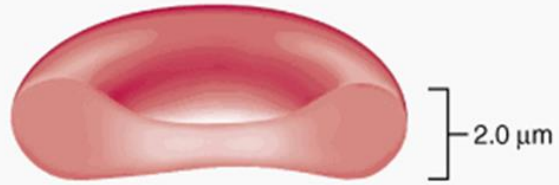




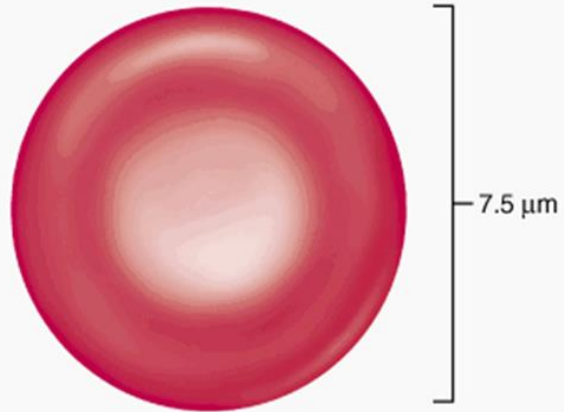
Anemia

Professor Nourz A. Elgheriani

Pediatric Hematology Oncology



Side view



Top view

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PLAN

- Definition
- Hemoglobin level according to age & sex
 - Morphological Classification
 - Pathophysiological Classification
 - Initial necessary investigation
 - Reticulocyte count
 - Peripheral blood film
 - Iron deficiency anemia
 - Megaloblastic Anemia
 - Hemolytic Anemia
(HS, G6PDD, Beta Thalassemia, SCA, AIHA)
 - Bone marrow failure

Anemia Definition

- Reduction of
Hemoglobin concentration (Hb),
Red blood cells (RBCs) mass &
Hematocrit
2 standard deviation below the mean for age.

WHO

(The normal range of hemoglobin varies with age)

Normal values

AGE	Hgb Mean/ (-2SD)	HCT% Mean/ (-2SD)	MCV Mean/ (-2SD)
Newborn	16.5 (13.5)	51 (42)	108 (96)
1 Month	13.9 (10.7)	44 (33)	101 (91)
2 Months	11.2 (9.4)	35 (28)	95 (84)
6 Months	12.6 (11.0)	36 (31)	76 (68)
> 6 Months	12.5 (11.0)	36 (33)	81 (70+ age per yr)
Adult	15.5 (13.5)	47 (40)	90 (80)
Male	14.0 (12.0)	41 (36)	90 (80)
Female			

Severity of anemia

according to hemoglobin concentration in grams/deciliter

- Mild > 9.5 gm/dl
 - Moderate
- Severe < 7 gm/dl

Rapid progressive decrease occurs in:

1. G6PD deficiency.
2. Autoimmune HA.
3. Severe hemorrhage.

Initial investigation for anemia

CBC

- Hb
- WBCs count
- Platelet count
- HCT
- MCV
- MCHC
- RDW
- Reticulocyte Count

- **Platelet count:**

Thrombocytopenia in:

Aplastic anemia
leukemia,
Bone marrow infiltration,
Hemolytic anemic syndrome,
Evan syndrome.

Thrombocytosis in:

Iron deficiency anemia
Sickle cell anemia

- **WBCs:**

segmented, lobulated neutrophils in megaloblastic anemia
(vitamin B12 & folate deficiency).

Increased in leukemia

Decreased in leukemia, aplastic anemia (bone marrow suppression).

Mean corpuscular volume (MCV)

- At birth the normal range is 98-123 (fl) femtoliter
- In old child and adults the normal range is 80-100
- The MCV is used to classify RBCs as:
 - Microcytic $< 80 \mu\text{m}^3$ [80 fL]) (femtoliter)
 - Normocytic = 80 to 100 μm^3 [80 to 100 fL])
 - Macrocytic $>100 \mu\text{m}^3$ [100 fL]

- **Mean corpuscular hemoglobin (MCH)**

is the average weight of hemoglobin/cell in picograms

(pg= 10^{-12} g)

- At birth the normal range is 31-37
- In adults the normal range is 26-34
- This is not used much because it does not take into account the size of the cell.

Mean corpuscular hemoglobin concentration (MCHC)

is the average concentration of hemoglobin

The normal range is 30-36 (g/dl)

The MCHC is used to classify RBCs as:

- Hypochromic (<31)
- Normochromic (30-36)
 - Hyperchromic,

not >37, just decreased amount of membrane.

Red cell distribution width (RDW)

is a measurement of the variation in RBC cell size.

Anisocytosis (red cells of unequal size)

- Standard deviation/mean MCV x 100
- is provided the more modern automated hematology analyzers particularly distinguishing between **iron deficiency anemia** (high RDW, normal to low MCV) and uncomplicated heterozygous **Thalassemia** (normal RDW, low MCV) (microcytic) & dimorphic cell populations (including patients who have had transfusions or have been recently treated for a nutritional deficiency)
- The range for normal values is **11.5-14.5%**
- A value > 14.5 means that there is increased variation in cell size above the normal amount
- A value < 11.5 means that the RBC population is more uniform in size than normal.

- Hematocrit (Hct) packed cell volume in %
normal range is 42-60 %
- RBCs indices (MCV, MCH, MCHC, RDW, HDW)
measured directly using an automated hemoglobin analyzer,
or is calculated by formulas.

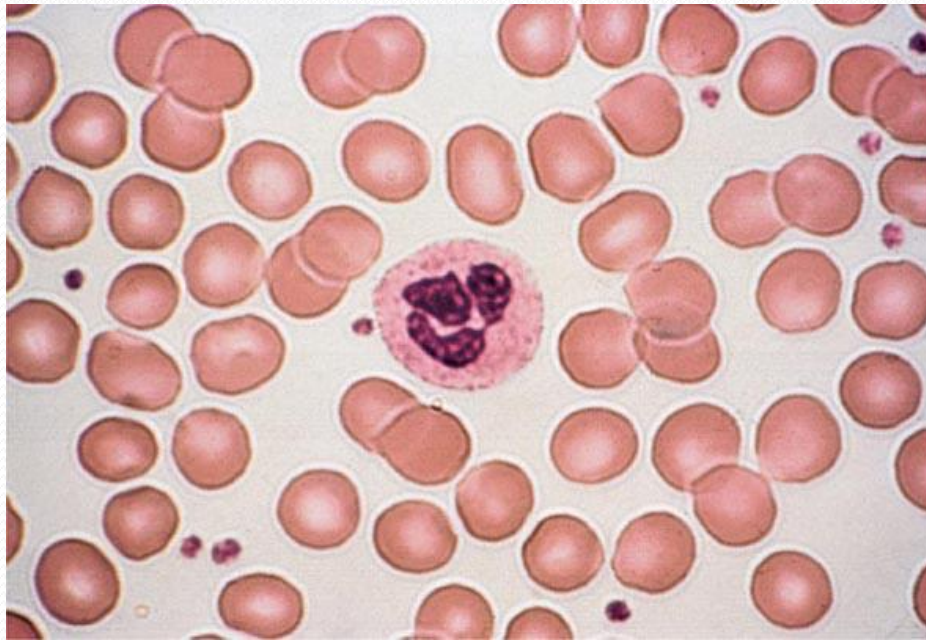


Peripheral blood film (PBF)

Is mandatory for any patient with low hemoglobin

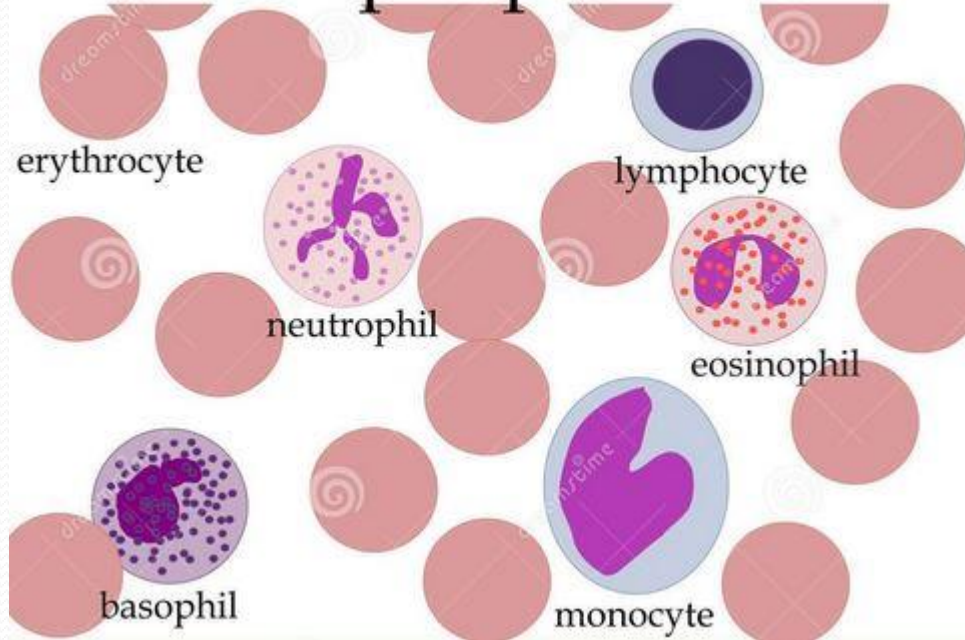
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Normal PBF

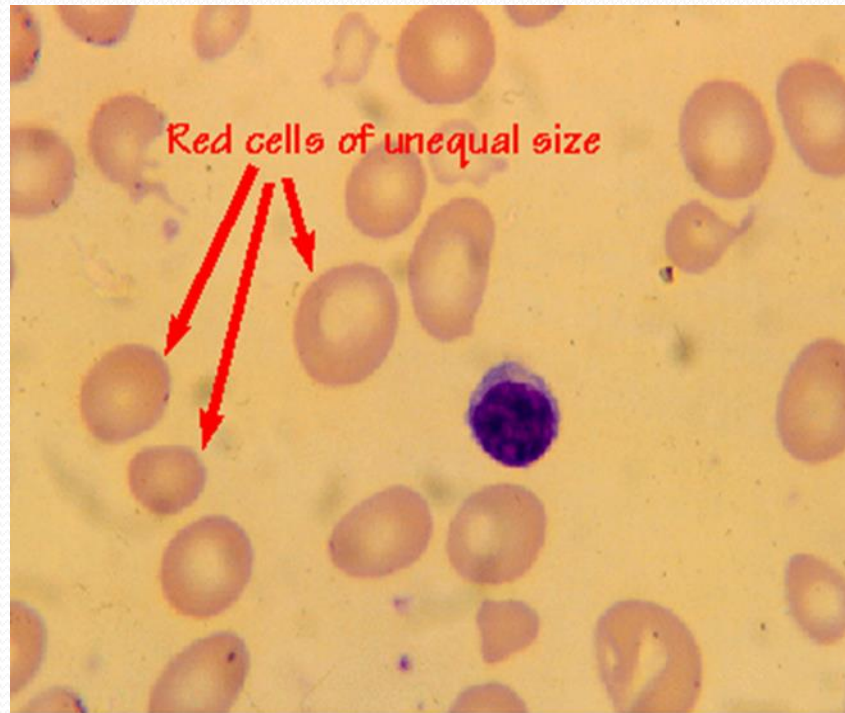


Normal PBF

Smear of peripheral blood



RBCs with 3 different sizes
Micro, Normo, Macro



Anisocytosis

means that the red cells are of unequal size.

- The red cell distribution width (RDW) is a quantitative measure of the degree of anisocytosis.
- The RDW is useful in the differential diagnosis of microcytic anemia.
- Most cases of iron deficiency have a raised RDW, and most cases of thalassemia trait have a near normal RDW.

1. Morphological Classification

- **Microcytic & Hypochromic**

- Iron deficiency A
- B-Thalassemias
- Chronic infection & dis.
- Lead poisoning
- Severe Malnutrition
- Copper deficiency
- Hemoglobin E
- Sideroblastic A
- Inborn error of Fe metabolism

- **Normocytic**

- Acute blood loss
- Other hemolytic A
- Infection
- Renal failure
- Liver disease
- Early iron deficiency
- Microangiopathy
- Dyserythropoietic A

- **Macrocytic**

- **Megaloblastic BM**

- Vit B12 & folate
- Drugs (methotrexate)

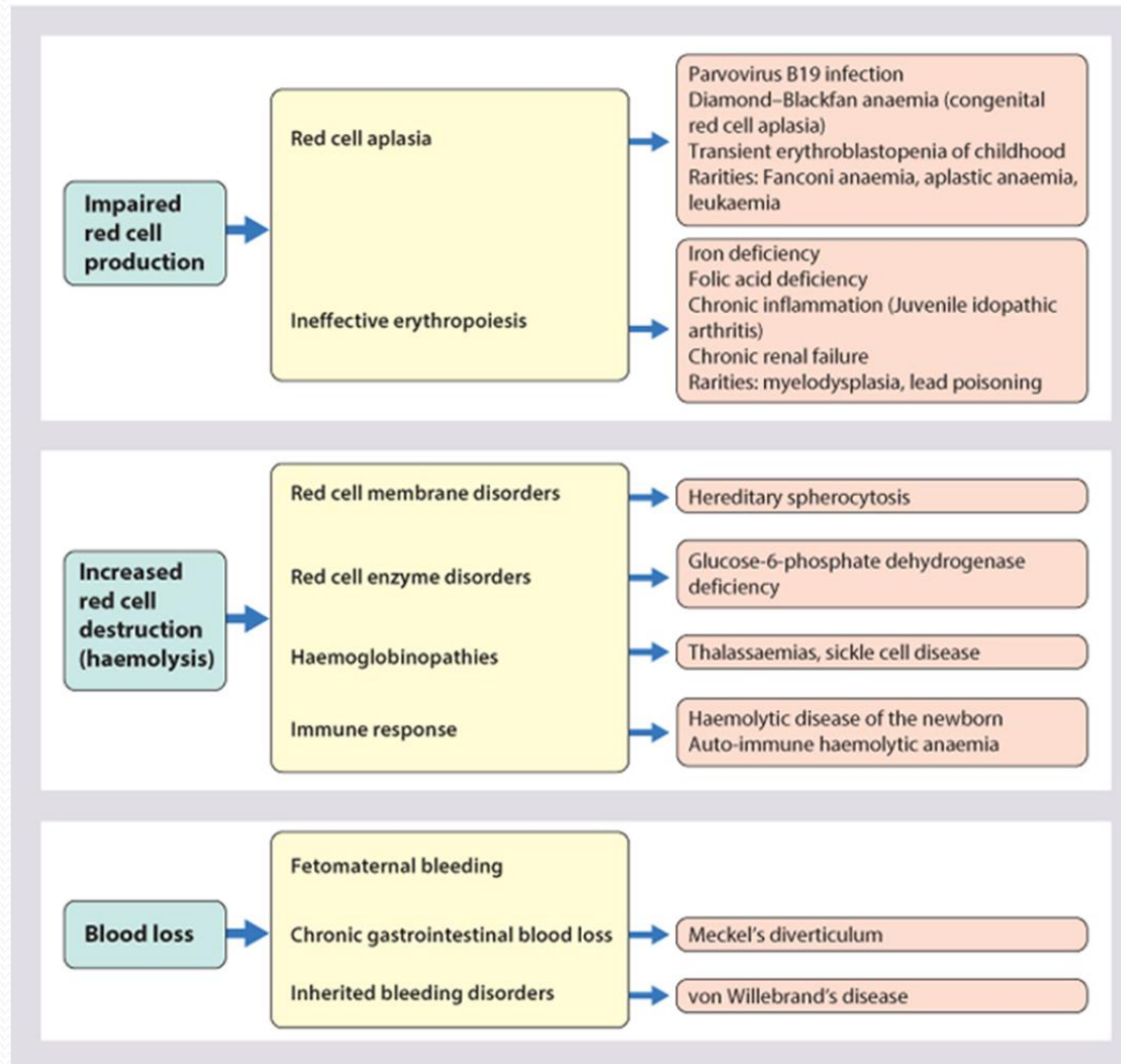
- **Non-megaloblastic BM**

- Normal newborn
- Acquired aplastic A
- Fanconi A
- Diamond Black Fan A
- Hypothyroidism
- Post-splenectomy
- Increased erythropoiesis
- Liver disease
- Obstructive jaundice
- Down Syndrome
- Reticulocytosis

2. Pathophysiological Classification

- 1. Decreased RBCs **Production**
- 2. Increased RBCs **Destruction** (Hemolysis)
- 3. Blood **loss** (GIT diseases, bleeding disorders - VWD)

Causes of anaemia in infants & children



Iron Deficiency Anemia

- Causes & Risk Factors
 - Clinical Features
 - Investigations
 - Treatment
 - Follow up


Causes of Iron Deficiency

- Deficient diet
- Decreased absorption
 - Celiac sprue
 - Zinc deficiency
 - Gastrointestinal
 - Menstrual
 - Blood donation
- Increased requirements
 - Pregnancy
 - Lactation
- Blood loss (chronic)
 - Hemoglobinuria
 - Iron sequestration
 - Pulmonary hemosiderosis
 - Idiopathic

Causes Of Iron Deficiency

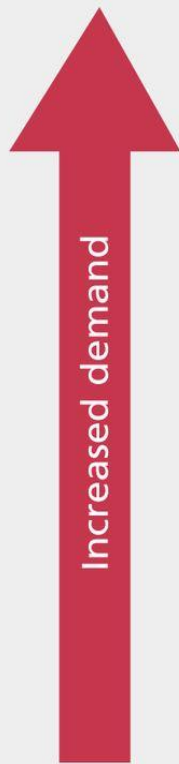
In Infants-

- **Low birth weight (preterm)**
- **Early cord clamping (as much as 80-100ml of blood may remain in placenta)**
- **If there's hemorrhage from the cord, placenta**
- **Later: poor intake(malnutrition), parasitic infestation**
- **Cow's milk – poor source and allergy may cause occult g.i bleeding**



Premature & LBW
Nutritional deficiency (Prolonged breast feeding with **Delayed weaning**)
Coeliac disease
Cow milk protein allergy
Lead Poisoning (pica)
Von willebrand disease, chronic GIT bleeding; ulcerative colitis.
Chronic infection

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Physiological state

- Pregnancy
- Childhood

Blood loss

- Menorrhagia
- Inflammatory bowel disease
- Peptic ulcer disease
- GI malignancies
- Blood donation

Poor intake

- Vegetarian/vegan diet (inadequate)

Poor absorption

- Gastric bypass surgery
- GERD/gastritis
- *Helicobacter pylori* infection
- Antacid/PPI use
- High caffeine consumption
- Celiac disease
- Parasitic infection

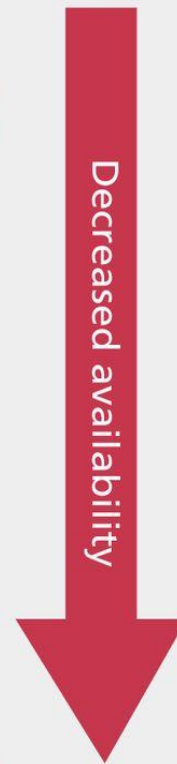


Table 1. Signs and Symptoms of Iron Deficiency

Common

- Fatigue
- Headache
- Exertional dyspnea
- Difficulty concentrating

Rare

- Pica
- Glossitis (tongue inflammation)
- Cheilosis
- Koilonychia (spoon nails)
- Dysphagia (difficulty swallowing)

Source: Reference 4.

Alarm:



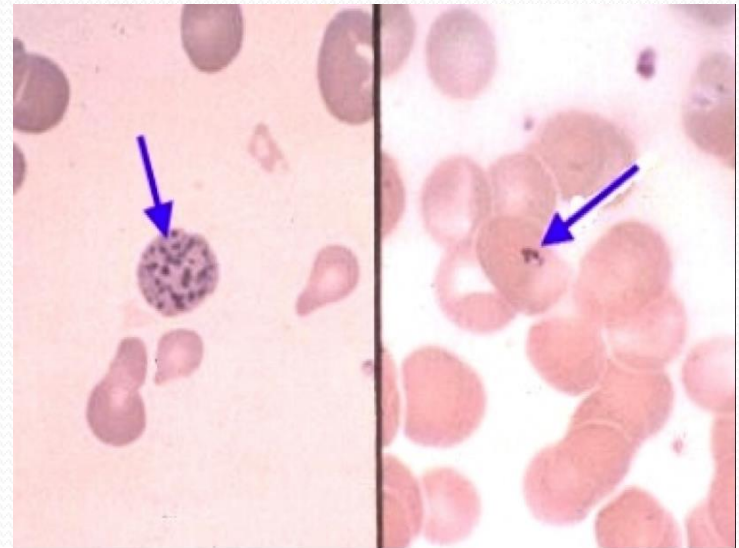
Iron deficiency anemia causes
decreased attention span & concentration
leading to **delay school performance**
which is **irreversible**.

So

Prophylaxis by **proper diet** and **iron supplement** for high risk
group and early treatment of underling disease is better to safe
patients

Pica: desire to eat unusual substance

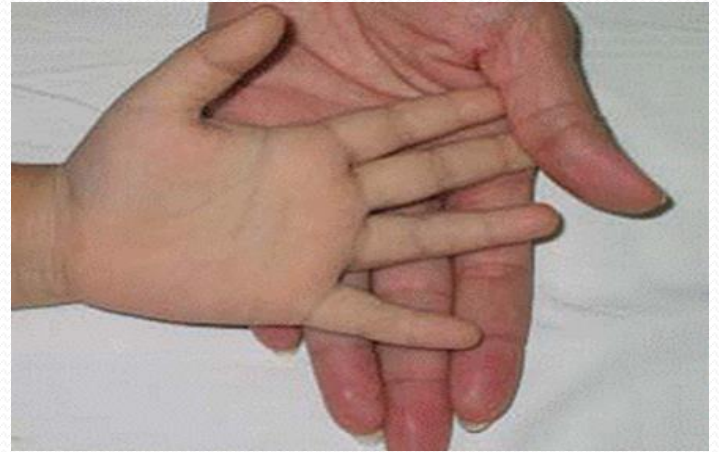
- pica, exposure to lead paint or lead dust
- Blood smear shows basophilic stippling.
- Blood lead is elevated.



Pallor



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Pallor



Koilonychia



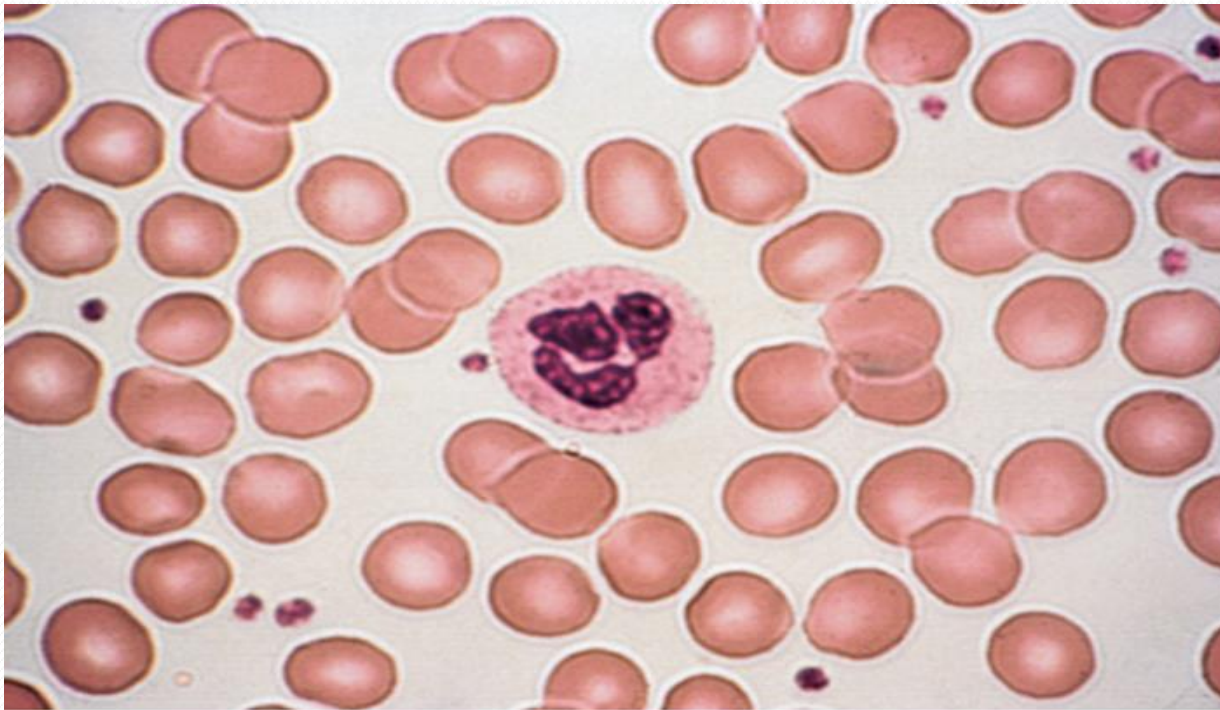
koilonychia nail in infant



Angular cheilitis induced by iron deficiency anemia



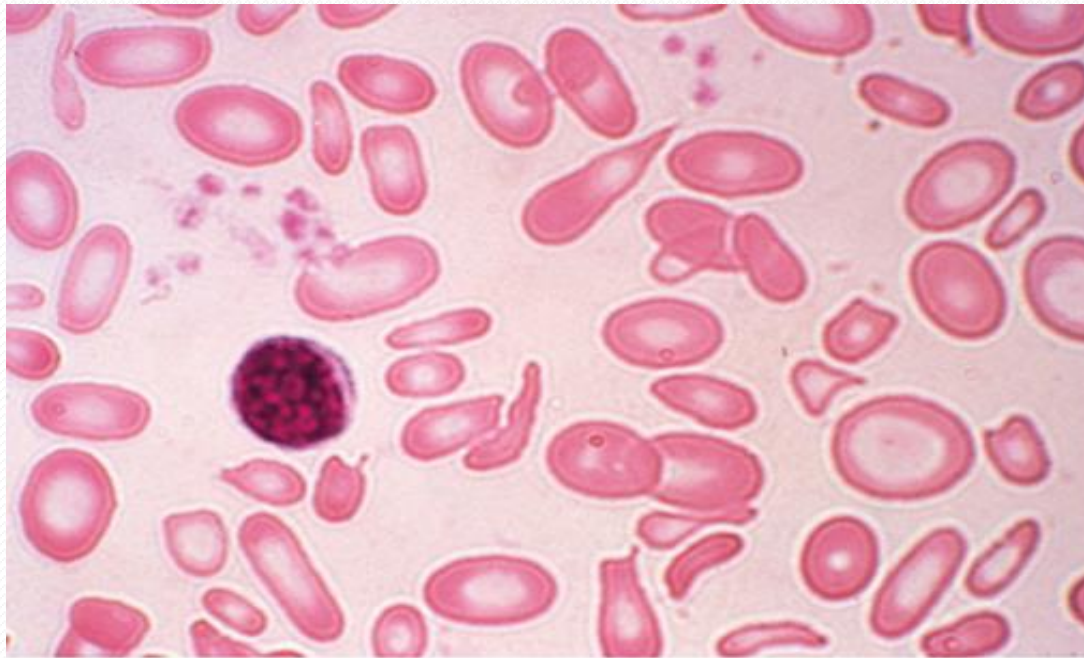
Normal peripheral blood film (PBF)



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

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Iron deficiency anemia
hypochromic microcytosis
with anisocytosis & poikilocytosis in PBF

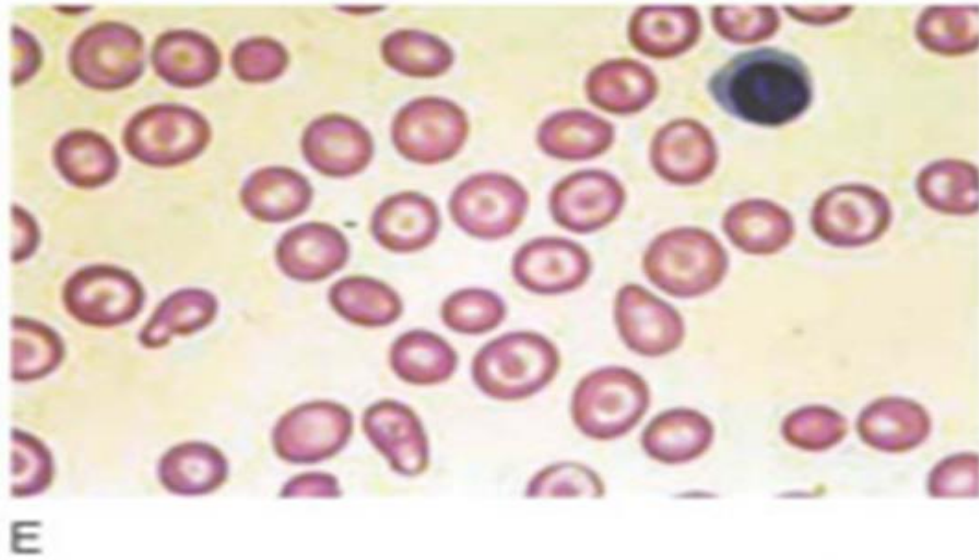


Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

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Target cell:

red cell with central area of Hb giving the appearance of a target. Seen in many conditions, including iron deficiency anemia, hemoglobinopathy (mainly thalassemia) and liver disease

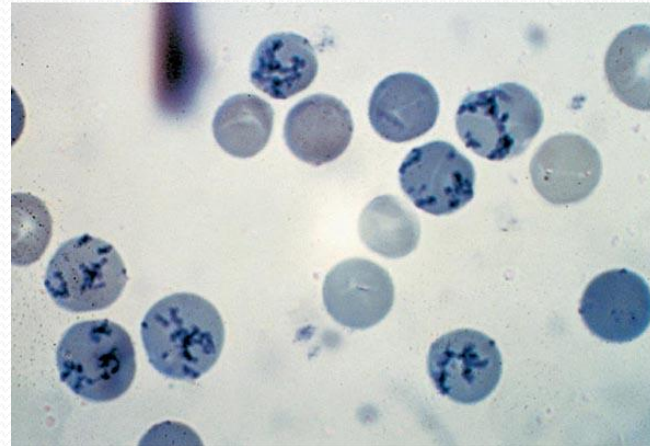
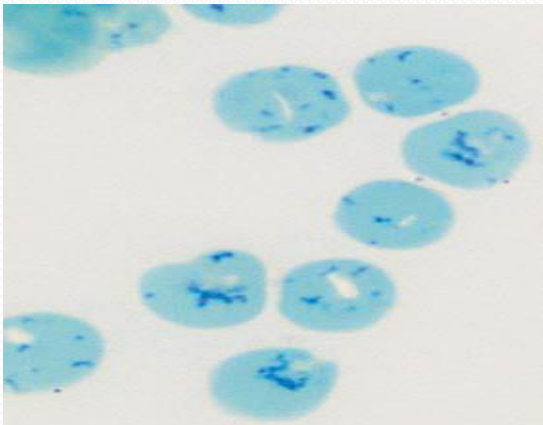


Reticulocyte

premature RBCs; larger than RBCs & has nucleus
Life span 2-4 days

normal Reticulocyte count is **0.5% to 1.5%**

methylene blue stain



Decreased Reticulocyte count:

Reticulocytopenia

Occurs in deficiency of iron, folic acid, B12,
bone marrow suppression such as aplastic anemia & leukemia.

Increased Reticulocyte count:

(Reticulocytosis)

in hemolytic anemia, hemorrhage & 3 days after iron therapy



Megaloblastic, Macrocytic Anemia

Vitamin B12 deficiency
Folic acid deficiency

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Macrocytic: $MCV > 100$

Megaloblastic: delayed nuclear maturation

Causes of **macrocytic & Non-megaloblastic** anemia

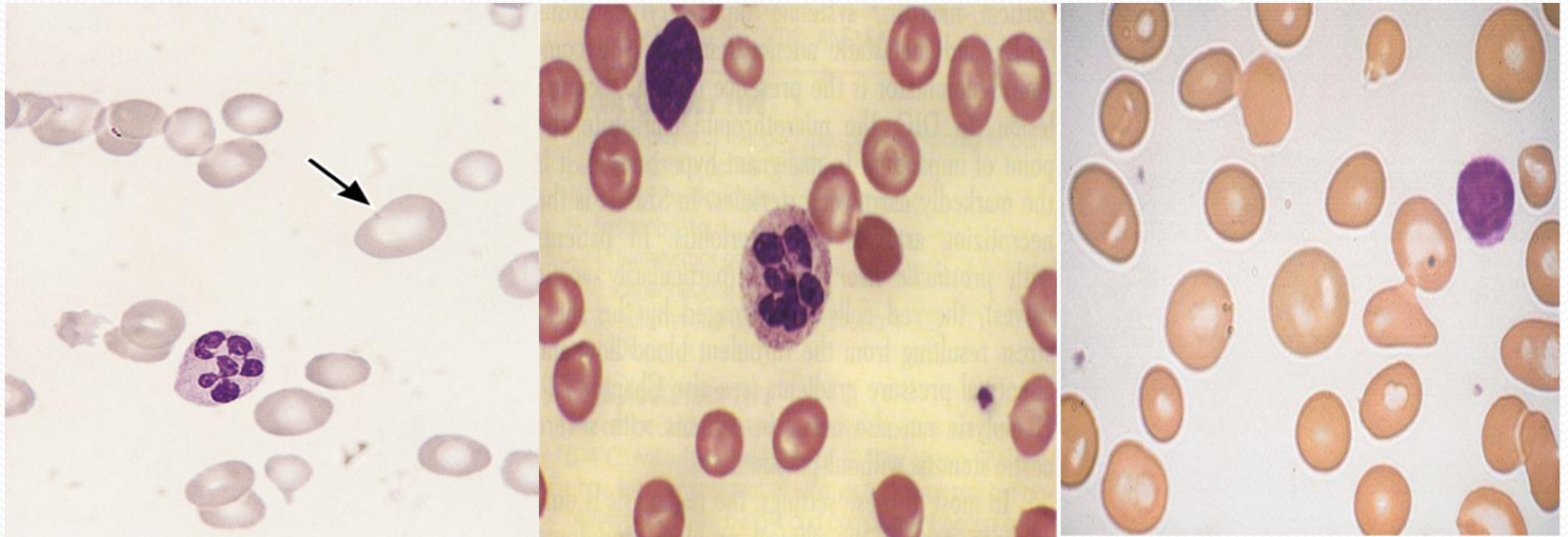
- Hypothyroidism
- Aplastic anemia
- Liver disease
- Alcoholism

Causes of **macrocytic & Megaloblastic** anemia

- B-12 and folate deficiency
- Myelodysplasia
- Drugs that block DNA synthesis (methotrexate)

PBF in B₁₂ & folate deficiency showed:

- Macrocytic RBCs
- Hyper segmented neutrophils: a neutrophil with six or more lobes. Usually (but not inevitably) means vitamin B₁₂ or folate deficiency



VITAMIN B-12

- **Dietary sources: meat, poultry, fish; typical intake 7-30 $\mu\text{g}/\text{day}$, 2-3 μg absorbed**
- **Stomach acid enhances absorption from food; intrinsic factor (from gastric parietal cells) facilitates absorption in ileum**
- **Absorbed vitamin bound to transcobalmin II and transported to marrow**
- **Normal body stores 2000-3000 μg (biologic half-life about 1 yr)**
- **Clinical disease associated with B-12 deficiency when stores < 20% of normal**

B-12 Absorption

- 1. Dietary B-12 transferred from food to binding protein (R-binder or haptocorrin) in stomach. Stomach acid needed.**
- 2. Intrinsic factor (IF) made by gastric parietal cells**
- 3. Pancreatic enzymes degrade R-binders in duodenum; B-12 transferred to IF**
- 4. B-12/IF complex binds to receptor (cubulin) in distal ileum**
- 5. B-12 absorbed, IF broken down**

NO INTRINSIC FACTOR → ↓↓ B-12 ABSORPTION

CAUSES OF B-12 DEFICIENCY

- **Insufficient intake (strict vegetarians/vegans)**
- **Failure of absorption**
 - Lack of intrinsic factor: autoimmune (“pernicious anemia”), gastrectomy, (rare) inherited deficiency of IF
 - Pancreatic insufficiency (rare cause)
 - Lack of ileal absorption: Crohn’s, small bowel resection
 - Competition for vitamin by intestinal bacteria (eg “blind loop” syndrome) or tapeworm
- **Genetic lack of transcobalmin II (rare)**
- **Destruction of B-12 by nitrous oxide**

AUTOIMMUNE DEFICIENCY OF B-12

“PERNICIOUS ANEMIA”

- **Antibodies to parietal cells (sensitive test)**
- **Antibodies to intrinsic factor (specific test)**
- **Permanent loss of B-12 absorption**
- **Achlorhydria, gastric atrophy (not corrected by B-12 replacement)**

B-12 DEFICIENCY

BLOOD AND MARROW FINDINGS

- **Megaloblastic anemia**
- **WBC and platelets may be low (pancytopenia)**
- **Low serum B-12 level, increased methylmalonate and homocysteine**
- **Marrow cellular with low G:E ratio, megaloblastic changes in rbc and granulocyte series**
- **Retic count not increased (*ineffective erythropoiesis*)**
- **High LDH and bilirubin due to red cell precursor breakdown in marrow (in advanced disease)**

Clinical features of B12 deficiency

Pallor

Neurological signs;
Ataxia, posterior column defect,
vibration sense defect.

Glossitis.

In severe cases may cause pancytopenia
because B12 & folic acid are required as well in synthesis of WBCs & platelet

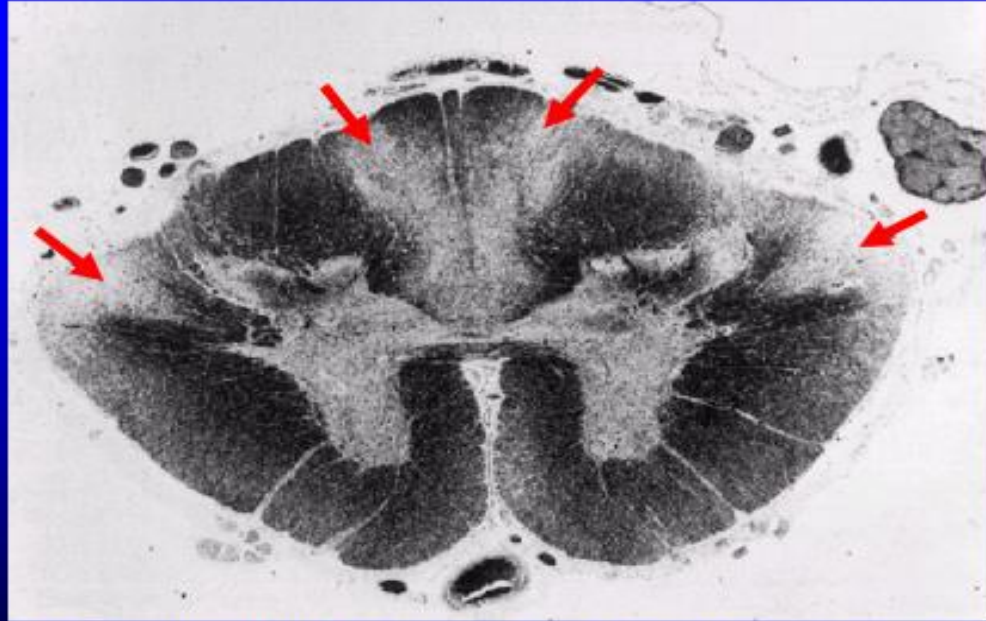


Figure 4.4. Degeneration of the posterior and lateral columns of the spinal cord in vitamin B12 deficiency. The arrows point to areas of demyelination and loss of nerve fibers. (From Kass, LS: Pernicious anemia. Phila., WB Saunders Co., 1976.)

FOLATE DEFICIENCY

Clinical and laboratory findings

- **Megaloblastic anemia with ineffective erythropoiesis (typically less severe than advanced B-12 deficiency)**
- **WBC and/or platelets may be low**
- **No neurologic injury**
- ***Mild maternal deficiency → neural tube defects***
- **Low serum folate level**
 - **RBC folate level does not provide additional useful information**
- **Increased serum homocysteine, normal methylmalonate**

- Goat milk is poor source of folic acid.
- Folic acid deficiency of mother cause neural tube defect in neoborn like hydrocephalus & meningomylocele).

- 
- Megaloblastic anemia due to B₁₂ & folic acid deficiency must be treated first by B₁₂ then folic acid to avoid deterioration In CNS manifestations.

B-12 AND FOLATE DEFICIENCY

Cause	B-12	Folate
Decreased intake	Strict vegetarians and vegans	Alcoholism Malnutrition
Malabsorption	Absence of intrinsic factor Blind loop Pancreatic insufficiency Resection of terminal ileum	Drugs Generalized malabsorption
Increased utilization/loss	Very rare	•Pregnancy •Hemolysis
Drug inhibition	Nitrous oxide	Methotrexate
Genetic defects	Transcobalmin II (rare)	Even rarer



Anemia

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Pediatric Hematology Oncology

PLAN

First Lecture

- Definition
- Hemoglobin level according to age & sex
 - Morphological Classification
 - Pathophysiological Classification
 - Initial necessary investigation
 - Reticulocyte count
 - Peripheral blood film
 - Iron deficiency anemia
 - Megaloblastic Anemia

Second Lecture

- Hemolytic Anemia
(HS, G6PDD, Beta Thalassemia, SCA, AIHA)
- Bone marrow failure
(Aplastic Anemia)



Hemolytic Anemia

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Hemolytic Anemia (RBCs destruction)

Hereditary:

Membrane defect: **Hereditary Spherocytosis**

Enzyme deficiency: **G6PDD**

Hemoglobinopathy: **Sickle cell disease
& Beta Thalassemia**

Acquired:

Autoimmune HA

Hemolysis is RBCs destruction

Sites of hemolysis

1. Intravascular:

Hemolysis occurs in side blood vessels, examples:

G6PDD, IgM & complement mediated Autoimmune Hemolytic Anemia
macroangiopathic anemia as in prostatic valve,
microangiopathic anemia as in DIC, HUS, HELLP.

2. Extravascular

Hemolysis occurs in Reticuloendothelial system (mononuclear phagocyte system) mainly in spleen, liver & lymph nodes by macrophages, monocytes, & Histiocytes, examples:

all other hemolytic anemia including IgG mediated hemolytic anemia

Causes of RBCs hemolysis

A. Intrinsic (corpuscular defects)

are often hereditary RBC disorders including:

- RBC **Enzyme** defects (e.g. G6PD deficiency, pyruvate kinase deficiency)
- RBC **membrane** defects (e.g. hereditary spherocytosis, elliptocytosis, ovalocytosis)
- **Hemoglobinopathy** (e.g. α & β thalassemia, hemoglobin SS, SC, SB thalassemia)

B. Extrinsic causes (extracorpuscular defects)

- **Immune** hemolysis: Rh incompatibility, ABO incompatibility, minor blood group incompatibility (e.g., Kell, Duffy), autoimmune HA, drug-induced (penicillin, antimalarial medications, sulfa medications, acetaminophen). Autoimmune diseases such as lupus, rheumatoid arthritis, Wiskott-Aldrich syndrome, ulcerative colitis. Various tumors, lymphoma, leukemia.
- **Infection** hemolysis: Infection such as hepatitis, cytomegalovirus (CMV), Epstein-Barr virus (EBV), typhoid fever, E. coli, mycoplasma pneumonia, streptococcus. DIC. HUS. Abetalipoproteinemia. Burn. Wilson disease. Vitamin E deficiency (very rare).
- **Mechanical** hemolysis: Hemangiomas (Kasabach-Merritt syndrome), heart valves

Pallor & Jaundice



Hereditary Spherocytosis

Clinical Manifestations

Jaundice with scleral icterus or pigment gallstones because of chronic hemolysis.

MEDVIZZ

A close-up photograph of a child's eyes. The sclera (whites of the eyes) are yellowed, a condition known as scleral icterus, which is a sign of jaundice.

Laboratory Evidence of hemolysis

- Decreased hemoglobin.
- Increased indirect (unconjugated) bilirubin.
- Increased reticulocytes (reticulocytosis).
- Deformed and fragmented erythrocytes on blood smear.
- Increased lactate dehydrogenase (LDH).
- Decreased haptoglobin.
- Increased urobilinogen.



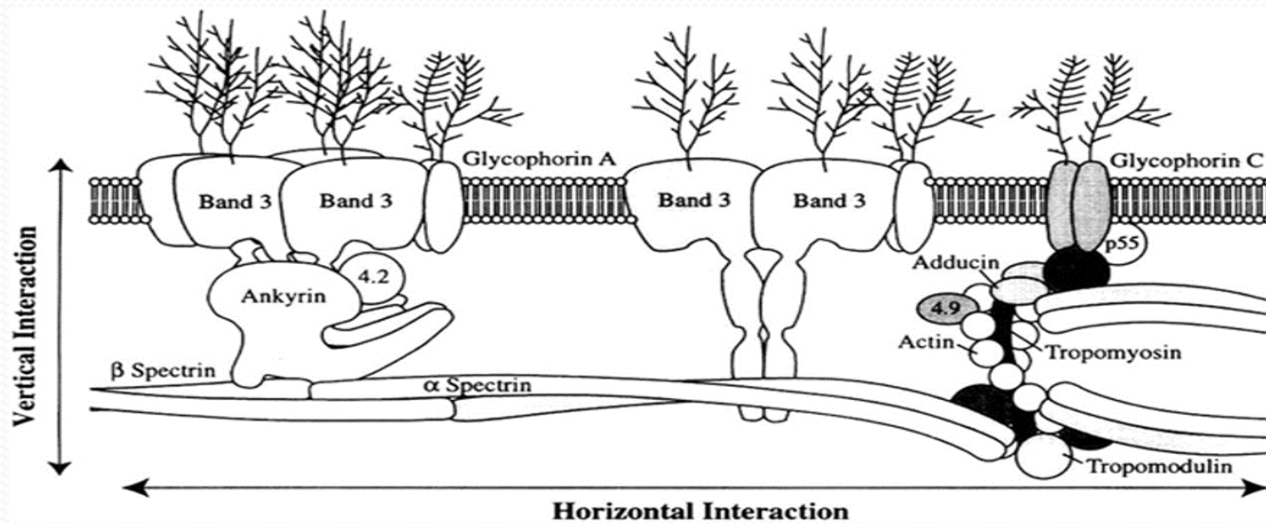
RBCs Membrane Defects

Hereditary Spherocytosis (HS)
is the most common

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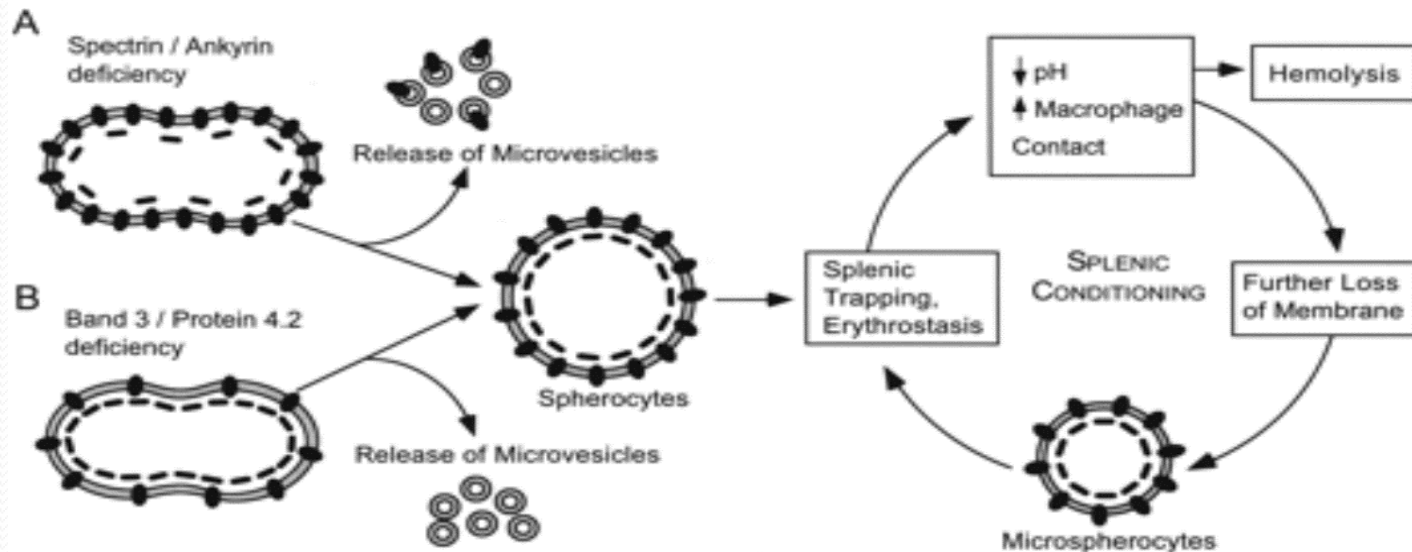
Normal Structure of RBC membrane:

- -Lipid bilayer(40%)
- -Membrane proteins (52%)
- -Carbohydrate (8%)



Hereditary Spherocytosis

is a deficiency of membrane surface area due to defects of the membrane proteins mainly; **Band 3**, **Ankyrin**, **alpha & beta Spectrin** that affect the cytoskeleton and lead to destabilization of the overlying lipid bilayer and release of lipid in micro vesicles causing reduction in surface area and subsequent spherocytosis which can not expand.



Hereditary Spherocytosis

Mode of inheritance

Clinical features

Investigation

Diagnostic test

Treatment



Hereditary Spherocytosis

Autosomal dominant

more in Northern European

Clinical features of HS

- *Onset **at any age** even at neonate
- *Positive family history of HS,
- *Frequent packed cell transfusion, *in patient or family*
- *Splenectomy, *in patient or family*
- *Folic acid therapy, *in patient or family*

- *Pallor, Jaundice, Splenomegaly
(spherocytes stuck in sinusoids of spleen and macrophages phagocytose them)
- *Later on:
 - Short stature,
 - Gall bladder stone (pigmented stone)
 - Signs of iron over load

Investigations of HS

1* Laboratory evidence of hemolysis (see slide 62)

2* Normal MCV but **high MCHC**

3* Peripheral blood film:

spherocytes and no central pallor

spherocytes are **not specific** for hereditary spherocytosis but present also in G6PDD & Rh & ABO incompatibility and other hemolytic anemia

4* Osmotic fragility test:

put RBCs in a hypotonic solution, will not expand if spherocytes.
indicates only the presence of spherocytes but does not mean HS.

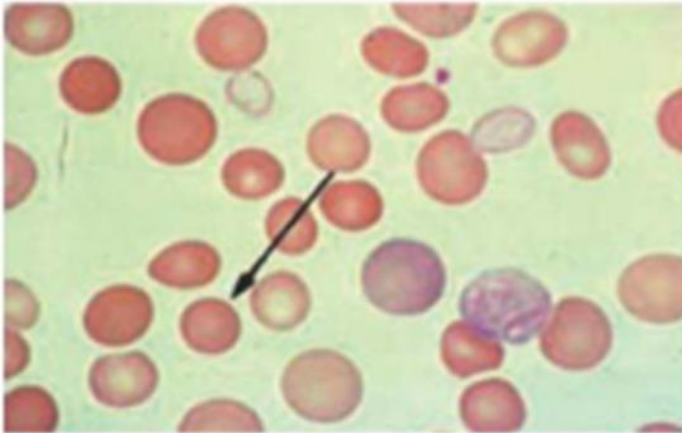
6* **Diagnostic tests are:**

Flow cytometry test using Eosin 5 Maleimide (EMA) staining for binding test for protein Band 3

Membrane studies for Spectrin, Ankyrin, and Band 3

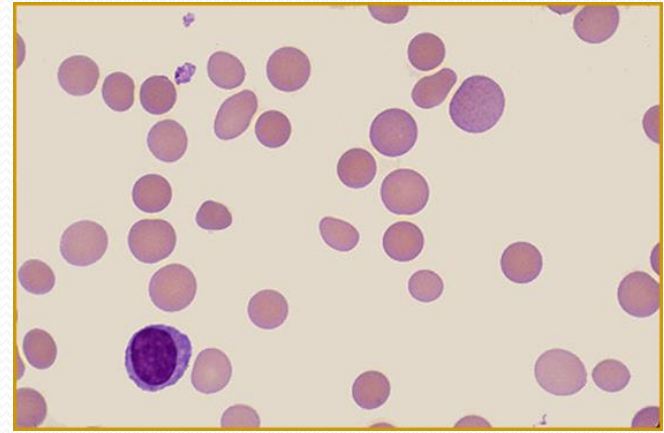
Molecular studies for gene detection which is the most accurate.

5* measure Serum Ferritin level (iron overload)

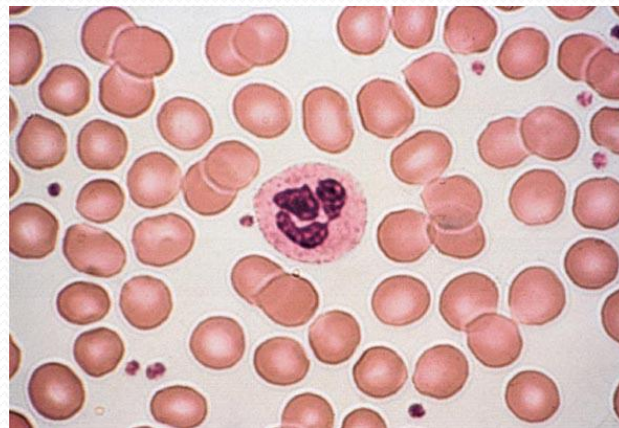


G

Spherocyte cells



Spherocyte cells



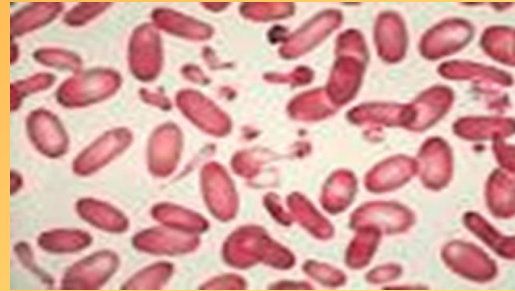
Normal RBCs

Treatment of Hereditary Spherocytosis

- Newborn may needs **Phototherapy** or **DVET**.
- **Packed cell transfusion**.
- **Erythropoietin** to stimulate RBCs synthesis by bone marrow.
- **Folic acid orally** because the demand for DNA synthesis of RBDs is increased.
- **Splenectomy** after age of 6 year .. Why? (capsulated organism) pneumococcal vaccine, H Influenza vaccine, Penicillin prophylaxis !!!) No cure but decreased hemolysis.
- * **Cholecystectomy** **if** there is symptomatic stones.
- * **Iron chelating agents** (see slide 125)

Another types of membrane defects

- **Hereditary Elliptocytosis**



- **Hereditary stomatocytosis**
(the cells contain high Na and low K concentrations)
- **Hereditary acanthocytosis**
- **Hereditary xerocytosis**

RBCs Enzyme Deficiency

- Glucose 6 Phosphate Dehydrogenase Deficiency **G6PDD**
 - Pyruvate Dehydrogenase Deficiency

Glucose 6 phosphate dehydrogenase deficiency

G6PDD

Mode of inheritance

Pathophysiology

Clinical features

Investigation

Diagnostic test (when to be done)

Treatment



Glucose 6 phosphate dehydrogenase deficiency G6PDD

X-Linked recessive
(Gene for G6PD is on **X chromosome**)

Pentose phosphate pathway

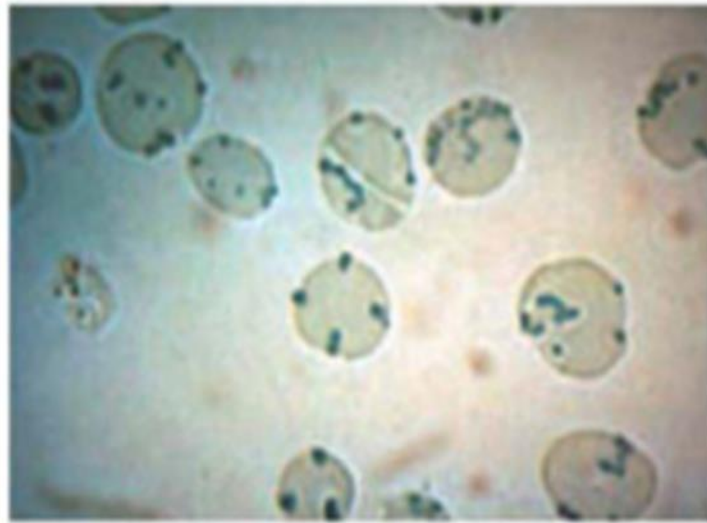
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G6PD Deficiency Pathophysiology

- Low G6PD activity results in low levels of NADPH and reduced glutathione, which are required to protect hemoglobin from oxidative damage.
- In the absence of adequate reducing ability (provided by G6PD), oxidizing agents convert hemoglobin to methemoglobin, then denature it, causing it to precipitate as **Heinz bodies**.
- The spleen pinches off the Heinz body and the overlying membrane, leaving a **"bite cell"** or **"blister cell"**

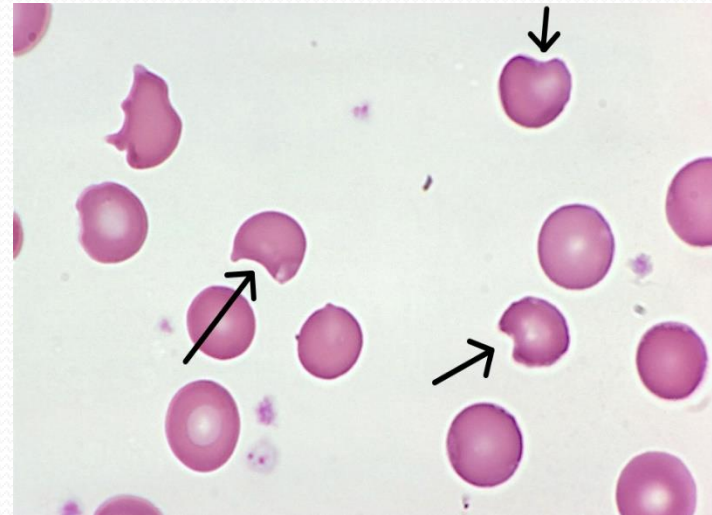
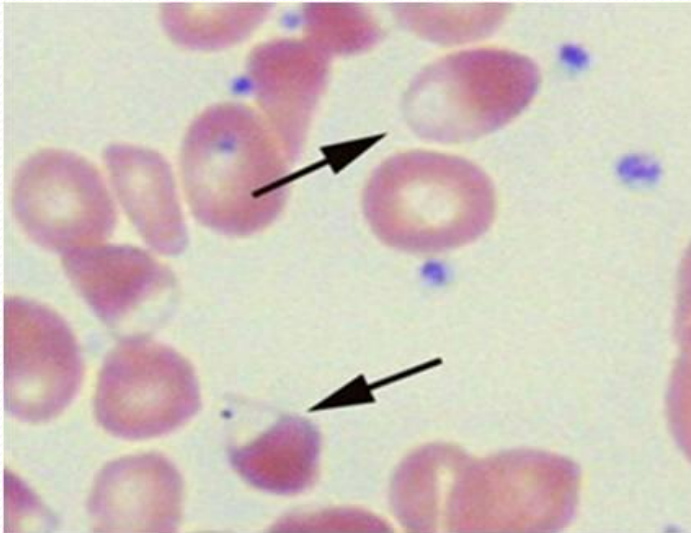
Heinz body

are inclusions within RBCs composed of **denatured hemoglobin**, resulting from exposure to oxidant in G6PD deficient person



H

Peripheral blood smear in G6PDD spherocytes, Bite cells, blister cells



G6PDD

Clinical features

- *Positive family history of G6PDD.
- *Normal person get hemolytic anemia when exposure to oxidative agent such as certain drugs, fava bean or get infection.
- *Onset at any age even at neonate.
- *Episodic hemolysis **24 h to 3 days after exposed to oxidant;**
 - ***Pallor.**
 - ***Jaundice.**
 - ***mild to moderate splenomegaly.**
- ***Dark urine:** because hemolysis is **intravascular** leads to presence of hemoglobin in urine (**hemoglobinuria**)
- *Rapid decrease in hemoglobin that may cause heart failure.

Favism

Hemolytic anemia in patient with G6PDD,
24 hours to 3 days after ingestion of **Fava bean**
presented with **pallor, jaundice, splenomegaly, & dark urine**



Drugs

cause acute hemolysis in G6PD deficient person

- **Antimalarial drugs:** Primaquine, Chloroquine.
- **Sulfonamides:** Sulfanilamide, Sulfamethoxazole, Mafenide.
- Thiazolesulfone.
- Methylene blue.
- Naphthalene.
- **Analgesics:** Phenazopyridine, Acetanilide.
- **Non-sulfa antibiotics:** Nalidixic acid, Nitrofurantoin, Dapsone, and Furazolidone.
- Doxorubicin, Rasburicase.
- High dose IV Vitamin C.
- Etc.

G6PDD Investigations

Laboratory evidence of hemolysis (see slide 62)

Peripheral blood smear:
spherocytes, bite cells, Heinz body

Diagnostic test is **estimation of G6PD in RBCs**

Where??? (in RBCs, not in the serum)

When??? (6 – 8 weeks after recovery of hemolysis)

G6PD levels may be normal with hemolysis.

Urine for **Hemoglobinuria** (causes dark urine)

• *Prevention*

*F*orbidden of the oxidant that cause hemolysis in G6PDD
Such as food (**fava beans**) & some **drugs**, etc.

- **Screening of other siblings**

- **Treatment at time of hemolysis**

Hospitalization

Packed cell transfusion

IV fluid

If there is **infection** treat it properly

- C6PDD confers protection against **Malaria**, in particular malaria caused by **Plasmodium falciparum**, the most deadly form of malaria.
- A similar relationship exists between malaria and **Sickle Cell Disease**.

Pyruvate Kinase deficiency

RBCs glycolysis disorder causing hemolytic anemia

Autosomal recessive inheritance

Less common than G6PDD

Hemoglobinopathy

Defect in hemoglobin

Genetic defect results in abnormal structure of the **globin chains** of the hemoglobin molecule.

- Sickle Cell Anemia & Trait
- Beta Thalassemia Major & Minor

Hemolysis is extravascular (in reticuloendothelial system mainly spleen)

Hemoglobin (Hb)

is the iron-containing protein found in all RBCs

It reversibly binds oxygen in lung
then transport and delivered O₂ to cells and tissues
& carries CO₂ in the opposite way

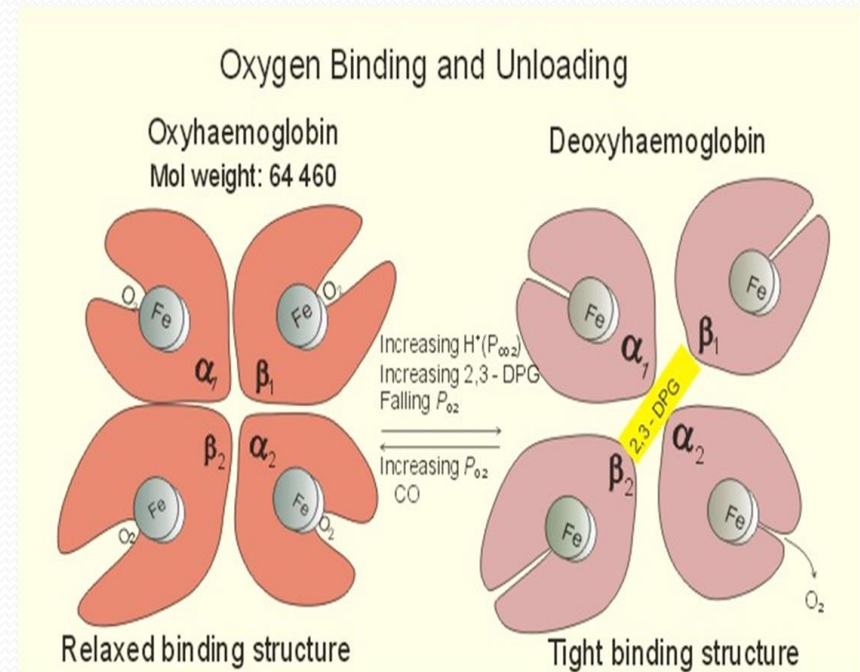
Hemoglobin consists of 2 portions

- **Heme** is a molecule contains an iron ion in the middle of a porphyrin ring
- **4 Globin chains**

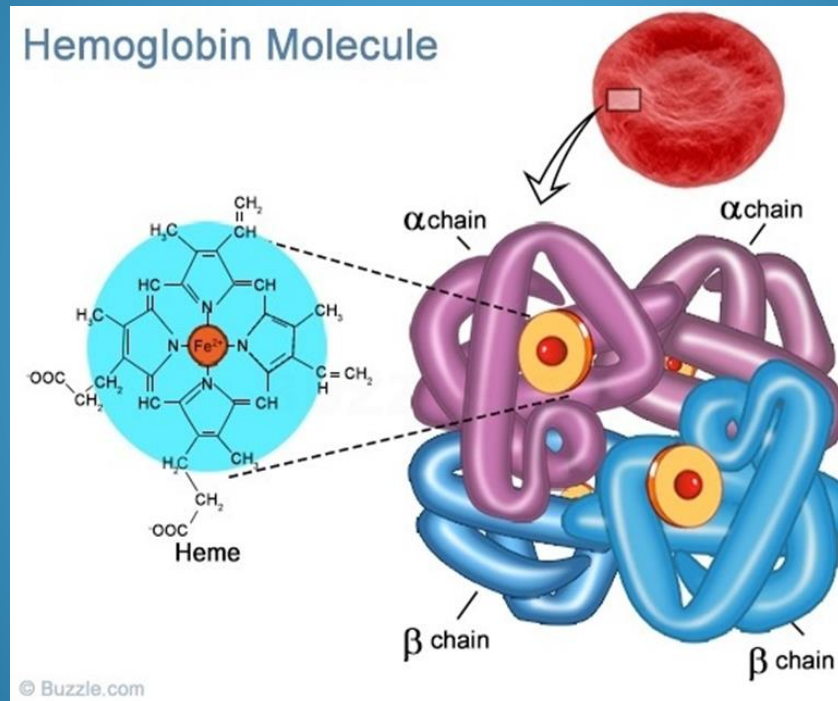
alpha, beta, gamma, delta.

The types of globin chains that are present are important in the function of hemoglobin and its ability to transport oxygen.

- Alpha chain on chromosome 16
- Beta chain on chromosome 11



Hemoglobin A₁ 96% - 98%



Normal Hemoglobin

Hemoglobin A1 (Adult 1)

Hemoglobin A2 (Adult 2)

Hemoglobin F (foetal): during foetal life and during first few months after delivery

Abnormal hemoglobin

Hemoglobin S

Hemoglobin C

Hemoglobin E

Hemoglobin D

Etc

Normal hemoglobin types

- Hemoglobin **A₁** (Adult Hb): $\alpha_2\beta_2$
about **96%-98%** of Hb in adults
it contains **two alpha** and **two beta** protein chains.
- Hemoglobin **A₂**: (Adult Hb): $\alpha_2\delta_2$
about **2% - 3%** of Hb in adults
it has **two alpha** and **two delta** protein chains.
- Hemoglobin **F** (fetal Hb): $\alpha_2\gamma_2$
about **1% - 2%** of Hb in adults
HbF has **two alpha** and **two gamma** protein chains.
This is the primary hemoglobin produced by the fetus during pregnancy;
its production usually falls shortly after birth, reaches adult levels by one year.

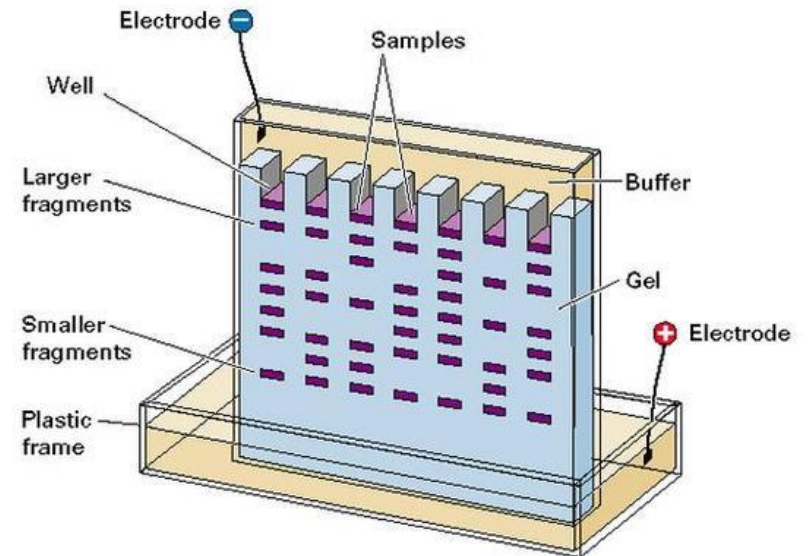
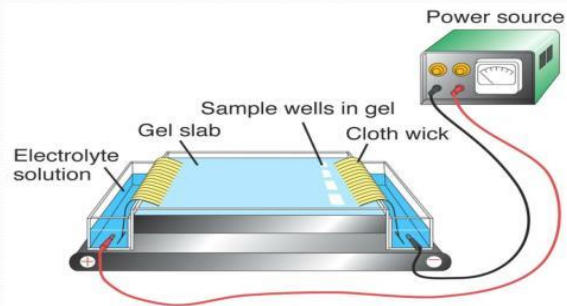
Abnormal hemoglobin types Hemoglobinopathy

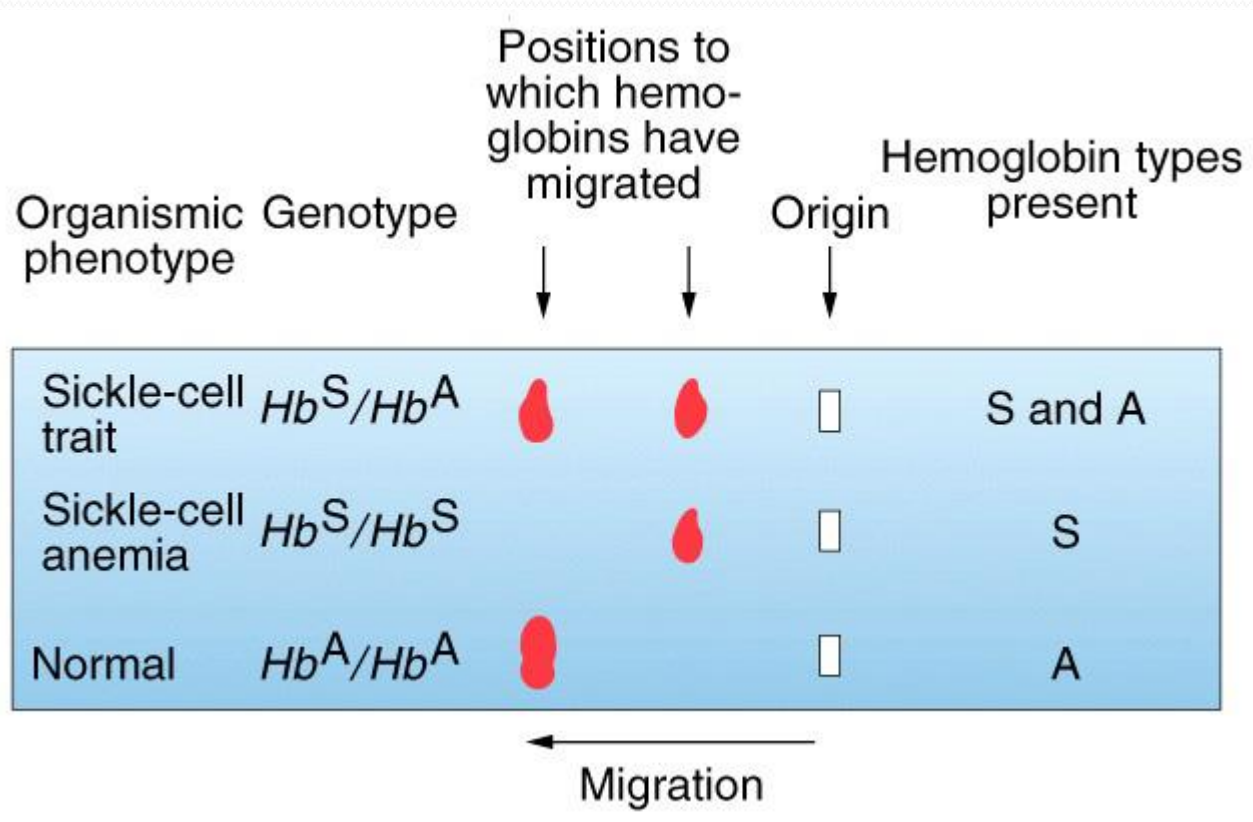
- Sickle cell disease Hemoglobin **S**:
 - SS** in sickle cell anemia (Homozygous)
 - SA** in sickle cell trait (Heterozygous)
- Beta Thalassemia Hemoglobin **F** after 1 year of age
 - B⁰/B⁰** complete absence of beta chain (B-Thalass. Major)
 - B⁺/B⁺ B⁰/B⁺** intermediate reduction of beta chain
 - B⁺/B B⁰/B** Little reduction of beta chain (B-Thalass. Minor)
- hemoglobin **C** which can cause a minor hemolytic anemia.
- hemoglobin **E** which may cause no or mild symptoms.
- Hemoglobin **D**
- **Etc**

Hemoglobin Electrophoresis

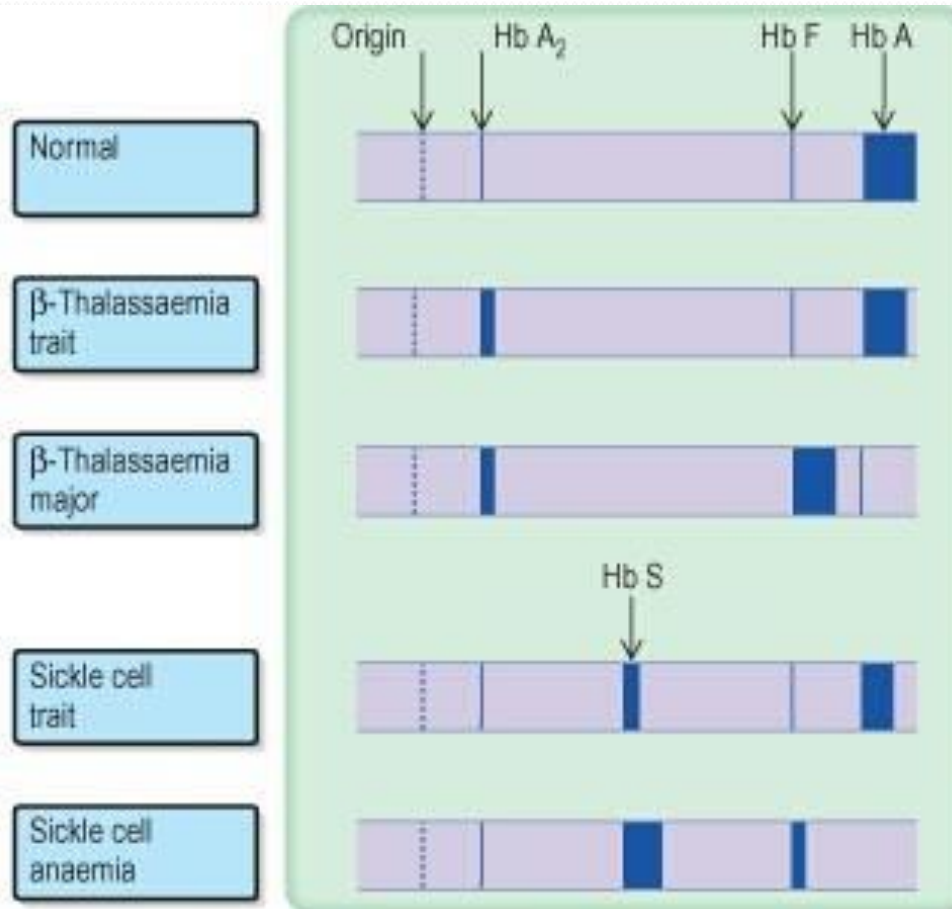
Diagnostic Test of Hemoglobinopathy

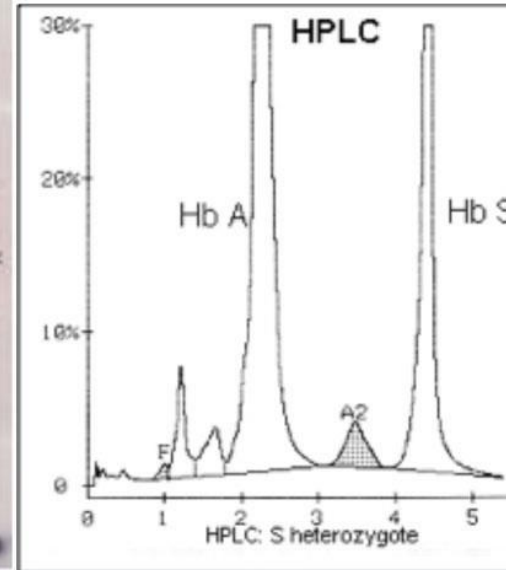
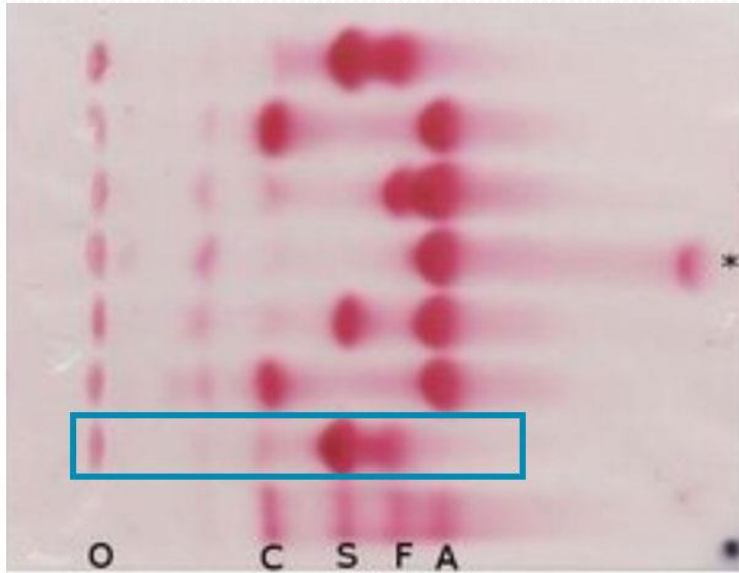
HEMOGLOBIN ELECTROPHORESIS





Hemoglobin Electrophoresis





Hemoglobinopathy

(Defect in hemoglobin)

Genetic defect results in abnormal structure of the **globin chains** of the hemoglobin molecule

- Sickle Cell Anemia & Trait
- Beta Thalassemia Major & Minor

Hemolysis is extravascular (in reticuloendothelial system mainly spleen)

Beta Thalassemia Major & Beta Thalassemia Minor

Mode of inheritance

Genetic

Clinical features

Investigations

Diagnostic tests

Differential diagnosis

Management

(packed cell transfusion, folic acid, iron chelating agent, Stem cell transplantation)



Beta Thalassemia Major & Minor

Mode of inheritance: **Autosomal Recessive**

Ethnicity:

Mediterranean, Africa, Southeast Asia

Beta Thalassemia

Reduction or absence of beta chain synthesis in hemoglobin,
caused by mutation in a gene on **chromosome 11**

It is ranging from:

Severe anemia (Homozygous Beta Thalassemia Major)

Absence of beta chain

Much reduction of beta chain

to:

Clinically asymptomatic (Heterozygous Beta Thalassemia minor)

Little reduction of beta chain

Beta Thalassemia **Major**

Clinical features

Onset: after 4 to 6 months of age. Why?

Pallor

Jaundice

Hepatosplenomegaly may leads to abdominal distension.

Failure to thrive

Later on:

Thalassemic Face: increased erythropoiesis cause expansion of bone marrow space leads to bony changes and prominence.

Short stature

Side effects of iron over load

Bone changes in Beta Thalassemia chipmunk

Beta Thalassemia Major – bone changes



Thalassemic face in B-thalassemia



Thalassemic Face

Oral manifestations

- Marked overdevelopment of maxilla and mandible.
- The osseous changes cause
 - prominent cheekbones,
 - sunken root of the nose,
 - labial inclination of the maxillary incisor,
 - inadequate lip seal.

These lead to the description “chipmunk” or “rodent facies”.

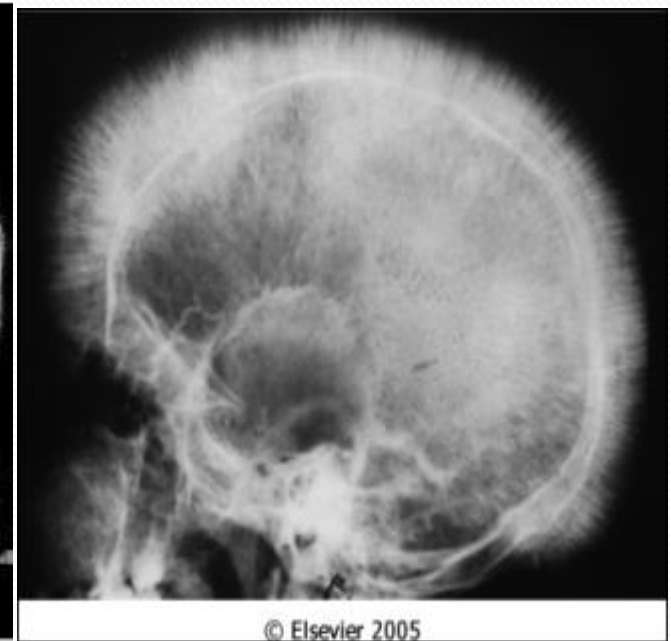
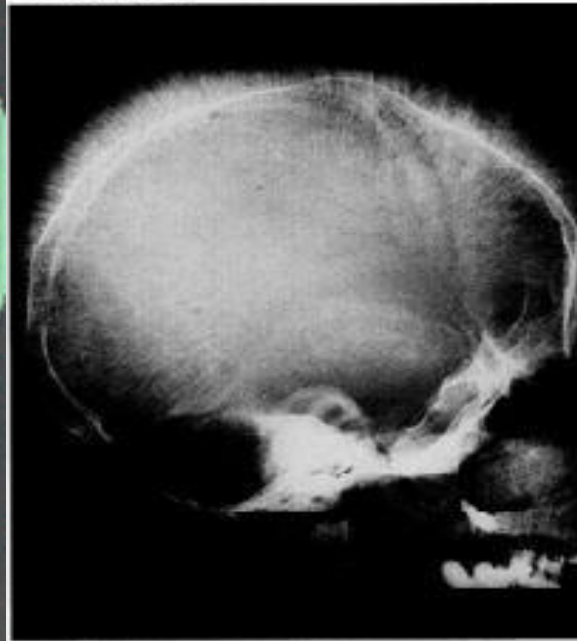
- The oral mucosa may be pale, owing to anaemia or yellow tinged due to jaundice.



Bone changes
Hair on end appearance



bone changes



Bone changes in Beta Thalassemia
Prominent maxillary bone & increased
tendency to dental caries



Beta Thalassemia **Minor** (Heterozygous)

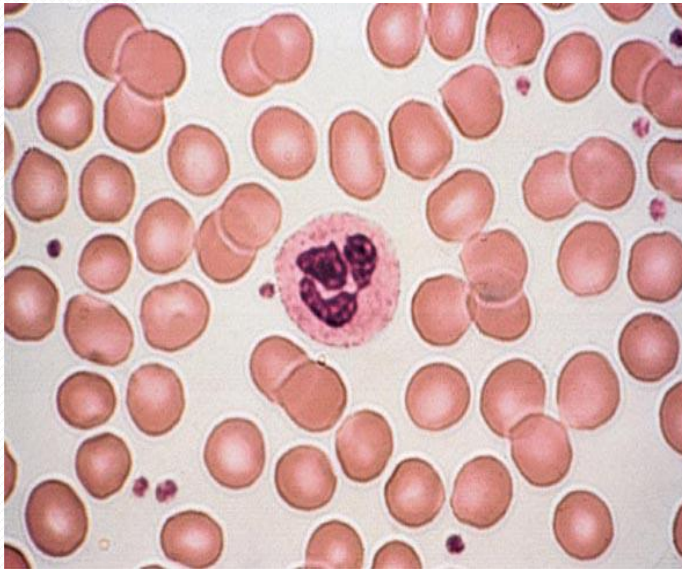
- Asymptomatic
- Symptoms only when patient has stress such as infection, pregnancy, etc.
- Hemoglobin usually more than 9 g/dl
- Mild microcytic hypochromic anemia
 - No organomegaly
- Differential diagnosis is iron deficiency anemia

Beta Thalassemia **Major** Investigations

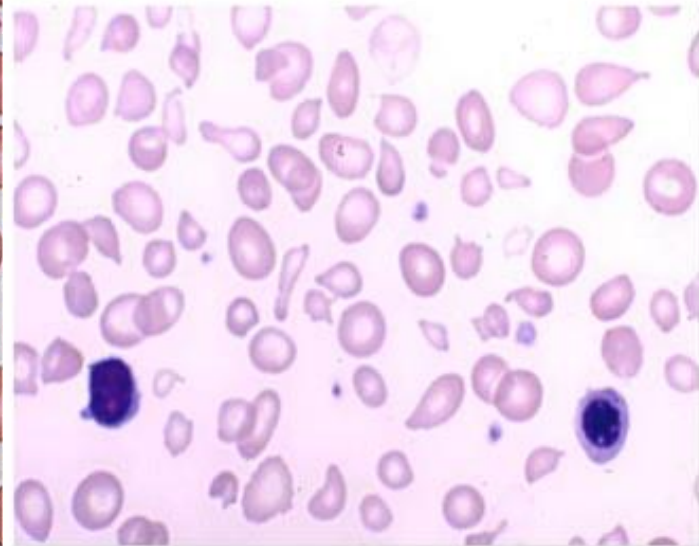
- * Laboratory evidence of hemolysis (see slide 62)
- * MCV, MCH, MCHC are low (sever microcytic hypochromic anemia)
- * Peripheral blood film:
 - Microcytic hypochromic RBCs,**
 - Poikilocytosis: different shapes of RBCs.
 - Anisocytosis: different size (less than that in iron deficiency anemia).
 - Target cells & nucleated RBCs.
- * Diagnostic tests is **Hemoglobin Electrophoresis**
 - DNA analysis** detect deletion & mutation in the beta globin producing genes

Peripheral blood film

Normal PBF



Beta Thalassemia PBF



Hemoglobin electrophoresis shows Beta Thalassemia Major (Homozygous)

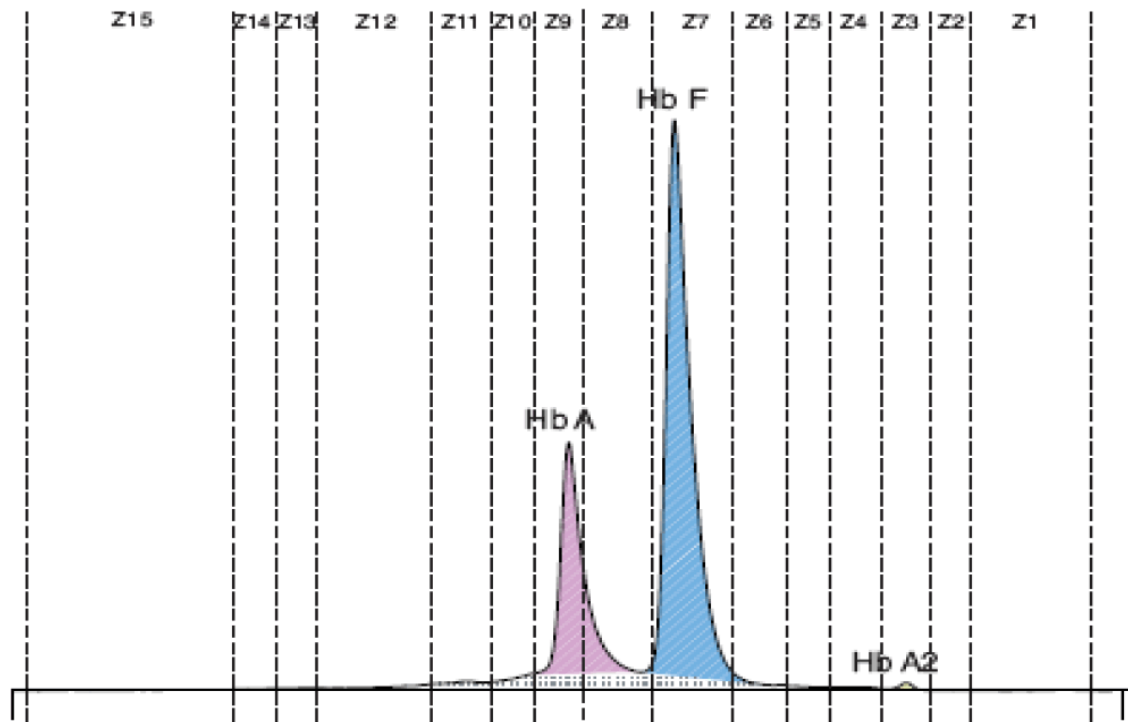


Figure 3. Electrophoretic profile of blood with homozygous beta thalassemia
[Hb A (%)=12 à 42%; Hb A2 (%)=3 à 6%; Hb F (%)=55 à 82%]

Beta Thalassemia Major Treatment

* Frequent Packed cell transfusion

* Folic acid

* Iron chelating agent (see slide 125)

* Splenectomy

* Stem cell transplantation

Sickle Cell Disease

Most common abnormal hemoglobin
(8% of the black population)

Autosomal Recessive

A single amino acid substitution:
valine for **glutaminic** acid
in the beta-polypeptide chain

Sickle cell disease

Autosomal Recessive

Homozygotes (two abnormal genes **HbSS**)

Sickle cell anemia

RBCs contain 90-100% Hb S

No or very little Hb A

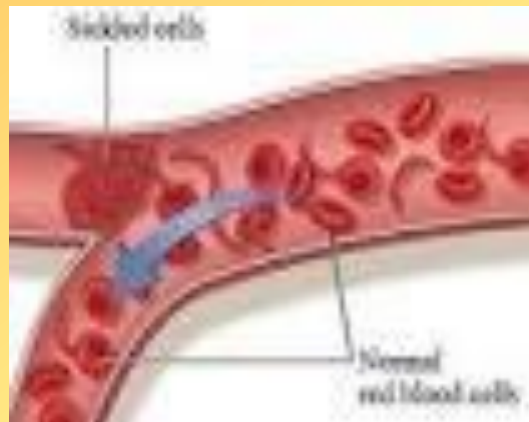
Heterozygotes (one abnormal gene **HbSA**)

Sickle cell Trait (carrier)

RBCs contain less than 40% Hb S



Sickle cells result in **increased blood viscosity**
& **impaired blood flow**
& **initiate thrombi**



Sickle cell crises



- **Vaso-occlusive crisis (painful crisis) Infarction:**
 - * hand – foot syndrome (dactylitis): painful hand-foot swelling
 - * bone crises: osteonecrosis
 - * CNS crises:
 - * pulmonary crises: ARDS: dyspnea, chest pain, severe hypoxemia
 - * priapism
 - * spleen (autosplenectomy)
- **Splenic sequestration crisis:**

pooling of large amount of blood in the spleen, presented with sudden onset of abdominal pain, splenomegaly and hypovolemic shock, treated by packed cell transfusion, Fluids Splenectomy.
- **Aplastic crisis (Erythroblastopenic crisis) :**

cessation of red cell production with Parvo 19 virus infection, needs good hydration, O2, Packed cell T.
- **Hyper-hemolytic crisis:**

unusual, in association with certain drugs or acute infections

Hand – Foot syndrome (dactylitis)



Sickle cell anemia

Clinical features

Onset after 4 to 6 months of age. Why?

Pallor, Jaundice

Spleen palpable only during first 2 years of age then **auto-splenectomy** occurs. Why?

Signs of high cardiac out put due to anemia

Failure to thrive, later on short stature

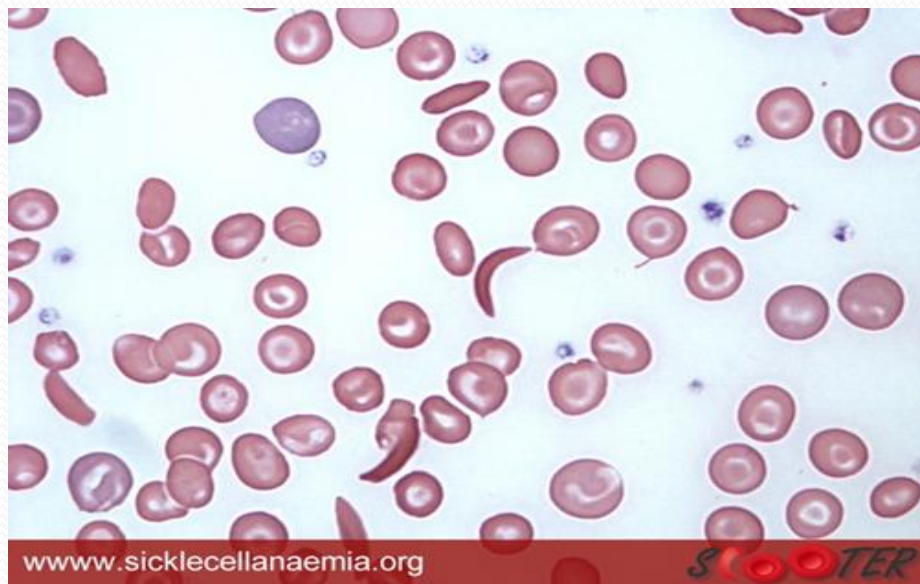
Signs & symptoms of crises

Sign & symptoms of infection by capsulated organisms

Sickle Cell Anemia Investigations

- Laboratory evidence of hemolysis (see slide 62)
- **CBC:**
Normocytic Normochromic Anemia (may neutrophilia & thrombocytosis)
- **Blood smear:**
Sickle cells, nucleated red cells, some Target cells
- **Sickling Test: +**
- **Hemoglobin electrophoresis:**
HbS migrates slower than HbA, giving the diagnostic SS pattern in SCA or SA pattern in SC-trait

Sickle cell anemia Hb SS



Infection/sepsis in SCA

Asplenia (Auto-splenectomy) after age of 2 year causes unfunctional spleen is a high risk for sepsis and may leads to serious infection in SCD. ARDS by capsulated bacteria; Pneumococcal, Hemophilus influenza, meningococcal, and Salmonella arthritis.

Prophylaxis

Vaccination is mandatory
Prophylactic penicillin is controversial.

Management

septic work up.
I.V. fluids.
Antipyretics
Antibiotic
PCT if Hb low
Hospitalization if toxic

**Vaso-occlusive crisis causes
Ischemia & infarction**

CNS manifested as Strokes

Admit to ICU

IV Fluid

O₂

prophylactic antibiotics

Packed cell transfusion

long term transfusion therapy

will need chelation for iron

overload if transfused more

than 1 year

Vaso-occlusive crisis
may cause severe pain called
Painful crisis

- Frequent occurrence
 - IV Fluid
- pain killer (Paracetamol)
 - O₂ only if needed(can suppress RBC production)
- Priapism is an emergency


- **Hydroxyurea in SCA**

increases Hgb F, which carries O₂ at lower O₂ tension, good efficacy, but has teratogen effects in pregnancy

- **Stem cell transplants**

curative, if compatible donor is found
reserved for severe patients with multiple strokes, frequent crises, and if long term transfusion therapy needed.

- **Gene therapy** (promising)



In Hereditary Hemolytic Anemia,
Family history is important

Professor Nourz A. Elgheriani

Iron overload

Frequent blood transfusion leads to increased iron in blood, all tissues & organs
(hemochromatosis, hemosiderosis)

Cardiac siderosis & Hepatic siderosis
are common causes of death in Beta thalassemia major

- Ferritin level to be estimated frequently
- MRI* for estimation of iron in heart and liver
- Iron chelation agent is necessary such as:
(oral Exjade, generic name: **Deferasirox**)
(subcutaneous Desferal, generic name: **Desferoxamine**)



Autoimmune Hemolytic Anemia

AIHA

Autoimmune hemolytic anemia AIHA

- It is type II hypersensitivity
- Production of autoantibodies (IgG or IgM) with or without complements that coat RBCs and lead to destruction of RBCs by macrophages in spleen and liver

- So the cause is outside the RBCs (extra-corporeal cause).

Warm Autoimmune Hemolytic Anemia

G = جمره

- IgG mediated hemolysis,
- IgG warm antibodies against Rhesus antigen.
- React at body temperature 37 degree centigrade
- Most common 70-80%
- Extravascular hemolysis mainly in spleen
- Macrophages in spleen consume RBCs changed to spherocytes
- More common in females
- Primary (idiopathic) or secondary due to: cancer as CLL, connective tissue disease as SLE, HIV, viral vaccines, drugs as ceftriaxone, piperacillin.
- Insidious to acute
- Anemia (severe)
- Fever, jaundice frequent
- Splenomegaly
- Hepatomegaly

Cold autoimmune HA (cold Agglutinin Disease)

- IgM mediated hemolysis bind to Complement
- React at low temperature 4 – 23 (room temp)
- Less common 20-30%
- More in elderly, causing acrocyanosis, cold painful blue swollen fingers.
- **Intravascular hemolysis** leads to hemoglobinuria & dark urine (similar to G6PDD).
- Primary (idiopathic) or secondary due to: infection (as CMV, EBV, IMN, Mycoplasma Pneumonia), Lymphoma, Drugs (Lenalidomide).
- Often chronic anemia
- 9-12 g/dL (less severe)
- Autoagglutination
- Hemoglobinuria, acrocyanosis and raynaud's with cold exposure
- No organomegaly
- Positive Direct Coombs test (DAT; Direct Antiglobin test)

M = مسقع

Clinical features of AIHA

Dramatic rapid **decrease of hemoglobin**

Signs & symptoms of anemia

Jaundice

Dark urine (cocacola like) if intravascular hemolysis by IgM bind

Complements

Massive **splenomegaly**

Mild hepatomegaly

History of drug ingestion or other underlining disease

Diagnosis of AIHA

Anemia, Jaundice, Splenomegaly

+

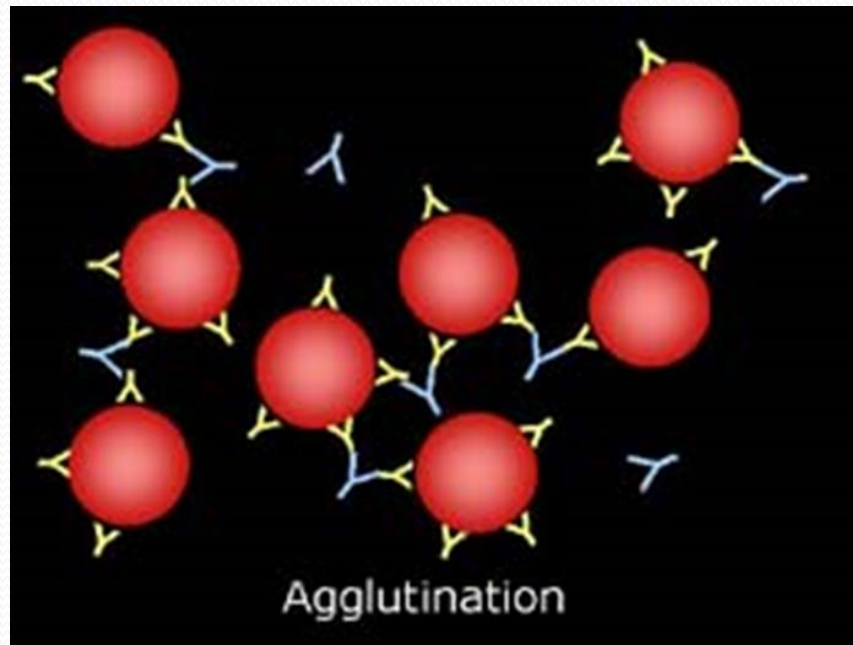
Following investigations:

- No family history
- Usually no previous hemolysis
- Laboratory evidence of hemolysis (see slide 62)
- Peripheral blood film: spherocytosis (do not indicate Hereditary Spherocytosis.)
- **Positive Coombs Test**
- Hemoglobinuria in case of cold AIHA (IgM) with dark urine and IV hemolysis)

Coombs Test

Direct Coombs Test (Direct Antiglobulin Test; DAT), testing the presence of antibodies (IgG) on RBCs.

Indirect Coombs Test, testing the presence of antibodies (IgG) in patient plasma.



Treatment of AIHA

- **Steroids** for extravascular hemolysis (warm, IgG mediated hemolysis) is the first line of treatment
- **Rituximab** anti-CD20 monoclonal antibody
- **Alemtuzumab (CD52)** against CD52
- Splenectomy
- Cyclosporine
- Azathioprine
- **Hematopoietic stem cell transplantation**
- Cold exposure avoidance for IgM AIHA and Keep patient warm
- Folic acid tablet
- Packed cell transfusion
- Treat underlying illness



Bone marrow failure



Aplastic Anemia

AA

**Pancytopenia
with hypocellular or acellular Bone Marrow**

Signs & symptoms of Aplastic A

Anemia: Pallor, signs of high cardiac output

**Leukopenia: Infections
like respiratory infection symptoms, Fever, etc.**

**Thrombocytopenia: Bleeding:
Petechial rash, Ecchymosis , Gums bleeding, Epistaxis**

No Organomegaly in AA



Types of Aplastic Anemia

- 1. Acquired AA**
- 2. Hereditary AA (Fanconi Anemia)**

Causes of Acquired AA

*** Idiopathic (majority) 2/3 of cases**

***Drug :**

**Acetazolamide, Carbamazepine, Gold, Hydantoin,
Chloramphenicol, Phenylbutazone,**

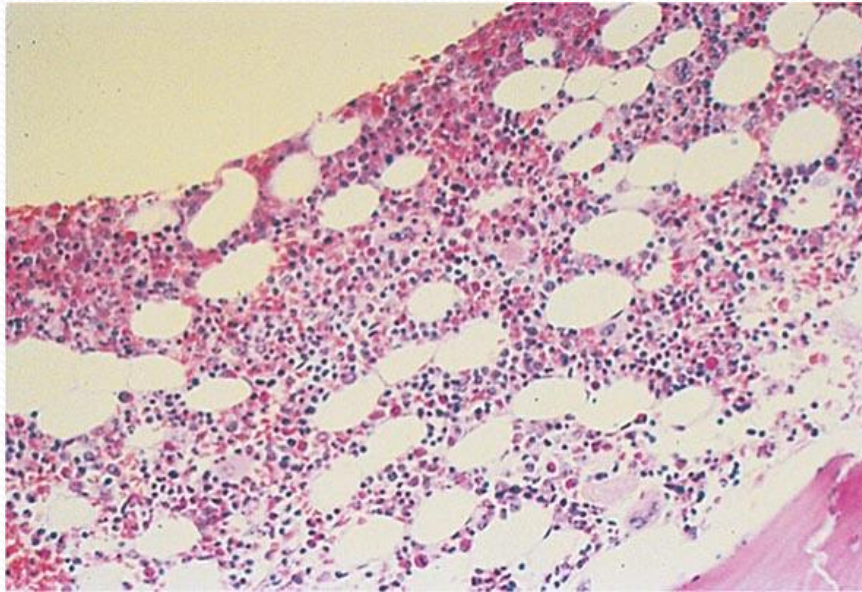
***Chemical (Benzene)**

***Radiation exposure**

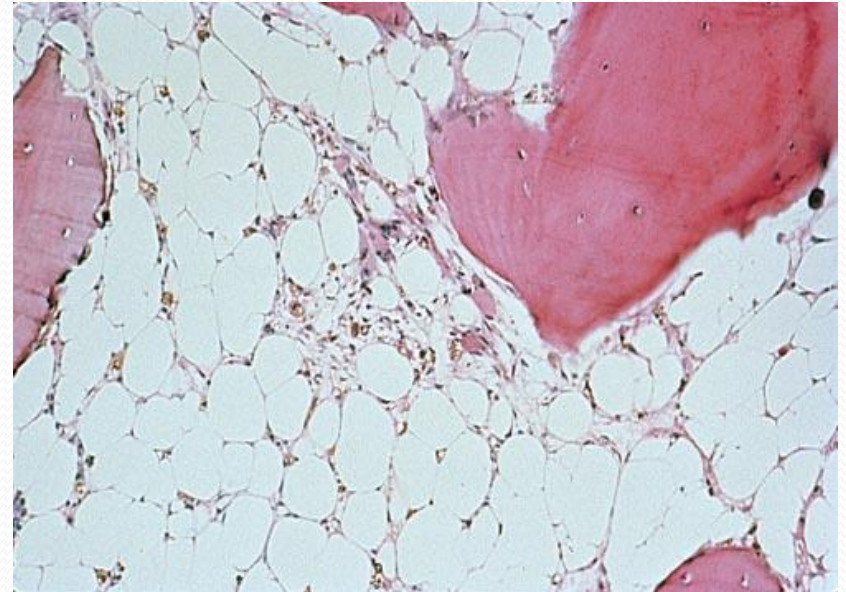
***Viral illness (Hepatitis B)**

Investigations of AA

- **Peripheral blood smear:**
pancytopenia,
decreased or absence of reticulocytes (Reticulocytopenia)
- **Bone marrow aspiration & biopsy:**
Hypocellular/aplastic bone marrow with increased fat spaces
- Tests for underlying cause (viral titres)



Normal Bone Marrow



Hypocellular Bone Marrow in Aplastic A

Fanconi anemia

Hereditary aplastic anemia
is an **autosomal recessive** disease.

Mutations in many genes can lead to Fanconi Anemia.

At birth only congenital abnormalities, but no pancytopenia

73 % have overt bone marrow disease by age 10 years, mean age 7 or 8

Fanconi Anemia associated anomalies

Congenital Abnormalities

- Hand and arm abnormalities (71%)
- Skeletal anomalies (hips, spine) (71%)
 - Skin discoloration (64%)
 - Short stature (63%)
 - Mental retardation (16%)
- Gastrointestinal difficulties (14%)
 - Hearing (11%)
 - No abnormalities (30%)

Haematopoietic Abnormalities

- Bone marrow failure
 - aplastic anemia
 - acute myeloid leukemia

susceptibility to infections

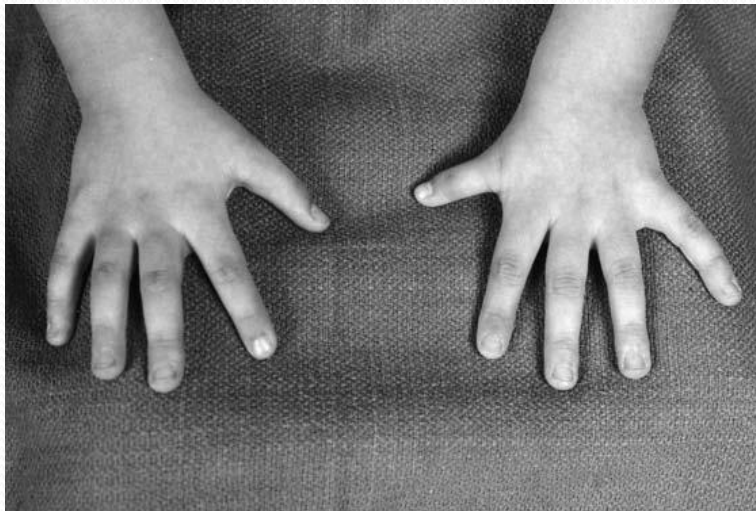
Predisposition to cancer

- cancers of rapidly dividing tissues
- (squamous cell carcinoma & GIT)

endocrine, gastrointestinal abnormalities



Absent Thumb



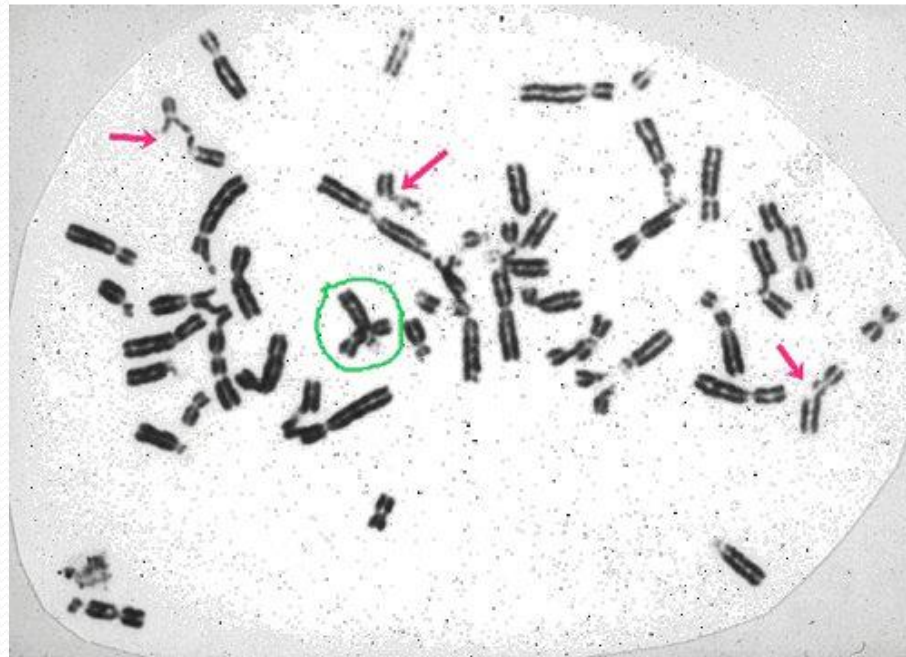
left thumb hypoplasia.




floating thumb

chromosome breakage test

is widely used as a standard diagnostic test for FA.
Lymphocytes are cultured in the presence of mitomycin C
and observed for excess chromosome breakage





Differential diagnosis of AA is

Acute Leukemia
(in AA there is no organomegaly)



THANK YOU

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