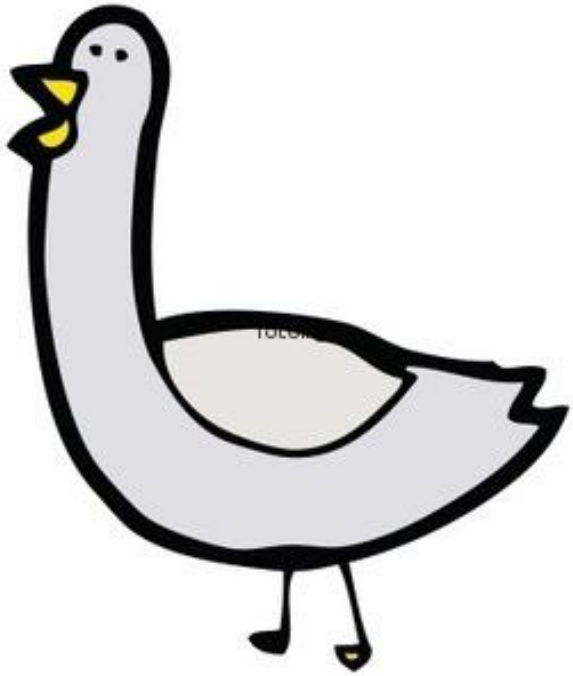
The 429's

Hematology

Final Edition



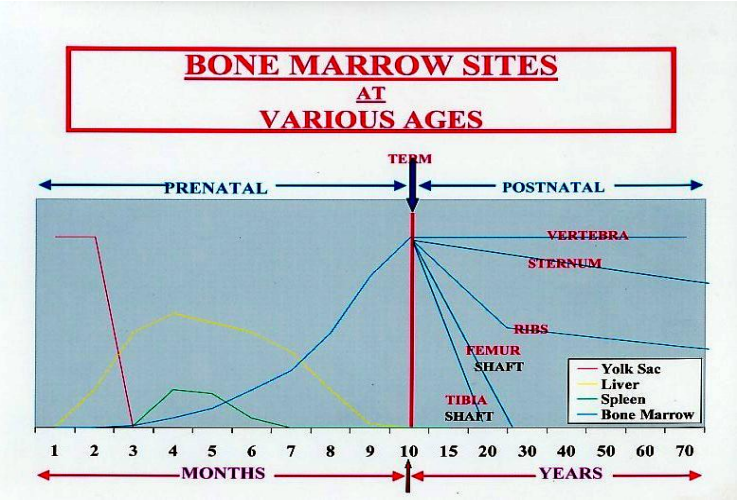
Edited by:

429 people

CONTENTS

 : NOT INCLUDED IN THE FINAL

HAEMATOPOIESIS AND CLASSIFICATION OF ANEMIA



Site of Hemopoisis

- **0-6** week's → Yolk sac
- **6** weeks – **6** months → liver + spleen (fetal life)
- **6** months – adult life → Bone Marrow

⌘ IN Fetus **ALL BONES** are involved
⌘ IN Adults **ONLY** flat bones + the PROXIMAL end of LONG LIMB BONES

- In Fetus the Marrow is Highly CELLULAR (**70%**)
- In Adults **50%** Marrow cells + **50%** FAT **(MCQ)**
  - The fat space can be replaced by marrow
  - In Adults when blood is needed in Extra amount the cellular component of the marrow increases + viscera are involved e.g. " Liver and Spleen "

- In evaluation of bone marrow, there are **2** parts :

❶**Aspiration**: it is a fluid sample from bone marrow. This part is good for looking at individual cells in detailed.

❷**Biopsy core**: as any other biopsy when taking it and it called Trephine biopsy. Good to see the architecture of bone marrow "fat, cellularity, and comparisons between them". Biopsy is simple, done under local anesthesia; pain full so should be done in need only and has few complications.

- No. 1 site for bone marrow biopsy is **iliac crest** ***"MCQ"***. Second site is sternum
- Bone marrow biopsy shows 2 parts: fat and cells. In comparison between fat and no. of cells we should know that cellularity depends on age of the patient e.g. in a 1 year old child, 70% of the sample contains marrow cells and 30% is fat. With increased age, no. of cells decreased and fat increased due to fat replacement as seen in adult where 50% of marrow space is occupied by fat.
- We comment also on different cell lines: *megakaryocytic line* for platelets production, *erythroid cell line* for RBCs production, *myeloid cell line* for neutrophil,

Megakaryocyte	**→ Platelet**	**Lymphoid**	**→ Lymphocte**
Erythroid	**→ RBC**	**Myeloid**	**→ Neutrophils + Basophils + eosinophils**

Stem cell

In the Bone Marrow a '***Pluripotent***' stem cell gives rise to all blood cells

Proliferation and differentiation are stimulated by different Growth Factors ,

Leukemia: is a disease of abnormality in differentiation!

Bone marrow stroma:

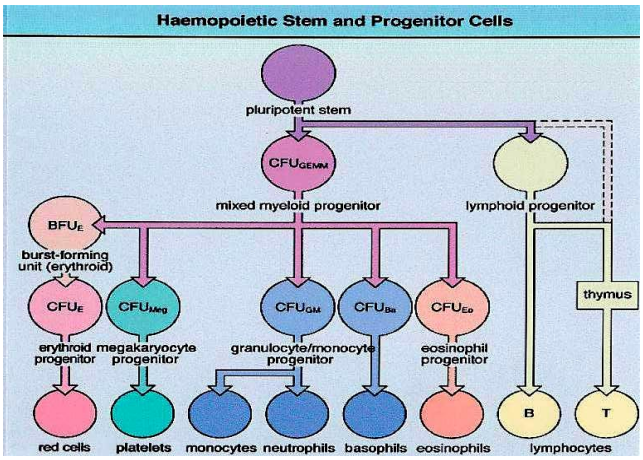
- Composed of stromal cells and micro vascular network.
- Stromal cells: fat cells, fibroblasts, macrophages, reticulum cells and endothelial cells.
- These cells secrete extracellular matrix and growth factors.

Growth factors:

- Hormones that regulate proliferation and differentiation of progenitor cells and the function of mature cells. *E.g. Granulocyte macrophage colony-stimulating factor "GM-CSF" Erythropoietin and Thromopoient*

Stem cells plasticity:

- Stem cells can give rise to various tissues e.g. hemopoietic stem cells and mesenchymal stem cells. ***Imp.***
- May help in treatment of mesenchymal tissue disease especially using embryonic stem cells.



- Monocytes & neutrophils both originate from a common progenitor
- One pronormoblast gives 16 reticulocytes that gives 16 red cells
- Targets for erythropoiesis:
  - Erythroid burst-forming units "BFU_E "
  - Erythroid colony-forming units "CFU_E"
  - Pronormoblast

Neutrophilia

- Acute infections:
  - Bacterial, viral, fungal, mycobacterial and rickettsial
- Physical stimuli:
  - Trauma, electric shock, anoxia, pregnancy
- Drugs and chemicals:
  - **Corticosteroids *"MCQ",*** aetiocholanolone, adrenaline, lead, mercury poisoning, lithium
- Hematological causes:
  - Acute haemorrhage, acute haemolysis, transfusion reactions, post-
  - splenectomy, leukaemia and myeloproliferative disorders.
- Malignant disease:
  - Carcinoma, especially of gastro-intestinal tract, liver or bone marrow
- Miscellaneous conditions:
  - Certain dermatoses, hepatic necrosis, chronic idiopathic leucocytosis

Lymphocytosis

Non-Malignant causes

- Virus infections:
  Infectious mononucleosis
  Infectious lymphocytosis
  Cytomegalovirus infection
  Occasionally mumps, varicella, hepatitis, rubella, influenza
- Bacterial Infections:
  Pertussis
  Occasionally cat-scratch fever, tuberculosis, syphilis, brucellosis
- Protozoal infections:
  Toxoplasmosis and occasionally malaria
- Other rare causes:
  Hyperthyroidism, congenital adrenal hyperplasia

Monocytosis

- Chronic bacterial infections:
  - Tuberculosis, subacute bacterial endocarditis, brucellosis
- Other Specific Infections:
  - Malaria, Kala-azar, trypanosomiasis, typhus, Rocky
  - Mountain spotted fever
- Malignant diseases:
  - Hodgkin's disease, carcinoma
- Leukaemia:
  - Acute myeloid leukaemia, chronic monocytic leukaemia
- Neutropenias:
  - Familial benign and severe neutropenia
  - Cyclical neutropenia
  - Drug-induced Agranulocytosis
- Miscellaneous:
  - Cirrhosis, systemic lupus erythematosus, rheumatoid arthritis

Eosinophilia

Note: Eosinophilia is highly related to thyroid-diseases (Iodine) → Due to drug hypersensitivity reactions. (vs Iodine)

- Allergic reactions:
  - Asthma, hay fever, urticaria, angioneurotic oedema
- Parasitic Infestation:
  - Tissue parasites – trichinosis, filariasis, visceral larva migrans, etc..
  - Intestinal parasites – Ascaris, Taenia, etc. (less regularly)
- Skin disorders:
  - Pemphigus, pemphigoid, eczema, psoriasis, (dermatitis herpetiformis)
- Drug hypersensitivity reactions:
  - Especially iodides, penicillin, allopurinol, gold salts, tartrazine
  - Loffler's pulmonary syndrome and Loffler's endomyocarditis
  - Tropical eosinophilia (probably filarial)
- Malignant diseases:
  - Especially Hodgkin's disease, carcinoma of ovary, lung stomach,
  - angioimmunoblastic lymphadenopathy.
- Following irradiation or splenectomy:
  - Hypereosinophilic syndromes
  - Eosinophilic leukaemia
- Miscellaneous Conditions:
  - Polyarteritis nodosa, ulcerative colitis, sardoidosis, scarlet fever,
  - pernicious anaemia, chronic active hepatitis, eosinophilic granuloma,
  - familial eosinophilia

Leukaemoid Reactions or LeucoerythroblasticAnaemia

Severe infections, especially in children:

a. Pneumonia, septicaemia, meningococcal meningitis
b. Infectious mononucleosis, pertussis

- Intoxications:
  Eclampsia, severe burns, mercury poisoning
- Neoplasia:
  Especially with bone-marrow infiltration
- Severe haemorrhage or haemolysis

Neutropenia

- Drugs:
  Selective neutropenia
  Agranulocytosis (Aplastic anaemia)
- Infections:
  Viral – including hepatitis, influenza, rubella
  Bacterial – typhoid fever, brucellosis, miliary tuberculosis
  Rickettsial and protozoal infections (Sometimes)
- Megaloblastic anaemia:
  Vitamin B^{12} or folate deficiency
-
-

- Chronic neutropenia:
  Chronic idiopathic neutropenia
  Immune neutropenia
  Congenital neutropenias
  Cyclical neutropenia
- Hypersplenism:
  Primary
  In association with cirrhosis, Felty's syndrome, etc.
- Ionizing radiation and cytotoxic drugs:
  Radiotherapy
  Alkylating agents, antimetabolites, others
- Malignant disease:
  Acute leukaemia
  Leuco-erythroblastic anaemia due to metastatic carcinoma, multiple myeloma or lymphoma
- Micscellaneous conditions:
  Systemic lupus erythematosus, myxoedema, hypopituitrism, iron deficiency, anaphylactic shock

Lymphopenia

Secondary Causes

- Loss:
  Mostly from gut as in intestinal lymphangiectasia, Whipple's disease and rarely Crohn's disease
  Thoracic-duct fistula
- Maturation:
  Primary, or secondary to gut disease
  Vit B12 or folate deficiency
  Zinc deficiency
- Pharmacological agents:
  Antilymphocyte globulin
  Corticosteroids
  Cytotoxic drugs
- Infections:
  Severe septicaemias
  Influenza, occasionally other virus infections
  Colorado tick fever
  Miliary tuberculosis
- Other miscellaneous conditions:
  Collagen vascular diseases, especially SLE
  Malignant disease
  Other conditions with lymhocytotoxins
  Radiotherapy
  Graft-versus-host disease

Anemia

is a Reduction of the Hemoglobin Concentration of the peripheral blood below the lower limit of the reference range for the Age and Gender.

Classification depends on size of RBCs (MCV) and concentration of Hb (MCH)

- Adult Males < 13.5 g/dl
- Adult Females < 11.5 g/dl

Pathophysiology:

Tissue Hypoxia → ↑ erythropoietin → ↑ erythrocyte.

Compensation physiological adjustment:

1. ↑ O2 delivery to the tissue by RBCs → ↑ 2,3-DBG → combine with Hb → ↓Affinity of Hb for O2 → ↑ O2 release → tissue.
2. Redistribustion of blood flow
3. Maintenance of total blood volume by expansion of the plasma volume.
4. ↑Cardiac output & rate of blood circulation
   - ↑stroke volume
   - ↑ heart rate (tachycardia)
   - ↑Hyperkinetic circulation.
5. ↑ Respiratory rate

Causes of Anemia

- Reduction of plasma volume (e.g. Dehydration)
- Increase in plasma volume (e.g. Pregnancy)
- Acute major blood loss

Clinical features

- It depends on speed of on set, severity and age
- If it is rapid it will have more symptoms
- Mild anemia usually has no symptoms at rest, However, when Hb = 9-10 it'll cause symptoms **(MCQ)**

Symptoms

- Shortness of breath
- Weakness (fatigue)
- Lethargy
- Palpitation :D يرفرف كالحمامة
- Headache
- Cardiac failure, angina, intermittent claudicating or confusion especially in elderly **(MCQ)**
- Pale skin
- Exercise induced dyspnea
- Chest pain
- Dizziness
- Cognitive problems
- Numbness or coldness in your extremities

Signs for anemia:

- **Koilonychias** (spoon shaped nails) in iron deficiency anemia
- **Jaundice** in hemolytic anemia due to increased level of bilirubin.
- **Leg ulcers** in sickle cell anemia due to blockage of vessel by sickled cells.

Other signs:

- Black and tarry stools (sticky and foul smelling)
- Maroon, or visibly bloody stools
- Tachycardia
- Tachypnea
- low blood pressure
- Heart murmur
- Enlargement of the spleen
- Constipation

Etiology

Anemia is produced by four main mechanisms. They can be divided into two main sub-divisions :

1. In the marrow
   - Actual diminution in productive marrow → Hypoplastic Anemia
   - Marrow unable to produce sufficient normal red cells usually due to deficiency of an essential factor e.g. iron and vitamin B12
2. In the circulation
   - Excessive loss of RBCs due to hemorrhage → Acute post hemorrhagic anemia
   - Excessive destruction of RBCs by the macrophage system in the spleen → Hemolytic Anemia

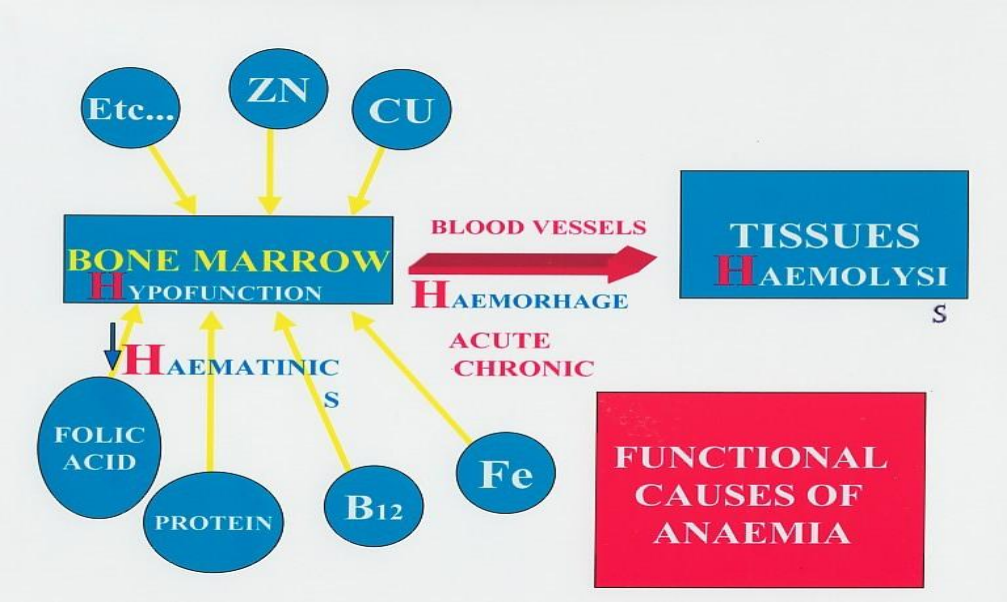


Table 2.4 Classification of anaemia.

Microcytic, hypochromic	Normocytic, normochromic	Macrocytic
MCV <80 fL	MCV 80–95 fL	MCV >95 fL
MCH <27 pg	MCH ≥27 pg	Megaloblastic: vitamin B_{12} or folate deficiency
Iron deficiency	Many haemolytic anaemias	Non-megaloblastic: alcohol, liver disease, myelodysplasia, aplastic anaemia, etc. (Table 4.11)
Thalassaemia	Anaemia of chronic disease (some cases)	
Anaemia of chronic disease (some cases)	After acute blood loss	
Lead poisoning	Renal disease	
Sideroblastic anaemia (some cases)	Mixed deficiencies	
	Bone marrow failure (e.g. post-chemotherapy, infiltration by carcinoma, etc.)	

MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

IRON DEFICIENCY ANEMIA

Iron deficiency is the most common cause of anemia in every country. **(MCQ)**

- It is the most important cause of **microcytic hypochromic** anemia (Decrease in MCV, decrease in MCH, decrease in MCHC). ***(MCQ)***

N.B: In early iron deficiency it's normocytic normochromic anemia.

- ✪ **microcytic**: because of a factor that stop the division at certain limit
- ✪ **hypochromic**: decreased Hb content

- The three main causes of hypochromic microcytic anemia are abnormalities in:

The haem part
❶ Iron. (e.g. Iron deficiency anemia)
❷Protoporphyrin. (e.g. sideroblastic anemia)
❸Globin. (e.g. thalassemia & lead poisoning because it inhibits haem and globin synthesis)

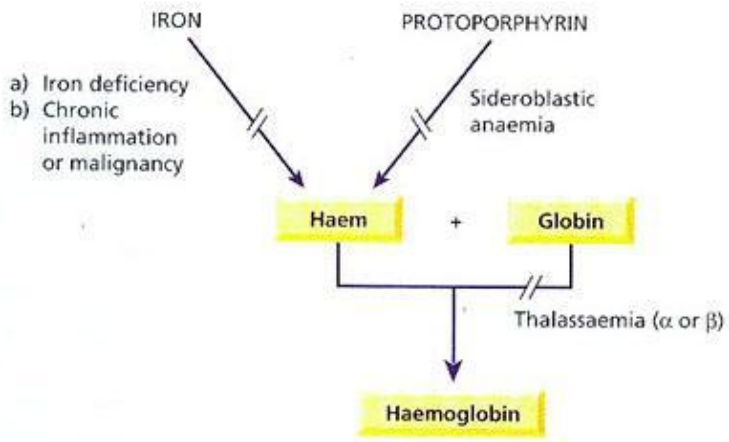


Fig. 3.1 The causes of a hypochromic microcytic anaemia. These include lack of iron (iron deficiency) or of iron release from macrophages to serum (anaemia of chronic inflammation or malignancy), failure of protoporphyrin synthesis (sideroblastic anaemia) or of globin synthesis (α- or β-thalassaemia). Lead also inhibits haem and globin synthesis.

Differential diagnosis in microcytic hypochromic anemia

- Iron deficiency.
- Thalassemia.
- Anemia of chronic disease.

Iron distribution and transport

Transferrin delivers iron to tissue.

- ✪ e.g. erythroblasts in bone marrow
- ✪ At the end of RBC's life → macrophages → iron released → plasma + combine transferring.

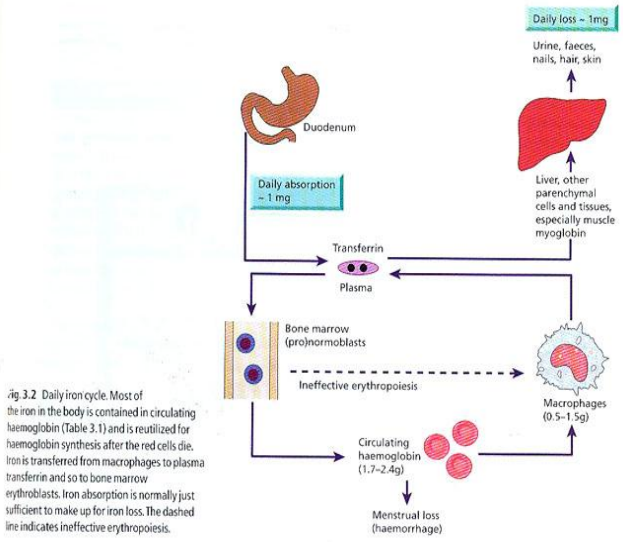


Fig. 3.2 Daily iron cycle. Most of the iron in the body is contained in circulating haemoglobin (Table 3.1) and is reutilized for haemoglobin synthesis after the red cells die. Iron is transferred from macrophages to plasma transferrin and so to bone marrow erythroblasts. Iron absorption is normally just sufficient to make up for iron loss. The dashed line indicates ineffective erythropoiesis.

Iron requirements:

- Varies depending on age, sex. It is increased in pregnancy, adolescent and menstruating females.
- It's about 1 mg/day .

Dietary iron

- Meat especially liver which is rich in iron.
- 5 -10% of taken iron is absorbed.

Iron absorption

Favored by	Reduced by
✪ **Dietary factors:** Increased Heme iron Increased animal iron Ferrous iron salts ✪ **Luminal factors:** Acid pH (e.g. gastric HCl) Low molecular weight soluble chelates (e.g. Vit. C, sugars, amino acids) ✪ **Ligand in meat (unidentified)** ✪ **Systemic factors:** Increased erythropoiesis Ineffective erythropoiesis Pregnancy Hypoxia	**Decreased heme iron** **Decreased animal iron** **Ferric iron salts** **Alkalis (e.g. pancreatic secretions)** **Insoluble iron complexes (e.g. phytates, tannates in tea الشاي الصعيدي : D)** **Iron overload** **Decreased erythropoiesis** **Inflammatory disorders**

N.B:

In hemolytic anemia and thalassemia there is:

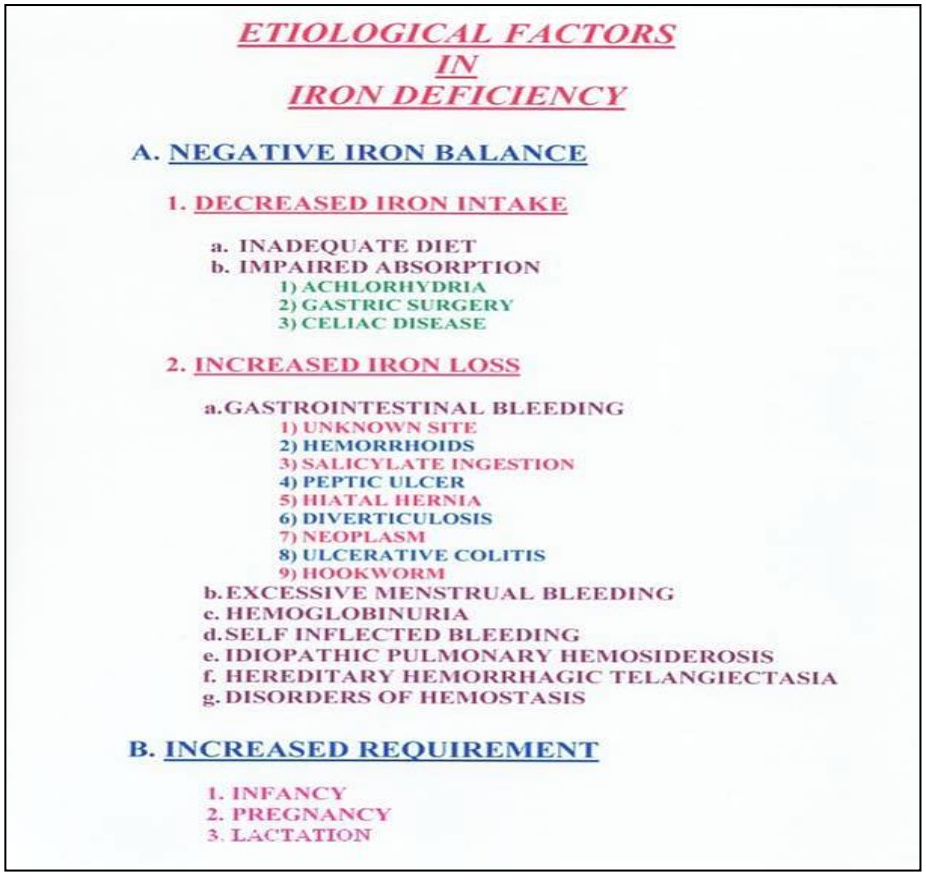
- Increased iron absorption.
- Increased erythropoiesis.
- Ineffective erythropoiesis.

Iron deficiency:

Caused by one of 2 cases:

❶Increased loss of iron from body. **"The most common"**

❷Decreased iron intake and its rare and mostly because of the removal of the stomach and duodenum.



Clinical features

General:

- Lethargy.
- Pallor.
- Headache.
- Palpitation (↑ H.R.).
- Dyspnea .

Specific:

- Painless glossitis. ***(MCQ)*** } Painless, not caused by infection
- Angular stomatitis.
- Brittle or spoon nails (Koilonychias).
- Pica (unusual dietary cravings وحم الحامل). ***MCQ*** 'usually eat mud'
- Irritability and delayed psychomotor development in children.
- Dysphagia , with pharyngeal web .
- Atrophic gastitis , ↓ gastric secretion which is reversible .

Laboratory findings:

- Hb level, RBC (decreased) + RBC indices.
  - **RBC indices**: (Decrease in MCV, decrease in MCH, decrease in MCHC). ***(MCQ)***
  - **RDW is increased** :(in Thalassemia it is normal or decreased→ to differentiate between them)

- Peripheral blood film: hypochromia, microcytosis, pencil shaped cells.

- Reticulocyte count: decreased.

Note: unless the cause is chronic hemorrhage then it is Increased

- Serum iron level is decreased.(**not diagnostic**)

- Total iron binding capacity is increased.

- Serum ferritin is decreased. 'the most import. Diagnostic tool' , reflects iron storage , if it is ↓ , this is microcytic anemia , if it is normal or ↑ it doesn't exclude the anemia **.** ***(MCQ)***

N.B. Refer to the slides

" Hemolysis and hemoglobinopathy

HEMOLYTIC ANEMIA

It's

1. A basic pathological change that leads in the reduction of life span of RBCs.
2. An increase in the rate of RBCs destruction.

First of all :

- Normal life span of RBCs is about 120 days
- The life span in hemolytic anemia is usually 30 days
- In hemolytic anemia the is usually an increase in the bilirubin witch will give some sighs of jaundice.

Red cell breakdown:

Extravascular	Intravascular
Spleen and liver [RES]	**Blood stream**
Destruction of Hb released by macrophages • Globin → amino acids • Iron → bind to transferrin • Heam → metabolized to bilirubin	**Free Hb released** • **Hbemia , Hburia** • **Haemosiderinuria** • **Methaemalbumiemia**

The urine will be black and feces will be light colored

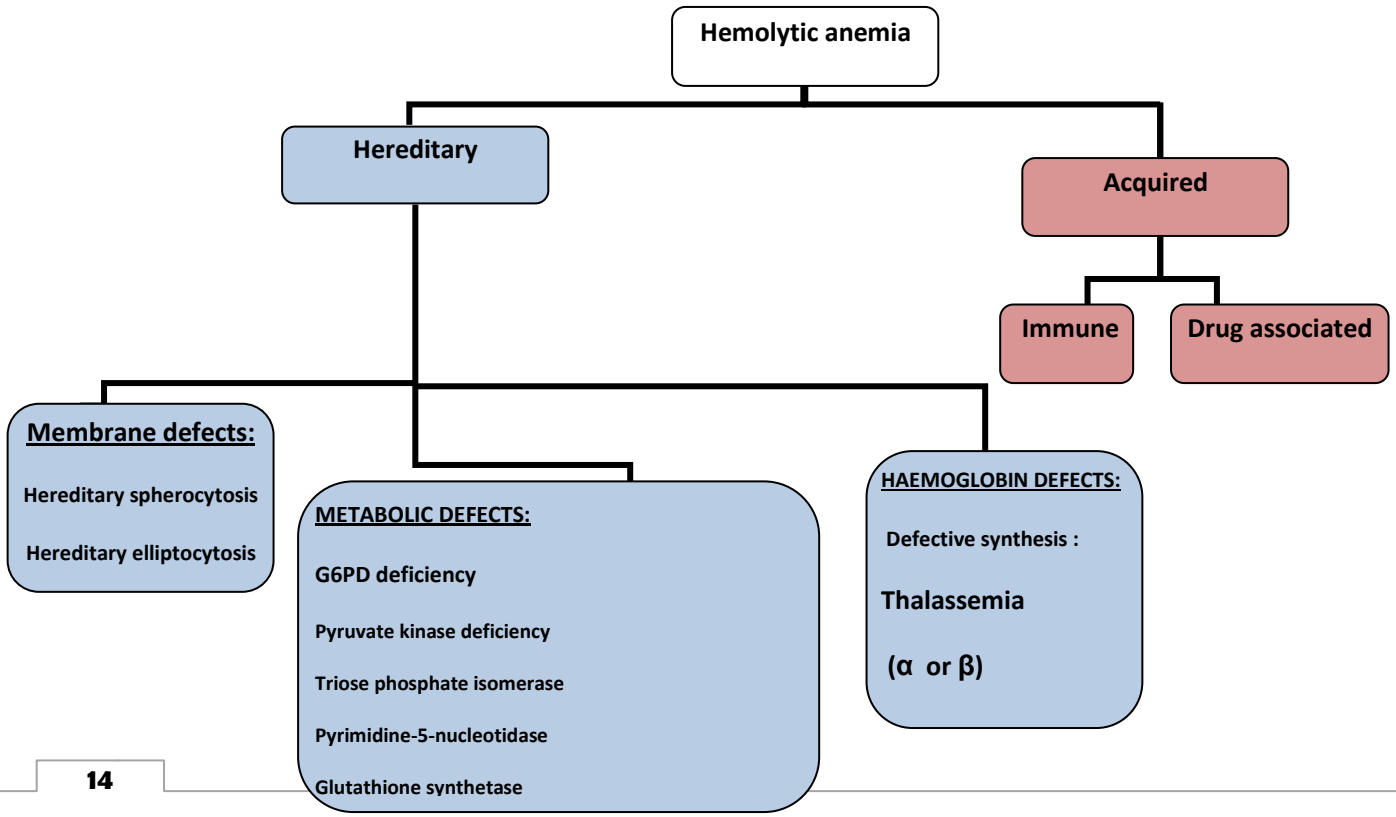
List 3 lab abnormalities in hemolytic anemia.

1. Increased unconjugated bilirubin
2. Increased hemoglobinuria
3. Increase reticulocyte (if there is no problem with the Bone marrow)
4. Decreased serum haptoglobin

What are some clinical effects of hypebilirubinemia?

Jaundice and pigment-containing gallstones

Classification of hemolytic anemia:



HEREDITARY SPHEROCYTOSIS (HS)

It is a hereditary hemolytic anemia due to **membrane defect**

Pathogenesis:

- ✓ HS is usually caused by defect in proteins (Ankyrin & spectrin) involved in the vertical interactions between the membrane skeleton and the lipid bilayer of the red cell.
- ✓ The loss of the membrane may be caused by the release of parts of the lipid bilayer that are not supported by skeleton.

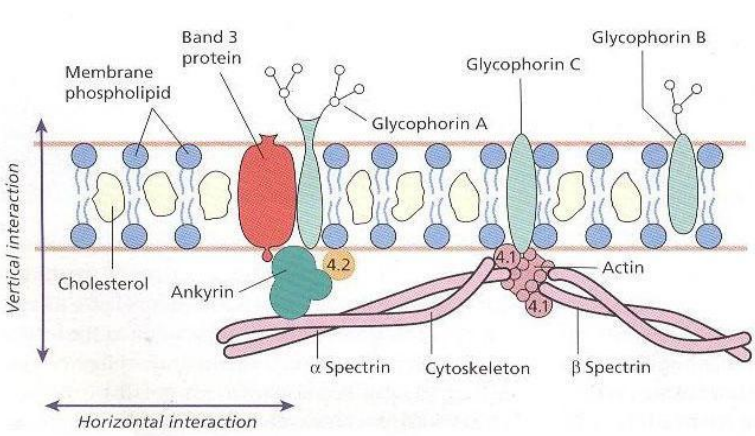


Fig. 2.12 The structure of the red cell membrane. Some of the penetrating and integral proteins carry carbohydrate antigens; other antigens are attached directly to the lipid layer.

- ✓ The marrow produce red cells of normal biconcave shape BUT they loss membrane and become more and more spherical (loss of the surface area relative to volume) as they circulate through spleen and the rest of reticuloendothelial system
- ✓ Ultimately the spherocytes are unable to pass through the splenic microcirculation where they die prematurely.

Clinical features:

- Autosomal **DOMINANT**.
- Anemia present at any age
- Splenomegaly.
- Jaundice due to presenceof unconjugated bilirubin.
- Aplastic crisis precipitated by parvovirus infection "by suppression of the bone marrow by the virus", causing a sudden increase in severity of anemia. " acute hemolytic + decrees erythpoisis"
- pigment gallstone "cholethiasis"

Laboratory findings:

- Anemia.
- **Reticulocytosis.**
- Blood film: spherocytes.
- Osmotic fragility: increased.

Treatment:

- Splenectomy → it can be effected in other types of hemolytic anemia
- Folic acid supplement.

What is the "osmotic fragility" test?
The osmotic fragility test is done to confirm the diagnosis of hereditary spherocytosis. A Patient's red blood cells are placed in different concentrations of saline solution for 24 hours. When red blood cells are placed in saline solution, they absorb water until the cell Membrane bursts. Spherocytes do not tolerate weak saline solutions, causing them to burst Sooner than normal cells.

What is HS?
The most common inherited intracorpuscular hemolytic anemia. It is characterized by spherical RBCs.
What is its mode of inheritance?
Autosomal dominant , rarely it may be recessive
What causes the anemia?
The abnormally shaped cells are trapped and destroyed in the spleen.
Why are the RBCs shaped like spheres?
Molecular defects in cytoskeletal proteins in the RBC (e.g., spectrin. ankyrin, and protein 4.2) cause the deformity.
What is the diagnostic test?
Increased erythrocyte osmotic fragility to hypotonic saline
What other abnormalities may contribute to the diagnosis?
In lab values?
Reticulocytosis, increased mean corpuscular hemoglobin concentration)MCHC), and unconjugated hyperbilimbinemia
On physical exam?

Defective red cell metabolism

Glucose 6-phosphate dehydrogenase deficiency

Definition

It is a hereditary hemolytic anemia due to metabolic defect.

Pathogenesis:

G6PD functions is

1. To reduce NADP → NADPH (needed for production of reduce glutathione GSH).
2. Oxidize glucose -6-phosphate
3. It is the only source of NADPH

Deficiency

1. Makes the RBCs susceptible to oxidant stress
2. Impairs NADPH and GSH synthesis

Note *that Hb and RBCs membrane are protected from oxidant stress agents by GSH Acute hemolytic anemia occurs in response to oxidant stress: drugs, fava beans or infections.*

Genetics:

X-Linked.
G6PD patients have resistance to falciparum malaria.
The gene is located on x-chromosome close to the factor VIII gene." **q2-8** "
It is found more in black people

Epidemiology:

The main races affected are West Africa, Middle East, Mediterranean and South East Asia. Most sever in Mediterranean and Middle East.
It usually effect tropical countries that malaria is found in
Note *It is thought to confer a selective protection against Plasmodium Falciprum Malaria*

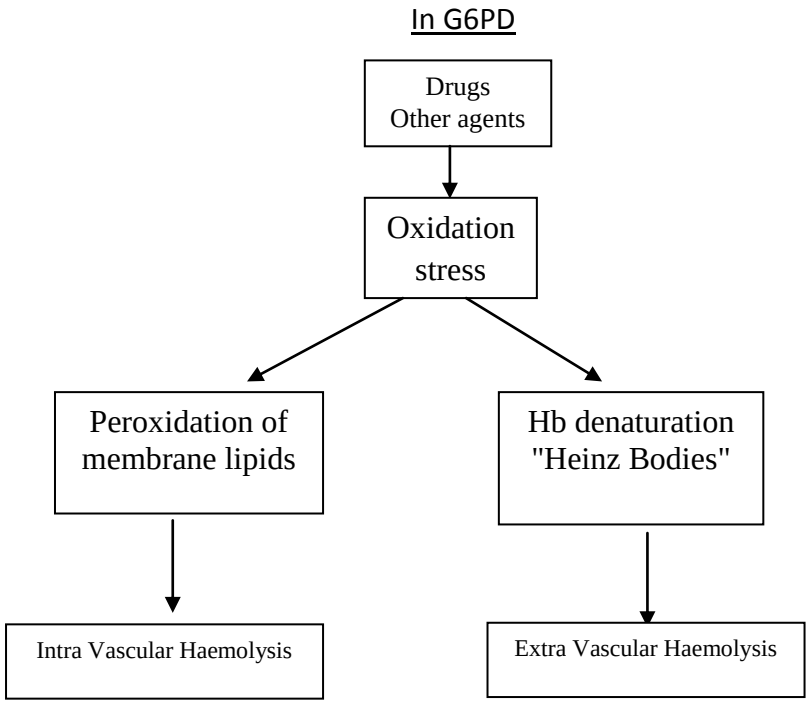
Favism

Fava beans ingestion is not always followed by a haemolytic attack in G6PD individuals.
The offending agent may be the glucoside divicine or its aglycone isouramil." Not protein "
Favism has been precipitated with fresh beans, dried beans, canned and frozen beans.
(It is commonest with fresh and raw beans)

Oxidative damage may depend on how much isouramil is released by glycosidases present in the beans or in the intestinal tract of the consumer.

Agents causing hemolytic anemia in G6PD Deficiency:

1- Antimalarial (Chloroquine) 2-sulphanamide Antibiotics (cotrimoxazole) 3- Infections



Note *that Heinz Bodies con be detected by using Methylin Blue*

Neonatal jaundice

The most comman case is G6PD
1/2 of children with G6PD will not have NNJ
Note *the jaundice is usually not caused by excess hemolysis But by G6PD deficiency which effects neonatal liver function*

Clinical features:

Features of intravascular hemolysis precipitated by infection, drugs, ingestion of fava beans.

- Anemia/ pallor.
- Hemoglobinuria. → is seen most frequently in children with favism.
- Renal failure → is common in adults
- Dark Urine
- Jaundice.
- Fever.
- Abdominal pain.
- Neonatal jaundice

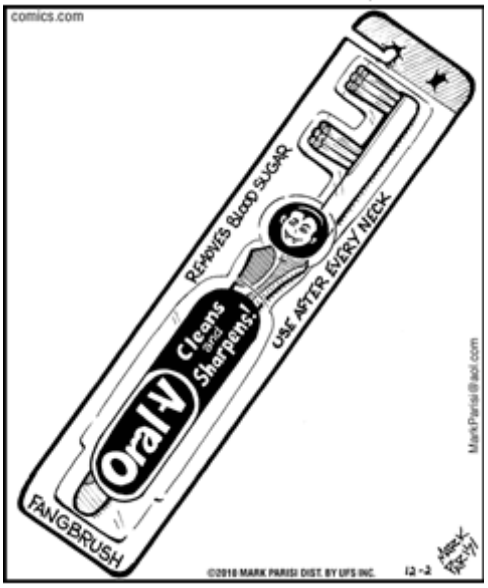
Laboratory findings:

- Anemia.
- Reticulocytosis.
- Peripheral blood: fragmented RBCs "bite cells-blister cells" → due to removal of Heinz bodies "HB". "HB on RBCs detected and removed by spleen, and fragmented RBCs formed".
- Heinz bodies: (oxidized, denatured Hb)
- Hemoglubinuria.
- Decrease haptoglobin.
- Hemoglobinemia.
- Hyperbilirubinemia.
- G6PD level: normal within acute phase because young RBC have higher level of enzyme "so we test for the disease after the acute phase".

So we take blood during crises and we test the enzyme between the crises

Treatment

- Stop offending drugs
- Treat infection if present
- Maintain high urine output
- Blood transfusion in sever anemia
- Phototherapy and exchange transfusion in neonatal jaundice



ACQUIRED HEMOLYTIC ANAEMIAS

Focus on red notes

Hemolysis:

- Premature destruction of RBCs.
- Hemolysis could be due to:
  a. Defect in the RBCs (intra - corpuscular) as in congenital hemolytic anemia.
  b. Defect in the surrounding environment (extra-corpuscular) as in acquired anemia.

Types:

- **Immune.**
- **Non- Immune.**

A. IMMUNE HEMOLYTIC ANEMIA:

Erythrocytes are destroyed due to deposition of immune-globulins and /or competent. It could be:

a. Alloimmune:

"Foreign" antibodies are destroying RBCs e.g. hemolytic transfusion reaction or hemolytic disease of the newborn.

*b. Autoimmune **(AIHA)**:*

Group of anaemias in which there is development of antibodies directed against antigens on the surface of the patients own RBCs. The antibodies are usually IgG or less commonly IgM and some bind complement. It classified, according to the temperature at which the antibody reacts with the RBCs, into:

1. **Warm: at 37° C. → IgG the majority**
2. **cold : < 37° C → IgM the Majority**

Mechanism of AIHA

Destruction of RBCs can be either: cell mediated or complement mediated.

a. **Cell mediated:** destruction is through macrophages with receptors for:
   i. Fc portion of immunoglobulin or
   ii. Complement bound to RBCs "through TAG".
b. **Complement mediated:** destruction or (Lysis) of the cell is directly by complement.

a. Cell mediated by macrophages with receptors for complement:

RBCs, with complement attach to macrophages through the complement receptor causing phagocytosis. The destruction is mainly extra vascular, in the liver because it contains a large number of macrophages with complement receptors. Spleen plays a lesser role unless it is enlarged. This mechanism is important in the cold type.

b. *Complement mediated:*

Direct lysis by complement, causes intravascular hemolysis. Seen in paroxysmal cold hemoglobin urea (a type of cold autoimmune hemolytic anemia).

Warm AIHA:

Etiology (classification):

a. **Idiopathic :** 30% of the cases *(Primary)*

b. Secondary:

1. Autoimmune diseases: e.g. systemic lupus erythematosis (SLE), rheumatoid arthritis.
2. Lympho-proliferative diseases: e.g. chronic lymphocytic leukemia, lymphoma.
3. Malignancies: e.g. ovarian carcinoma.
4. Post viral infections.
5. drugs: e.g. methyldopa (aldomet)

Antibody features:

- Warm antibody.
- Usually IgG.
- Usually has Rh specificity

Clinical features:

- Lethargy, Pallor.
- Jaundice.
- Splenomegaly, hepatomegaly.

Laboratory features:

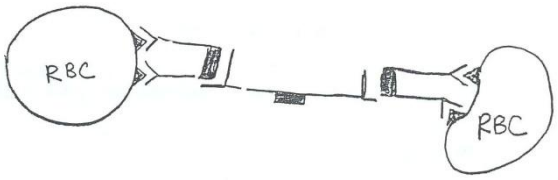
- **peripheral blood:**
  - Anemia
  - Polychromasic
  - Spherocytes
  - Nucleated RBCs
- Reticulocyte count: increased.
- **Bone Marrow:** erythroid hyperplasia.
- **Chemistry:** increased Bilirubin, mostly indirect.
- Coombs test (direct ant globulin test): to detect antibodies covering the RBCs by using an anti–immunoglobulin (i.e. antibody vs. the attached anti- immunoglobulin). This reaction causes agglutination.

**COOMB'S TEST (ANTIGLOBULIN TEST) **

Red cell with Anti – immunoglobulin

Bound antibody to

Membrane antigen



Agglutination

Cold AIHA

Antibody characteristics:

* Usually IgM.
* React with RBCs (agglutinate) on cooling.
* Complement mediated.
* Complement can be detected using complement specific Coombs.
* Specificity for RBCs surface antigens (group) can be anti P, anti I, anit I depending on associated disease.
* Antibody usually binds RBCs in peripheral circulation (10-20^{o}C) causing activation of complement.

Classification of Cold Agglutinin syndrome:

* Idopathic (Cold hemagglutinin disease) (CHAD):
* Secondary:

Cold Hemagglutinin Disease:

Idiopathic:

Seen in elderly, run a chronic course. Usually a benign course. But many terminate in a B cell lymphoma. Monoclonal proliferation of B cells.

Laboratory Findings:

* Hemoglobin: low.
* Agglutination of RBCs: (clumping) seen in the tube or on film.
* MCV ↑ MCH ↑.
* Coomb's test: + for complement but not IgM because IgM dissociates from the cells in vitro.
* Antibody, Screen for specificity e.g. Anti I.
* Serum protein electrophoresis for mononclonal band (para-protein) in CHAD.

B. NON IMMUNE HEMOLYTIC ANAEMIAS

Hemolytic anaemias due to mechanisms or agents other than antibodies + / or complement e.g.:

- Mechanical (traumatic) (fragmentation)
- **Toxins (drugs)**
- **Infections (septicemia)**
- Splenomegaly (hypersplenism)
- Burn (physical)
- Renal failure and liver failure
- Chemical
- **DIC**

Mechanical (traumatic) (fragmentation):

This is due to direct trauma (stress) to the RBCs causing fragmentation of the RBCs and intravascular hemolysis. The fragmented cells can be seen on peripheral blood smears and are called (schistocytes).

Types:

1. cardiac – most common type

Due to:

a. prosthetic valves
b. Patches "injured endothelium".
c. Valvular disease e.g. stenosis.

2. **Microangiopathic:** mechanical hemolysis due to contact between the RBCs and the abnormal intema of thrombosed, narrowed, necrotic small vessels or fibrin strand formation.
   Caused by many diseases e.g. DIC (disseminated intravascular coagulation), malignant hypertension, disseminated malignancies especially mucin secreting Adeno-carcinomas (thrombotic thrombocytopenic purpura), hemolytic uremic syndrome (HUS). Causes intravascular hemolysis→

   - Hemoglobinurea
   - Hemoglobinemia.
   - Decreased haptoglobin.
   - Fragmented RBCs (schistocytes) on peripheral blood film.
   - Hemosidrinurea.

Infection:

Many infectious agents can cause hemolytic anaemias due to various mechanisms, e.g.

- **Malaria: due to:**
  - Direct invasion causing intravascular lysis, or /and extra vascular lysis.
  - Immune complex formation.
  - Splenomegaly.

Black water fever: Is an example of severe intra-vascular hemolysis due to falsiparum sp.

- **Clostridium perfringens:** due to action of lipase and proteinase enzymes produced by the organism.
- **Meningococcal:** due to DIC

Chemical:

E.g. some toxins e.g. spider venom, snake venom, bacterial toxins, arsenic (As), (Cu).

Physical:

Burn: characterized by the presence of many spherocytes in the peripheral blood.

Splenomegaly:

Renal Failure and Liver failure:

Due to change in the metabolic structure of the RBCs.

INTRODUCTION TO MACROCYTIC ANEMIA

Normal adult red cell values:

	Male	Female
Hb(g/dl)	**13.5-17.5**	**11.5-15.5**
PCV (%)	**40-52**	**36-48**
Red cell cont	**4.5-6.5**	**3.9-5.6**
MCH (pg)	**27-34**	
MCV (fl)	**80-95**	
MCHC (g/dl)	**30-35**	
Reticulocyte count	**25-125**	

Note:

- Hb , PCV and Red cell count are the only three values that differ in male with female.
- MCV "size of RBCs" : If
  - less than 80, microcytic anemia occur
  - more than 95 , macrocytic anemia occur
  - Between 80-95 normocytic normochromic anemia
- MCH "concentration of Hb" : If less than 26, hypochromic anemia occur.

  No hyperchromic condition!

- In children Hb is 15-21 because of the low O_2
- After birth physiological jaundice happens

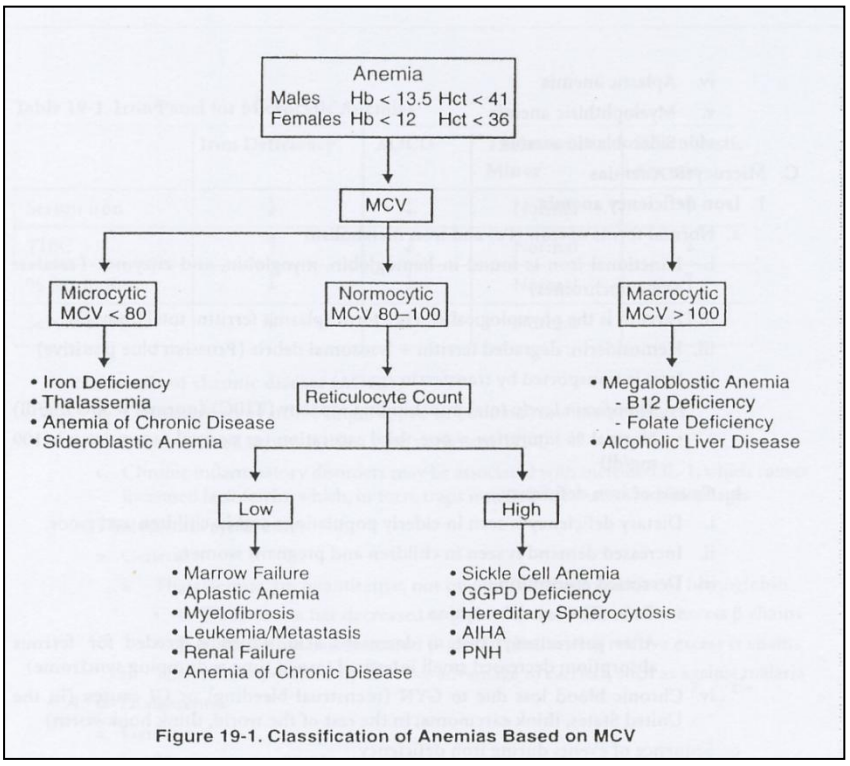


Figure 19-1. Classification of Anemias Based on MCV

MACROCYTIC ANEMIA

- Large RBCs.
- Based on appearance of developing erythroblast (with delayed nucleus maturation) macroctic anemia is divided into :

Megaloblastic	Non – Megaloblastic
• Delay of nucleus maturation in bone marrow erythroblast • Defect DNA synthesis due to Vit. B_{12} or Folate deficiency	alcoholism, liver disease, aplastic anemia, reticlocytosis, hypothyroidism myeloma , pregnancy and newborn

MEGALOBLASTIC ANEMIA:

- Not only RBCs and bone marrow becomes larger but **all body cells** because of the deficiency in Vit. B_{12} or Folate those are required in DNA synthesis.
- Anemia's characterized by delayed maturation of the nucleus compared to the cytoplasm.

B12	Folate
Found in animal sources	In plant sources
Absorption in terminal ileum and intrinsic factor is required from gastric juices. (gastric resection and Crohn's)	Absorption in jejunum
Deficiency also causes neural symptoms	Deficiency in pregnant causes neural tube defects in child.

Laboratory findings:

- Anemia, macrocytic.
- Peripheral blood: macrocytosis, ovalocytosis.
- Decrease WBC: with **hypersegmented neutrophils** "more than 5 lobes".
- Decrease platelets.
- Bone marrow: hyper cellular, Megaloblastic erythropoiesis – immature nuclei with normal hemoglobinization "hemoglobinization = cytoplasm".
- Increase bilirubin; increase Lactate dehydrogenase "LDH" due to marrow cell breakdown.
- Decrease serum B12 serum Folate and RBC Folate.

Vitamin B12:

- Source: food of animal origin.
- Absorption after combination with intrinsic factor (synthesized by gastric parietal cells), the complex is absorbed in the distal ileum.
- Transport: the absorbed Vit. B12 is attached to transcobalamin II which transports Vit. B12 to the bone marrow.
- Deficiency:
  - Causes:
    - Dietary → strict vegetarians.
    - Pernicious anemia: Gastric mucosa (chief and parietal cells) destruction due to autoimmune disease lead to
      - No intrinsic factor → no B_{12} absorption
      - No HCL (achlorohydria) → decrease iron absorption
    - Gastrectomy.
    - Intestinal disorders: e.g. chron's disease, ileac resection, parasites " fish tapeworm"

- Notes:
  - Deficiency takes 2 years at least to develop
  - It is not destroyed by heat " cooking"
  - Treatment: IM Vit B_{12}

Folate:

- Source: not synthesized in the body and thus obtained in diet only.
- Absorbed in the upper small intestine.
- Deficiency:
  - Dietary.
  - Mal-absorption.
  - Drugs: e.g. anticonvulsant.
  - Increased requirements:
    e.g. pregnancy, hemolytic anemia. << leads to folate def. because high eryhtropoiesis needs lots of folate.

- Notes:
    - Deficiency takes only months to develop
    - Easily destroyed by heat
    - The requirement is increased in Pregnant and infant
    - The main defferent between the Vit B_{12} and Folate deficncy is that Folate don't have neurological symptoms

Clinical features: *"MCQ"*

General:

- Pallor, lethargy, etc …. .

Specific:

- Jaundice.
- Glossitis**: painful**.
- Angular stomatitis.
- Purpusa: due to thrombocytopenia.
- Neuropathy: sensory and motor, especially in the lower limbs.
- Neural tube defect.
- Psychological impairment.
- Ulceration of the tinge

MEGALOBLASTIC ANEMIA

What is the cause?

Deficiency of vitamin B12 or Folate

How do these deficiencies lead to anemia?

Both vitamin 12 and Folate are necessary for DNA synthesis. Decreased DNA synthesis subsequently leads to decreased RBC production.

What is a Megaloblastic?

An erythroid precursor cell found in the bone marrow

What are typical lab findings?

Pancytopenia, decreased vitamin B12, decreased Folate

What is seen on histology?

Oval macrocytosis, hypersegmented neutrophils (> 5 lobes), and megaloblastic hyperplasia of bone marrow

FOLATE DEFICIENCY

What are some causes of Folate deficiency؟

Poor diet, pregnancy, spme, drug effects، and Giardia lamblia infection

In which populations is diet-related Folate deficiency often found؟

Chronic alcoholics and fad dieters

What drugs are associated with decreased Folate؟

Phenytoin, oral contraceptives, and methotrexate

How does hemolytic anemia cause a relative deficiency of Folate؟

The compensatory accelerated eythropoiesis uses up the body stores of Folate.

VITAMIN B12 DEFICIENCY

What is the most common cause؟

Pernicious anemia

Define pernicious anemia.

An autoimmune disorder with failure of production of intrinsic factor (IF) due to Anti-IF antibodies

How is IF related to vitamin B12؟

IF is essential for the absorption of vitamin B12 in the distal ileum.

What are the 4 clinical findings of pernicious anemia؟

1 .Yellow skin .2 Stomatitis .3 Clossitis .

4Subacute combined degeneration of the spinal cord

How does subacute combined degeneration of the spinal cord manifest؟

Ataxic gait, hyperrefiexia, and impaired vibratory and positional sensation

What abnormal antibodies are found؟

Anti-IF and autiparietal cell antibodies

What are the results of a Schilling test؟

Decreased vitamin B12 absorption corrected by adding IF

What type of gastritis is present؟

Chronic fundal gastritis (Type A(

What feared entity may Type A gastritis progress to؟

Gastric cancer

What are some other causes of vitamin B12 deficiency؟

Intestinal bacterial overgrowth, gastric resection, vegetarian diet, intestinal malabsorption, *D. latum* infestation

What are 2 causes of excess bacteria in the intestine؟

Broad-spectrum antibiotics and blind- loop syndrome

HEMOGLOBIN SYNTHESIS

- Normal adult blood contains three types of hemoglobin : A, A2, F.
- The genes for alpha (**α**) globin and Zeta (**ζ**) is on chromosome **16**, for **ε** (epsilon), **γ**(gamma) **, β** and **δ** (delta) are on chromosome **11**.
- In Yolk Sac → **ζ** and **ε** (Zeta and Epsilon)
- Liver → **β** " very low in fetal life"
- **δ** → very little and stats before birth
- Gower I , II and Portland → all are embryonic hemoglobin's
- Hemoglobin F is made after 8 weeks
- Last hemoglobin made is A
- The age of maturation of hemoglobin is 1 year

No β in fetus

	HbA	HbF	HbA_2
Structure	$\alpha_2 \beta_2$	$\alpha_2 \gamma_2$	$\alpha_2 \delta_2$
Normal	96-98	0.5-0.8	1.5-3.2
At Birth	15-40	50-85	<0.3

Hb Bart's …. **4 gamma** chains… At birth normally less than 0.5

Examples of abnormal hemoglobin:

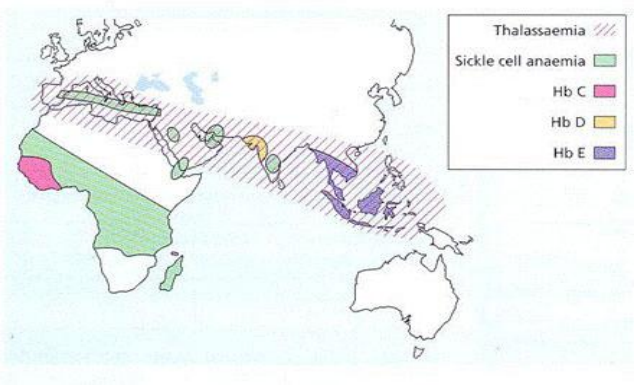
- **Hb S**: Alpha2 (α_2) Beta2 (β_2) in amino acid **6** Glutamine → Valine = Sickle cell disease
- **Hb C**: Alpha2 (α_2) Beta2 (β_2) in amino acid **6** Glutamine → Lysine.
- **Hb O Arab**: Alpha2 (α_2) Beta2 (β_2) in amino acid : **121** Glutamine → Lysine
- **Hb Lepore** has 2 normal alpha and 2 delta beta fusion chain → heavy Hb So it precipitates.

Hemoglobinopathies:

- Diseases caused by reduced or abnormal synthesis of hemoglobin.
- Synthesis of abnormal hemoglobin like sickle cell disease.
- Reduced rate of synthesis of normal alpha (α) or β globin chain (Alpha (α) and β thalassemia).

Epidemiology:

- Affects 10% of the world's population.
- Occurs in tropical and subtropical areas.
- β thalassemia is more common in the Mediterranean region.
- Alpha (α) thalassemia is more common in the Far East.



Disorders:

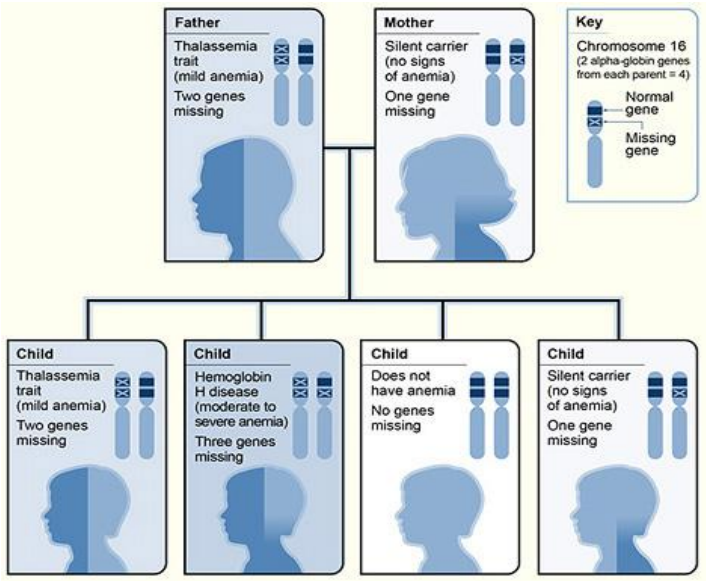
1. Thalassemia.
2. Sickle cell anaemia.

1- THALASSEMIA:

Thalassemias are a heterogeneous group of genetic disorders, which result from a reduced rate of synthesis of alpha (α) or β chains.

A- ALPHA THALASSEMIA:

- Caused by gene deletion.
- Since there are four copies of alpha (α) globin gene, the clinical severity depends on the numbers of genes missing.



HYDROPS FETALIS

- There is loss of all 4 genes; there is complete suppression of alpha (α) chains → incompatible with life → intrauterine death.
- High Hb. Bart's

HEMOGLOBIN H DISEASE

- There are 3 gene deletions.
- Moderately severe anemia 7 – 11 g / d1.
- Microcytic hypochromic anemia.
- Splenomegaly.
- Hb H is formed (β4) and can be seen in the peripheral blood by reticulocyte stain or can be detected by Hb electrophoresis.
- Neonates have high (>0.5%) Hb. Bart's (4gamma) and adults have high Hb H (4 beta chains) (note: B-globin synthesis starts only at birth--> so think of Hb-bart as the fetal equivalent of Hb-H(Hb- H ---> Four beta chains Hb-Bart---> Four gamma chains)
- Heinz bodies can be found by methyl v. stain.
- When stained by brilliant cresyl blue stain precipitated Hb. H in the RBC give them "golf-ball" appearance.

ALPHA THALASSEMIA TRAIT

- Loss of 2 genes.
    - (on the same chromosome--> Asian , on different chromosomes --> African)
- No anemia but the RBC count is increased and there is microcytosis (decreased MCV) and hypochromia (decrease MCV).
    - Increased RBC and normal or decreased RDW----->Imp. In differentiation with iron deficiency anemia.
- Hemoglobin electrophoresis is normal.
- Diagnosis is based on α/β chain synthesis. Normal ratio is 1:1. It is reduced in alpha thalassemia and increased in B thalassemia.

SILENT CARRIER

- Loss of one gene. (we have three fully functioning genes----> alpha chains are not affected)

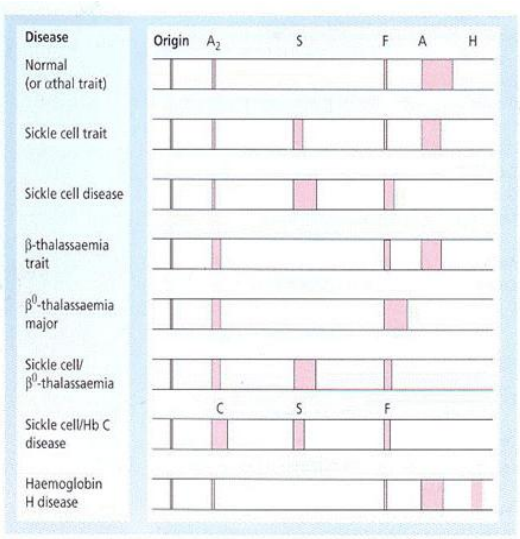
B- BETA THALASSEMIA:

Inheretance:

25% offspring's if parents are carriers of the β thalassemia trait.

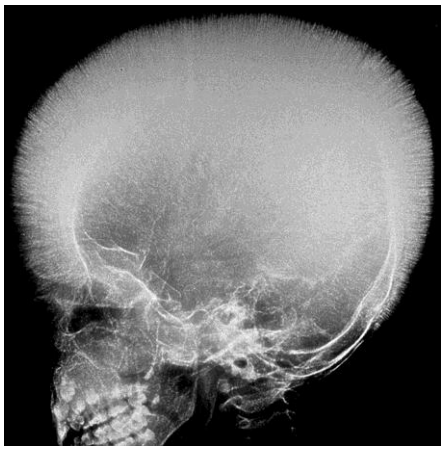
Pathogenesis

- No B chain (β0) or small amounts (β+) are synthesized "autosomal recessive".
- Alpha (α) chain production in excess → precipitate in RBCs → hemolysis (Type of hemolytic anemia)
- Production of gamma (γ) chains → reduced severity of the disease
  (severity depends on Hb F : more Hb F → less severe [more gamma chains to compensate] so, less Hb F → more severe
- The defect is gene mutation rather than a gene deletion.
- HbF is increased especially in β0



Clinical features

1. **Anemia:** Severe appears 3-6 month after birth (switch from gamma to beta chains).
2. **Hepatomegaly – Splenomegaly:** due to hemolysis, extramedullary hematopoiesis and iron overload "see no. 5 for more".
3. **Jaundice:** Due to hemolysis, liver damage.
4. **Bone expansion**: due to marrow hyperplasia results in thalassemia facies, boozing of skull, Broad maxilla, and depressed nose bridge and **hair-on-end** appearance on x – ray.



5. **Iron overload:** due to hemolysis, increased absorption and multiple transfusions results in:
    a. Hepatomegaly and liver damage.
    b. Splenomegaly.
    c. Endocrine organs failure: growth failure, delayed puberty, diabetes mellitus, hypothyroidism.
    d. Cardiac complications: usual cause of death if not treated "arrhythmia due to accumulation of iron in heart → death if not treated".
6. **Increased susceptibility to infections:** usually bacterial infection especially after splenectomy → Pneumococcal, Meningococcal and Hemophilus. ***MCQ***

Note: We cannot do a splenectomy on a child (<5 yrs) because the spleen gets rid of capsulated organisms.

Laboratory diagnosis :

Serum ferritin is increased

> 500mg/dL.

Important to differentiate between thalasemia and IDA

1- **Anemia:**
- Hypo chromic, microcytic (low MCH, low MCV).
- Reticulocytosis.
- On peripheral blood smear: nucleated RBC "normoblasts", target cells, polychromasia, basophilic stippling.

2- **Hemoglobin Electrophoresis:**
    a. **HbA:** absent. ***MCQ***
    b. **HbF:** Increased.
    c. **HbA2:** Normal, decreased, increased. "Imp. in treatment of B thal. Trait".
    d. Alpha / Beta globin chain ratio is increased.

❖ *BETA THALASSEMIA TRAIT (THALASSEMIA MINOR):*
- Common, asymptomatic.
    - One gene is still functioning (the other is defective)
- Laboratory findings:
    - Microcytosis (low MCV), Hypochromia (low MCH).
    - Mild anemia. "or no"
    - Increased RBC count.
    - Increased HbA2 level. > 3.5% but never exceeds ten percent (10%)

Diagnosis of B thal. Trait is important for per- marital, counseling and prenatal diagnosis. If both parents with B thal. Trait, 25% chance of a child with thal. Major.

Prenatal diagnosis:

a) Chorionic villous sampling.
b) Amniotic fluid analysis.
c) Fetal blood sampling.

❖ *THALASSEMIA INTERMEDIA:*

- Moderate severity, Hb7 -10 g / d1.
- caused by a variety of genetic defects e.g.:
  - ✪ Homozygous B thalassemia with increased HbF-gamma (γ).
  - ✪ Homozygous mild B thalassemia – mild defect in B chain synthesis.
  - ✪ Hemoglobin lepore: abnormal hemoglobin caused by crossing – over of the B and delta (δ) genes with production of a B delta (δ) protein chain.

2- SICKLE CELL DISEASE:

Sickle cell disease is group of hemoglobin disorders in which the sickle B –globin gene is inherited e.g.:

- Homozygous sickle cell anemia (HbSS): the most common.
- Double heterozygote HbSC. "rare" (SC)
- Double heterozygote HbS B thal. . "less common" (SB. Thal)

HbS	**β**	**6**	**Glu→ Val**
HbC	β	6	Glu→Lys
HbE	β	26	Glu→Lys
Hb O Arab	β	121	Glu→Lys
Hb D Pungab	β	121	Glu→Gln

Hemoglobin S:

- The most common Hb Abnormality
- Hb S (α2β2): Substitution of valine for glutamic acid in position 6 in the β chain. ***MCQ***
- Substitution of single base replacement of Adenine by Thiamin ---> Different physical and chemical properties (insolubility)
- Insoluble: when exposed to low O2 → crystal formation. ***MCQ***
- Symptoms appear around six months of age (because of inhibition of gamma chains → shift to Adult hemoglobin (A) with normal alpha and abnormal BETA chain)

Pathogenesis:

Deoxygenated sickle hemoglobin polymerizes into long fibers. The RBCs sickle:

- Hemolysis due to fragility.
- Blockage of vessels → infarct.
- High viscosity.

Epidemiology:

- Widespread.
- 1/4 in West Africa. Protection against malaria is afforded by carrier state.
- In Saudi Arabia: common especially in Eastern Province, Jizan and Al-Ola area.

Factors effecting sickling

1. Oxygen tension
    i. 50-60 mmHg for SS
    ii. 20-30 mmHg for AS
2. pH
    i. Inhibited at Alkine
    ii. Exacerbated by acidification
3. concentration of HbS
4. presence of other Hb

Clinical features:

a. **Severe hemolytic anemia:** however the symptoms are mild compared to the Hb level because HbS has a lower affinity for Q2 than A2. The symptoms are pallor, jaundice … etc. ***MCQ***

b. Crises: ***MCQ***

i. Vasooclusive.

ii. Visceral.

iii. Aplastic.

iv. Hemolytic.

c. **Other:**

i. Susceptibility to infection, e.g. Lobar pneumonia and Salmonella osteomyelitis. ***MCQ***

ii. Gall stones.

iii. Liver damage.

iv. Papillary necrosis of the kidney → hematuria.

v. Leg ulcers.

vi. Splenomegaly in early childhood and autosplenectomy in later life (asplenia). ***MCQ***

vii. Retinopathy.

viii. Osteomylitis → infection of bones lead to deformation → hand foot syndrome

ix. Hand

Note: normally, in non-sickled patients, Osteomyelitis is caused by Staph. aureus

❶ **Vasoocclusive:**

- Painful.
- Precipitated by infection, acidosis, dehydration, pyrexia, deoxygenation, exposure to cold, pregnancy.
- Organ infarcts e.g. bone (femoral head necrosis), lungs, spleen, liver, brain (stroke).
- Painful dactylitis – small infarcts of the small bones of the hands and feet.

❷ **Visceral Crises (Sequestration):**

- Pooling of blood.
- Sickling inside the organs e.g. spleen, liver and lungs (acute chest syndrome). ***MCQ***

❸ **Aplastic Crises:**

- Due to parvovirus infection or folate deficiency.
- Sudden fall in Hb, associated with **decreased** reticulocyte count.

❹ **Hemolytic Crises:**

- Increased rate of hemolysis.
- Decreased Hb with **increased** reticulocyte count.

Factors predisposing crises:

1. Infection
2. Pyrexia
3. Exposure to cold
4. Dehydration
5. Pregnancy

Laboratory findings:

- Hb: Usually 6 -9 g /l.
- Peripheral blood smear: Sickle cells, target cells.
- Sickling test: Positive (test of solubility of Hb when deoxygenated). ***MCQ***
- Hb electrophoresis: The predominant Hb is HbS. HbF (5 – 15%). Higher values of HbF associated with milder disease. ***MCQ***

❖ *SICKLE CELL TRAIT:*

- No anemia.
- Normal RBC morphology on peripheral blood smear.
- Very minimal infarction e.g. kidney → hematuria. ***MCQ***
- HbS level = 25 – 45%. (if its more than 50% its HbS severe SS)
- Sickling increase when exposed to low O2 e.g. anesthesia, pregnancy, high altitude.

Blood Groups

Blood group	Antigen(s) present on the red blood cells	Antibodies present in the serum	Genotype(s)
A	A antigen	Anti-B	AA or AO
B	B antigen	Anti-A	BB or BO
AB	A antigen and B antigen	None	AB
O	None	Anti-A and Anti-B	OO

ABO genotype in the offspring		ABO alleles inherited from the mother		
		A	B	O
ABO alleles inherited from the father	A	A	AB	A
	B	AB	B	B
	O	A	B	O

❖ Red cell antigens:

- The ABO and Rh groups are the most important blood grouping in the clinical side.
- AB group can receive blood from any other group
- O ˉ can give any group
- In RH Grouping D→ R + (meaning Dominant) d→ r – (Recessive)

❖ Blood group antibodies:

- Naturally occurring, most important: Anti A (found in blood group B) and Anti B (Found in blood group A)
- Blood group AB has no antibodies (Anti A & B) → can receive blood from any other group
- Blood group O has both antibodies (Anti A & B) → can't receive blood from any other blood groups except from O.

➲ Antibodies usually are IgM.

BLOOD TRANSFUSION

❖ Preparations to store blood:

- ACD → 21 day
- CPDA → 28 days
- CPDA-1 → 35 days
- CPDA-2 → 42 days

All are Anticoagulation
store at 1-6 °

- 42 days the max time for blood to stay in the blood bank

- Platelets → 5 days store at room temperature (20-24 °)
- Platelets will loss there activity and reduce in number if they are put in a freezer

❖ Lab tests before blood Transfused:

1. Blood type
2. Screening for antibodies that may produce adverse effects if transfused.
3. Screening for possible infectious agents that could be transmitted with transfusion.

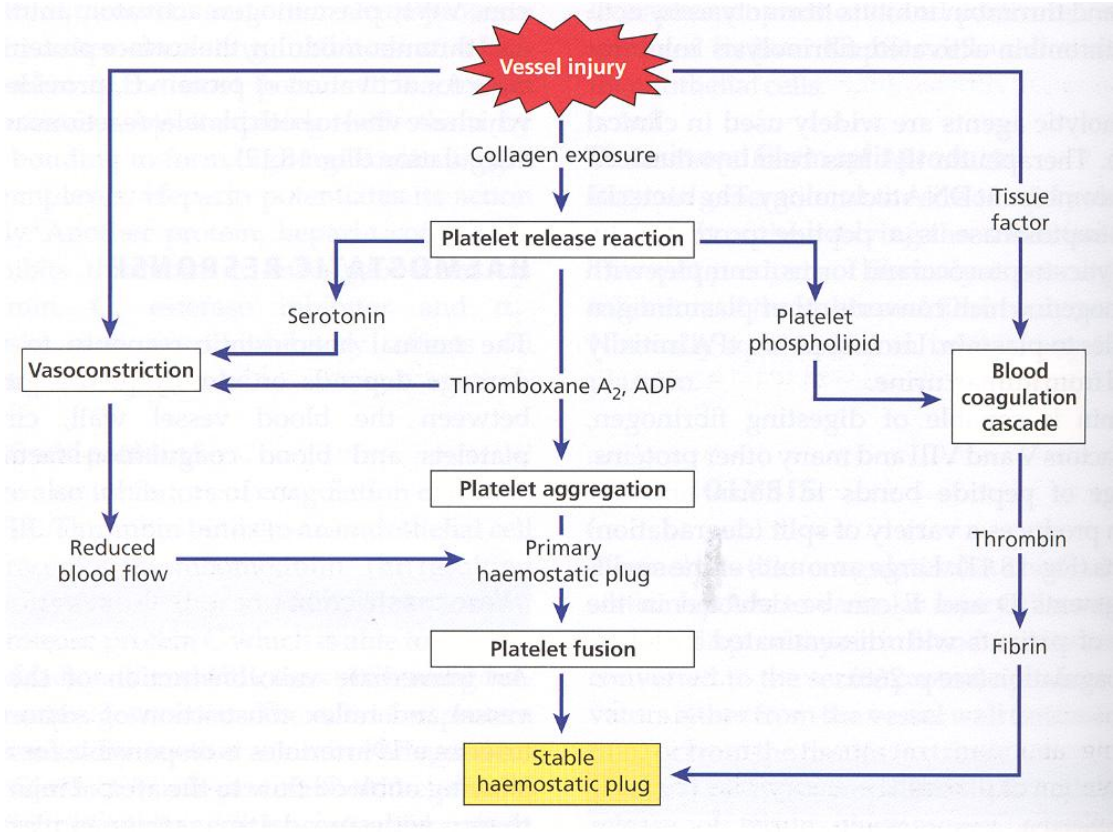
❖ Blood components:

*** Iron chelation is a therapy used for the prevention of iron overload.**

- RBC: iron chelation* is needed with regular transfusion.
- Fresh Frozen Plasma: Replacement of coagulation factors e.g. liver disease. [stored at -30°)
- Albumin: Used in hypovolemic shock as a volume expander and in hypoalbuminemia.
- Cryoprecipitate: is factor VIII + fibrinogen. Used in treatment of hemophilia
- & vW disease (However, factor VIII concentrate is now used to treat hemophilia A and vW disease).

Immediate transfusion reactions	Delayed reactions
Hemolytic reaction against ABO –RH antigens.	Heamolytic deases of nonates
Allergic reaction against protein.	Graft Versus Host Disease (GVHD)
Fibrile reaction against platelets.	Transfusion-associated graft versus host disease (TAGVHD)
Transfusion related acute lung injury (TRALI)	Post-transfusion purpura
Infectious reactions (bacterial or viral contamination)	Iron overload.
Circulatory overload.	Hemolytic disease of the newborn (HDN)
Citrate toxicity.	
Air embolism.	
Alloimmunization	

PLATELETS PHYSIOLOGY



Production:

Hematopoietic stem cell → Megakaryoblasts → Megakaryocyte $\xrightarrow[\text{cytoplasm}]{\text{fragmentation of}}$ Platelets.

Megakaryocyte : is the largest cells in the bone morrow

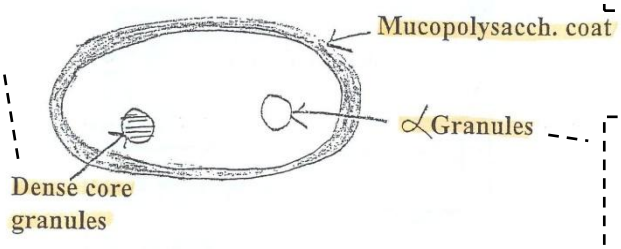
Circulation:

- Normal count is **150-400x10^9/L**.
- Normal life span 7 – 10 days.
- About $\frac{1}{3}$ are trapped in the spleen.

Structure:

Glycoprotein content which are important for interaction of platelets with each other or aggregating agents.

Dense granules: secrete:"Nucleotides (**ADP**), **Ca^{+2}, serotonin**"



Alpha (α) granules: secrete **Fibronigen**, Factor V, **vWF**, Fibronectin,β-thromboglobin, heparin antagonist (PF4), thrombospondin

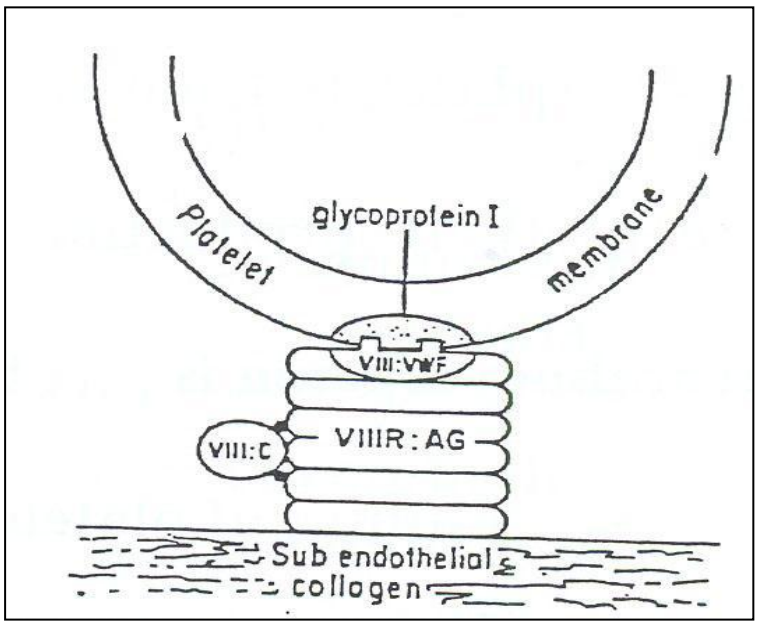
- Young platelets are larger than old ones.

Function:

Formation of mechanical plug during normal homeostatic response to vascular injury. The main steps involved are: adhesion, release, aggregation.

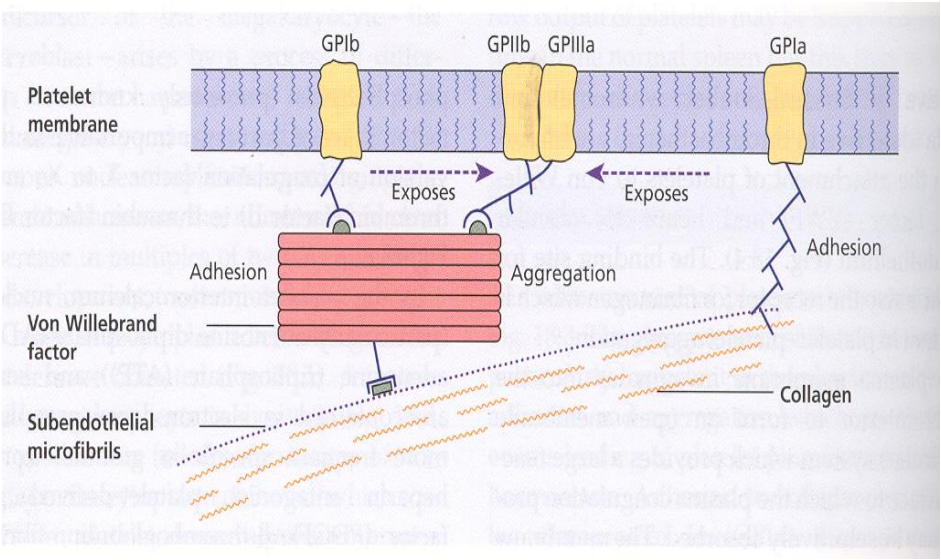
❶Platelets adhesion:

- Adhesion of platelet to sub-endothelial collagen.
- Dependent on the vW factor (Von Willebrand part of factor VIII).
- Also dependent on glycoproteins. ***MCQ***



Note: Glycoproteins

- Glycoproteins **Ib**, **IIb**, and **IIIa** need vW factor to connect to collagen; however, **Ia** glycoprotein does not because it has a direct connection with collagen.
- **IIb** and **IIIa** join platelet to each other and fibrinogen.
- Deficiency of **IIb, IIIa** is the most common and lead to *Glanzman disease "congenital"*.
- Deficiency in **Ib** lead to *Bernard Soluier's syndrome "congenital"*



❷Release (secretion):

Collagen exposure results in the release of granules contents (ADP, serotonin, and fibrinogen). Collagen and thrombin activate prostaglandin synthesis.

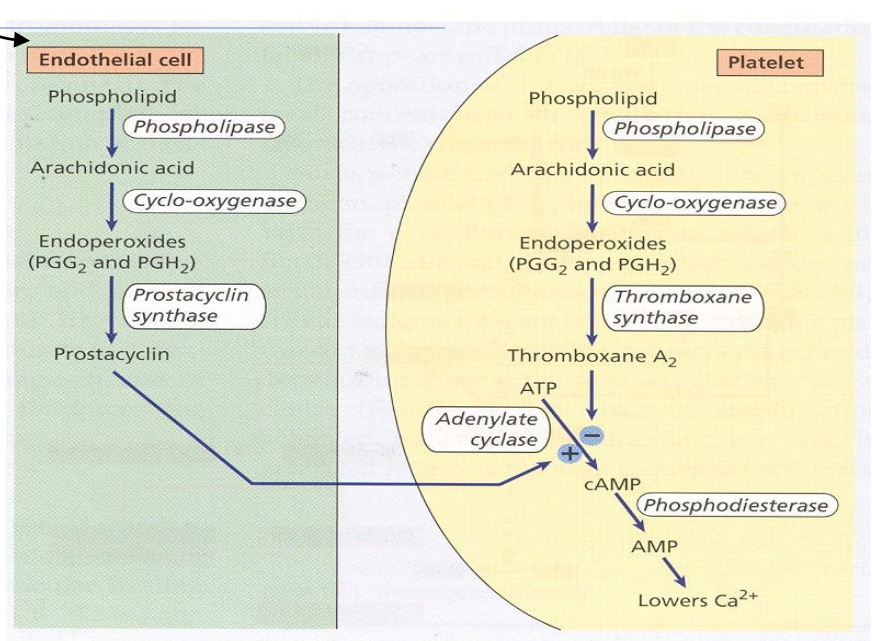
membrane phospholipids → arachidonic acid ---(cyclo-oxygenase)--→ ---(thrombpxane synthase)--→ thromboxane A2

Thromboxane A2: Potentiates aggregation and vasoconstrictor.

❸Aggregation:

Release ADP + thromboxane A2 → aggregation. → more secretion → secondary aggregation → formation of platelet mass or plug.

Mechanism that prevent platelet aggregation without injury [through endothelial]



❖ PLATELETS DISORDERS:

Platelet disorders are the most common cause of bleeding. The disorder could be decrease in number (thrombocytopenia) or defect in function.

A-1- DECREASED PLATELETS NUMBER OR THROMBOCYTOPENIA:

Loss of platelets from the circulation faster than the rate of their production by the bone marrow.

So Thrombocytopenia is due to:

a) Failure of platelets production, most common cause. Megakaryocytes are decreased in the bone marrow e.g. drugs.
b) Increase rate of removal of platelets from the circulation.

Megakaryocytes are increased or normal in the bone marrow i.e. production is normal but platelets are destroyed e.g. by antibodies.

c) Combination of A&B.

Causes:

Congenital	Acquired
◎ Megakaryocytic hypoplasia.	⌘ Immuno thrombocytopenia
◎ TAR Syndrome.	⌘ Thrombotic thrombocytopenic purpra
◎ Wiscott Aldrich syndrome	⌘ DIC
	⌘ Drugs
	⌘ Infections
	⌘ Splenomegaly.
	⌘ Bone marrow suppression or infiltration
	⌘ A plastic anemia

⌘ Bone Marrow Suppression:

Due to:

- effect of infections (viral) or toxins
- replacement e.g. by malignancy [leukemias or metastatic tumors]
- fibrosis of the bone marrow e.g. due to irradiation

⌘ Drugs:

- Due to suppression e.g. phenylbutazone, Gold, Thiazide.
- Other mechanisms of action are immune, or by causing direct aggregation of platelets.
- May be accompanied by other sings e.g. fever, joint pain, rash, leucopenia.

⌘ Aplastic Anemia :

means shut down of the bone marrow

⌘ Splenomegaly:

- Normally 1/3 of body platelets are in the spleen and 2/3 in the peripheral circulation.
- With spleen enlargement, up to 80 - 90% of body platelets will pool in the spleen → decreased platelets in the peripheral circulation.
- This spleen enlargement could be due to many causes e.g. portal hypertension,thalassemia, gaucher's disease ,malaria, kala-azar, lymphomas, etc.
- Life span of the platelet is normal.

NOTE : one of the treatments of thrombocytopenia is by removing the spleen. But in some cases it can lead to thrombocytosis

⌘ Infection:

- Decreased platelets can be seen with many infections, e.g. intra–uterine infections: best examples are congenital **syphilis**, toxoplasmosis, rubella, herpes cytomegalovirus (CMV), Also seen with other infections e.g. influenza, chickenpox, rubella, infectious mononucleosis.
- The infection's effect is through suppression of bone marrow, immune mediated or due to DIC in fulminant infections.

Manifestations:

☠Usually bleeding not significant until count is < 80,000.
☠Presents as skin purpura, ecchymoses, mucosal membrane bleeding, and prolonged bleeding after trauma.
☠The most common site of bleeding is in the retina
☠If the Thrombocytopenia is severe, intracranial hemorrhage can be seen.

Investigations:

In addition to history and examination, the following tests are required:

- ✓ Platelet count
- ✓ Blood smear
- ✓ Bleeding time: To measure Platelet plug formation in vivo. Normal is 2.5-10 min.
- ✓ Bone marrow examination.
- ✓ Aggregation studies: Detect aggregation using aggregation agents ADP, adrenaline, collagen, ristocetin.

Note:

- Musculoskeletal bleeding is a manifestation in coagulation disorders.
- Mucocutanous bleeding is a manifestation of platlet disorders.
- When both are present >> vWF deficiency or afibrogenemia.

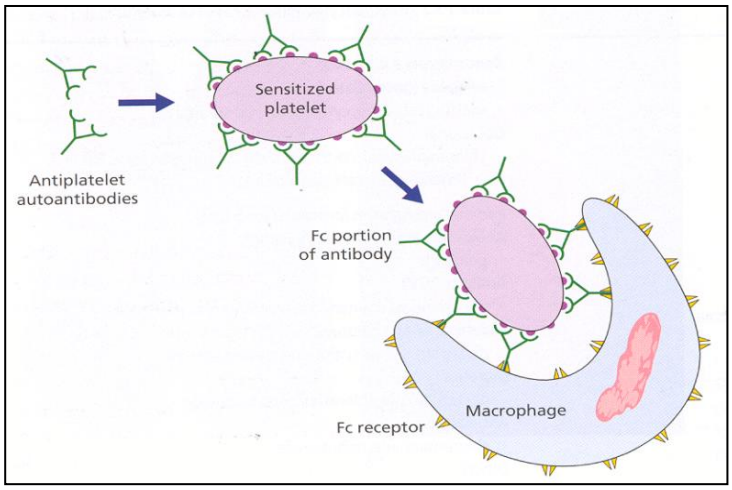
A-2-IMMUNOTHROMBOCYTOPENA (ITP): MCQ

It's an autoimmune disorder characterized by **platelets bound antibodies.**

Pathogenesis:

❶ Autoimmune. Antibodies are formed against antigens on platelet surface.

❷ Premature removal of platelets from the circulation by macrophages of the R-E system and destroyed mainly in the spleen.



Etiology:

Idiopathic.

Classification:

- **Acute:** usually in children, self limiting, and preceeded by infection usually viral.
- **Chronic:** usually in adults, more common in female.

A- ACUTE IMMUNOTHROMBOCYTOPENIA:

- Self limiting usually weeks.
- In children.
- Usually preceeded by viral infection.
- Bone marrow shows normal or increased megakaryocytes.
- Due to immune complexes bound to platelets.

(Complex = viral antigen –antibody complex). These complexes are removed by the reticulo-endothelial system (RE system).

- 5-10% can go into chronic ITP.
- 90% recover.

B- CHRONIC IMMUNOTHROMBOCYTOPENIA:

Clinical:

- Usually adults, young female 15 -50 yrs.
- Insidious onset.
- Chronic: last months or years.
- No precipitating (cyclical) course with periods in which platelets number return to normal.

Manifestations:

- Skin purpura, superficial bruising, epistaxis, and menorrhagia.
- Mucosal hemorrhage is seen in severe cases and intra – cranial hemorrhage is rare.
- Splenomegaly: 10% of cases

Laboratory findings:

- Thrombocytopenia with giant forms. Count usually 10 -50,000.
- Bone marrow shows normal or increased megakaryocytes.
- Platelet bound IgG is +ve.
- Platelet survival studies show decreased life span.

Clinical features of immune thrombocytopenia

Degree of Thrombocytopenia	Symptoms	Physical findings
Mild (>50 000/mm^3)	None	None
Moderate (30-50 000/mm^3)	Bruising with minor trauma	Scattered ecchymoses at trauma site
Severe (10-30 000/mm^3)	Spontaneous bruising. menorrhagia	Petechiae and purpura, more prominent on extremities
Marked (<10 000mm^3)	spontaneous brusing, mucosal bleeding, risk for CNS bleeding	Generalized purpura, epistaxis, GU bleeding CNS symptoms

B-DEFECTIVE PLATELET FUNCTION:

A defect in function is suspected if there is **prolonged bleeding time with or without skin or mucosal hemorrhage** in the presence of **normal platelet count**.

Disorders:

Congenital	Acquired
◎ Storage granules defect	⌘ Drugs
◎ Bernard Soluier's	⌘ Uremia
◎ Glanzman disease	⌘ Myeloproliferative disorders
◎ Storage granules defect	⌘ Multiple Myeloma

◎ GLANZMAN'S DISEASE (THROMBASTHENIA):

- Autosomal recessive inheritance.
- Normal platelets count and appearance.
- No clumps are seen on peripheral blood film (i.e. no platelets clumps).
- Due to decreased surface membrane glycolproteins **IIb** +**IIIa** → failure of primary aggregation. ***MCQ***
- Platelets do not aggregate with all aggregation agents but they aggregate with ristocetin. ***MCQ***
- Bleeding time is prolonged.

MCQ: in prolonged bleeding time, the first test we perform is platelet aggregating test

◎Bernard Soluier's syndrome:

- Due to deficiency of surface membrane glycolprotein **Ib** → failure of adherence.
- Platelets do not aggregate with all aggregation agents including ristocetin.

⌘ ACQUIRED DISORDERS OF PLATELET FUNCTION:

Causes:

- Drugs e.g. aspirin
- Myeloproliferative disorder.
- Paraproteinemias e.g. multiple myeloma.
- Cardiopulmonary bypass.
- Autoimmune diseases e.g. SLE
- Uremia (renal failure)

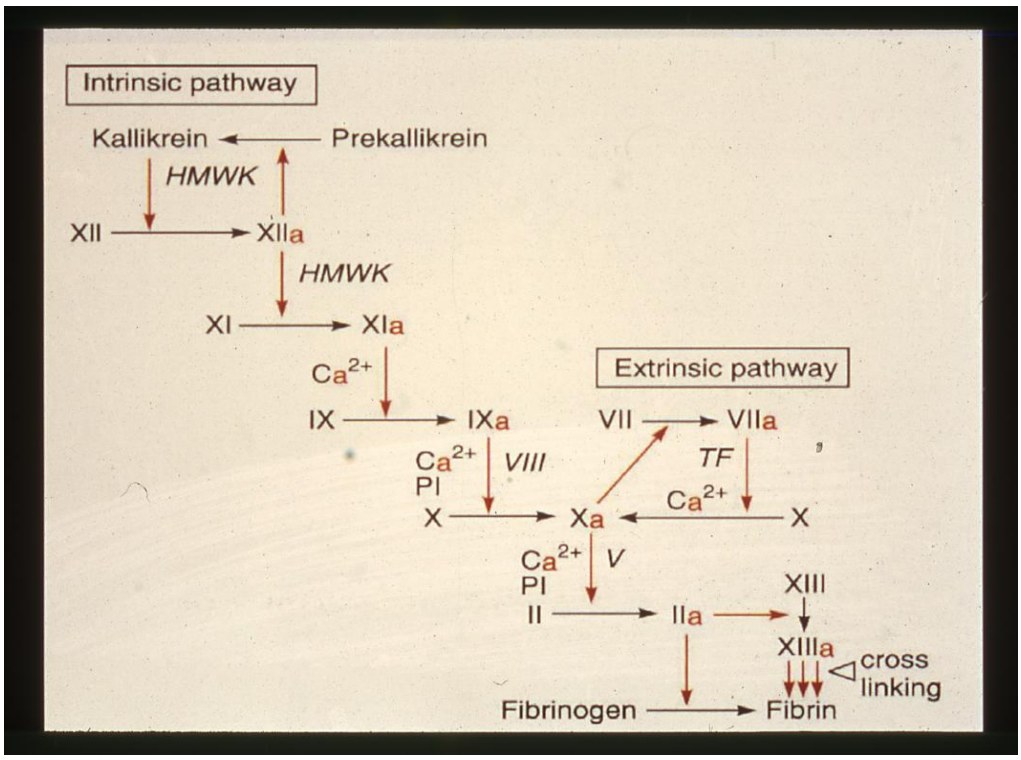
✪ Asprin is the best example which is the most common cause of acquired platelet function disorder.

✪ It irreversibly affects the cyclooxygenase enzyme. The effect last 4 -7 days and it takes about 10 days before the platelets are replaced.

✪ Presents as increased bleeding time but purpura is unusual.

COAGULATION

Haemostasis Plasma Coagulation Factor					
F I	Fibrinogen	**F IX**	Christmas Factor	**AT III**	**Anti thrombin III**
F II	Prothrombin	**F X**	Stuart-Power Factor		
F III	Tissue Factor	**F XI**	Plasma Thromboblastin – antecedent		
F IV	Calcium Ions	**F XII**	Hageman factor	TM	**Thrombomodulin**
F V	Proaccelerin	**F XIII**	Fibrin Stabilising Factor	TFPI	**Tissue Factor Pathway inhibitor**
F VII	Proconvertin	PC	Protein C		
F VIII	Antihaemophilic	PS	Protein S		



- The coagulation factors have a specific sequence leading to activation of Thrombin {Factor II-A} , This thrombin in-turn activates fibrin, from fibrinogen.
- Factor XIII converts soluble fibrin into insoluble fibrin through cross

Tests for coagulation:

Extrinsic Factors	*Common Factors*	*Intrinsic Factors*
VII	**X**, **V**, **II**(Prothrombin), I(Fibrinogen)	**VIII, IX, XI, XII**

1. Prothrombin time (PT):

- Tests the extrinsic & common pathways = Factors (**VII**) & (**X**, **V**, Prothrombin & fibrinogen).
- Normal = 10 - 14 sec.
- Test:

  Plasma + calcium + brain extract (thromboplastin).

Note:

- **This test should proceed any surgery**
- **Any disorder in any factor(common and extrinsic) cause prolonged PT.**

Activated Partial Thromboplastin time (APTT) or (PTT):

- Tests intrinsic pathways (**VIII, IX, XI, XII**,) & common pathway (**X, V**, prothrombin, and fibrinogen).
- Normal = 30 - 40 sec.
- Test:

  Plasma + calcium + phosholipid + kaolin (activates contact factors)

Note:

- **Any disorder in any of these factors (Common & intrinsic) cause prolonged APTT.**
- **Kaolin is used to hasten the test. (to decrease the duration of testing**)

3. Thrombin time:

- Test for fibrinogen
- Normal =10 - 12 sec.

4. Specific factor assay:

- **Function Assay:**
  - ✓ Based on PTT or PT

5. Immunological Assay:

Available for some coagulation factors.

Note: Test 4 and 5 are not routinely made but are specific.

Hereditary coagulation disorders:

Hereditary deficiencies of coagulation factors are rare. However, the following disorders are more common:

- **Hemophilia A** (Factor **VIII** deficiency)
- **Hemophilia B** (Christmas disease) (Factor **IX** deficiency)
- **von Willebrand's disease**.

A- Hemophilia-A

This is the most common hereditary disorder of blood coagulation.

Inheritance:

X – Linked

Defect:

The absence or low level of factor **VIII** (clotting activity [**VIII**: C]) *MCQ*

Table 20.1 Correlation of coagulation factor activity and disease severity in haemophilia A or haemophilia B

Coagulation factor activity (percentage of normal)	Clinical manifestations
<1	Severe disease Frequent spontaneous bleeding episodes from early life Joint deformity and crippling if not adequately treated
1–5	Moderate disease Post-traumatic bleeding Occasional spontaneous episodes
5–20	Mild disease Post-traumatic bleeding

"I diet all year, and —BOOM!—one hemophiliac and it's back to square one!"

Clinical features:

The clinical severity of the disease correlates well with the extent of factor deficiency.

- **Severe (Factor level= <1% of normal):**

- Profuse post – circumcision bleeding.
- Recurrent painful hemarthroses (I.e., bleeding into joints most commonly **knees**) with progressive joint deformity & crippling.
- Muscle hematomas.
- Prolonged bleeding after dental extraction.
- Hematuria
- Spontaneous intra-cerebral hemorrhage, **uncommon.**

- **Moderate (Level of factor = <1 - 5% of normal):**

- Most post – traumatic bleeding.
- Occasional spontaneous bleeding.

- **Mild (Level of factor = < 5 - 20% of normal):**

- Post – traumatic bleeding.
- Spontaneous bleeding is rare.

Laboratory features: ***MCQ***

Abnormal	**Normal**
- APTT : Prolonged	PT: normal
- Factor VIII - C: Decreased	Bleeding time: normal

Treatment:

❖ **Principles of treatment:**

- **For spontaneous bleeding:**

bleeding is controlled if the level of factor VIII is raised to above 20%.

- **For major surgery or serious post – traumatic bleeding**:

the level should be raised to **100%.**

❖ **Complication of treatment:**

- Disease transmission:
  - HIV infection
  - Hepatitis
- Development of antibodies (inhibitors to factor **VIII**):
  - Seen in 5-10% of patients
  - The antibodies make the patients resistant to treatment & make him require larger doses.

Haemophilia B (Christmas disease)

The inheritance and clinical features of factor **IX** deficiency are identical to those of hemophilia A.

Lab findings :

It's identical to Hemophilia A except factor IX deficiency instead of VIII

Treatment:

Give factor **IX**

VON WILLEBRAND'S DISEASE MCQ

Inheritance:

Autosomal **DOMINANT**.

Defect:

❶ Reduced level of factor vWF (von Will brand's factor). This result in rapid loss of factor VIII: C and abnormal coagulation.
❷ Defective platelet – related activity of (Von Will brand's factor). This affect platelet's adherence to sub-endothelial collagen & platelet aggregation induced by ristocetin. Thus vW disease has two manifestations:

- low **VIII** C level
- low vW factor

Classification:

Type 1: partial quantitative deficiency

Type 2: qulititative deficiency

Type 3: complete quantitative deficiency

Clinical features:

- Operative & post – traumatic bleeding.
- Mucous membrane bleeding e.g., epistaxes, menorrhagia. ***MCQ***
- Hemarthroses & muscle hematomas are rare in contrast to hemophilia A.

Laboratory Features:

- **Abnormal:**
  Bleeding time: prolonged
  PTT: prolonged

  Normal:
  PT

- Factor Assay: low levels:
  - ✓ VWF
  - ✓ VIII C

  Unlike Glansman's disease !

  - Platelet aggregation: defective with ristocetin. Normal with other reagents.

Note:

Ristocetin (+) ----------> Glansman's Disease

Ristocetin (-) ----------> Von Willebrand's

Treatment:

- Cryoprecipitate
- Factor VIII concentrate
- Desmopressin

imp.

Table 20.2 Main clinical and laboratory findings in haemophilia A, factor IX deficiency (haemophilia B, Christmas disease) and von Willebrand's disease

	Haemophilia A	Factor IX deficiency	Von Willebrand's disease
Inheritance	Sex-linked	Sex-linked	Dominant (incomplete)
Main sites of haemorrhage	Muscle, joints, post-trauma or postoperative	Muscle, joints, post-trauma or postoperative	Mucous membranes, skin cuts, post-trauma or postoperative
Platelet count	Normal	Normal	Normal
Bleeding time ✓	Normal	Normal	Prolonged
Prothrombin time ✓	Normal	Normal	Normal
Partial thromboplastin time ✓	Prolonged	Prolonged	Prolonged or normal
Factor VIII ✓	Low	Normal	May be moderately reduced
Factor IX ✓	Normal	Low	Normal
VWF ✓	Normal	Normal	Low
Ristocetin-induced platelet aggregation ✓	Normal	Normal	Impaired

VWF, von Willebrand factor.

DISSEMINATED INTRAVASCULAR COAGULATION (DIC):

Definition: Widespread intravascular deposition of fibrin with consumption of coagulation factors and platelets. This occurs as a consequence of many disorders which release pro-coagulant material into the circulation or cause widespread endothelial damage or platelet aggregation.

- It is **acquired** (more common than hereditary)
- It consumes all **clotting factors** including **platelets** and coagulation factors

Forms of DIC:

- Chronic, less server course.
- Fulminate hemorrhagic course.

Pathology:

- Deposition of fibrin in the microcirculation.
- Formation of large amounts of fibrin monomers.
- Increased fibrinolysis with release of fibrin split products (FSPs) or fibrin degradation products (FDPs).
- These FDPs interfere with fibrin polymerization, thus causing coagulation defect. ***MCQ***
- Depletion of fibrinogen and other factors.
- Increase consumption of platelets.

Causes:

- Due to release of pro-coagulant material: e.g. in amniotic fluid embolism, premature separation of placenta, mucin secreting adenocarcinoma, M3 AML, falciparum malaria, hemolytic transfusion reaction, and snake bites.
- Widespread endothelial damage: e.g. gram negative septicemia, meningococcal septicemia.
- Widespread intravascular platelet aggregation: e.g. some bacterial or viral infections.
- Hypothermia, Heat Stroke (Hajj) , Acute hypoxia

Laboratory Features:

- Platelet count: Decreased
- Fibrinogen level: Decreased
- FDPs: Increased
- PT: Prolonged
- PTT: Prolonged
- (Thrombin time) TT: Prolonged
- Blood film: Features of microangiopathic hemolytic anemia: there is fragmentation of RBCs when they passed through fibrous strands in small vessels.

Factor XIII Defiency:

- Rare Autosomal Recessive Disorder
- Sever secondary haemorrhagic tendency and poor wound healing
- PT, aPTT, and fibrinogen level are normal
- BT and Platelet function studies are normal
- Clot solubility test is abnormal
- Deficiency of F XIII leads to fibrin being left soluble and makes fibrinolysis easier in the vessels ------> Recurrent (**secondary**) bleeding in patients with injury

Hemostasis tests typical results in acquired bleeding disorders

	Platelet count	Prothrombin time	Activated partial thromboplastin time	Thrombin time
Liver disease	Low	Prolonged	Prolonged	Normal (rarely prolonged)
Disseminated intravascular coagulation	Low	Prolonged	Prolonged	Grossly prolonged
Massive transfusion	Low	Prolonged	Prolonged	Normal
Oral anticoagulants	Normal	Grossly prolonged	Prolonged	Normal
Heparin	Normal (rarely low)	Mildly prolonged	Prolonged	Prolonged
Circulating anticoagulant	Normal	Normal or prolonged	Prolonged	normal

Note:

- **The last factor to aggregate in the blood vessel is Fibrinogen (Factor I)**
- **M3 type of Acute Myelocytic Leukemia (AML) which has granulations and aeur bach rods, will burst causing a combination of DIC and Leukemia {highly dangerous}**
- **Snake venom works as an active coagulation factor.**

MYELOPROLIFERATIVE DISORDERS

Group of closely related disorders:

1. **Polycythemia Vera**
2. **Chronic Idiopathic Myelofibrosis**
3. **Essential Thrombocythaemia**

} Non-Leukemia

4. Chronic Granulocytic Leukemia
5. Chronic Myelogenous Leukaemia "Most Important"
6. Chronic Neutrophilic Leukaemia
7. Chronic Eosinophilic Leukaemia
8. Chronic Myeloproliferative Disease, Unclassifiable

POLYCYTHEMIA

- *Definition:* Polycythemia indicates increased red cell mass in the relation to plasma volume.
- Increased packed cell volume of RBCs (PCV) or (Hematocrit).
  PCV Male > 55%

  Female > 47%
- Increased Hb. & RBC count

Causes:

- **Relative:** the red cell mass is normal. But the plasma volume is decreased e.g. due to dehydration**.**
- **Absolute:** increased red cell mass (total red cell volume).

☒ **Primary**
  - Congenital → Truncation of the Erythropoietin "EPO" receptor
  - Acquired → Polycythaemia Vera

☒ **Secondary**
  - Congenital → e.g., high oxygen affinity Hb, autonomous high EPO production
  - Acquired → e.g., hypoxemia, renal disease

A-Polycythemia Vera (primary):

Clinical features:

- More common in elderly males
- Headache, Lethargy, Dyspnea
- Weight Loss, Night Sweats.

Presentation: "viscosity-stasis"

- Vascular:
  - Intermittent claudication
  - Cerebral ischemia
  - Cardiac ischemia
- Hemorrhage:
  - Gastrointestinal
  - Cerebral hemorrhage
- Plethora "احمرار":
  - Red pace
  - Conjunctival congestion
- Pruritis:
  - Increase with warm environment e.g. hot bath.
  - Iron deficiency anemia: Due to bleeding.
- Peptic Ulcer →"5-10% of patients and can lead to iron deficiency"
- Splenomegaly→"2/3 of patients"
- Gout
- Hypertension→"1/3 of patients"
- Thrombosis

Laboratory Features:

1. Blood parameters:

a. hemoglobin:

Male > 17.5g

Female > 15. 5g

b. packed cell volume (PCV):

(Hematocrit) Male > 55%

Female > 47%

c. RBC Count:

Male > 6 × 10 /1

Female > 5.5 × 10 /1

d. WBC Count: increased

e. Platelets: increase and defected in function.

2. Total red cell mass: increase

Female > 36 ml / kg

Male > 32ml / kg

Note 3 : if Oxygen saturation is ↓ then it is due to secondary erythrocytosis

3. Arterial O_2 saturation: Normal.

This is important to differentiate P.R.V from secondary polycythemia due to hypoxia.

4. Neutrophil Alkaline Phosphatase (NAP score): increased

Note 4 : NAP is ↓ in CML and Proxysmal nocturnal hemoglobinuria.

5. Serum Uric Acid: increase

6. Erythropoietin(EPO) hormone Level: Normal or decreased.

This is important to differentiate from polycythemia secondary to inappropriate erythropoietin e.g. in tumors.

Note 6 : If Erythropoietin is increased then it is indicative of secondary Polycythemia.

7. Blood Viscosity: increase

8. Bone Marrow

- Hyper cellular with increased erythropoiesis.
- Absent iron stores.

9. Serum B_{12} level: increased

10. JAK mutation is detected by genetic studies

Complications:

1. Thrombosis and hemorrhage.
2. Myelofibrosis :30% of cases
3. Acute leukemia, usually AML type: 15% of cases.

Treatment

- Venesecton
- Radioactive Phosphorus (P32)
- Chemotherapy: e.g. Hydroxyurea

It is usually discovered accidentally

B-Secondary Polycythemia:

Due to:

1. **Idiopathic:**

   Not increase in erythropoietin, but not enough features to be P.R.V.

2. **Increased erythropoietin:**
   a. **Compensatory:** Due to hypoxia, lung disease, cardiac disease, high altitudes, smoking, or high affinity hemoglobin.
   b. **Inappropriate:** In tumors e.g. hepatocellular carcinoma, cerebelar hemangioma, massive uterine fibroids.
   c. **Renal Disease:** Renal artery stenosis, renal carcinoma, renal cysts.

Acute Leukemia

A malignant disease of the bone marrow , characterized by increased immature haemopoietic cells which are known as **blast cells.**

Classification of leukemia :

- Clinically :
  - **Acute** : rapid progression of the disease . May lead to death in weeks if left untreated.
  - **Chronic** : slow progression of the disease. Typically it takes months or years.

- Histopathologically:
  - **Lymphoid :** the cancerous change takes place in a type of marrow cell that normally goes on to form lymphocytes
  - **Myelogenic :** the cancerous change takes place in a type of marrow cell that normally goes on to form red blood cells, some other types of white cells, and platelets.

Note :

Normally , less than 5% of bone marrow is blasts.

<20% indicates pre-acute leukemia

>20% indicates acute leukemia

Acute leukemia	Chronic leukemia
Rapid proression	Slower progression
>20 % blasts	>5% blasts
Immature cells	Mainly , Mature cells

Etiology:

Factors associated with development of acute leukemia

1- Environment:
   e.g. Ionizing raditation or smoking
   There is increased incidence of leukemia in survivors of the atomic bombs in japan.
   Also , there is higher incidence in patients treated with radiation.
2- Chemicals :
   Best example is **Alkylating agents** which are used to treat malignant neoplasms.
3- Congenital:
   Down's syndrome
4- Marrow failure syndrome
   Bloom's syndrome

Clinical features :

Decreased RBCs + WBCs + platlets are called pancytopenia

- Bone marrow failure :
  Pancytopenia:
  - ❖ Anemia : pallor , lethargy , and dyspnea
  - ❖ Neutropenia: infection ; fever ,mouth ulcers , pharyngeal ulcers , skin infection .
  - ❖ Thrombocytopenia: Bleeding , usually purpura.

- Tissue involvement:
  - ❖ Liver and spleen :
    - ✓ Hepatomegaly
    - ✓ splenomegaly
  - ❖ Bone :
    - ✓ Bone pain
    - ✓ Lytic lesions on X-ray.
    - ✓ Usually in ALL
  - ❖ Lymph nodes:
    - ✓ Lymphadenopathy – enlargement of lymph nodes -
  - ❖ Meninges:
    - ✓ Usually in ALL
  - ❖ Testicular
    - ✓ Usually in ALL
  - ❖ Skin and gum:
    - ✓ Usually in AML

Laboratory findings :

- Peripheral blood :
  - ❖ WBC could be increased or decreased or normal
  - ❖ Anemia
  - ❖ Decreased platelets
- Bone marrow:
  - ✓ Increased blasts >20% of cells " thickening of the bone marrow"

Classification of acute leukemia :

- Acute lymphoblastic leukemia (ALL)
- Acute myeloblastic – Non lymphoblastic - leukemia (AML)

Acute lymphoblastic leukemia:

- Mostly , seen in children
- Tissue involvement is seen in bones, meninges , and testis. **IMP!**
- Increased risk of relapse .
- Cytochemical stains of blasts :
  - ✓ Perioxidase (sudanblack) : negative
  - ✓ PAS: positive
- Immunological test:
  - ✓ Terminal deoxynucleotide transferase (tdt) : positive.

Site of relapse:

- ☒ Bone marrow
- ☒ Meninges
- ☒ Testis

Classification :

- Morphologic (FAB – French American British)
- Immunologic

Morphologic (FAB) :

- ✓ Three stages based on blasts morphology : L1 , L2 , and L3 .

	L1	L2	L3
Frequency in children	80%	20%	
In adults	50%	50%	3% of all
Morphology	× Homogenous × Small blasts × Little cytoplasm × Nucleoli: not prominent	× Hetergenous × Large blasts × More cytoplasm	× Vaculated cytoplasm × **Burkitt -cell type (v.imp)**

Immunological classification :

1- **B ALL**
   - × Rare , only 3% of all cases
   - × Poor prognosis
   - × Associated with L3 morphology (Burkitt-cell type)
   - × Subtypes :
     - ☒ Early pre-B ALL (pre pre-B ALL):
       - CD19 : positive
       - CD 10: negative
       - Mu immunoglobin is not present in the cytoplasm (negative)

- ☒ Pre-B ALL
  - CD19: positive
  - CD10 : positive
  - Mu immunoglobim is present in the cytoplasm of >20% of cells

2- **T ALL**
- 10-20% of the cases
- Better prognosis than B ALL
- CD7 : positive
- CD1a: positive
- CD3: positive
- Associated with:
  - Older age group.
  - More commen in boys.
  - Mediastinal mass due to thymus enlargement.
  - High incidence of CNS relapse and high WBC presentation.

3- **Common ALL (CALL)**
- Most common type (65%) of the cases
- CD19:positive
- CD10:positive
- Associated with CALL antigen (CALLA) IMP !
- Associated with good prognosis

Acute Myeloid Leukaemia

Note: Genetics

- In Acute myeloid leukemia there's (8, 21) translocation
- While in chronic myelogenous leukemia (9, 22) translocation [Philadelphia chromosome]

- A clonal malignant disease characterised by:
  - ✓ Proliferation of abnormal blasts in the BM.
  - ✓ Impaired production of normal blood cells.
- Mainly seen in adults.
- Slightly more common in males than females.
- Primary AML : occurs de novo and have a better prognosis
- Secondary AML : can develop from myeloproliferative diseases or follow previous treatment have worse prognosis.
- Tissue involvement : gum hypertrophy and infiltration (MCQ) , skin involvement , and CNS disease .
- Disseminated intravascular coagulation (DIC) is a characteristic M3. **V.IMP !**
- Auer rods (which is a needle like structure of accumulated granules) are a diagnostic feature in acute myeloid leukemia. (MCQ)
- Cytochemical stains of blasts :
  - ✓ Perioxidase (sudanblack) : positive
  - ✓ PAS: negative

Morphologic classification (FAB) :

M1: Myeloblastic without maturation

M2:myeloblastic with maturation

M3: Hypergranular promyelocytic (very high auer rods) **IMP!**

M4:myelomonocytic

M5: Monocytic **IMP !**

- ✓ M5a : poorly differentiated
- ✓ M5b : well differentiated

M6: erythroleukemia

M7: megakaryoblastic (increased megakaryocyte) **IMP !**

<u>How to differentiate between ALL & AML :</u>

- Morphology
- Cytochemistry
- Immunological markers

Morphologically:

	AML	ALL
Cytoplasm	More	less
Size	Larger	Smaller
Azurophilic granules	+	-
Nucleoli	Larger / more	Smaller / less
Auer rods	+	-

Notice that AML is + and larger in everything , while ALL in smaller and - !

Tests:

	AML	ALL
Sudan black (perioxidase)	+	-
PAS	-	+
Tdt	-	+

<u>Monoclonal Antibodies :</u>

- ✓ Myeloid markers : e.g. CD13 , CD33
- ✓ T-cell lymphoblasts : e.g. CD13
- ✓ B-cell lymphoblasts: e.g. CD19
- ✓ Common ALL antigen : CALLA (CD10) **IMP!**

INFECTIOUS MONONUCLEOSIS

Etiology:

Infection with Epstein – Barr (EB) virus, (a virus from the herpes group of viruses).

Pathogenesis:

- ✓ EB virus enters into epithelial cells of oropharynx or into B lymphocytes of Waldeyers' ring.
- ✓ B-lymphocytes proliferation.
- ✓ T-lymphocytes proliferation and T-lymphocytes will appear in the peripheral blood as atypical lymphocytes.
- ✓ T-lymphocytes are of the cytotoxic type. They will attack the B-lymphocytes causing severe pharyngitis.
- ✓ Involvement of other lymphoid tissues.
- ✓ Viremia.
- ✓

Clinical Features:

- Incubation period 5 - 8 weeks.
- More common in females.
- Usually in young patients 15 - 25 years.

Features:

- ✓ Fever.
- ✓ Pharyngitis, follicular tonsillitis (sore throat).
- ✓ Lymphadenopathy: usually cervical, but can be generalized.
- ✓ Splenomegaly: in about 50%.
- ✓ Hepatomegaly.
- ✓ Rash.
- ✓ Bleeding: in severe cases.
- ✓ Tachycardia and ECG abnormalities.
- ✓ CNS symptoms e.g. convulsion, rare.
- ✓ Eye symptoms: photophobia, conjunctivitis.
- ✓ Acute abdomen: acute abdominal pain due to involvement of mesenteric lymph nodes.

Laboratory Finding:

- ✓ **Leukocytosis with lymphocytosis:** WBC usually 10 - 20,000/ul with lymphocytes forming >50 %. *Atypical lymphocytes are seen.
- ✓ **Anemia:** can be due to cold autoimmune hemolytic anemia.
- ✓ **Thrombocytopenia:** Autoimmune.
- ✓ **Liver enzymes:** increase due to hepatitis.
- ✓ **Serological tests (antibodies):** Types of antibodies seen in infectious mononucleosis are:
    A. EB virus specific.
    B. Heterophile.
    C. Autoimmune.

B. Virus specific antibodies:

1. **IgM:** Develop early in the disease and lasts for few months**.**
2. **IgG :**
    a. One type against capsid antigen (VCA). Appears early in the acute phase and used to diagnose new infections.
    b. Against nuclear antigen (EBNA). Develops after the acute phase and persists for life. Usually indicated old infection.

B. **Heterophile antibodies:**

Antibodies that are produced as a result of the infection but react with an antigen different from the causative agent.

***Paul Bunnel Test:**

- To detect Antibodies that can agglutinate sheep red cells.
- Can also be positive in other diseases e.g. serum sickness or leukemia (Forsemann antibody). To differentiate it from IM (Infectious mono). Guinea pig kidney cells are used. Forsemann antibodies react with the kidney cells. IM antibodies do not.

***Monospot test:**

- Replaced the Paul Bunnel test.
- Serum mixed with guinea pig kidney cells and then with horse RBCs.

C. Autoimmune antibodies:

1. Autoimmune cold hemolytic anemia.

2. Immune thrombocytopenia.

Differential diagnosis:

A similar clinical syndrome and atypical lymphocytes can be seen in other disease e.g. toxoplasmosis and cytomegalovirus. However, these can be differentiated by serology. (IM) is (+) while these diseases are (-).

Course and Prognosis:

- Most patients recover in 4 - 6 weeks.
- Unusual complications are hepatitis, encephalitis, hepatic failure, glottic edema and splenic rupture.

CHRONIC LYMPHOCYTIC LEUKEMIA

Neoplastic proliferation of mature lymphocytes usually B-lymphocytes. Usually seen in the elderly. More common in the west and more common in males. Account for about 25% of all leukemias.

***Clinical features:* MCQ**

- **Accidental:** about 25% of cases are diagnosed on routine blood exams.
- **Pallor:** Due to anemia.
- Lymphadenopathy.
- Splenomegaly.
- Hepatomegaly.
- **Bruising and purpura:** due to thrombocytopenia.

- Herpes Zoster and simplex infection.
- Pruritis.
- Skin infiltration.
- Depressed immunity, both cellular and humeral.

Laboratory features:

1. **Lymphocytosis:** > 5,000/ul is required for diagnosis the lymphocytes are mature, small round lymphocytes. Another feature seen on peripheral blood is smudge "مكسرة" (smear) cells.
2. **Anemia:** can be due to:
    a. Warm autoimmune hemolytic anemia (seen in 10% of cases).
    b. Bone marrow failure (indicates poor Prognosis).
3. **Thrombocytopenia:** can be due to:
    a. Autoimmune (seen in 5 % of cases).
    b. Bone Marrow failure (indicates poor prognosis).
4. **Bone marrow and Lymph node:** infiltration by mature lymphocytes.
5. **Immunoglobulins:**
    - Monoclonal band on serum protein electrophoresis.
    - ↓ Immunoglobulin levels.
6. **Uric Acid: increased**

Prognosis:

Related to:

1. Stage:
2. Age: worse if > 70 years.
3. WBC: worse if > 50 years.
4. Pattern of bone marrow involvement: diffuse involvement.

Staging Systems:

- RAI system: 5 stages
  - 0: Lymphocytosis
  - I: Lymphocytosis + Lymphadenopathy
  - II: Lymphocytosis + Splenomegaly +/ or Hepatomegaly + or - Lymphedenopathy.
  - IV: lymphocytosis + Anemia (not autoimmune) + or - Lymphadenopathy.
  - IV: Lymphocytosis + thro mbocytopenia (not autoimmune) + or - Lymphadenopathy.

- **International system:**
  - **Stage A.** lymphocytosis + < 3 areas of nodal involvement (nodal includes liver, spleen and lymph nodes).
  - **Stage B.** 3 or more nodal areas involvement.
  - **Stage C.** Anemia + or thrombocytopenia

Course and prognosis:

1. Stage O or may remain stable for many years and they may not require treatment.
2. Advances stages may terminate in marrow failure with consequent infection or in a high grade type of lymphoma (Rechter's syndrome).

CHRONIC MYELOCYTIC LEUKEMIA (CML)

= CHRONIC GRANULOCYTIC LEUKEMIA (CGL) = Chronic Myelogenous Leukaemia

Chronic Myeloproliferative Disease

1. Chronic Myelogenous Leukaemia :[Ph chromosome, t(9;22)(q34;q11), BCR/ABL- positive]
2. Chronic Neutrophilic Leukaemia
3. Chronic Eosinophilic Leukaemia (and the hypereosinophilic syndrome)
4. Polycythaemia Vera
5. Chronic Idiopathic Myelofibrosis (with extramedullary haematopoiesis)
6. Essential Thrombocythaemia
7. Chronic Myeloproliferative Disease, Unclassifiable

Chronic Myelocytic leukemia : Characterized by increase in body granules.

Clincal feature:

- Seen in middle aged people.
- Symptoms:
  - Anemia
  - splenomegaly

  } Most common !
  - Symptoms due to the raised metabolic rate
  - Haemorrhagic Manifestations, especially bruising
  - Acute abdominal pain
  - Bone or joint pains
  - Menstrual disturbances
  - Neurological symptoms
  - Priapism
  - Gout
  - Skin disorder
  - Disturbances of vision or hearing
  - Accidental discovery on routine blood examination

Laboratory Findings :

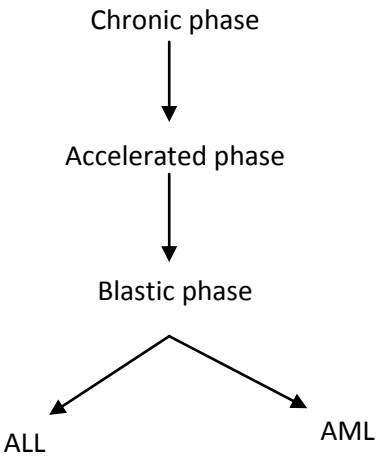
- Peripheral blood :
  - Leukocytosis (50,000 – 300,00)
  - Differential count of WBC shows mature and immature forms.
  - Most of the cells are "segmented" neutrophils and myelocytes.
  - Increased eosinophils and basophils.
  - Increased platelets .
  - Anemia

- Decreased Neutrophil Alkaline Phosphate (NAP) IMP!
- Increased serum B12 and B12 binding capacity
- Bone marrow examination:
  - Hyper cellular with increase granulocytic proliferation.
- Philadelphia chromosome :
  - It is an important characteristic in CML IMP !
  - Positive in 90-95% of the cases
  - It is a translocation between chromosome 9 and 22 IMP !
- C-abl oncogene :
  - This proto-oncogene is found on chromosome 9
  - What happens is that this proto-oncogene (c-ABL) is moved to BCR gene on chromosome 22
  - When the c-ABL and BCR are united on the same chromosome 22 (Philadelphia chromosome) they increase the activity of the enzyme tyrosine kinase , that's why one of the treatments we use is tyrosine kinase inhibitor.

Note 4 : NAP is ↓ in CML and Proxysmal nocturnal hemoglobinuria.

And ↑in polycythemia vera

Evolution of the disease:



Accelerated phase :

- Blasts 10% to 19% of peripheral blood white cells or bone marrow cells
- Peripheral blood basophiles at least 20%
- Persistent thrombocytopenia (<100X10^9/L) unrelated to therapy or persistent thrombocytosis (> 1000X109L) unresponsive to therapy
- Increasing spleen size and increase WBC count unresponsive to therapy
- Cytogenetic evidence of clonal evolution (i.e., the appearance of an additional genetic abnormality that was not present in the initial specimen at the time of diagnosis of chronic phase CML

- Cytogenetic evidence of clonal evolution (i.e., the appearance of an additional genetic abnormality that was not present in the initial specimen at the time of diagnosis of chronic phase CML.

Blastic phase:

- Diagnosis is established if one or more of following is present:
  - Blasts 20% or more of peripheral blood white cells or bone marrow cells
  - Extramedullary blast proliferation
  - Large foci or clusters of blasts in bone marrow biopsy

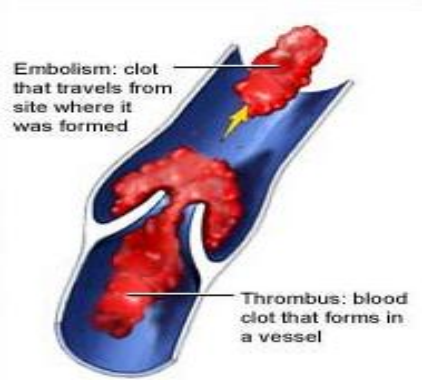
Treatment of chronic myeloid leukemia :

- Hydroxyurea (HU) - chemotherapy -:

	WBC/*u*l	HU (mg/kg BW/DAY)
×	>50,000	50
×	15-50,000	30 – 40
×	<15,000	25

- Gleevec (tyrosine kinase inhibitor)
- Busulphan
- Alpha – Interferon:
  - 5 MU/DAY
  - 7.5 MU/DAY if WBC > 10,000/mcl
  - 10 MU/DAY if WBC > 20,000/mcl
  - 3 MU/DAY if cytopenia develops
- BMT, ABMT & STEM CELL HARVEST

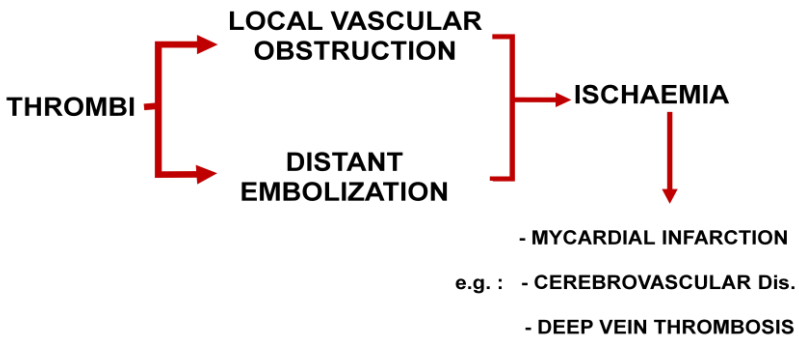
Thrombosis



the formation of an abnormal clot (or thrombus) in the circulatory system

- Thrombi are solid plugs formed basically of platelets & fibrin.
- It can be arterial or venous.
- It becomes more common with age.
- It is frequently associated with risk factors, e.g. surgical operations or pregnancy.

CLINICAL SIGNIFICANCE :



PATHOGENESIS :

Atherosclerosis of Arterial Wall + Plaque Rupture +Endothelial injury = Exposure of the Blood to Sub endothelial Collagen & Tissue Factor ->Initiate the Formation of Platelet Nidus -> Platelet Adherence & Platelet Aggregation

Regrowth of Endothelium , Repair at the Site of Arterial Damage & the Incorporated Thrombus -> Vessel Wall Thickening & Blocking Arteries Locally

- Emboli of Platelet & Fibrin may break away from the 1ry thrombus to cause Occlusion of Distal Arteries

e.g. Carotid Artery Thrombus – Cerebral Thrombosis – Transient Ischemic Attacks (TIAs)

Heart Valve & Chamber Thrombus – Systemic Emboli & Infarcts

- Platelet Deposition & Thrombus Formation are important in the pathogenesis of Atherosclerosis.
- Platelet Derived Growth Factor (PDGF) stimulates Migration & Proliferation of Smooth Muscle Cells & Fibroblasts in the Arterial Intima.

Risk Factors :

- Positive Family History
- Cigarette Smoking
- ECG abnormalities
- Elevated Factor VII
- Elevated Fibrinogen
- Lupus Inhibitors
- Collagen Vascular Diseases
- Behçet diseaseDisease
- Polycythaemia
- Male Gender
- Hyperlipidaemia
- Hyperhomocysteinaemia
- Low Serum Folate, Vit B_{12}, Vit B_6
- Hypertension
- Diabetes Mellitus
- Gout

Virchow's Triad

3 components are important in thrombus formation:

1. Slowing down of the blood flow (stasis)*
2. Hypercoagulability*
3. Damage of vessel wall**

* 1 & 2 are most important for venous thrombosis

** 3 is more important in arterial thrombosis

HYPERCOAGULABLE DISORDERS (THROMBOPHILIA)

It's about inherited & acquired haemostatic disorders which predispose to thrombosis.

"HERIDITARY THROMBOPHILIA"

- **It is known also as:**
- **It should be suspected in young patients with:**

1. **Spontaneous Thrombosis**
2. **Recurrent Deep Vein Thrombosis**
3. **Unusual Site of Thrombosis**

   e.g. Axillary, Splanchnic or Sagittal Vein

Hereditary Risk Factors:

- **Factor V Leiden**
- **Prothrombin G20210a Variant**
- **Protein C Deficiency**
- **Protein S Deficiency**
- **Antithrombin Deficiency**
- **Hyperhomocyseinaemia**
- **Dysfibrinogenaemia**
- **Heparin Cofactor Deficiency**
- **Factor XII Deficiency**
- **Dysplasminogenaemias**
- **Plasminogen Activator Deficiency**
- **Plasminogen Activator Inhibitor Excess**

Believe it or not, FXII deficiency causes thrombosis.

Hereditary and Acquired risk factors :

- Raised Plasma Levels
- - Factor VII, VIII, IX or XI - Fibrinogen - Homocysteine
- Glucosylceramide Deficiency
- Coagulation Factor IX Concentrates
- Lupus Inhibitor

- Oral Contraceptives (Oestrogen Therapy)
- Heparin Induced Thrombocytopenia
- Pregnancy & Puerperium
- Surgery especially Abdominal & Hip
- Major Trauma
- Malignancy
- Myocardial Infarction
- Thrombocythaemia

RISK FACTORS RELATED TO STASIS:

- **Cardiac Failure**
- **Stroke**
- **Prolonged Immobility**
- **Pelvic Obstruction**
- **Nephrotic Syndrome**
- **Dehydration**
- **Hyperviscosity, Polycythaemia**
- **Varicose Veins**

RISK FACTORS RELATED TO UNKNOWN FACTORS:

- Age
- Obesity
- Sepsis
- Paroxysmal Nocturnal Haemoglobinuria
- Behcet's Disease مرض بهجت
- Kamal E. Higgy

Treatment :

- Antiplatelet agents
    - Inhibit platelet functions (aspirin, ticlopidine, dipyridamole)
- Thrombolytic therapy
    - E.g. streptokinase, recombinant tPA
- Heparin and Low Molecular Weight Heparins
- Oral Anticoagulant therapy (e.g Warfarin)
- New drugs *(Orgaran, Hirudin, Argatroban, Debagatran)*

HEPARIN LABORATORY MONITORING :

- Aptt ratio 1.5 -2.5
- For i.v. Heparin repeat aptt evry 4-6 hours
- For sc heparin repeat aptt / 3 - 4 weeks
- Aptt for sc heparin 4-6 hours after the last injection
- Sc dose can be given / 8-12 hours
- Lmw heparin is given /24 hours
- Maintain heparin level between 0.2 & 0.4 u/ml
- Maintain anti-xa between 0.4 & 0.6 u/ml

WARFARIN LABORATORY MONITORING:

- PROTHROMBIN TIME (PT)
- PT RATIO
- THROMBOTEST
- INR = (PT RATIO)^{ISI}
- INR= LOG (MACHINE INR / MANUAL INR)