IN THE NAME OF GOD
Diagnostic Approach to Anemic Patients
✓ Hg f = %90 in 24 wk
  %70 in term
  %2 in 1 year
✓ HgA = 16-20 wk DNA assay
  %5-10 in 24 wk fetal
  %30 in term
✓ HgA2 = %1 in term
  %2-3.4 in 1 year
✓ Hg A / HgA2 = 30
Definition

- Reduction in RBC mass or Hb concentration.
- -2SD < normal state
- 2.5% of normal population = Anemic
- Body oxygen metabolism & accompanying CV compensation + Hb concentration + adjust with age & sex: Functionally anemic
<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>HB (g/dl)</th>
<th>HCT (%)</th>
<th>MCV (µ³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Lower Limit</td>
<td>Mean</td>
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<tr>
<td>0.5-1.9</td>
<td>12.5</td>
<td>11.0</td>
<td>37</td>
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<td>2-4</td>
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<td>11.0</td>
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<td>8-11</td>
<td>13.5</td>
<td>12.0</td>
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<tr>
<td>12-14</td>
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<td>Female</td>
<td>13.5</td>
<td>12.0</td>
<td>41</td>
</tr>
<tr>
<td>Male</td>
<td>14.0</td>
<td>12.5</td>
<td>43</td>
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<tr>
<td>Female</td>
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<td>12.0</td>
<td>41</td>
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<tr>
<td>Male</td>
<td>15.0</td>
<td>13.0</td>
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<td>18-49</td>
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<tr>
<td>Female</td>
<td>14.0</td>
<td>12.0</td>
<td>42</td>
</tr>
<tr>
<td>Male</td>
<td>16.0</td>
<td>14.0</td>
<td>47</td>
</tr>
</tbody>
</table>
ANEMIA

HEMOGLOBIN AND INDICES
RETIC COUNT AND MORPHOLOGY

Inadequate response (RPI < 2)
Hypochromic, Microcytic
Iron deficiency
- Chronic blood loss
- Poor diet
- Cow's milk protein intolerance
- Menstruation
Thalassemia
- β major, minor
- α minor
Chronic inflammatory disease
Cooper deficiency
Sluder's anemia
Aluminum, (?) lead intoxication
Hereditary pyropoikilocytoses
Hemoglobin CC
Hemoglobin EE

Normochromic, Normocytic
Chronic inflammatory disease
- Intention
- Collagen-vascular disease
- Inflammatory bowel disease
Recent blood loss
Malignancy/marrow infiltration
Chronic renal failure
Transient erythroblastopenia of childhood
Marrow aplasia/hypoplasia
HIV infection
Hemophagocytic syndrome

Macrocvtic
Vitamin B12 deficiency
- Pernicious anemia
- Ileal resection
- Strict vegetarian
- Abnormal intestinal transport
- Congenital intrinsic factor or transcobalamin deficiency
Folate deficiency
- Malnutrition
- Malabsorption
- Antimetabolite
- Chronic hemolysis
- Phenylketonuria
- Trimethoprim/sulfa
Hypothyroidism
Orotic aciduria
Chronic liver disease
Lesch-Nyhan syndrome
Down syndrome
Marrow failure
- Myelodysplasia
- Fanconi anemia
- Congenital dyserythropoietic anemia
- Aplastic anemia
- Pearson syndrome (mitochondrial disorder)
- Diamond-Blackfan syndrome
Drugs
- Alcohol
- Azidothymidine (zidovudine)

Adequate response (RPI > 3)
R/O blood loss
Hemolytic Disorders
Hemoglobinopathy
- Hemoglobin SS, SC, S-β thalassemia
Enzymopathy
- G6PD deficiency (loite cells)
- Pyruvate kinase deficiency
Membranopathy
- Hereditary spherocytosis
- Elliptocytosis
- Ovalocytosis
Extrinsic factors
- DIC, HUS, TTP
- Abetalipoproteinemia
- Burns
- Wilson disease
- Vitamin E deficiency
Immune hemolytic anemia
- Autoimmune
- Isoimmune
- Drug-induced
Classification

- 1- Physiologic Classification (Best)
- 2- Red Cell Size Classification
A. RBC Production Disorders (rate < expected for degree of anemia)

B. Erythroid maturation & Ineffective erythropoiesis Disorders

C. Hemolytic Anemia
RBC Production Disorders (rate < expected for degree of anemia)

- Marrow failure
- Impaired EPO production
Marrow failure

1. Aplastic anemia
2. Pure red cell aplasia
3. Marrow replacement
Marrow replacement

- Malignancies
- Osteopetrosis
- Myelofibrosis
  - vitamin D deficiency
  - Pancreatic insufficiency - marrow hypoplasia syndrome
Impaired EPO production

• 1. Chronic renal disease
  2. Hypothyroidism, Hypopituitarism
  3. Chronic inflammation
  4. Protein malnutrition
  5. HB mutants with decreased affinity for oxygen
Erythroid maturation & Ineffective erythropoiesis Disorders

- Cytoplasmic maturation abnormalities
- Nuclear maturation abnormalities
Cytoplasmic maturation abnormalities

- Iron deficiency
- Thalassemia syndrome*
- Sideroblastic anemias
- Lead poisoning
Nuclear maturation abnormalities

- Vitamin B$_{12}$ deficiency
- Folic acid deficiency
Hemolytic Anemia

- 1. Defects of HB
  - a. Structural mutants
  - b. Synthetic mutants (Thalassemia syndrome)

- 2. RBC membrane defects

- 3. Red cell metabolism defects

- 4. Antibody-mediated
Hemolytic Anemia (cont)

- 5. Mechanical injury to the erythrocyte
- 6. Thermal injury to the erythrocyte
- 7. Oxidant-induced red cell injury
- 8. Infectious agent-induced red cell injury
- 9. PNH
- 10. Red cell membrane plasma-induced abnormalities
Red cell Size Classification of Anemia

A. Microcytic

- 1. Iron Def.
- 2. Chronic Lead poisoning
- 3. Thalassemia syndrome
- 4. Sideroblastic anemia
- 5. Chronic inflammation
- 6. Some congenital hemolytic anemias with unstable HB
Important Historical Factors

Age:

- Nutritional IDA is never responsible for anemia in term infants before 6 months of age; rarely seen in premature infants prior to the time they have doubled their birth weight.
- In neonatal period: recent blood loss, isoimmunization, initial manifestation of CHA or congenital infection.
- First detected in 3-6 months: congenital disorder of HB synthesis or HB structure.
Important Historical Factors

- **Gender**: X-linked disorders in males (G6PD-d, PKD)
- **Race**: HB S & C more common in blacks, B-Thalassemia more common in whites, α-Thalassemia trait most common among black & yellow race.
- **Neonatal**: hyperbilirubinemia: CHA,
- **Diet**: document sources of iron, vit B12, folic acid, or vit E in diet.
  - Pica, geophagia, pagophagia => IDA
- **Drugs**: - Oxidant-induced hemolytic anemia
  - Phenytoin- induced megaloblastic anemia
  - Drug- induced Aplastic anemia
<table>
<thead>
<tr>
<th>Important Historical Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infection:</strong> Hepatitis-induced Aplastic anemia,</td>
</tr>
<tr>
<td>■ Infection-induced red cell aplasia or hemolytic anemia</td>
</tr>
<tr>
<td><strong>Inheritance:</strong></td>
</tr>
<tr>
<td>FH of anemia, Jaundice,</td>
</tr>
<tr>
<td>Gallstones, or Splenomegaly.</td>
</tr>
<tr>
<td><strong>Diarrhea:</strong></td>
</tr>
<tr>
<td>Small bowel disease with malabsorption of folate or vit B12.</td>
</tr>
<tr>
<td>IBD with blood loss.</td>
</tr>
<tr>
<td>Exudative enteropathy with blood loss.</td>
</tr>
<tr>
<td>Skin</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Petechiae, Purpura</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Carotenemia</td>
</tr>
<tr>
<td>Jaundice</td>
</tr>
<tr>
<td>Cavernous Hemangioma</td>
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<tr>
<td>Ulcer on Lower Extremity</td>
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<tr>
<td>Key Physical Findings</td>
</tr>
<tr>
<td>-----------------------</td>
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<tr>
<td>Eyes</td>
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<td>Key Physical Findings</td>
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<tr>
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<tr>
<td><strong>Glossitis</strong></td>
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<tr>
<td><strong>Angular stomatitis</strong></td>
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<tr>
<td><strong>Mouth</strong></td>
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<tr>
<td><strong>Angular stomatitis</strong></td>
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<tr>
<td><strong>Chest</strong></td>
</tr>
<tr>
<td><strong>Hands</strong></td>
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<tr>
<td><strong>Hands</strong></td>
</tr>
<tr>
<td><strong>Spoon nails</strong></td>
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<tr>
<td><strong>Spleen</strong></td>
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</tbody>
</table>
Paraclinical Approach

Initial laboratory tests:
- HB & HCT
- Red cell indices
- Platelet count
- WBC & diff
- Reticulocyte count
- Peripheral blood smear
## Complete Blood Count

- Hemoglobin **Hb**
- Hematocrit **Hct**
- Mean corpuscular hemoglobin **MCH**
- MCH concentration **MCHC**
- Mean corpuscular volume **MCV**
- RBC distribution width **RDW**
- Erythrocyte count **RBC**
- Leukocyte count
- Platelet count
- Reticulocyte count
Complete Blood Count

- **Directly measured indices:**
  - Hb (more reliable, more directly related to O2 carrying capacity)
  - MCV
  - RBC count

- **Calculated indices:**
  - Hct = MCV x RBC
  - MCH = Hb / RBC
  - MCHC = Hb / Hct = Hb / (MCV x RBC)

- **RDW:**
  - Coefficient of variation of the erythrocyte volume distribution = cell size variability
Coulter Counter

- Most widely used method.
- Directly measure the MCV and compute the HCT from the MCV and RBC.
- Cold agglutinins in high titer tend to cause spurious macrocytosis with low red cell counts and very high MCHCs. Warming either the blood or the diluent eliminates this problem.
Red cell volume distribution width (RDW)

- Index of the variation in red cell size.
- Detect Anisocytosis.
- Derived from RBC histogram.
- \( RDW = \frac{SD}{MCV} \times 100 \)
- Because RDW reflects the ratio of SD and MCV, a wide red cell distribution curve in a patient with a markedly increased MCV may still generate normal RDW.
- Normal range = 11.5-14.5 in adults
- For infants and children = 1.5-15
<table>
<thead>
<tr>
<th>RDW</th>
<th>MCV</th>
<th></th>
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<tbody>
<tr>
<td>Normal</td>
<td>Low</td>
<td>Heterozygous α-</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and β-Thalassemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aplastic Anemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lead poisoning</td>
</tr>
<tr>
<td>High</td>
<td>IDA</td>
<td>Early IDA</td>
<td>Newborn, Prematurity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vitamin B12 def.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Folic acid def.</td>
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</tr>
</tbody>
</table>

- **MCV & RDW**

- **MCV**: Low, Normal, High
- **RDW**: Normal, High
Blood Film

- The single most useful procedure in the initial evaluation of the patient with anemia.

- Can be classified red cell hemolytic disorders by predominant morphology:
Normal Peripheral Blood Smear

G.v.e.F
Hb 13.7
MCV 77
RDW 13.4
RBC 5.35

Homogeneous picture of red blood cells with almost no variability in size. Central pallor should be about one-third of the diameter of the cell.
Normal Peripheral Blood Smear
Reticulocyte count

- **Increased:** (RI\(\geq\)3%)
  - Chronic blood loss
  - Hemolysis
- **Normal or decreased:** (RI\(\leq\)1.5%)
  - Impaired red cell formation

- Must be adjusted for the level of anemia to obtain the *Reticulocyte Index*:
  \[ \text{Retic count} \times \frac{\text{Patient’s HCT}}{\text{Normal HCT}} \]
Outline of Approach

I- Initial Screening & presumptive diagnosis

A- Careful History and Physical Examination

B- Initial Lab Tests
Outline of Approach

II- Confirmatory Studies:

- Direct antiglobulin test
- G6PD screening test
- Osmotic fragility
- HB Electrophoresis
- BMA/ biopsy
- Bilirubin, LDH, B12, Folate, Haptoglobin, Ferritin, Iron, TIBC, ....
Initial evaluation of anemia

WBC – Absolute neutrophil count – Platelets – Blood smear

↑ WBC
± shift to left
(see ‘Leukocytosis’, p 38)

↓ WBC + ↓ ANC

↓ WBC +/or ANC
↓ Platelets
NI WBC/ANC
Blood smear
DCT

↑ Platelets
(see ‘Thrombocytosis’, p 62)

Borderline Platelets
(see ‘Pancytopenia’, p 12)

Borderline WBC/ANC

+ DCT
Microangiopathic changes

NI WBC, ANC, platelets

Clinical evidence of acute infection or autoimmune disease

Otherwise well

Persistent ↓ ANC
± chronic infections ± failure to thrive

Drug usage

Shwachman-Diamond syndrome

Drug induced

Acute bacterial infection

Acute or chronic viral illness

Collagen vascular disorder

Evans syndrome

↓ MCV

Decreased
(see ‘Microcytic anemia’, p 8)

Normal
(see ‘Normocytic anemia’, p 8)

Increased
(see ‘Macrocytic anemia’, p 10)

R/O DIC if ↓ platelets

Specific Dx and Rx

Possible corticosteroids
Microcytic anemia

- Fe intake or blood loss
  - RBC, RDW, MCHC
  - Anisocytosis
  - Mild ovalocytosis
  - Dimorphic population

- Family Hx of anemia or thalassemia
  - Mediterranean/Asian/African ancestry

- Chronic illness or infection
  - Target cells
  - Family Hx
  - African/Asian ancestry

- Pb level
  - Rare disorders

- RBC indices
  - Blood smear

- Trial oral Fe
  - RBC, NI or minimal
  - RDW
  - Hb ≥9 g/dl (1.4 mmol/l)
  - No hepatosplenomegaly
  - Target cells
  - Basophilic stippling

- Hb electrophoresis

- Hb <9 g/dl (1.4 mmol/l)
  - Severe hypochromia
  - Anisopoikilocytosis
  - Target cells
  - Normoblastemia

- Fe, TIBC, TS, ferritin
  - Thalassemia intermedia or major
  - Hb H disease

- Anemia of infection or chronic disease
  - Hb C or E
  - Sickle thalassemia
  - Unstable hemoglobinopathy

- Pb poisoning

- Sideroblastic anemia
  - Protein calorie malnutrition
  - Metabolic defects of Fe absorption and metabolism

Iron deficiency anemia

- Thalassemia trait

(see 'Thalassemia', p 24)

(see 'Presumed iron deficiency anemia which fails to respond to oral iron', p 22)

Continue Fe therapy

- ° Indicates a diagnosis or test that is considered significant or critical.
Normocytic anemia

Evaluate clinical and laboratory evidence of blood loss
  Indirect bilirubin
  Reticulocyte count
  Blood smear

Ni reticulocytes
Ni indirect bilirubin
No blood loss

Blood loss
  + reticulocytes
  ± indirect bilirubin
  Ni SMER or polychromasia
  and/or dimorphic population

↑ reticulocytes
↑ indirect bilirubin
No blood loss

Clinical evaluation
  Smear: no PMN hypersegmentation or macro-ovalocytosis

Pregnant
  Viral or bacterial infection

Hb ≥ 9 g/dl, not ill appearing
  Hb < 9 g/dl and/or ill appearing

Observe and repeat Hb in 3-4 weeks
  Fe/TIBC, ferritin
  ↑ ferritin
  Reassess

Hb
  ↓ Fe and TIBC
  ↓ ferritin

Anemia of pregnancy

Anemia of infection

Anemia of renal disease

Anemia of chronic disease

TEC
  DBA
  Acquired pure RBC aplasia

IDA
  Early megaloblastic anemia or combined
  IDA + megaloblastic anemia

Ensure patient is stable

No further evaluation

Further Dx and Rx

R/O IDA
(see “Macrocytic anemia”, p 10)
Macrocystic anemia

RBC indices
Blood smear

Macro-ovalocytosis
PMN hypersegmentation

Megaloblastic anemia ⊗

Serum B₁₂
RBC folate ⊗

↓ B₁₂ level
↓ folate ⊗
NI B₁₂ and folate

NI Hgb or only mildly ↓

Anemia
Bone marrow aspirate and biopsy ⊗

Pure RBC aplasia ± congenital anomalies

Hypocellular ↑ Hgb F

Spurious ↑ MCV ⊗

Drug-induced megaloblastic anemia

Rare disorders ⊗

Reticulocytosis ⊗

Diamond-Blackfan anemia ⊗

Fanconi anemia ⊗

Rare findings ⊗

Cold agglutinins
Hypergycemia
Leukocytosis

Drugs ⊗
Congenital heart disease
Down syndrome
Hypothyroidism
Liver disease
Asplenia

B₁₂ deficiency
Folate deficiency

Identify cause ⊗
R/O pernicious anemia
Rx B₁₂ deficiency

Consider if drug can be discontinued

Evaluate for hemolysis, blood loss, recovering aplasia

Corticosteroid Rx

Confirmed with lymphocyte chromosomal analysis ± DNA studies
Neonatal anemia due to impaired RBC production

Reticulocytes <2%
Ni indirect bilirubin
CBC – RBC indices – Blood smear

↓ MCV

Mediterranean, Asian or African ancestry
± Obstetrical complications
Twin gestation

NI MCV

Sick infant

NI MCV

Pancytopenia

No evidence of underlying disease

Evidence of infection

BM aspirate

BM aspirate and biopsy

Pure RBC aplasia

Normal

Bone marrow replacement

Megaloblastic changes

α-Thalassemia trait
Hb H disease

Blood loss and resulting iron deficiency

Acute and chronic disease

Infection

Neuroblastoma
Congenital leukemia
LCH
Osteopetrosis

DBA Rare diagnoses
Megaloblastic anemia

(see 'Thalassemia', p 24)
Thalassemia

**History**
- Laboratory criteria: 
  - CBC: hypochromic microcytic anemia
  - Target cells on blood smear

**Physical examination**
- Age of manifestation, clinical presentation, other cause of anemia excluded

**α-Thalassemia**
- Intrauterine death
- Stillborn
- Hydrops fetalis
- Neonatal anemia
- Signs of hemolysis
- Mild anemia or normal

**Hb analysis**
- Severe anemia in late infancy
- Anemia beyond infancy
- Mild, asymptomatic anemia

**β-Thalassemia**
- No HbA/HbF
- Hb Barts (γ4) 80–90%
- Hb Portland (εγ2)
- Hb Barts 20–30%
- In later childhood, HbH (β4) 4–20%

**Genotype**
- α-globin gene: --/--
- α-globin gene: α-/--
- α-globin gene: α+/-
- β-globin gene: β+/β+
- β-globin gene: β-β- (dominant)
- β-β- + α-globin gene triplication
- β-β- + HPFH mutation or β-thalassemia
- δβ/δβ or δβ/δβ+ - α-Thalassemia trait

**Therapy**
- Prenatal transfusion
- Regular transfusion
- Iron elimination therapy
- BMT
- Family studies and counseling
- Transfusion in hemolytic/aplastic crisis ± splenectomy
- Folic acid
- Family studies and counseling
- Regular transfusion
- Iron elimination therapy
- BMT
- Family studies and counseling
- No or irregular transfusion
- Splenectomy
- Family studies and counseling
- Family studies and counseling
Newborn screening for hemoglobinopathies

Population hemoglobinopathy screening

Hemoglobin phenotype

FA FAS FAC FA 'X' FS FSC FSA F 'X' F only AF

Unusual phenotype

Confirmatory testing Family studies

Normal phenotype Trait phenotype HbSS HbS-HPFH HbSβ+ T HbSC HbSβ+ T β-Thalassemia major HPFH Extreme prematurity

Both parents HbS donors (e.g., HbAS) Parental HbF donor and HbS donor Parental microcytosis/ elevated HbA2 Parental Hbc donor and HbS donor Parental microcytosis/ elevated HbA2

Both parents thalassemia minor (↑HbA2) Parental HgbF donor Parents normal

Genetic counseling No further testing required Refer to comprehensive sickle cell center
Thank you for your attention
Introduction

- The most common nutritional deficiency in children throughout the world.
- Higher incidence in infancy.
- 5.5% in school children, 5-8 yrs.; 2.6% in preadolescent; 25% in pregnant teenage girls.
- Higher in black children.
- Inversely proportional to economic status.
Introduction

- Iron lack the glitter of gold and silver but outshines both in biologic importance.
- Vital to the function of a number of critical enzymes (T).
- Human existence is inextricably linked to iron.
Nonhematological Manifestation

- I. Gastrointestinal tract
- II. Central nervous system
- III. Cardiovascular system
- IV. Musculoskeletal system
- V. Immunologic system
- VI. Cellular changes
- VII. Other tissues
Etiologic Factors (T)

- **Dietary**
  - Requirements
  - Food Iron Content

- **Growth**

- **Blood Loss**

- **Impaired Absorption**
Infants at risk for IDA (T)

- Increased iron needs
- Blood loss
- Dietary factors
Differential Diagnosis (T)

- Hemoglobinopathies
- Heme synthesis disorders caused by chemicals
- Sideroblastic anemias
- Chronic infections or other inflammatory states
- Malignancy
- Hereditary Orotic aciduria
- Hypo- or Atransferrinemia
- Copper deficiency
- Inborn error of metabolism
Differential Diagnosis

Further evaluation in children with:
- No suspicious history of Fe deficiency
- Severe anemia
- Atypical hematological findings
- < 6mo of age
- No response to iron trial

R/O blood loss, inflammation

RBC count (> 5 million/ul in thal trait)

MCV/RBC ratio (<13 thal trait, >15 Fe def)

Peripheral blood smear:
- Hypochromia, anisocytosis (greater in Fe deficiency)
- Poikilocytosis, target cells, cigar cells, basophilic stippling
- (greater in thalassemia trait)
Severe Iron Deficiency

12 yo. F
Irregular
Periods
X 1yr

Hb 5.8
MCV 59
RDW 25.2
RBC 3.50
Retic 2.2
Pit 891K
Ferritin 1

40x

Marked Hypochromia. Central pallor is great than one-third of the diameter of the cell.

BJCRH, 2003
Severe Iron Deficiency

Large central pallor and variability in sizes.
Treatment

- Nutritional Counseling
- Oral Iron Medication
- Parenteral therapy
- Blood Transfusion
- Partial Exchange Transfusion
Thalassemia Syndrome
Characteristics: Thalassemia

- Hereditary disorders that can result in moderate to severe anemia
- Basic defect is *reduced production* of selected globin chains
Demographics: Thalassemia

- Found most frequently in the Mediterranean, Africa, Western and Southeast Asia, India and Burma
- Distribution parallels that of Plasmodium falciparum
Hemoglobin

Two α and two β globin chains (α₂β₂) make up Hemoglobin A
Globin Chain Genes

Chromosome 16

Chromosome 11
Developmental Switching of Human Hemoglobin

Olivieri, N. NEJM
Normal Hemoglobins

- Hb A = $\alpha_2\beta_2$
- Hb F = $\alpha_2\gamma_2$
- Hb A$_2$ = $\alpha_2\delta_2$
- Hb H = $\beta_4$
- Hb Bart’s = $\gamma_4$
Hemoglobin Variants

- Hemoglobin S ($\alpha_2\beta_2^6$ Glu-Val)
  - predisposes to sickling
- Hemoglobin C ($\alpha_2\beta_2^6$ Glu-Lys)
  - predisposes to cellular dehydration
- Hemoglobin E ($\alpha_2\beta_2^{26}$ Glu-Lys)
  - microcytosis, slight anemia
Cellulose
Acetate
pH 8.6

AA (Normal)
Elevated A2
AS
AC
SS
S/βthal
SC
Secondary Laboratory Investigation
Cellulose Acetate Hb Electrophoresis

Normal

- $A_2/C$
- $S$
- $F$
- $A^+$
Secondary Laboratory Investigation
Cellulose Acetate Hb Electrophoresis

Normal
Hb SS

- A₂/C  S  F  A +
Secondary Laboratory Investigation
Cellulose Acetate Hb Electrophoresis

- $A_2/C$
- $S$
- $F$
- $A+$

Normal
Hb SS
Hb AS
Secondary Laboratory Investigation
Cellulose Acetate Hb Electrophoresis

- $A_2/C$
- $S$
- $F$
- $A^+$

Normal
Hb SS
Hb AS
Hb SC
Secondary Laboratory Investigation
Cellulose Acetate Hb Electrophoresis

- $A_2/C$
- $S$
- $F$
- $A+$

<table>
<thead>
<tr>
<th>Normal</th>
<th>Hb SS</th>
<th>Hb AS</th>
<th>Hb SC</th>
<th>Hb CC</th>
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</tbody>
</table>
Secondary Laboratory Investigation
Cellulose Acetate Hb Electrophoresis

- A$_2$/C  S  F  A+

Normal

Hb SS
Hb AS
Hb SC
Hb CC
HB AD
α- and β-Thalassemias

- The most common monogenic diseases
- Widespread throughout Mediterranean, Africa, Middle East, Indian subcontinent and Burma, SEA, Southern China, Malaysia, and Indonesia
- Gene frequency 3-10 percent
  - mutations tend to be very regionally specific
<table>
<thead>
<tr>
<th>Condition</th>
<th>CBC Description</th>
<th>Hemoglobin Description</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal CBC</td>
<td>Normal CBC</td>
<td>Rare live births</td>
</tr>
<tr>
<td>(\alpha) Thal-1 trait</td>
<td>Normal</td>
<td>Mild microcytic anemia or no anemia</td>
<td></td>
</tr>
<tr>
<td>(\alpha) Thal-2 trait</td>
<td>CBC Normal</td>
<td>Lowish MCV</td>
<td></td>
</tr>
<tr>
<td>Hb H Disease</td>
<td>Lowish MCV</td>
<td>Moderate microcytic anemia</td>
<td></td>
</tr>
<tr>
<td>Hydrops fetalis</td>
<td>Moderate microcytic anemia</td>
<td>Hydrops fetalis</td>
<td></td>
</tr>
</tbody>
</table>
### Classification & Terminology

#### Alpha Thalassemia

<table>
<thead>
<tr>
<th>Category</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>( \alpha\alpha/\alpha\alpha )</td>
</tr>
<tr>
<td>Silent carrier</td>
<td>(-\alpha/\alpha\alpha)</td>
</tr>
<tr>
<td>Minor</td>
<td>(-\alpha/-\alpha)</td>
</tr>
<tr>
<td>Hb H disease</td>
<td>(--/-\alpha)</td>
</tr>
<tr>
<td>Barts hydrops fetalis</td>
<td>(--/--)</td>
</tr>
</tbody>
</table>
β-Thalassemia

- Due to mutations in the β-globin gene leading to decreased production of normal chains
- Varying degrees of microcytic anemia
- Heterozygous (thalassemia minor)
  - mild anemia, microcytosis, RBC > 5 X 10^6
- Homozygous or compound heterozygotes (thalassemia major or intermedia)
  - moderate to severe hemolytic anemia with splenomegaly
## Classification & Terminology

### Beta Thalassemia

<table>
<thead>
<tr>
<th>Type</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>Normal</td>
<td>$\beta/\beta$</td>
</tr>
<tr>
<td>Minor</td>
<td>$\beta/\beta^0$</td>
</tr>
<tr>
<td></td>
<td>$\beta/\beta^+$</td>
</tr>
<tr>
<td>Intermedia</td>
<td>$\beta^0/\beta^+$</td>
</tr>
<tr>
<td>Major</td>
<td>$\beta^0/\beta^0$</td>
</tr>
<tr>
<td></td>
<td>$\beta^+/\beta^+$</td>
</tr>
</tbody>
</table>
Excess free α-globin chains

Hemolysis

Ineffective erythropoiesis

Membrane binding of IgG and C3

Formation of heme and hemichromes

Denaturation
Degradation

Iron-mediated toxicity

Removal of damaged red cells

Increased erythropoietin synthesis

Reduced tissue oxygenation

Anemia

Splenomegaly

Erythroid marrow expansion

Increased iron absorption

Iron overload

Skeletal deformities, osteopenia
Thalassemia “Trait”

Hoffbrand and Pettit
CBC in Thalassemia Trait

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>7.2</td>
</tr>
<tr>
<td>RBC</td>
<td>6.9 X 10⁶</td>
</tr>
<tr>
<td>Hb</td>
<td>15.8</td>
</tr>
<tr>
<td>Hct</td>
<td>47.6</td>
</tr>
<tr>
<td>MCV</td>
<td>69</td>
</tr>
<tr>
<td>RDW</td>
<td>14</td>
</tr>
<tr>
<td>Plt</td>
<td>351,000</td>
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</tbody>
</table>
β-thalassemia major

Hoffbrand and Pettitt
<table>
<thead>
<tr>
<th>Parameter</th>
<th>α-Thal</th>
<th>β-Thal</th>
<th>IDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (mg/dl)</td>
<td>12±0.6</td>
<td>M=12.6±1.4</td>
<td>10.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F=10.8 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>5.6 ± 0.5</td>
<td>M=5.8 ± 0.6</td>
<td>4.67 ± 0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F= 5.1 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>72.2 ± 3.3</td>
<td>55 - 69</td>
<td>67 ± 6.6</td>
</tr>
<tr>
<td>MCH</td>
<td>23.2 ± 3.3</td>
<td>20.2 ± 2</td>
<td>21.8 ± 2.9</td>
</tr>
<tr>
<td>Hb A2</td>
<td>N or ↓</td>
<td>5.2 ± 0.8</td>
<td>N or ↓</td>
</tr>
<tr>
<td>Hb F</td>
<td>&lt;1%</td>
<td>2.1 ± 1.2</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>
Summary

- Thalassemias and hemoglobinopathies are paradigms for disease that result from abnormal protein interactions.
- They are quite common and can have serious clinical consequences.
- Knowledge of the genetics of these disorders allows effective genetic counseling and prenatal screening.
- Insight into the pathogenesis of these disorders has lead to rational drug development.
- Gene augmentation therapy holds the hope of eventual “cure”
THANK YOU
SEE YOU LATER