoluble inclusions in erythroid precursors
  - highly toxic
  - intramedullary death of erythroid precursors: ineffective erythropoiesis

16

Membrane Defects in β-Thalassemia

Excess cellular Fe and unstable unpaired α-globin chains cause

- membrane lipid oxidation
- membrane protein damage
- decreased RBC deformability
- removal from the circulation

Membrane damage leads to PS exposure and hypercoagulability

17

Ineffective Erythropoiesis

- High degree of erythropoietic activity
- Death of erythroid precursors in BM
- Blood tests look like hemolysis, but retics not increased for degree of anemia
  - ↑ or high nl LDH, indirect bilirubin
  - ↓ haptoglobin
- Thal major and intermedia
  - both ineffective erythropoiesis & hemolysis

18

β-Thalassemia Mutations

β^0-thal mutations

- totally abolish expression of affected gene by critical point mutation or deletion

β^+-thal mutations

- partially abolish gene expression
- mild, moderate, severe-depending on amount of Hb A produced

19

Clinical Classification of β-Thalassemia*

β-Thalassemia trait

- uncomplicated heterozygous β-thalassemia
- β-thalassemia minor

β-Thalassemia intermedia

- no firm definition; many different genotypes

β-Thalassemia major

- Cooley's anemia
- homozygous or compound heterozygous β-thalassemia

*genotype-phenotype correlations often difficult to make: 100s of mutations, frequent interactions, role of other modifying genes and environment.

20

Clinical Diagnosis of β-Thalassemia

β-Thal trait

- microcytosis
- hypochromia
- +/- mild anemia
- elevated level of HbA_2 (>3.5%)

β-Thal intermedia

- microcytic anemia
- +/- Tx requirement
- high Hb F
- bone disease, iron loading, splenomegaly, pulmonary hypertension
- many different genotypes

β-Thalassemia major

- transfusion-dependent microcytic anemia
- very high Hb F (approaching 100%)
- bone disease, iron loading, splenomegaly, pulmonary hypertension
- many different genotypes

21

Beta-Thalassemias

Genotype	Phenotype	Hematologic Findings
Heterozygous [illegible]	Silent carrier/ thal minima	Normal
Heterozygous [illegible]	thal minor	Mild hypochromic anemia
Homozygous or compd hetero. [illegible]	thal intermedia	Moderate hemolysis & ineffective erythropoiesis
Homozygous or compd hetero. [illegible]	thal major	Severe hemolysis & ineffective erythropoiesis

22

Clinical Features of β-Thal Syndromes

	Major	Intermedia	Minor
Severity	4+	2+	1
Splenomegaly	4+	2-3+	0
Skeletal changes	2-4+	0-1+	0
Hemoglobin	<7 g/dL	7-10 g/dL	>10 g/dL
Hypochromia	4+	3+	2+
Microcytosis	3+	2+	1+
Nucleated RBCs	3+	0-1+	0

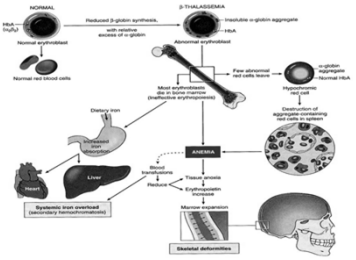
23

Hb Fractions in β-Thal Syndromes

	Nl	Minor	Intermedia	Major
Hb A	97%	>90%	15-65%	0%
Hb A2	2.2-3.5%	3.5-8%	5.4-10%	1-5.9%
Hb F	<1%	1-2%	30-75%	>94%

24

Pathophysiology of β-Thalassemia



25

β-Thalassemia Major: Clinical Features

Hematologic
- Severe microcytic anemia
- Splenomegaly
- Extramedullary hematopoiesis

Thromboembolism

Skeletal changes
- Expanded marrow cavity
- Thalassemic facies
- Osteopenia,
- Thin cortex

Growth retardation

26

β-Thalassemia Major: Clinical Features

Cardiopulmonary
- Myocardial Fe overload with arrhythmia; CHF
- Hemolytic PHT

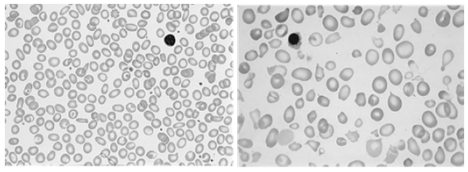
Liver
- Hepatic iron-loading with fibrosis, cirrhosis
- Pigmented gall stones

Endocrinopathies
- diabetes mellitus
- hypoparathyroidism
- hypogonadism and delayed puberty

Transfusion related
- Infection
- alloimmunization

27

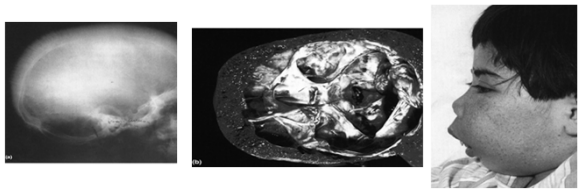
Peripheral Smear in β-Thalassemia



Thalassemia minor **Thalassemia major**

28

Skull and Face in Poorly Treated β-Thalassemia

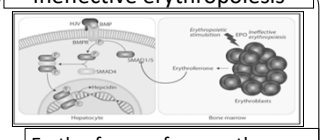


(Weatherall & Clegg, The Thalassaemia Syndromes, 2001)

29

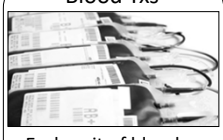
Iron Overload in Thalassemia Major

Ineffective erythropoiesis



Erythroferrone from erythro-blasts suppresses hepcidin

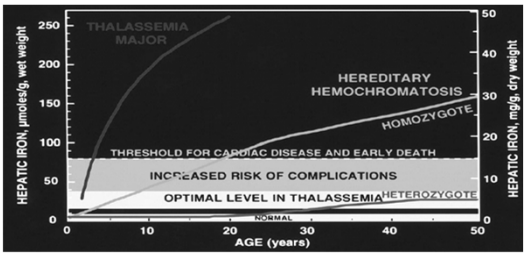
Blood Txs



Each unit of blood contains ≈ 225 mg Fe

30

Hepatic Iron and Organ Damage



(Olivieri & Brittenham, Blood 89: 739, 1997)

31

β-Thalassemia Major: Prognosis

- No Rx:
  - death by age 5 from infections, cachexia
- Episodic blood Tx's:
  - survival into 2nd decade
- Aggressive blood Tx's:
  - death ~age 20 from iron overload (cardiac)
- Aggressive blood Tx's plus iron chelation:
  - prolonged survival

32

β-Thalassemia Major: Treatment

- Management in comprehensive center:
  - endocrinology
  - cardiology
  - social services
- Hypertransfusion beginning 2nd or 3rd year:
  - maintain Hb 9-10.5 g/dL
- Splenectomy for increasing Tx requirement

33

β-Thalassemia Major: Treatment

- Fe chelation starting after age 3 years-
  - keep liver Fe <5 mg/g dry weight
- Also:
  - Consider stem cell transplantation
  - Increase synthesis of fetal Hb with hydroxyurea or other agents
  - Genetic counseling
  - Prenatal diagnosis

34

Iron Chelators

Deferoxamine
- Given by prolonged infusion

Deferasirox
- Once daily oral dosing
- Can remove cardiac Fe

Deferiprone
- Orally active; limited approval in US
- Removes cardiac iron

35

Potential Toxicity of Iron Chelation

- Skin reactions
- Bone, bone marrow, hepatic, GI, otologic, renal, retinal damage
- Yersinia infection
- Growth delay
- Agranulocytosis (deferiprone)

36

Alpha Thalassemia

- Decreased synthesis of α-globin chains
- Excess of beta-like globin chains
- Potential formation of abnl Hbs:
  - Hemoglobin Barts: γ_4
  - Hemoglobin H: β_4

37

Gene Deletion α-Thalassemia*

α^+-thalassemia

- deletion of a single gene on one chromosome 16 allele

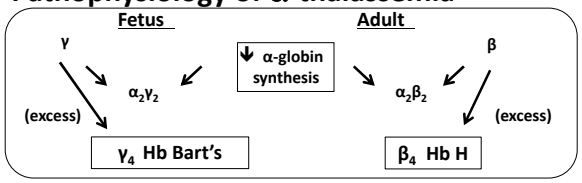
α^0 thalassemia

- deletion of both genes on one chromosome 16 alelle

*Point mutations less common cause of α-thalassemia; often associated with severe defect in α-globin synthesis

38

Pathophysiology of α-thalassemia

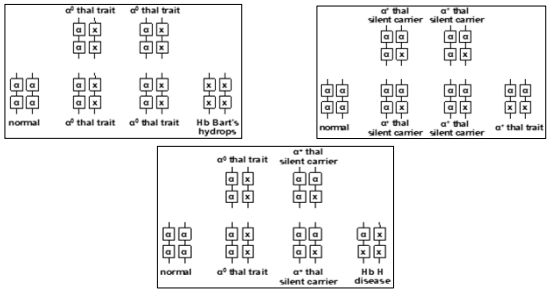


- High O_2 affinity- tissue hypoxia
- Instability of homotetramers
- Inclusion bodies; membrane damage
- Shortened RBC survival- hemolysis
- Splenomegarly- hypersplenism.

(Weatherall and Proven, Lancet 2000;355:1169-1175)

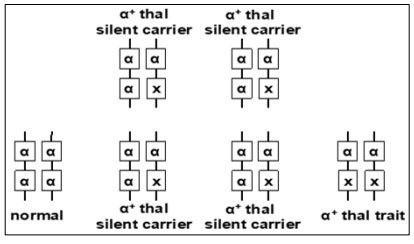
39

Genetics of α-Thalassemia: SE Asia



40

Genetics of α-Thalassemia: Africa



41

Alpha-Thalassemias

Geno-type	Pheno-type	Hb Barts (γ_4)	Hb H (β_4)	Heme Findings
αα/αα	Normal	---	---	Normal
αα/α-	Silent carrier	---	---	Normal
αα/-- or α-/α-	α-thal trait	2-10% newborn	---	Mild anemia
α-/--	Hb H disease	20-40% newborn	5-40% adults	Hemolysis, ineff. erythro.
--/--	Hydrops fetalis	~100% cord blood	---	Anemic stillborn

42

α-Thalassemia 'Silent Carrier'

- heterozygous α^+ thalassemia
- 3 of 4 alpha genes present and functional
- +/- mild anemia
- ↓ MCV (age dependent)

43

Alpha-Thalassemia Trait

2 of 4 alpha genes present and functional
- Homozygous α^+ thal (α-/α-): ~7% of Africans
- Heterozyg. α^0 thal ($\alpha\alpha$/--): common SE Asia

Clinical features:
- +/- mild anemia
- MCV <78 fL
- Hb Barts (γ_4) 2-10% in newborns

44

Alpha-Thalassemia Trait

- Often Dx of exclusion
  - Compatible ethnicity and clinical picture
  - Exclude Fe def, β-thal, hereditary sideroblastic anemia
- Molecular diagnosis available thru referral labs
- Do not confuse with Fe def or treat with iron

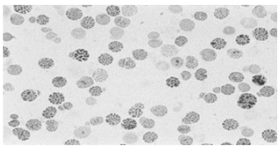
45

Hemoglobin H Disease

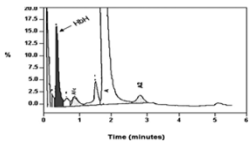
- Genotype α-/-- (SE Asia)
  - α^+-thal one allele
  - α^0-thal other allele
- 20-40% Hb Barts (γ_4) in newborn
- 5-40% Hb H (β_4) in adults
  - visualized by brilliant cresyl blue
  - Hb electrophoresis
  - HPLC

46

Diagnosis of Hemoglobin H Disease



RBC inclusions generated by brilliant cresyl blue



Fast moving peak on HPLC

47

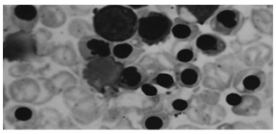
Hemoglobin H Disease

- Clinical features
  - hemolytic anemia of varying degrees
  - microcytosis
  - splenomegaly
  - ineffective erythropoiesis
  - Fe-loading

48

Hemoglobin Bart's Hydrops Fetalis

- Homozygous α^0-thalassemia (- -/- -)
- No functional α-globin genes: Hb Barts (γ_4)
- Eclampsia in mother
- Stillbirth
- Erythroblastosis in infant



49

Perspective
blood
Blood. 2017;129(10):1251-1259

An international registry of survivors with Hb Bart's hydrops fetalis syndrome

Duantida Songdej,[1,2] Christian Babbs,[1] and Douglas R. Higgs,[1] in collaboration with the BHFS International Consortium
[1]Medical Research Council Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom; and [2]Division of Hematology/Oncology, Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Intra-uterine Rx

- RBC transfusion
- Hematopoietic stem cell transplant
- Improved perinatal care

Long-term complications in survivors

- Growth retardation and neurodevelopmental delay
- Lifelong transfusion
- Associated congenital abnormalities

50

RBC Indices in Alpha-Thalassemia

	NI	Silent Carrier	Trait	Hb H Disease	Hydrops Fetalis
Hb (g/dL)	M: 14-18 F: 12-16	M: 13-16 F: 10-14	M: 12-15 F: 10-14	M: 9-13 F: 7-11	M: 3-8 F: 3-8
MCV (fL)	79-99	67-95	64-79	53-69	126-146
MCH (pg)	27-35	22-30	21-25	16-20	22-42

51

Atypical α-Thalassemias

α-Thalassemia-mental retardation syndromes

- ATR-16 (alpha thal. retardation associated with Chr. 16): large deletions involving α-globin genes
- X-linked- mutations in *ATRX* on Chr. X, which encodes a chromatin-associated protein

α-thalassemia-MDS

- acquired α-thalassemia in myelodysplastic syndrome

52

Management of α-Thal Syndromes

Hb Bart's

- Screening, genetic counseling, intrauterine transfusions

Hb H disease

- Regular medical follow-up
- Blood Tx and Rx of Fe overload as needed

Mild α-thalassemias

- Dx important for genetic counseling and avoiding misguided Rx with iron

53

Other conditions affecting globin chain synthesis

- Hemoglobin Lepore
  - Fusion of β and δ globin genes
  - ↓ synthesis of β-like globins
  - Homozygote: β-thal major phenotype
    - 8-30% Hb Lepore
    - 70-92% Hb F
  - Heterozygote: β-thal minor phenotype

54

Other conditions affecting globin chain synthesis

- Hb Constant Spring
  - non-deletional form of α-thalassemia
  - mutation in stop codon of α2-globin
  - poor output (1% of nl) of α-globin with 31 additional amino acids
  - homozysity leads to Hb H type clinical picture but nearly nl MCV

55

Other conditions affecting globin chain synthesis

- Hereditary persistence of fetal Hb
  - Up-regulation of γ chain synthesis
  - Almost 100% Hb F in homozygotes
  - Clinically silent
  - Causes:
    - deletions involving β and δ genes
    - ↓ expression of KLF1 transcription factor that activates BCL11A Hb F suppressor

56

Hemoglobin E (β26 glu→lys)

- Second most prevalent Hb variant: 30,000,000 worldwide; >80% in SE Asia
- Beta-thalassemia-like hemoglobinopathy (decreased β^E-mRNA production)
- RBC cytoplasm: precipitated α-chains, increased oxidant stress
- Carriers clinically silent; low MCV

57

Hb E Disorders

Condition	*Genotype*	*Clinical*
Hb E Trait	A/E	30% Hb E ± ↓ MCV
Hb E Disease	E/E	90% Hb E, ↓ MCV
Hb E-β-thal	E/beta$^{0,+}$	Hb E 40-85%, Hb F 10-60%, ↓ MCV, Hb
Hb SE disease	S/E	resembles Hb S-β$^+$ thal

58

Hemoglobin E/β-Thalassemia

- SE Asia
- Hb E 60-85%, Hb F 15-40%
- Mild to moderate microcytic hemolytic anemia
- Ineffective erythropoiesis and iron-loading

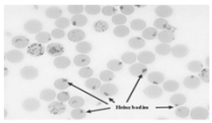
59

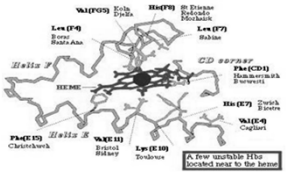
Unstable Hemoglobin Disease

- Congenital Heinz body anemia
- Rare autosomal dominant mutations → defective binding of heme by globin
- About 200 'unstable' variants: phenotype heterogeneous
- Heinz bodies, peroxidant membrane damage, hemolysis

60

Unstable Hemoglobins





Heinz bodies

- RBC inclusions of denatured Hb
- Stain: new methylene blue or bromocresol green
- Detect in RBC hemolysate: heat stability test or isopropanol stability test

61

Unstable Hemoglobins

Diagnosis

- Normocellular to microcytic hemolytic anemia
- ± Distinct electrophoretic pattern
- Heinz bodies by stain, heat stability or isopropanol stability
- Mutation detection
- Hb Köln most common (β98 Val➔Met)
  - anemia; retics 10-25%, splenomegaly

62

Unstable Hemoglobins

Hb Köln

- Most common
- β98 Val➔Met destabilizes heme pocket
- Anemia; retics 10-25%, splenomegaly

Hb Zurich

- β98 Val➔Met
- Increased affinity for CO
- Smokers protected from hemolysis

63

Unstable Hemoglobins

Treatment

- avoid oxidant drugs
- blood Tx's
- splenectomy in severe cases

64

Hemoglobin M Disorders

Hereditary methemoglobinemia and cyanosis

Autosomal dominant

Amino acid substitution in heme pocket and allows Fe oxidation (ferrous heme ⍰ ferric heme)

Clinical: asymptomatic cyanosis, slate grey/brownish skin, no dyspnea, nl life expectancy

65

Hemoglobin M Disorders

Diagnosis

- abnormal pulse oximeter saturation
- distinguish from other methemoglobinemias
- Hb delectrophoresis, Hb spectra
- Methemoglobin < 30%
- Cyanosis not reversible with Vit C, Meth Blue

Treatment: major hazard is misdiagnosis and untoward treatment

66

Other Forms of Methemoglobinemia

Congenital deficiency of *CYB5R3*

Type I: most common congenital methbemia

- Autosomal recessive; defective enzymatic reduction of Fe^{+3} to Fe^{+2} only in RBCs
- Methemoglobin usually < 30%
- Rx cyanosis: methylene blue or ascorbic acid

Type II: 10-15% of cases

- CYB5R3 deficiency in all cells
- Mental retardation and developmental delay
- Methylene blue improves cyanosis, not CNS

67

Other Forms of Methemoglobinemia

Oxidation Fe^{+2} to Fe^{+3} Hb by drugs or chemicals

Offending agents

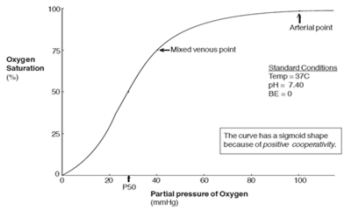
- Nitrites, trinitrotoluene, sulfanilamide, PAS, dapsone, primaquine, chloroquine, lidocaine, naphthoquinone, resorcinol, phenylhydrazine

Clinical

- Methemoglobin > 30% symptoms; > 50% lethal
- Emergency treatment: 1-2 mg/kg methylene blue as 1% solution IV over 10-15 minutes

68

Hemoglobin O_2 Dissociation Curve



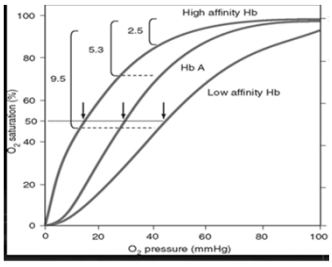
Arterial point:
pO_2 100, SaO_2 98%

Mixed venous:
pO_2 40, SaO_2 75%

p50:
pO_2 26, SaO_2 50%

69

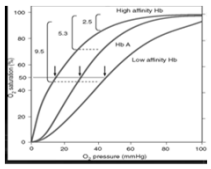
Hemoglobins with Altered O_2 Affinity



70

Hemoglobins with High O_2 Affinity

- Familial erythrocytosis; autosomal dominant
- α or β-chain can be affected
- ± distinct electrophoretic pattern
- Left shift O_2 dissociation curve (low P50)



71

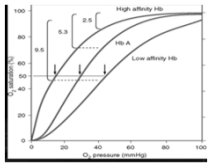
Hemoglobins with High O_2 Affinity

- Normal 2,3-DPG levels
- Diagnosis
  - Erythrocytosis in familial pattern
  - low P_{50}
  - Hb electrophoresis or HPLC
  - PCR or gene sequencing
- Treatment
  - Polycythemia mild; phlebotomy not necessary

72

Hemoglobins with Low O_2 Affinity

- Asymptomatic cyanosis
- Right shift in O_2 dissociation curve (high P50)
- Hb electrophoresis, HPLC
- No Rx required



73