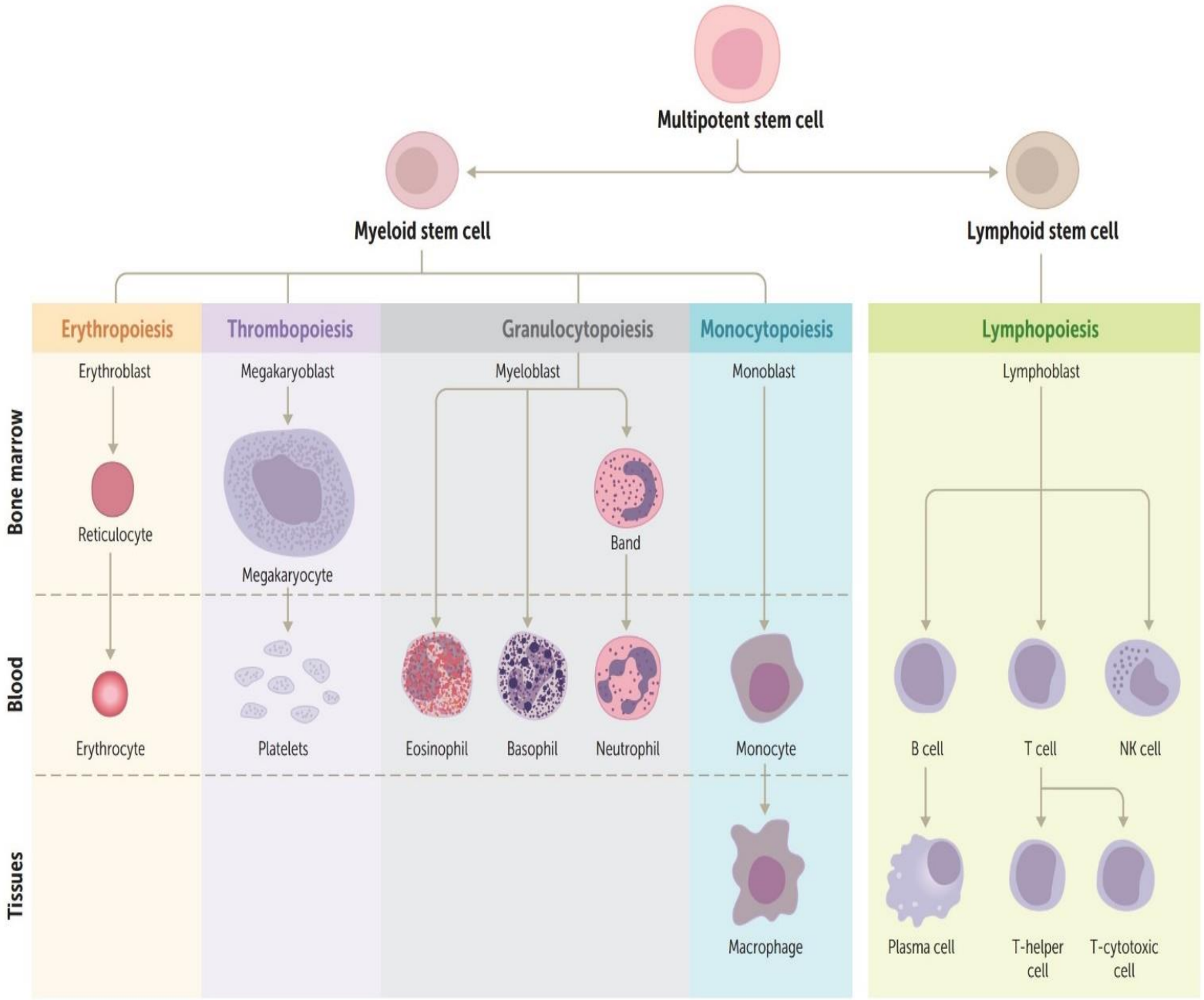
CHAPTER 1

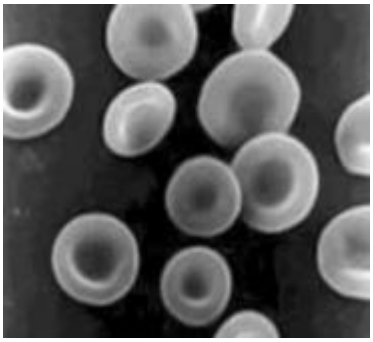
Pathophysiology

Hematopoiesis



Erythrocyte

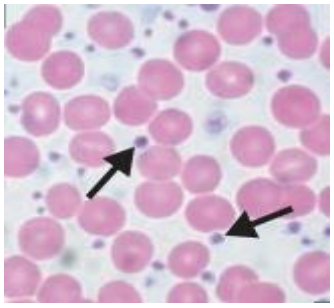
- Carries O_2 to tissues and CO_2 to lungs.
- Anucleate and lacks organelles; biconcave, with large surface area-to-volume ratio for rapid gas exchange.
- Life span of 120 days.
- Source of energy is glucose (90% used in glycolysis, 10% used in HMP shunt).
- Membrane contains Cl/HCO_3 antiporter, which allows RBCs to export HCO_3 and transport CO_2 from the periphery to the lungs for elimination.
- Eryth = red; cyte = cell.
- Erythrocytosis = polycythemia = ↑ hematocrit.
- Anisocytosis = varying sizes.
- Poikilocytosis = varying shapes.
- Reticulocyte = immature RBC; reflects erythroid proliferation.
- Bluish color on Wright-Giemsa stain of reticulocytes represents residual ribosomal RNA.



Thrombocyte (platelet)

- Involved in 1° hemostasis.
- Small cytoplasmic fragment derived from megakaryocytes.
- Thrombopoietin stimulates megakaryocyte proliferation.
- Life span of 8–10 days.

- When activated by endothelial injury, aggregates with other platelets and interacts with fibrinogen to form platelet plug.
- Contains dense granules (Ca, ADP, Serotonin, Histamine; CASH) and α granules (vWF, fibrinogen, fibronectin, platelet factor 4).
- Approximately 1⁄3 of platelet pool is stored in the spleen.
- Thrombocytopenia or ↓ platelet function results in petechiae.
- vWF receptor: GpIb.
- Fibrinogen receptor: GpIIb/IIIa.



Leukocyte

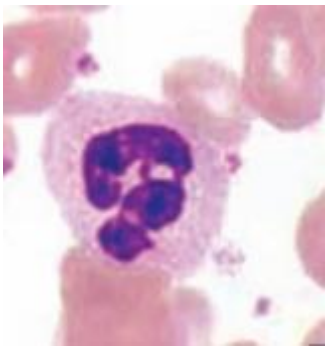
- Divided into granulocytes (neutrophil, eosinophil, basophil) and mononuclear cells (monocytes, lymphocytes).
- Leuk = white; cyte = cell.
- WBC differential from highest to lowest (normal ranges per USMLE):

- Neutrophils (54 – 62%).
- Lymphocytes (25 – 33%).
- Monocytes (3 – 7%).
- Eosinophils (1 – 3%).
- Basophils (0 – 0.75%).
- Neutrophils Like Making Everything Better.

Neutrophil

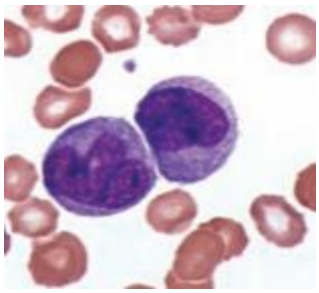
- Phagocytic multilobed nucleus.
- Acute inflammatory response cells. Increased in bacterial infections.
- Specific granules contain leukocyte alkaline phosphatase (LAP), collagenase, lysozyme, and lactoferrin.
- Azurophilic granules (lysosomes) contain proteinases, acid phosphatase, myeloperoxidase, and β-glucuronidase.

- Hypersegmented neutrophils (nucleus has 6 lobes) are seen in vitamin B_{12}/ folate deficiency.
- ↑ band cells (immature neutrophils) reflect states of ↑ myeloid proliferation (bacterial infections, CML).
- Important neutrophil chemotactic agents: C5a, IL-8, LTB4, kallikrein, platelet-activating factor.



Monocyte

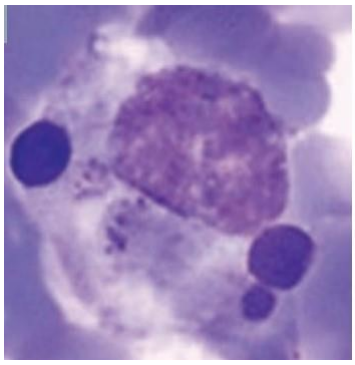
- Monocyte: in the blood.
- Mono = one (nucleus); cyte = cell.
- Large, kidney-shaped nucleus. Extensive "frosted glass" cytoplasm.
- Differentiates into macrophage in tissues.



Macrophage

- Macro = large; phage = eater.
- Macrophages differentiate from circulating blood monocytes. Activated by γ-interferon.
- Long life in tissues.
- Phagocytoses bacteria, cellular debris, and senescent RBCs.
- Can function as antigen-presenting cell via MHC II.
- Important component of granuloma formation (TB, sarcoidosis)

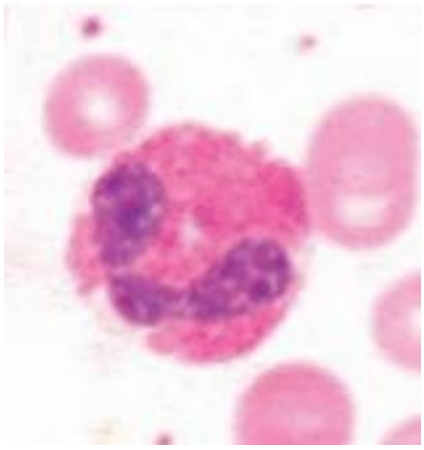
- Lipid A from bacterial LPS binds CD14 on macrophages to initiate septic shock.
- Macrophage naming varies by specific tissue type (Kupffer cells in liver, histiocytes in connective tissue, Langerhans cells in skin, osteoclasts in bone, microglial cells in brain).



Eosinophil

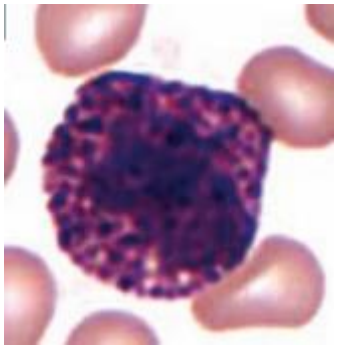
- Eosin = pink dye; philic = loving.
- Bilobate nucleus Packed with large eosinophilic granules of uniform size.
- Defends against helminthic infections (major basic protein).
- Highly phagocytic for antigen antibody complexes.
- Produces histaminase and major basic protein (MBP), a helminthotoxin.
- Causes of eosinophilia = NAACP:

- Neoplasia
- Asthma
- Allergic processes
- Chronic adrenal insufficiency
- Parasites (invasive)



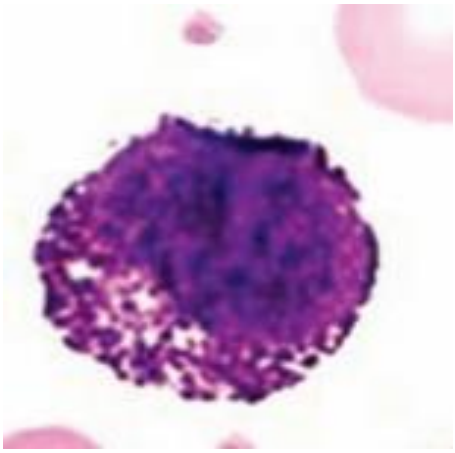
Basophil

- Basophilic: staining readily with basic stains.
- Mediates allergic reaction. Densely basophilic granules contain heparin (anticoagulant) and histamine (vasodilator).
- Leukotrienes synthesized and released on demand.
- Basophilia is uncommon, but can be a sign of myeloproliferative disease, particularly CML.

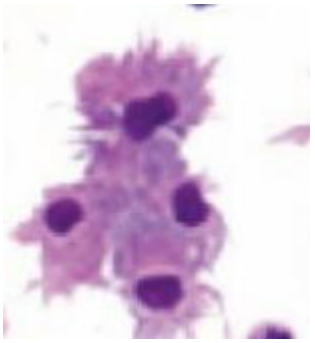


Mast cell

- Mast cells contain basophilic granules and originate from the same precursor as basophils but are not the same cell type.
- Mediates allergic reaction in local tissues.
- Can bind the Fc portion of IgE to membrane.
- IgE crosslinks upon antigen binding → degranulation → release of histamine, heparin, tryptase, and eosinophil chemotactic factors.
- Involved in type I hypersensitivity reactions.
- Cromolyn sodium prevents mast cell degranulation (used for asthma prophylaxis).

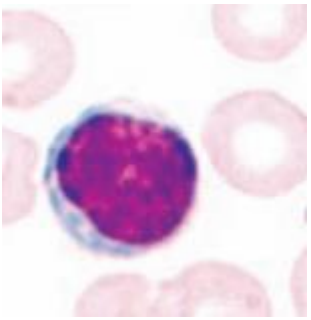


Dendritic cell



- Highly phagocytic antigen-presenting cells (APC). Called Langerhans cell in the skin.
- Functions as link between innate and adaptive immune systems.
- Expresses MHC class II and Fc receptors on surface.

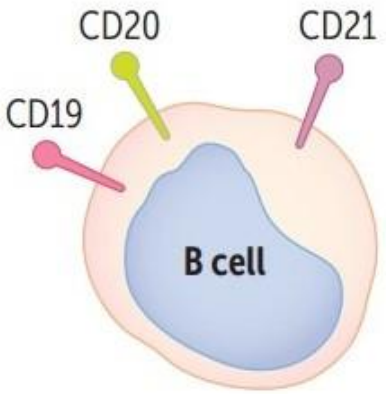
Lymphocyt



- Refers to B cells, T cells, and NK cells.
- B cells and T cells mediate adaptive immunity.
- NK cells are part of the innate immune response. Round, densely staining nucleus with small amount of pale cytoplasm.

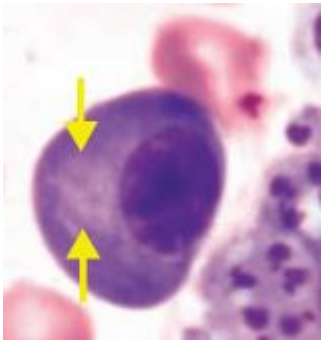
B cell

- B = Bone marrow.
- Part of humoral immune response.
- Originates from stem cells in bone marrow and matures in marrow.
- Migrates to peripheral lymphoid tissue (follicles of lymph nodes, white pulp of spleen, unencapsulated lymphoid tissue).
- When antigen is encountered, B cells differentiate into plasma cells (which produce antibodies) and memory cells.
- Can function as an APC via MHC II.



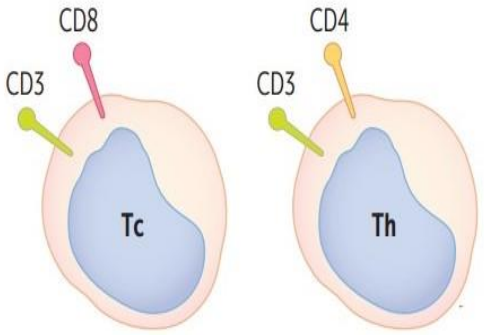
Plasma cell

- Produces large amounts of antibody specific to a particular antigen.
- "Clock-face" chromatin distribution and eccentric nucleus, abundant RER, and well-developed Golgi apparatus (yellow arrows).
- Found in bone marrow and normally do not circulate in peripheral blood.
- Multiple myeloma is a plasma cell cancer.



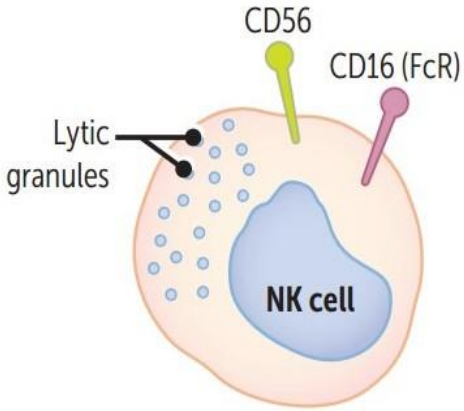
T cell

- T is for Thymus.
- Originates from stem cells in the bone marrow but matures in the thymus.
- Mediates cellular immune response.
- T cells differentiate into cytotoxic T cells (express CD8, recognize MHC I), helper T cells (express CD4, recognize MHC II), and regulatory T cells.
- CD28 (costimulatory signal) necessary for T-cell activation.
- The majority of circulating lymphocytes are T cells (80%).
- CD4 helper T cells are the primary target of HIV.
- MHC × CD = 8 (MHC 2 × CD4 = 8, and MHC 1 × CD8 = 8).



Natural killer cells

- Important in innate immunity, especially against intracellular pathogens.
- Larger than B and T cells, with distinctive cytoplasmic lytic granules (containing perforin and granzymes) that, when released, act on target cells to induce apoptosis.
- Distinguish between healthy and infected cells by identifying cell surface proteins (induced by stress, malignant transformation, or microbial infections).



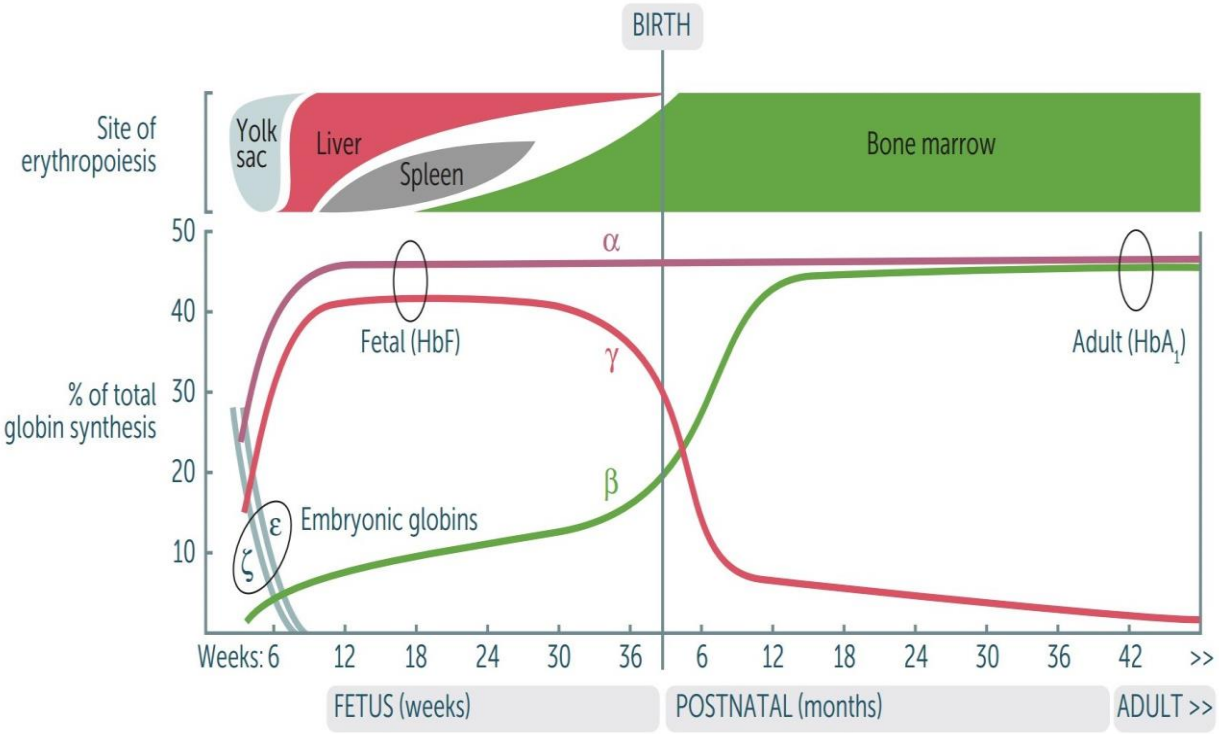
Fetal erythropoiesis

- <u>Fetal erythropoiesis occurs in:</u>

- Yolk sac (3-8 weeks)
- Liver (6 weeks-birth)
- Spleen (10-28 weeks)
- Bone marrow (18 weeks to adult)
- Young Liver Synthesizes Blood.

Hemoglobin development

- Embryonic globins: ζ and ε.
- Fetal hemoglobin (HbF): $\alpha_2\gamma_2$.
- Adult hemoglobin (HbA1): $\alpha_2\beta_2$.
- HbF has higher affinity for O_2 due to less avid binding of 2,3-BPG, allowing HbF to extract O_2 from maternal hemoglobin (HbA_1 and HbA_2) across the placenta.
- From fetal to adult hemoglobin → Alpha Always; Gamma Goes, Becomes Beta.



Hemeostasis

- Integrity of the blood vessel is necessary to carry blood to tissues.
- Damage to the wall is repaired by hemostasis, which involves formation of a thrombus (clot) at the site of vessel injury.
- Hemostasis occurs in two stages:

A. Primary hemostasis: forms a weak platelet plug and is mediated by interaction between platelets and the vessel wall.

B. Secondary hemostasis: stabilizes the platelet plug and is mediated by the coagulation cascade.

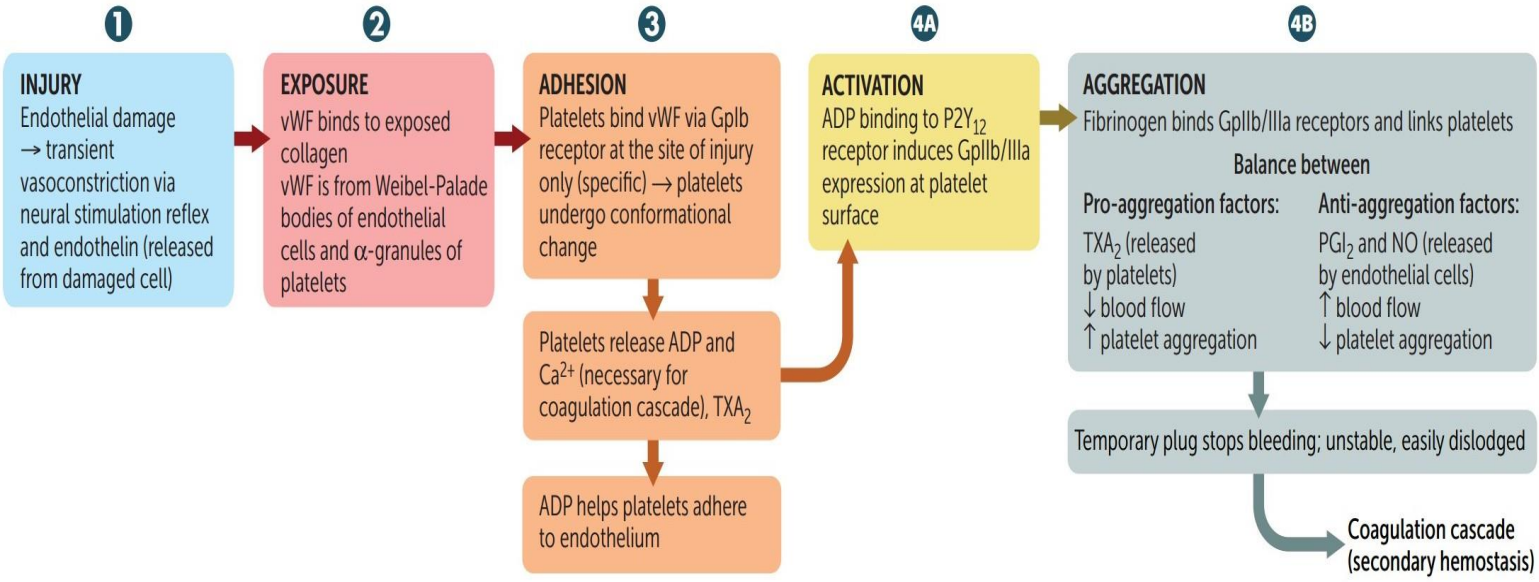
Primary hemeostasis

A. Step 1: Transient vasoconstriction of damaged vessel
- Mediated by reflex neural stimulation and endothelin release from endothelial cells

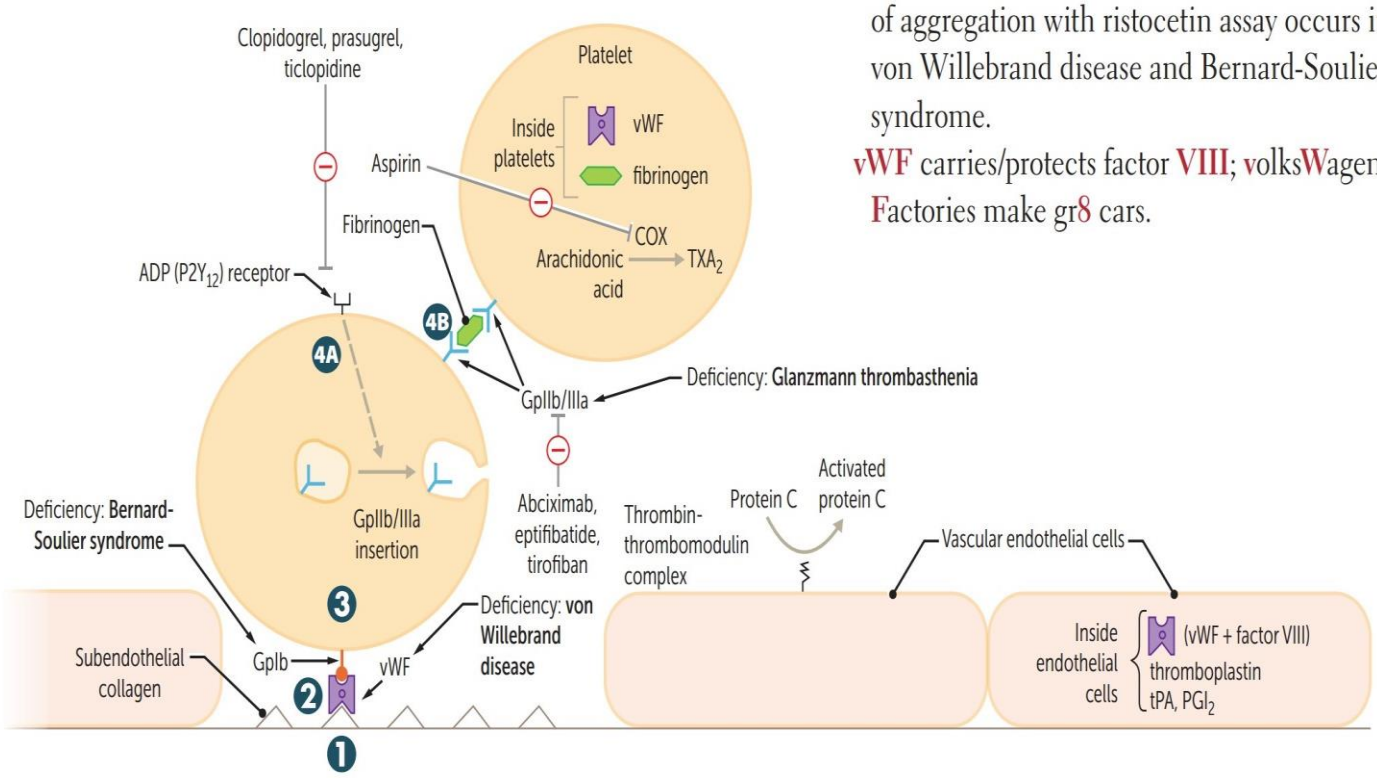
B. Step 2: Platelet adhesion to the surface of disrupted vessel
- Von Willebrand factor (vWF) binds exposed subendothelial collagen.
- Platelets bind vWF using the GPIb receptor.
- vWF is derived from the Weibel-Palade bodies of endothelial cells and α-granules of platelets.

C. Step 3: Platelet degranulation
- Adhesion induces conformational changes in platelets and degranulation with release of multiple mediators.
- ADP is released from platelet dense granules; promotes exposure of GPIIb/IIIa receptor on platelets.
- TXA_2 is synthesized by platelet cyclooxygenase (COX) and released; promotes platelet aggregation.

D. Step 4: aggregation
- Platelets aggregate at the site of injury via GPIIb/IIIa using fibrinogen (from plasma) as a linking molecule, results in formation of platelet plug.
- Platelet plug is weak; coagulation cascade (secondary hemostasis) stabilizes it.



Formation of insoluble fibrin mesh.
Aspirin irreversibly inhibits cyclooxygenase, thereby inhibiting TXA_2 synthesis.
Clopidogrel, prasugrel, and ticlopidine inhibit ADP-induced expression of GpIIb/IIIa by irreversibly blocking $P2Y_{12}$ receptor.
Abciximab, eptifibatide, and tirofiban inhibit GpIIb/IIIa directly.
Ristocetin activates vWF to bind GpIb. Failure of aggregation with ristocetin assay occurs in von Willebrand disease and Bernard-Soulier syndrome.
vWF carries/protects factor VIII; volksWagen Factories make gr8 cars.



Disorders of primary hemeostasis

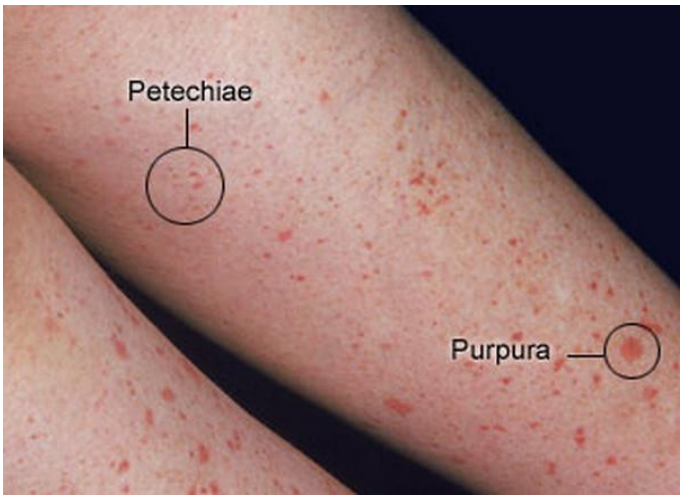
- Usually due to abnormalities in platelets; divided into quantitative or qualitative disorders.

- Clinical features include mucosal and skin bleeding:

- Symptoms of mucosal bleeding include:
- Epistaxis (most common overall symptom).
- Hemoptysis.
- GI bleeding.
- Hematuria.
- Menorrhagia.

- Symptoms of skin bleeding include:
  - Petechiae (1-2 mm).
  - Purpura (> 3 mm).
  - Ecchymoses (> 1 cm).
  - Easy bruising.
  - Petechiae are a sign of thrombocytopenia and are not usually seen with qualitative disorders.

- Intracranial bleeding occurs with severe thrombocytopenia.



- Useful laboratory studies include:

1. Platelet count: normal 150,000 to 400,000 platelets per microliter (mcL); < 50,000 leads to symptoms.
2. Bleeding time: normal 2-7 minutes; prolonged with quantitative and qualitative platelet disorders.
3. Blood smear: used to assess number and size of platelets.
4. Bone marrow biopsy: used to assess megakaryocytes, which produce platelets.

Immune thrombocytopenic purpura (ITP)

- Most common cause of thrombocytopenia in children and adults.
- ITP is characterized by the autoimmune destruction of platelets by anti-platelet antibodies, likely IgG autoantibodies against the platelet membrane glycoproteins GPIIb/IIIa.
- Autoantibodies are produced by plasma cells in the spleen.
- Antibody-bound platelets are consumed by splenic macrophages, resulting in thrombocytopenia.
- Divided into acute and chronic forms:

A. Acute form:

- Arises in children weeks after a viral infection or immunization.
- Self-limited, usually resolving within weeks of presentation.

B. Chronic form:

- Arises in adults, usually women of child-bearing age.
- May be primary or secondary (SLE).
- May cause short-lived thrombocytopenia in offspring since antiplatelet IgG can cross the placenta.

- laboratory findings include:

- ↓ platelet count with prolonged bleeding time: often < 50,000 platelets per microliter (mcL).
- Normal PT/PTT: Coagulation factors are not affected.
- ↑ megakaryocytes on bone marrow biopsy.

- Treatment:

- Initial treatment is corticosteroids. Children respond well; adults may show early response, but often relapse.
- IVIG is used to raise the platelet count in symptomatic bleeding, but its effect is short-lived.
- Splenectomy eliminates the primary source of antibody and the site of platelet destruction (performed in refractory cases).

Microangiopathic hemolytic anemia

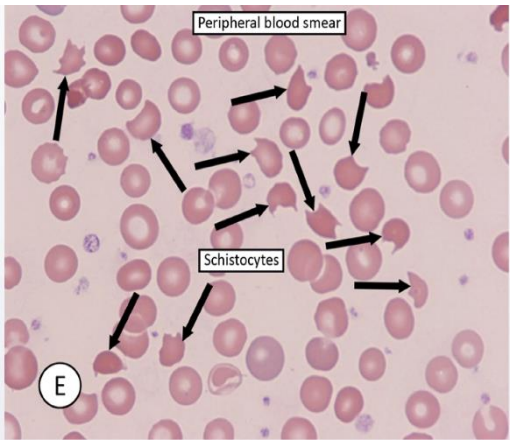
- Pathologic formation of platelet microthrombi in small vessels:
  - Platelets are consumed in the formation of microthrombi.
  - RBCs are "sheared" as they cross microthrombi → hemolytic anemia with schistocytes.
- Seen in thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS).

A. TTP is due to decreased ADAMTS13 (vWF metalloprotease), an enzyme that normally cleaves VwF multimers into smaller monomers:
  - Large, uncleaved multimers lead to abnormal platelet adhesion, resulting in microthrombi.
  - Decreased ADAMTS13 is usually due to an acquired autoantibody; most commonly seen in adult females.

B. HUS is due to endothelial damage by drugs or infection:
  - Classically seen in children with E coli O157:H7 dysentery, which results from exposure to undercooked beef.
  - E coli verotoxin damages endothelial cells resulting in platelet microthrombi.

- Clinical findings (HUS and TTP) include:
  - Fever.
  - Skin and mucosal bleeding.
  - Microangiopathic hemolytic anemia.
  - Renal insufficiency (more common in HUS): thrombi involve vessels of the kidney.
  - CNS abnormalities (more common in TTP): Thrombi involve vessels of the CNS.
- Laboratory findings include:
  - Thrombocytopenia with ↑ bleeding time.
  - Normal PT/PTT (coagulation cascade is not activated).
  - Anemia with schistocytes.
  - ↑ Megakaryocytes on bone marrow biopsy.
  - The hemolytic anemia causes decreased hemoglobin and haptoglobin levels as well as increased serum lactate dehydrogenase and unconjugated bilirubin levels. The bleeding time may also be increased due to the reduced number of platelets.

- Treatment involves plasmapheresis and corticosteroids, particularly in TTP.



Thrombotic microangiopathies Disorders overlap significantly in symptomatology.

	Thrombotic thrombocytopenic purpura	Hemolytic-uremic syndrome
EPIDEMIOLOGY	Typically females	Typically children
PATHOPHYSIOLOGY	Inhibition or deficiency of ADAMTS13 (a vWF metalloprotease) → ↓ degradation of vWF multimers → ↑ large vWF multimers → ↑ platelet adhesion and aggregation (microthrombi formation)	Commonly caused by Shiga-like toxin from EHEC (serotype O157:H7) infection
PRESENTATION	Triad of thrombocytopenia (↓ platelets), microangiopathic hemolytic anemia (↓ Hb, schistocytes, ↑ LDH), acute kidney injury (↑ Cr)	
DIFFERENTIATING SYMPTOMS	Triad + fever + neurologic symptoms	Triad + bloody diarrhea
LABS	Normal PT and PTT helps distinguish TTP and HUS (coagulation pathway is not activated) from DIC (coagulation pathway is activated)	
TREATMENT	Plasmapheresis, steroids, rituximab	Supportive care

Qualitative platelet disorders

A. Bernard-Soulier syndrome:

- It is due to a genetic GP1b deficiency; platelet adhesion is impaired.
- Labs: abnormal ristocetin test, large platelets.

B. Glanzmann thrombasthenia: due to a genetic GIIb/IIIa deficiency; platelet aggregation is impaired.

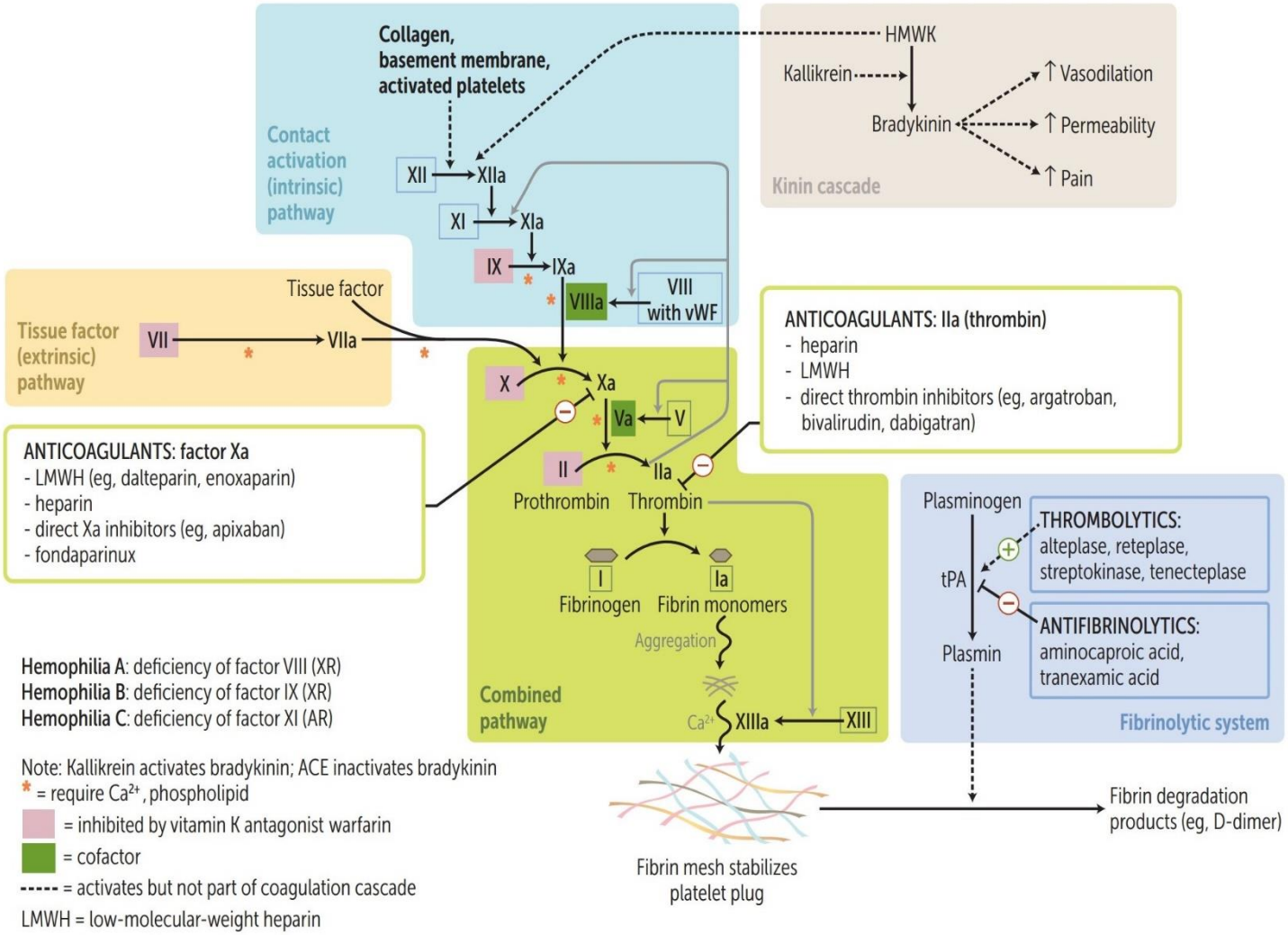
C. Aspirin irreversibly inactivates cyclooxygenase; lack of TXA_2 impairs aggregation.

D. Uremia disrupts platelet function; both adhesion and aggregation are impaired.

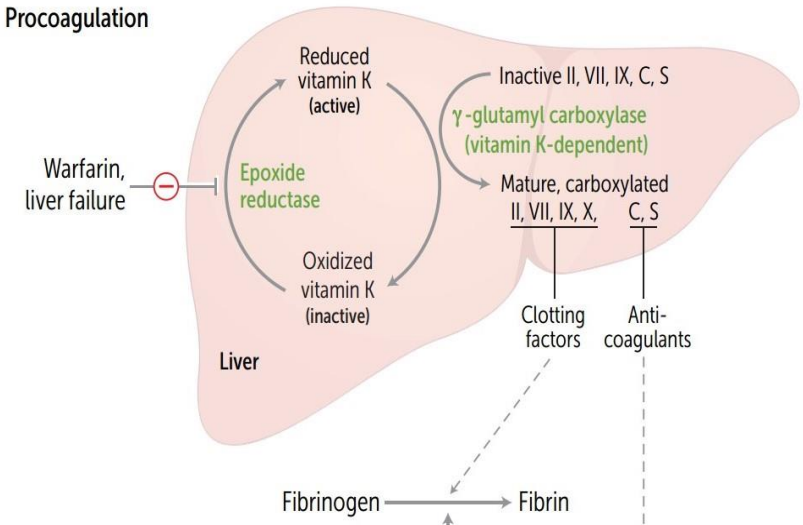
Secondary hemeostasis

- Stabilizes the weak platelet plug via the coagulation cascade:
- Coagulation cascade generates thrombin, which converts fibrinogen in the platelet plug to fibrin.
- Fibrin is then cross-linked, yielding a stable platelet-fibrin thrombus.
- Factors of the coagulation cascade are produced by the liver in an inactive state.
- Activation requires:
- Exposure to an activating substance:
  - Tissue thromboplastin activates factor VII (extrinsic pathway).
  - Subendothelial collagen activates factor XII (intrinsic pathway).
- Phospholipid surface of platelets.
- Calcium (derived from platelet dense granules).

Coagulation and kinin pathways



Procoagulation



Vitamin K deficiency: ↓ synthesis of factors II, VII, IX, X, protein C, protein S.

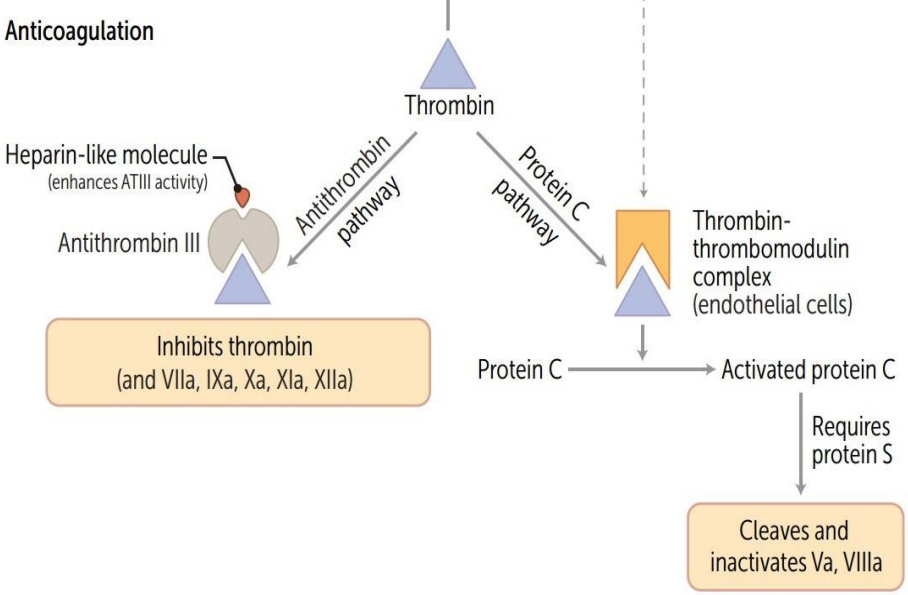
Warfarin inhibits vitamin K epoxide reductase. Vitamin K administration can potentially reverse inhibitory effect of warfarin on clotting factor synthesis (delayed). FFP or PCC administration reverses action of warfarin immediately and can be given with vitamin K in cases of severe bleeding.

Neonates lack enteric bacteria, which produce vitamin K. Early administration of vitamin K overcomes neonatal deficiency/coagulopathy.

Factor VII (Seven)—Shortest half life.

Factor II (Two)—Longest (Tallest) half life.

Anticoagulation



Antithrombin inhibits thrombin (factor IIa) and factors VIIa, IXa, Xa, XIa, XIIa.

Heparin enhances the activity of antithrombin.

Principal targets of antithrombin: thrombin and factor Xa.

Factor V Leiden mutation produces a factor V resistant to inhibition by activated protein C.

tPA is used clinically as a thrombolytic.

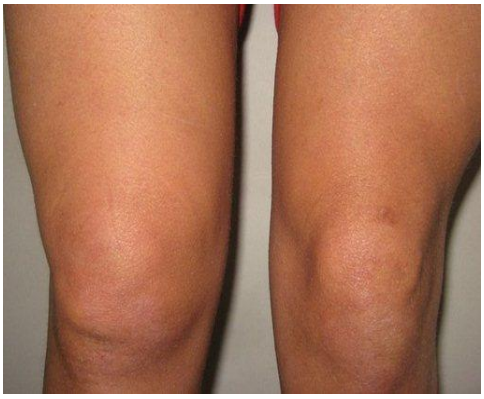
Disorders of secondary hemeostasis

- Usually due to coagulation factor abnormalities.
- Coagulation disorders can be due to clotting factor deficiencies or acquired factor inhibitors.
- Diagnosed with a mixing study, in which normal plasma is added to patient's plasma:
  - Clotting factor deficiencies should correct (the PT or PTT returns to within the appropriate normal range).
  - Factor inhibitors will not correct.
- Clinical features include deep tissue bleeding into muscles and joints (hemarthrosis) and re-bleeding after surgical procedures (circumcision and wisdom tooth extraction).
- Laboratory studies include:
  - Prothrombin time (PT): measures extrinsic (factor VII) and common (factors II, V, X, and fibrinogen) pathways of the coagulation cascade. (Play Tennis outside [extrinsic pathway]).
  - Partial thromboplastin time (PTT): measures intrinsic (factors XII, XI, IX, VIII) and common (factors I, V, X, and fibrinogen) pathways of the coagulation cascade. (Play Table Tennis inside).

Hemophilia A

- Genetic factor VIII (FVIII) deficiency:
  - X-linked recessive (predominantly affects males).
  - Can arise from a new mutation (de novo) without any family history.
- Presents with deep tissue, joint, and post-surgical bleeding.
- Clinical severity depends on the degree of deficiency.
- Laboratory findings include:
  - ↑ PTT; normal PT.
  - ↓ FVIII.
  - Normal platelet count and bleeding time.
- Treatment involves recombinant FVIII.
- Factor VIII is synthesized by the liver and stored in endothelial cells. Desmopressin acetate (DDAVP) is a synthetic vasopressin analog that releases von Willebrand factor and factor VIII from the endothelium. It is used for the treatment of mild-to-moderate hemophilia A.

- In the absence of factors VIII or IX, activation of factor X and subsequent conversion of prothrombin into thrombin does not occur. Administration of thrombin, however, will make up for the deficiency and result in blood clotting.



Hemophilia B (Christmas disease)

- Genetic factor IX deficiency.
- Resembles hemophilia A, except FIX levels are decreased instead of FVIII.
- Hemophilia A and B are indistinguishable clinically, as both demonstrate similar symptoms, similar inheritance patterns, and isolated prolongation of the PTT (PT and bleeding time are normal).

Coagulation factor inhibitor

- Acquired antibody against a coagulation factor resulting in impaired factor function; anti-FVIII is most common.
- Clinical and lab findings are similar to hemophilia A.
- PTT does not correct upon mixing normal plasma with patient's plasma (mixing study) due to inhibitor; PTT does correct in hemophilia A.

Vitamin k deficiency

- Disrupts function of multiple coagulation factors (general coagulation defect).
- Vitamin K is activated by epoxide reductase in the liver.
- Activated vitamin K gamma carboxylates factors II, VII, IX, X, and proteins C and S; gamma carboxylation is necessary for factor function.

- Deficiency occurs in:

- Newborns: due to lack of GI colonization by bacteria that normally synthesize vitamin K; vitamin K injection is given prophylactically to all newborns at birth to prevent hemorrhagic disease of the newborn.

- Long-term antibiotic therapy: disrupts vitamin K-producing bacteria in the GI tract.

- Malabsorption: leads to deficiency of fat-soluble vitamins, including vitamin K.

❖ N.B:

- Most coagulation factors are synthesized in the liver. Not surprisingly, liver dysfunction commonly results in acquired coagulation disorders.
- Factor VII, part of the extrinsic pathway, has the shortest half-life, so the PT is the first to be prolonged in liver disease.
- The vitamin K-dependent coagulation factors (II, VII IX, X) are initially produced by the liver in an inactive form, and are then activated by vitamin K-dependent carboxylation.
- Thus, failure of a prolonged PT to correct with vitamin K supplementation indicates liver disease.

DISORDER	PT	PTT	MECHANISM AND COMMENTS
Hemophilia A, B, or C 	—	↑	Intrinsic pathway coagulation defect (↑ PTT). ▪ A: deficiency of factor VIII; X-linked recessive. ▪ B: deficiency of factor IX; X-linked recessive. ▪ C: deficiency of factor XI; autosomal recessive. Hemorrhage in hemophilia—hemarthroses (bleeding into joints, eg, knee A), easy bruising, bleeding after trauma or surgery (eg, dental procedures). Treatment: desmopressin + factor VIII concentrate (A); factor IX concentrate (B); factor XI concentrate (C).
Vitamin K deficiency	↑	↑	General coagulation defect. Bleeding time normal. ↓ activity of factors II, VII, IX, X, protein C, protein S.

Mixed platelet and coagulation disorders

Von willbrand disease

- Genetic vWF deficiency.
- Most common inherited coagulation disorder.
- Multiple subtypes exist, causing quantitative and qualitative defects; the most common type is autosomal dominant with decreased vWF levels.
- Presents with mild mucosal and skin bleeding; low vWF impairs platelet adhesion.
- vWF serves as a carrier for factor VIII and prolongs its half-life. The half-life of factor VIII bound to vWF is 12 hours, while the half-life of free factor VIII is 2 hours. Decreased levels of vWF, therefore, lead to functional deficiency of factor VIII. This causes prolonged bleeding after tooth extraction and other minor surgeries, as well as prolonged PTT.
- Laboratory findings include:

- ↑ bleeding time.
- ↑ PTT, normal PT.
- Decreased FVIII half-life (vWF normally stabilizes FVIII); however, deep tissue, joint, and postsurgical bleeding are usually not seen.
- Abnormal ristocetin test: Ristocetin induces platelet aggregation by causing vWF to bind platelet GPIb; lack of vWF → impaired aggregation → abnormal test.

- Treatment is desmopressin (ADH analog), which increases vWF release from Weibel-Palade bodies of endothelial cells.

Disseminated intravascular coagulation (DIC)

- Pathologic activation of the coagulation cascade → Widespread microthrombi result in ischemia and infarction.
- Consumption of platelets and factors results in bleeding, especially from IV sites and mucosal surfaces (bleeding from body orifices).
- Almost always secondary to another disease process:

- Obstetric complications: Tissue thromboplastin in the amniotic fluid activates coagulation (retained dead fetus in the uterus).
- Sepsis (especially with N. Meningitidis): Endotoxins from the bacterial wall and cytokines (TNF and IL-1) induce endothelial cells to make tissue factor.

- Adenocarcinoma: Mucin activates coagulation.
- Acute promyelocytic leukemia: Primary granules activate coagulation.
- Snake bite: Venom activates coagulation.

DIC	TTP- HUS
Patients bleed	Usually do not bleed
Coagulation cascade is activated	Only platelets are activated
PT and PTT are prolonged	Normal PT and PTT
Low fibrinogen and increased FDP	Normal fibrinogen

- Laboratory findings include:

- ↓ platelet count and prolonged bleeding time.
- ↑ PT/PTT.
- ↓ fibrinogen.
- Microangiopathic hemolytic anemia.
- Elevated fibrin split products, particularly D-dimer.
- Elevated D-dimer is the best screening test for DIC. Derived from splitting of cross-linked fibrin.

- Treatment involves addressing the underlying cause and transfusing blood products and cryoprecipitate (contains coagulation factors), as necessary.

Mixed platelet and coagulation disorders

DISORDER	PC	BT	PT	PTT	NOTES
von Willebrand disease	—	↑	—	—/↑	Intrinsic pathway coagulation defect: ↓ vWF → ↑ PTT (vWF acts to carry/protect factor VIII). Defect in platelet plug formation: ↓ vWF → defect in platelet-to-vWF adhesion. Autosomal dominant. Mild but most common inherited bleeding disorder. No platelet aggregation with ristocetin cofactor assay. Treatment: desmopressin, which releases vWF stored in endothelium.
Disseminated intravascular coagulation	↓	↑	↑	↑	Widespread activation of clotting → deficiency in clotting factors → bleeding state. Causes: Snake bites, Sepsis (gram ⊖), Trauma, Obstetric complications, acute Pancreatitis, Malignancy, Nephrotic syndrome, Transfusion (SSTOP Making New Thrombi). Labs: schistocytes, ↑ fibrin degradation products (D-dimers), ↓ fibrinogen, ↓ factors V and VIII.

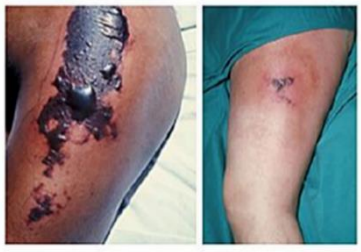
Hereditary thrombosis syndromes leading to hypercoagulability

- Due to excessive procoagulant proteins or defective anticoagulant proteins; may be inherited or acquired.
- Classic presentation is recurrent DVTs or DVT at a young age.
- Usually occurs in the deep veins of the leg; other sites include hepatic and cerebral veins.

Protein C or S deficiency

- Autosomal dominant which decreases negative feedback on the coagulation cascade.
- Proteins C and S normally inactivate factors V and VIII.
- Increased risk for warfarin skin necrosis:

- Warfarin-induced skin necrosis is a rare but important complication of warfarin initiation. It is thought to be due to a transient hypercoagulable state that can occur during the first few days of warfarin therapy.
- The overall anticoagulant effect of warfarin is due primarily to its inhibition of the vitamin K-dependent gamma-carboxylation of clotting factors II, VII, IX, and X (Vitamin K-dependent clotting factors).
- However, warfarin also decreases carboxylation of proteins C and S, which normally exert an anticoagulant effect (through proteolysis and deactivation of factors V and VIII).
- Protein C has a short half-life, so its anticoagulant activity is reduced quickly when warfarin therapy is initiated, by about 50% within the first day.
- During this time, the vitamin K-dependent clotting factors II, IX, and X continue to exert a procoagulant effect as they have longer half-lives (factor VII has a short half-life similar to protein C).
- This difference in half-lives translates into a transient hypercoagulable state → Thrombosis and clot can interrupt blood flow to the skin and lead to skin necrosis.
- For this reason, overlapping coadministration of heparin ("heparin bridge") is commonly used when warfarin is initiated.
- The risk of warfarin-induced skin necrosis is increased in patients with a preexisting protein C deficiency, as well as in those started on a large loading dose of warfarin.



Factor V Leiden

- It is a mutated form of factor V that lacks the cleavage site for deactivation by proteins C and S.
- Most common inherited cause of hypercoagulable state.
- The major clinical manifestations of factor V Leiden include deep vein thrombosis (DVT), cerebral vein thrombosis, and recurrent pregnancy loss.
- The resulting hypercoagulable state predisposes to deep vein thromboses, which are the source of most pulmonary emboli.

Prothrombin gene mutation

- Mutation in 3' untranslated region → ↑ production of prothrombin → ↑ plasma levels and venous clots.
- Increased prothrombin results in increased thrombin, promoting thrombus formation.

Antithrombin deficiency

- Inherited deficiency of antithrombin.
- ATIII deficiency decreases the protective effect of heparin-like molecules produced by the endothelium, increasing the risk for thrombus.
- Heparin-like molecules normally activate ATIII, which inactivates thrombin and coagulation factors.
- Can also be acquired (renal failure/nephrotic syndrome) → antithrombin loss in urine → ↓ inhibition of factors IIa and Xa.
- Has no direct effect on the PT, PTT, or thrombin time but diminishes the increase in PTT following heparin administration.
- In ATIII deficiency, PTT does not rise with standard heparin dosing:
  - Pharmacologic heparin works by binding and activating ATIII.
  - High doses of heparin activate limited ATIII; Coumadin is then given to maintain an anticoagulated state.

Anemias

- Reduction in circulating red blood cell (RBC) mass.

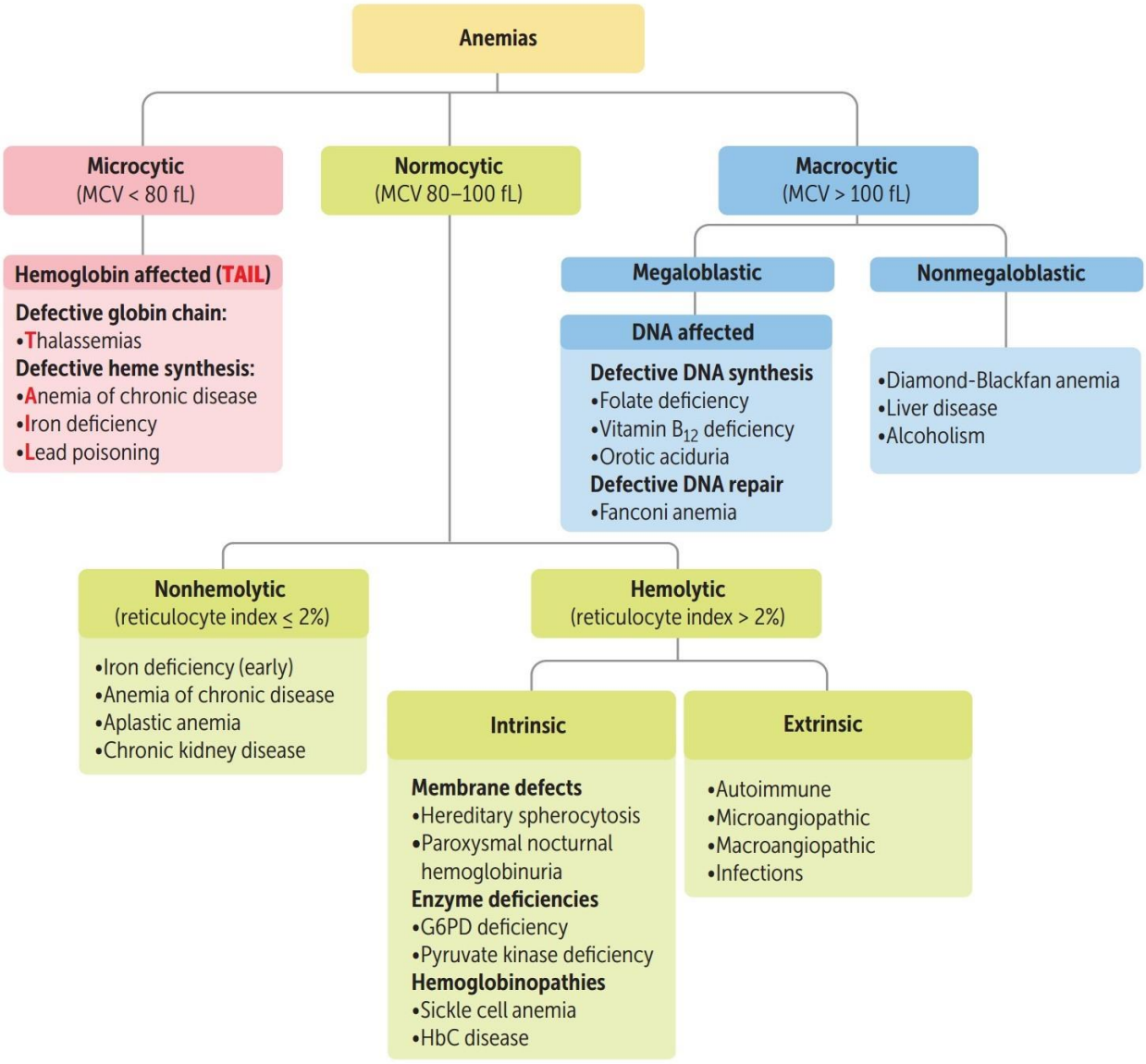
- Presents with signs and symptoms of hypoxia:

- Weakness, fatigue, and dyspnea.
- Pale conjunctiva and skin.
- Headache and lightheadedness.
- Angina, especially with preexisting coronary artery disease.

- Hemoglobin (Hb), hematocrit (Hct), and RBC count are used as surrogates for RBC mass, which is difficult to measure.

- Anemia is defined as Hb < 13.5 g/dL in males and < 12.5 g/dL in females (normal Hb is 13.5-17.5 g/dL in males and 12.5-16.0 g/dl. in females).

- Based on mean corpuscular volume (MCV), anemia can be classified as microcytic (MCV < 80),



Microcytic (MCV < 80 fL), hypochromic anemia

- Anemia with MCV < 80.

- Microcytic anemias are due to decreased production of hemoglobin:

- RBC progenitor cells in the bone marrow are large and normally divide multiple times to produce smaller mature cells (MCV = 80-100).

- Microcytosis is due to an "extra" division which occurs to maintain hemoglobin concentration.

- Hemoglobin is made of heme and globin. Heme is composed of iron and protoporphyrin. A decrease in any of these components leads to microcytic anemia.

- Microcytic anemias include:

1. Iron deficiency anemia.
2. Anemia of chronic disease (late).
3. Sideroblastic anemia.
4. Thalassemia.

A. Iron deficiency anemia:

- Due to decreased levels of iron.

- ↓ iron → ↓ heme → ↓ hemoglobin → microcytic anemia.

- Most common type of anemia.

- Lack of iron is the most common nutritional deficiency in the world, affecting roughly 1/3 of world's population.

- Iron is consumed in heme (meat-derived) and non-heme (vegetable-derived) forms:

- Absorption occurs in the duodenum.

- Enterocytes transport iron across the cell membrane into blood via ferroportin.

- Transferrin transports iron in the blood and delivers it to liver and bone marrow macrophages for storage.

- Stored intracellular iron is bound to ferritin, which prevents iron from forming free radicals.

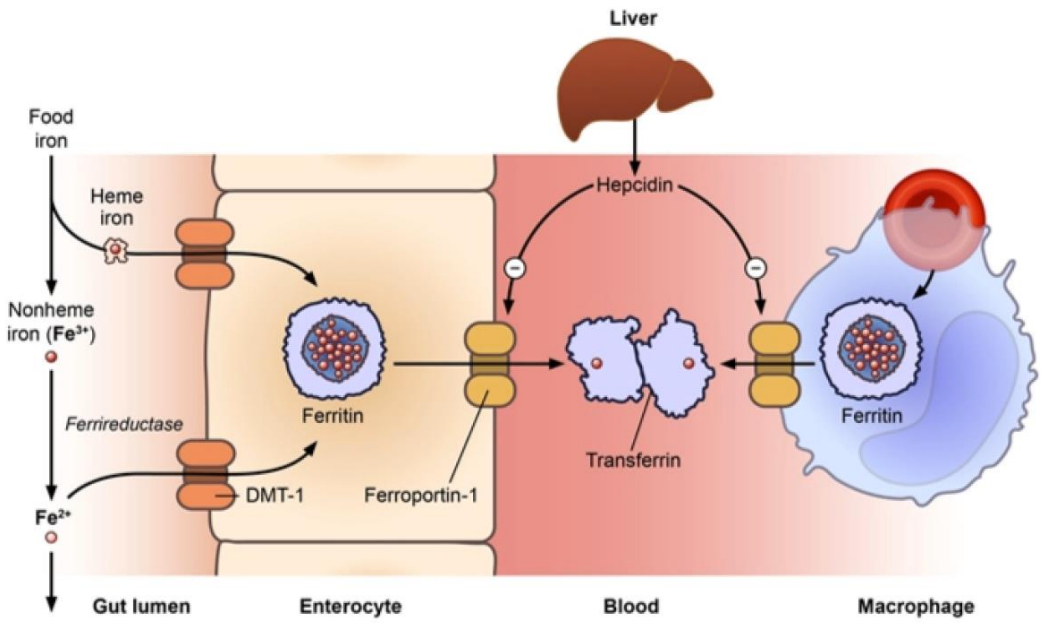
- Laboratory measurements of iron status:

- Serum iron: measure of iron in the blood.

- Total iron-binding capacity (TIBC): measure of transferrin molecules in the blood.

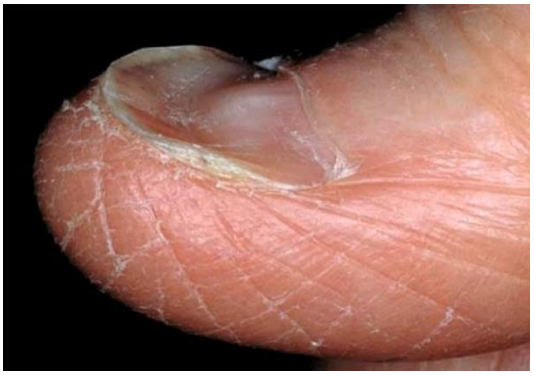
- Saturation: percentage of transferrin molecules that are bound by iron.

- Serum ferritin: reflects iron stores in macrophages and the liver.



- Iron deficiency is usually caused by dietary lack or blood loss:
- Infants: breast-feeding (human milk is low in iron).
- Children: poor diet.
- Adults (20-50 years): peptic ulcer disease in males and menorrhagia or pregnancy in females.
- Elderly: colon polyps/carcinoma in the Western world; hookworm (Ancylostoma duodenale and Nicator americanus) in the developing world.
- Other causes include malnutrition, malabsorption, and gastrectomy (acid aids iron absorption by maintaining the Fe^2 state, which is more readily absorbed.
- Blood loss (especially in the gastrointestinal tract) must be ruled out in a patient with iron-deficiency anemia.

- Stages of iron deficiency:
- Storage iron is depleted: ↓ ferritin; ↑ TIBC.
- Serum iron is depleted: ↓ serum iron; ↓ % saturation.
- Normocytic anemia (early): Bone marrow makes fewer, but normal-sized RBCs.
- Microcytic, hypochromic anemia: Bone marrow makes smaller and fewer.



- Clinical features:

- Fatigue, conjunctival pallor.
- Pica (consumption of nonfood substances).
- Spoon nails (koilonychia).
- May manifest as glossitis, cheilosis.
- Plummer-Vinson syndrome: Triad of iron deficiency anemia, esophageal webs, and glossitis. Presents with anemia, dysphagia, and beefy-red tongue.

- Laboratory findings include:

- Microcytic, hypochromic RBCs with ↑ red cell distribution width (RDW).
- ↓ ferritin; ↑ TIBC.
- ↓ Serum iron; ↓ % saturation.
- ↑ Free erythrocyte protoporphyrin (FEP).

- Treatment: Treatment involves supplemental iron (ferrous sulfate).

❖ N.B:

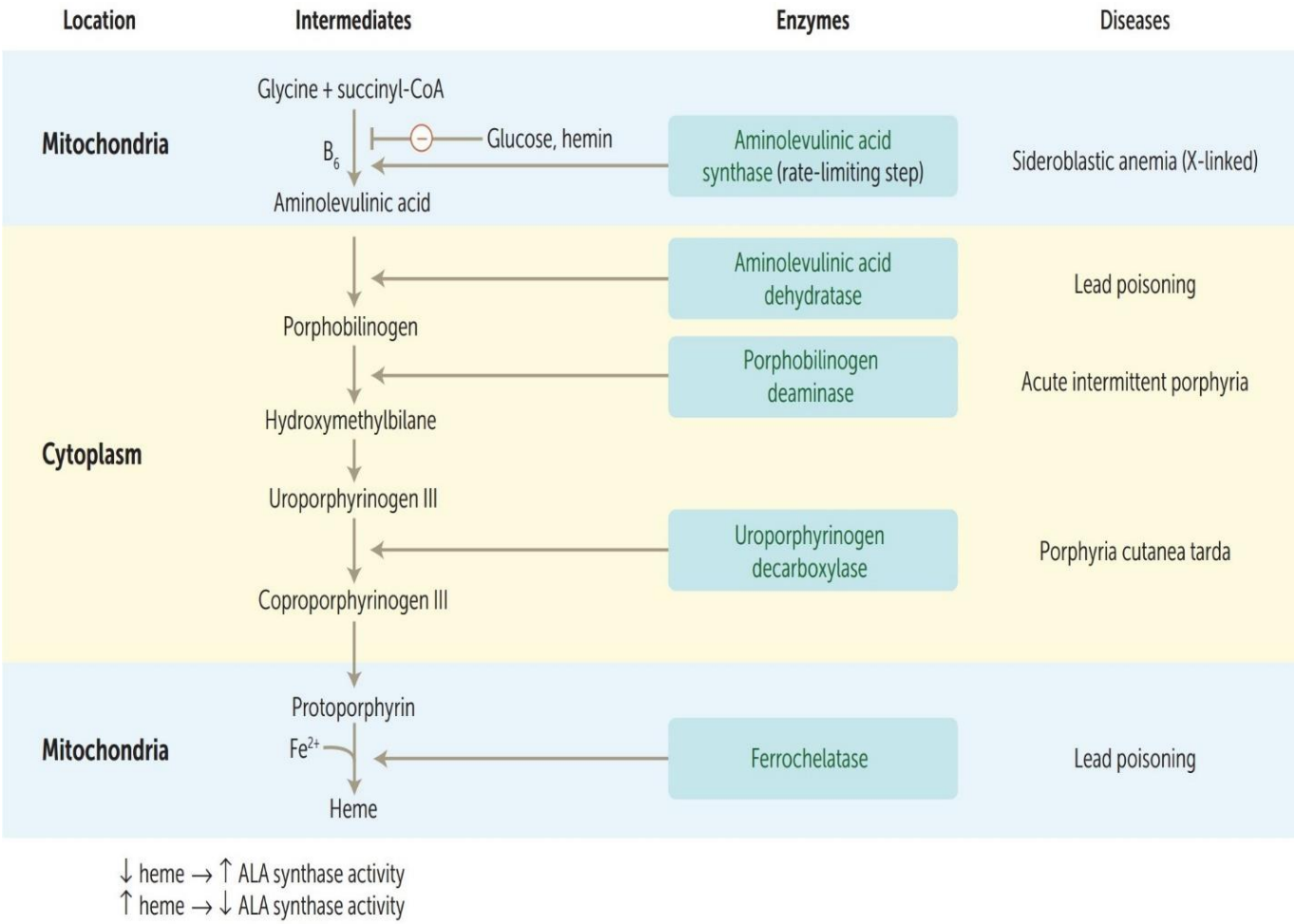
- Iron-deficient individuals on replacement therapy should experience hemoglobin level increases of approximately 2 g/dL per week for the first three weeks.
- This increase in hemoglobin results from enhanced erythropoiesis and the accelerated release of both mature red blood cells (RBCs) and reticulocytes into the bloodstream
- Increased bone marrow erythropoiesis results in an accelerated release of immature red blood cells (reticulocytes) into the bloodstream. Reticulocytes contain bluish cytoplasm and reticular precipitates of residual ribosomal RNA.
- It lacks a cell nucleus but retains a basophilic, reticular (mesh-like) network of residual ribosomal RNA. The ribosomal RNA appears blue microscopically after the application of the Wright-Giemsa stain.
- After spending a day or so in the bloodstream, the reticulocytes are transformed into mature red blood cells that have a lifespan of approximately 120 days.

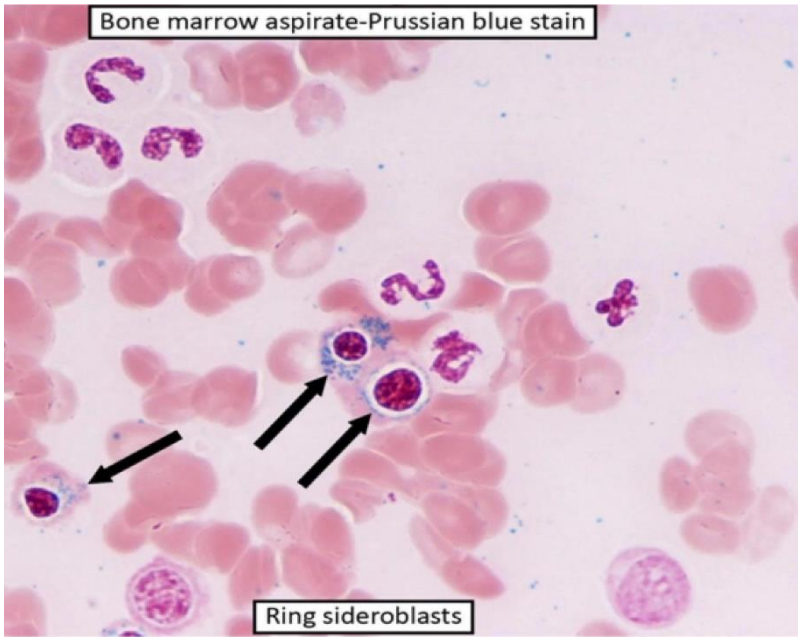
B. Sideroblastic anemia:

- Anemia due to defective protoporphyrin synthesis.
- ↓ protoporphyrin → ↓ heme → ↓ hemoglobin → microcytic anemia.
- Protoporphyrin is synthesized via a series of reactions:

- Aminolevulinic acid synthetase (ALAS) converts succinyl CoA to aminolevulinic acid (ALA) using vitamin B_6 as a cofactor (rate-limiting step).
- Aminolevulinic acid dehydratase (ALAD) converts ALA to porphobilinogen.
- Additional reactions convert porphobilinogen to protoporphyrin.
- Ferrochelatase attaches protoporphyrin to iron to make heme (final reaction; occurs in the mitochondria).

- Iron is transferred to erythroid precursors and enters the mitochondria to form heme. If protoporphyrin is deficient, iron remains trapped in mitochondria.
- Iron-laden mitochondria form a ring around the nucleus of erythroid precursors; these cells are called ringed sideroblasts (hence, the term sideroblastic anemia).





- Sideroblastic anemia can be congenital or acquired:

- Congenital defect most commonly involves ALAS (rate-limiting enzyme).

- Acquired causes include:
  - Alcoholism: mitochondrial poison.
  - Lead poisoning: inhibits ALAD and ferrochelatase.
  - Vitamin B6 deficiency: required cofactor for ALAS; most commonly seen as a side effect of isoniazid treatment for tuberculosis.

- Laboratory findings include:

- ↑ ferritin, ↓ TIBC, ↑ serum iron, and ↑ % saturation (iron-overloaded state).

- Ringed sideroblasts (with iron-laden, Prussian blue-stained mitochondria) seen in bone marrow.

- Treatment:

- Pyridoxine (B_6, cofactor for δ-ALA synthase).

C. Lead poisoning:

- Lead inhibits ALA dehydratase and ferrochelatase → ↓ heme synthesis.

- Lead toxicity is most prevalent among impoverished children residing in deteriorating urban housing built before 1978. Young children are particularly susceptible to lead poisoning via inhalation and ingestion of lead-based paint dust or chips due to normal crawling and mouthing behaviors. The incomplete blood-brain-barrier in children is vulnerable to the neurotoxic effects of lead, which include long-standing behavioral problems and developmental delay or regression.

- Lead poisoning can occur in adults as well (especially those who work in battery manufacturing) who inhale particulate lead while working.

- Symptoms of LEAD poisoning:

- Lead Lines on gingiva (Burton lines) and on metaphysis of long bones on x-ray.

- Encephalopathy (cognitive impairment) and Erythrocyte basophilic stippling.

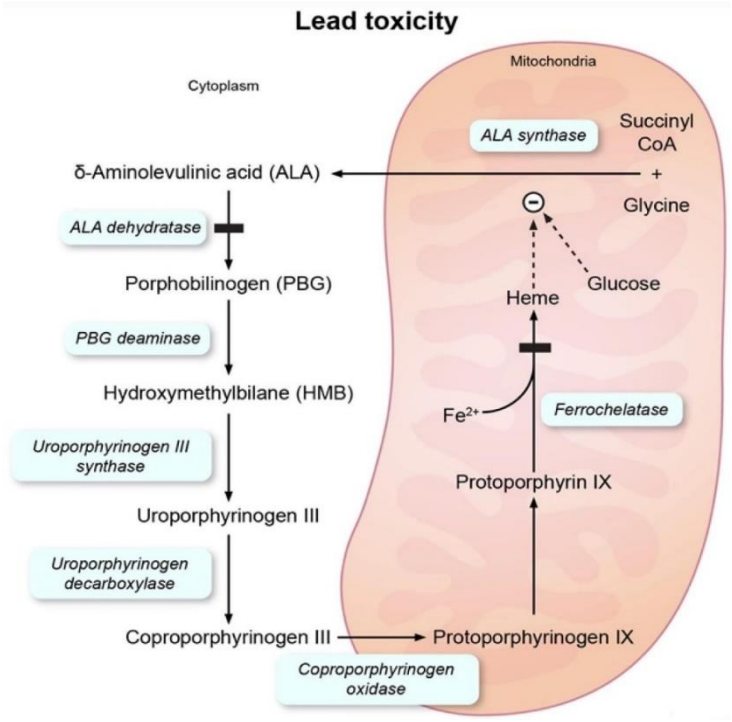
- Abdominal colic and constipation and sideroblastic Anemia.

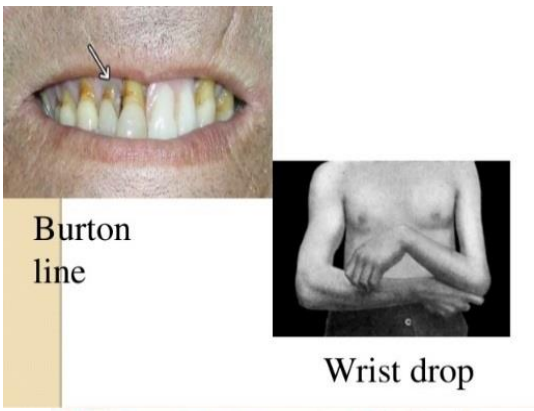
- Drops: wrist and foot drop.

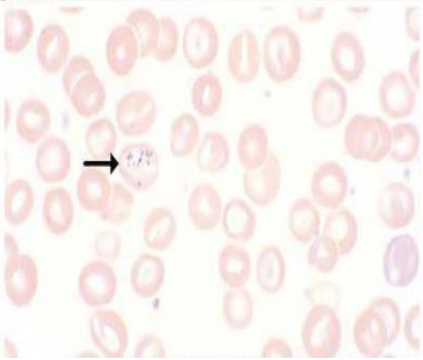
- The classic diagnostic finding on peripheral blood smear is coarse basophilic stippling on a background of hypochromic microcytic anemia. Basophilic stippling results from the abnormal aggregation of ribosomes (lead inhibits rRNA degradation).

- Diagnosis is made by measuring the patient's blood lead level.

- Dimercaprol and EDTA are 1st line of treatment. Succimer used for chelation for kids (It "sucks" to be a kid who eats lead).







D. Thalassemia:

- Anemia due to decreased synthesis of the globin chains of hemoglobin.
- ↓ globin → ↓ hemoglobin → microcytic anemia.
- Inherited mutation. Divided into α- and β-thalassemia based on decreased production of alpha or beta globin chains.
- Normal types of hemoglobin are HbF ($\alpha_2\gamma_2$), HbA_1 ($\alpha_2\beta_2$), and HbA_2 ($\alpha_2\delta_2$).
- α-Thalassemia:

- It is usually due to gene deletion; normally, 4 alpha genes are present on chromosome 16.
- One gene deleted: asymptomatic.
- Two genes deleted: mild anemia with ↑ RBC count; Cis deletion is associated with an increased risk of severe thalassemia in offspring:
  - Cis deletion is when both deletions occur on the same chromosome; seen in Asians.
  - Trans deletion is when one deletion occurs on each chromosome; seen in Africans, including African Americans.
- Three genes deleted: severe anemia; β chains form tetramers (HbH) that damage RBCs; HbH is seen on electrophoresis.
- Four genes deleted: lethal in utero (hydrops fetalis); γ chains form tetramers (Hb Barts) that damage RBCs; Hb Barts is seen on electrophoresis.

NUMBER OF α-GLOBIN GENES DELETED	DISEASE	CLINICAL OUTCOME
1 (α α/α –)	α-thalassemia minima	No anemia (silent carrier)
2 (α –/α –; *trans*) or (α α/– –; *cis*)	α-thalassemia minor	Mild microcytic, hypochromic anemia; *cis* deletion may worsen outcome for the carrier's offspring
3 (– –/– α)	Hemoglobin H disease (HbH); excess β-globin forms β_4	Moderate to severe microcytic hypochromic anemia
4 (– –/– –)	Hemoglobin Barts disease; no α-globin, excess γ-globin forms γ_4	Hydrops fetalis; incompatible with life

- β-Thalassemia:

- It is usually due to gene mutations (point mutations in promoter or splicing sites); seen in individuals of African and Mediterranean descent.

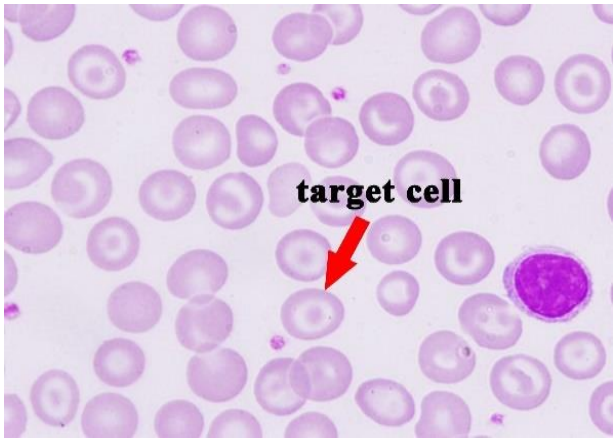
- Beta-thalassemia is caused by mutations that result in defective transcription, processing and translation of beta-globin mRNA. This leads to deficiency of the beta-globin chains required for normal hemoglobin synthesis.

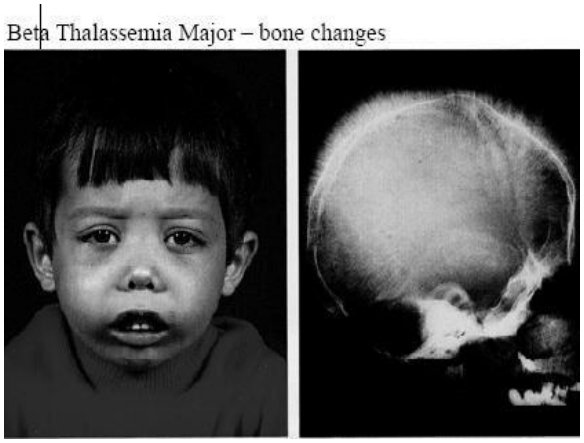
- Two genes are present on chromosome 11; mutations result in absent (β^0) or diminished ($ß^+$) production of the β-globin chain:

A. ß-Thalassemia minor (ß/ß⁺):
   - It is the mildest form of disease and is usually asymptomatic with an increased RBC count.
   - Microcytic, hypochromic RBCs and target cells are seen on blood smear.
   - Hemoglobin electrophoresis shows slightly decreased HbA with increased HbA_2 (5%, normal 2.5%) and HbF (2%, normal 1%).

B. Thalassemia major ($\beta^0\beta^0$):
   - It is the most severe form of disease and presents with severe anemia a few months after birth; high HbF ($\alpha_2\gamma_2$) at birth is temporarily protective (symptomatic only after 6 months, when fetal hemoglobin declines).
   - α Tetramers aggregate and damage RBCs, resulting in ineffective erythropoiesis and extravascular hemolysis (removal of circulating RBCs by the spleen)
   - Massive erythroid hyperplasia ensues resulting in:
     1. Expansion of hematopoiesis into the skull (reactive bone formation leads to 'crewcut' appearance on x-ray) and facial bones ('chipmunk facies), extra medullary hematopoiesis with hepatosplenomegaly.
     2. Risk of aplastic crisis with parvovirus B19 infection of erythroid precursors.
   - Chronic transfusions are often necessary; leads to risk for secondary hemochromatosis.
   - Smear shows microcytic, hypochromic RBCs with target cells and nucleated red blood cells.
   - Electrophoresis shows little or no HbA_1 with increased HbA_2, and HbF.

C. HbS/β-thalassemia heterozygote:
   - Mild to moderate sickle cell disease depending on amount of β-globin production.





❖ N.B:

1. Target cells form when erythrocytes have reduced cell volume (thalassemia, iron deficiency) or excessive membrane (obstructive liver disease, postsplenectomy).

- Patients who undergo splenectomy usually develop target cells because the spleen is the primary organ that prunes excessive red cell membrane.

2. The presence of erythroid precursor cells in the liver and spleen is indicative of extramedullary hematopoiesis, a condition characterized by erythropoietin-stimulated, hyperplastic marrow cell invasion of extramedullary organs. Extramedullary hematopoiesis is most frequently caused by severe chronic hemolytic anemias, such as β-thalassemia.

- Extramedullary hematopoiesis can cause a range of skeletal abnormalities. The expanding mass of progenitor cells in the bone marrow thins the bony cortex and impairs bone growth. Pathologic fractures are common in the most symptomatic of children. Maxillary overgrowth and frontal bossing are associated with the characteristic "chipmunk facies" observed in the pediatric population.

Macrocytic (MCV > 100 fL) anemia

- Anemia with MCV > 100 most commonly due to folate or vitamin B12 deficiency (megaloblastic anemia).

- Folate and vitamin B12 are necessary for synthesis of DNA precursors:

- Folate circulates in the serum as methyltetrahydrofolate (methyl THF); removal of the methyl group allows for participation in the synthesis of DNA precursors.

- Methyl group is transferred to vitamin B12 (Cobalamin).

- Vitamin B_{12} then transfers it to homocysteine, producing methionine.

- Lack of folate or vitamin B12 impairs synthesis of DNA precursors:

- Impaired division and enlargement of RBC precursors leads to megaloblastic anemia.

- Impaired division of granulocytic precursors leads to hypersegmented neutrophils.

- Megaloblastic change is also seen in rapidly-dividing (intestinal) epithelial cells.

- Other causes of macrocytic anemia (without megaloblastic change) include alcoholism, liver disease.

1. Megaloblastic anemia:

- Impaired DNA synthesis → maturation of nucleus of precursor cells in bone marrow delayed relative to maturation of cytoplasm → RBC macrocytosis, hypersegmented neutrophils (containing nuclei with >5 lobes), glossitis.

A. Folate deficiency:

- Dietary folate is obtained from green vegetables and some fruits.

- Absorbed in the jejunum.

- Folate deficiency develops within months, as body stores are minimal.

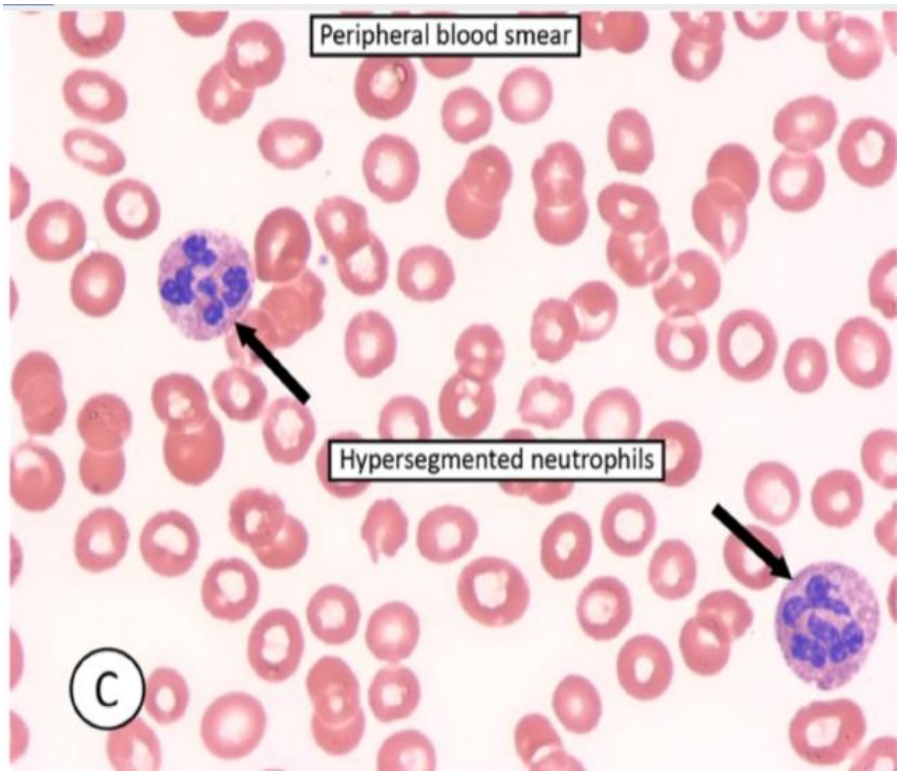
- Causes include poor diet (alcoholics and elderly), increased demand (pregnancy, cancer, and hemolytic anemia), and drugs (methotrexate, trimethoprim, phenytoin).

- Alcoholism is one of the most common causes of folate deficiency anemia due to poor dietary intake and impaired folate absorption, utilization, and enterohepatic recycling.

- Patients with sickle cell disease (SCD) or other hemolytic anemias are predisposed to develop folic acid deficiency due to increased erythrocyte turnover.

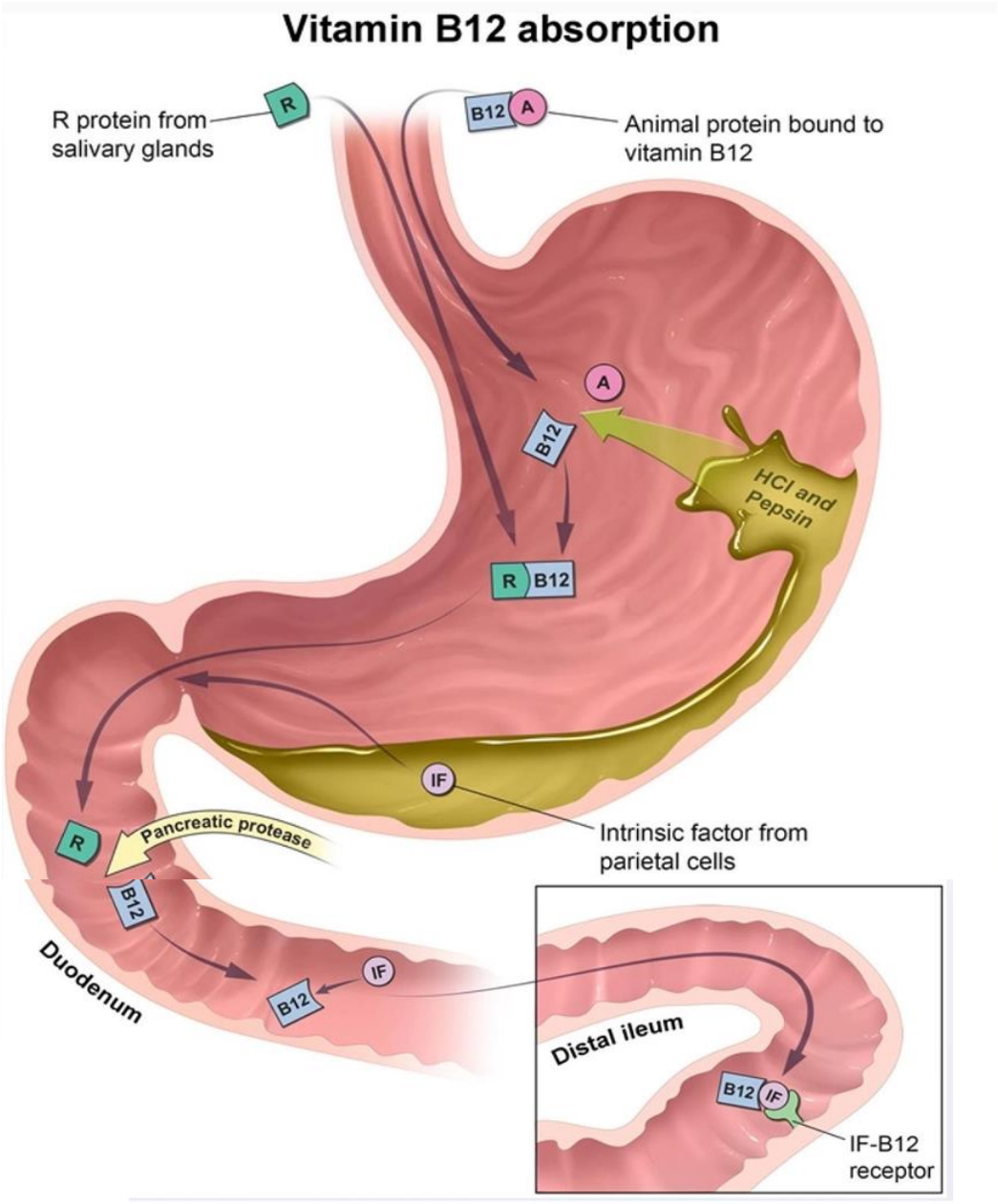
- Clinical and laboratory findings include:

- Macrocytic RBCs and hypersegmented neutrophils (> 5 lobes).
- Glossitis.
- ↓ serum folate.
- ↑ serum homocysteine (increases risk for thrombosis).
- Normal methylmalonic acid.
- No neurologic symptoms (vs B12 deficiency).



B. Vitamin B12 deficiency:

- Dietary vitamin B12 is complexed to animal-derived proteins:
  - Salivary gland enzymes (amylase) liberate vitamin B_{12}, which is then bound by R-binder (also from the salivary gland) and carried through the stomach.
  - Pancreatic proteases in the duodenum detach vitamin B_{12} from R-binder.
  - Vitamin B12 binds intrinsic factor (made by gastric parietal cells) in the small bowel; the intrinsic factor-vitamin B12 complex is absorbed in the ileum.



- Causes of vitamin B12 deficiency:
  - Vitamin B12 deficiency is less common than folate deficiency and takes years to develop due to large hepatic stores of vitamin B12.
  - Pernicious anemia is the most common cause of vitamin B12 deficiency. Autoimmune destruction of parietal cells (body of stomach) leads to intrinsic factor deficiency
  - Gastrectomy.
  - Pancreatic insufficiency.
  - Damage to the terminal ileum (Crohn disease or Diphyllobothrium latum [fish tapeworm]).
  - Dietary deficiency is rare, except in vegans.

- Clinical and laboratory findings include:

- Macrocytic RBCs with hypersegmented neutrophils.

- Glossitis.

- ↓ serum vitamin B12.

- ↑ serum homocysteine (similar to folate deficiency), which increases risk for thrombosis.

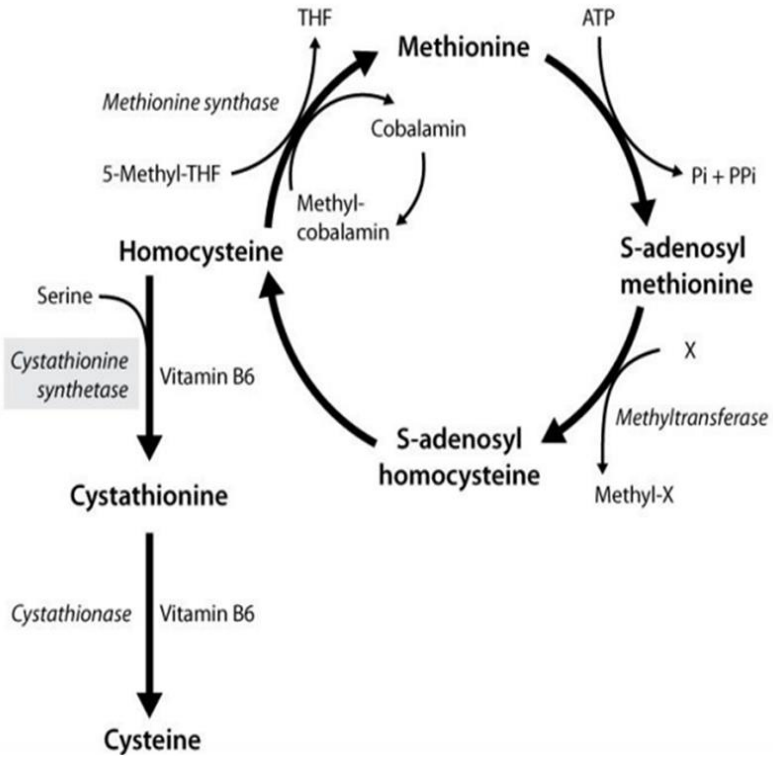
- ↑ methylmalonic acid (unlike folate deficiency).

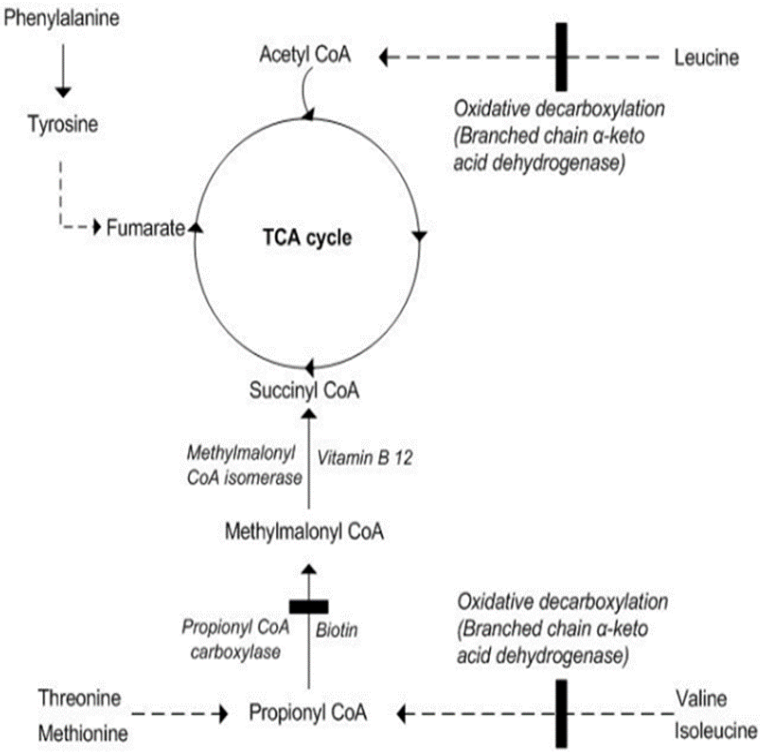
- Subacute combined degeneration of the spinal cord. Vitamin B12 is a cofactor for the conversion of methylmalonic acid to succinyl CoA (important in fatty acid metabolism).

- Vitamin B12 deficiency → accumulation of methylmalonic acid (which is toxic to myelin sheath) → patchy demyelination of:
  - Spinocerebellar tracts → Ataxic gait.
  - Corticospinal tract (UMNL Signs).
  - Dorsal columns (impaired vibratory and proprioception sensation).

- The reticulocyte count increases dramatically once vitamin B12 replacement therapy is initiated in an individual with pernicious anemia.

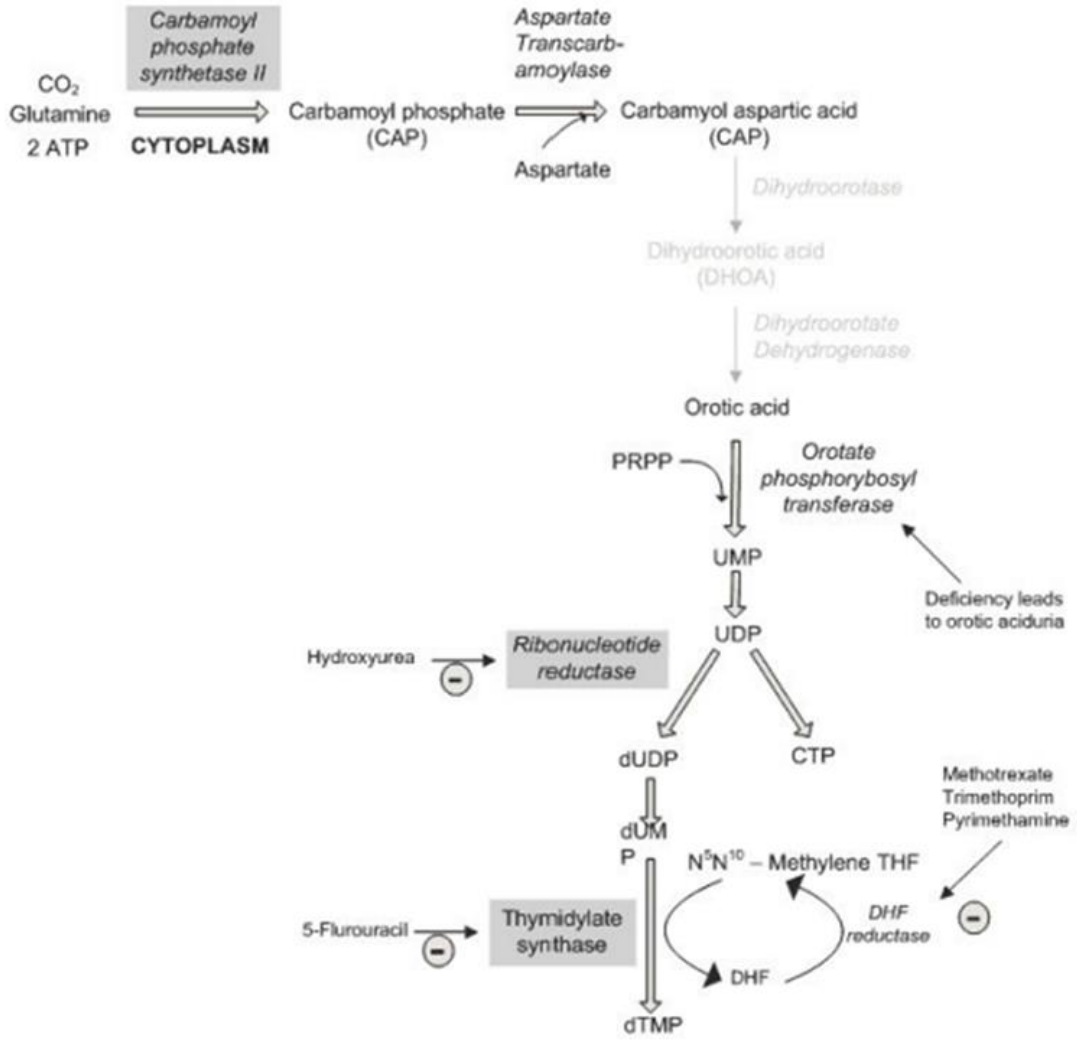
- If megaloblastic anemia due to vitamin B_{12} deficiency is mistakenly treated with folate alone, the neurologic dysfunction can worsen.





C. Orotic aciduria:

- Inability to convert orotic acid to UMP (de novo pyrimidine synthesis pathway) because of defect in UMP synthase.
- Autosomal recessive.
- Presents in children as failure to thrive, developmental delay, and megaloblastic anemia refractory to folate and B_{12}.
- No hyperammonemia (vs ornithine transcarbamylase deficiency → ↑ orotic acid with hyperammonemia).
- Orotic acid in urine.
- Treatment: uridine monophosphate to bypass mutated enzyme.



2. Nonmegaloblastic anemia:

- Macrocytic anemia in which DNA synthesis is unimpaired.

- Causes: alcoholism, liver disease.

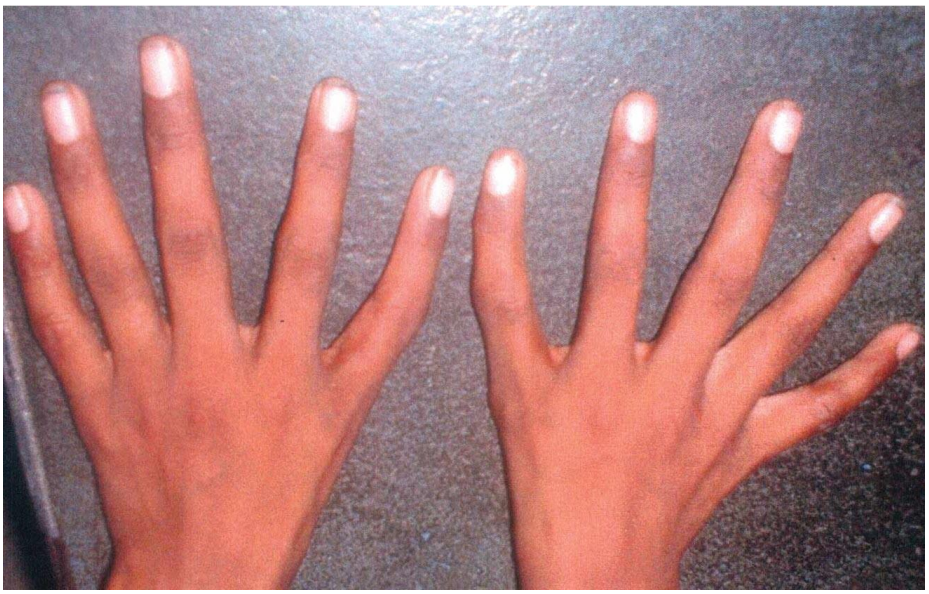
- RBC macrocytosis without hypersegmented neutrophils.

- Diamond-Blackfan anemia:

- Rapid-onset anemia within 1st year of life due to intrinsic defect in erythroid progenitor cells that affect ribosomal synthesis.

- ↑ % HbF (but ↓ total Hb).

- Short stature, craniofacial abnormalities, and upper extremity malformations (triphalangeal thumbs) in up to 50% of cases.



Normocytic, normochromic anemia

- Anemia with normal-sized RBCs (MCV = 80-100).
- Due to increased peripheral destruction (hemolytic) or underproduction (nonhemolytic).
- The hemolytic anemias are further classified according to the cause of the hemolysis (intrinsic vs extrinsic to the RBC) and by the location of the hemolysis (intravascular vs extravascular).
- Reticulocyte count helps to distinguish between these two etiologies.
- Reticulocytosis:

- Normal reticulocyte count (RC) is 1-2%.
- RBC lifespan is 120 days; each day roughly 1-2% of RBCs are removed from circulation and replaced by reticulocytes.
- A properly functioning marrow responds to anemia by increasing the RC to >3%.
- RC, however, is falsely elevated in anemia.
- RC is measured as percentage of total RBCs; decrease in total RBCs falsely elevates percentage of reticulocytes.
- RC is corrected by multiplying reticulocyte count by Hct/45:
  - Corrected count > 3% indicates good marrow response and suggests peripheral destruction.
  - Corrected count < 3% indicates poor marrow response and suggests underproduction.

1. Hemolytic, normocytic anemia:

- The hemolytic anemias are further classified according to the cause of the hemolysis (intrinsic vs extrinsic to the RBC) and by the location of the hemolysis (intravascular vs extravascular). Both result in anemia with a good marrow response.

A. Normocytic anemias with predominant extravascular hemolysis:

- Extravascular hemolysis involves RBC destruction by the reticuloendothelial system (macrophages of the spleen, liver, and lymph nodes).
- Macrophages consume RBCs and break down hemoglobin:

- Globin is broken down into amino acids.
- Heme is broken down into iron and protoporphyrin; iron is recycled.
- Protoporphyrin is broken down into unconjugated bilirubin, which is bound lo serum albumin and delivered to the liver for conjugation and excretion into bile.

- Clinical and laboratory findings include:

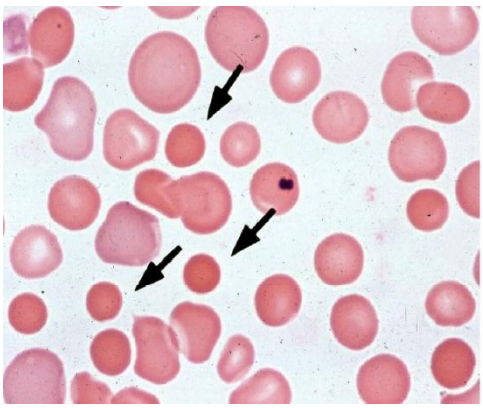
- Anemia with splenomegaly, jaundice due to unconjugated bilirubin, and increased risk for bilirubin gallstones.
- ↑ LDH, no hemoglobinuria/hemosiderinuria.
- Marrow hyperplasia with corrected reticulocyte count > 3%.

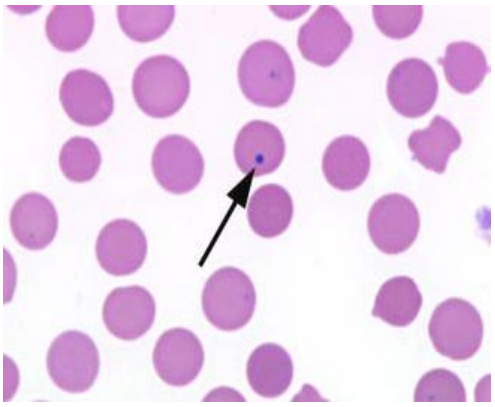
i. Hereditary spherocytosis (Intrinic):

- Inherited defect of RBC cytoskeleton-membrane tethering proteins.
- Most commonly involves spectrin, ankyrin, or band 3.
- Membrane blebs are formed and lost over time.
- Loss of membrane renders cells round (spherocytes) instead of disc-shaped.
- Spherocytes are approximately two-thirds the diameter of normal RBCs, are more densely hemoglobinized at the periphery, and often lack a zone of central pallor. Spherocytes also stain a deeper red than do normal RBCs when viewed on Wright stain.
- Spherocytes are less able to pass through splenic sinusoids and are consumed by splenic macrophages, resulting in anemia.
- Clinical and laboratory findings include:

- Spherocytes with loss of central pallor.
- ↑ RDW and ↑ mean corpuscular hemoglobin concentration (MCHC).
- Splenomegaly (work hypertrophy).
- Jaundice with unconjugated bilirubin, and increased risk for bilirubin gallstones (extravascular hemolysis).
- Increased risk for aplastic crisis with parvovirus B_{19} infection of erythroid precursors.

- Diagnosed by osmotic fragility test, which reveals increased spherocyte fragility in hypotonic solution.
- Treatment is splenectomy: anemia resolves, but spherocytes persist and Howell jolly bodies (fragments of nuclear material in RBCs) emerge on blood smear.



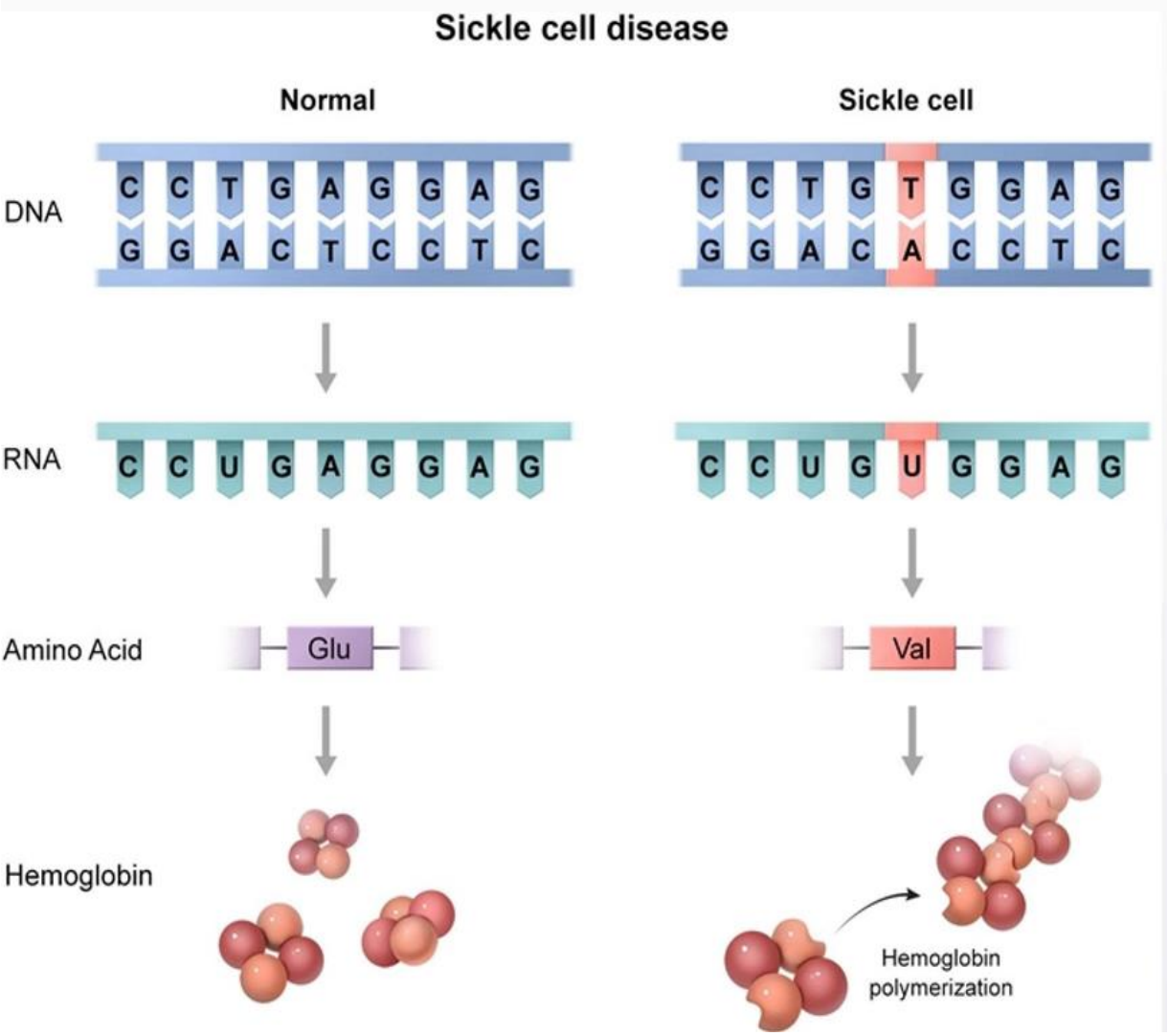


ii. Sickle cell anemia (Intrinsic):

- Autosomal recessive mutation in β chain of hemoglobin; a single amino acid change replaces normal glutamic acid (hydrophilic) with valine (hydrophobic).

- Gene is carried by 10% of individuals of African descent, likely due to protective role against falciparum malaria.

- Sickle cell disease arises when two abnormal β genes are present; results in > 90% HbS in RBCs.



- HbS polymerizes when deoxygenated; polymers aggregate into needle-like structures, resulting in sickle cells:

- Increased risk of sickling occurs with hypoxemia, dehydration, and acidosis.

- HbF protects against sickling; high HbF at birth is protective for the first few months of life.

- Treatment with hydroxyurea increases levels of HbF.

- Cells continuously sickle and de-sickle while passing through the microcirculation, resulting in complications related to RBC membrane damage:

- Extravascular hemolysis: Reticuloendothelial system removes RBCs with damaged membranes, leading to anemia, jaundice with unconjugated hyperbilirubinemia, and increased risk for bilirubin gallstones.

- Intravascular hemolysis: RBCs with damaged membranes dehydrate, leading to hemolysis with decreased haptoglobin and target cells on blood smear.

- Massive erythroid hyperplasia ensues resulting in:
  - Expansion of hematopoiesis into the skull ('crewcut' appearance on x-ray) and facial bones ('chipmunk facies').

  - Extramedullary hematopoiesis with hepatosplenomegally.

  - Risk of aplastic crisis with parvovirus B_{19} infection of erythroid precursors.

- Irreversible sickling leads to complications of vaso-occlusion:

A. Dactylitis: Swollen hands and feet due to vaso-occlusive infarcts in bones; common presenting sign in infants.

B. Autosplenectomy (Shrunken, fibrotic spleen). Consequences include:
  - Increased risk of infection with encapsulated organisms such as Streptococcus pneumoniae and Haemophilus influenzae, Neisseria meningitidis, salmonella typhi (most common cause of death in children); affected children should be vaccinated by 5 years of age.

  - Increased risk of Salmonella osteomyelitis.

  - Howell-Jolly bodies on blood smear.

C. Acute chest syndrome:
  - Vaso-occlusion in pulmonary microcirculation.

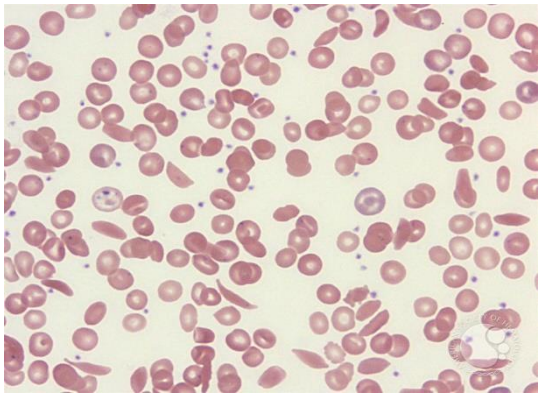
  - Presents with chest pain, shortness of breath, and lung infiltrates.

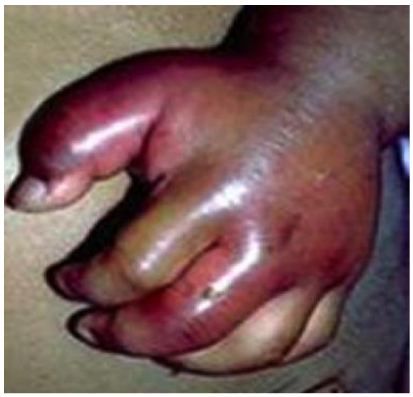
  - Often precipitated by pneumonia.

- Most common cause of death in adult patients.

D. Pain crisis: Patients with SCD may experience pain from hypoxic tissue injury.

E. Renal papillary necrosis: results in gross hematuria and proteinuria.





❖ Sickle cell trait:

- It is the presence of one mutated and one normal β chain; resulls in < 50% HbS in RBCs (HbA is slightly more efficiently produced than HbS).

- Generally asymptomatic with no anemia; RBCs with < 50% HbS do not sickle in vivo except in the renal medulla.

- Extreme hypoxia and hypertonicity of the medulla cause sickling, which results in microinfarctions leading to microscopic hematuria and, eventually, decreased ability to concentrate urine.

- Laboratory findings:

- Sickle cells and target cells are seen on blood smear in sickle cell disease, but not in sickle cell trait.

- Metabisulfite screen causes cells with any amount of HbS to sickle; positive in both disease and trait.

- Hb electrophoresis confirms the presence and amount of HbS:

- Disease: 90% HbS, 8% HbF, 2% HbA_2 (no HbA_1).
- Trait: 55% HbA_1, 43% HbS, 2% HbA_2.

F. Hemoglobin C:

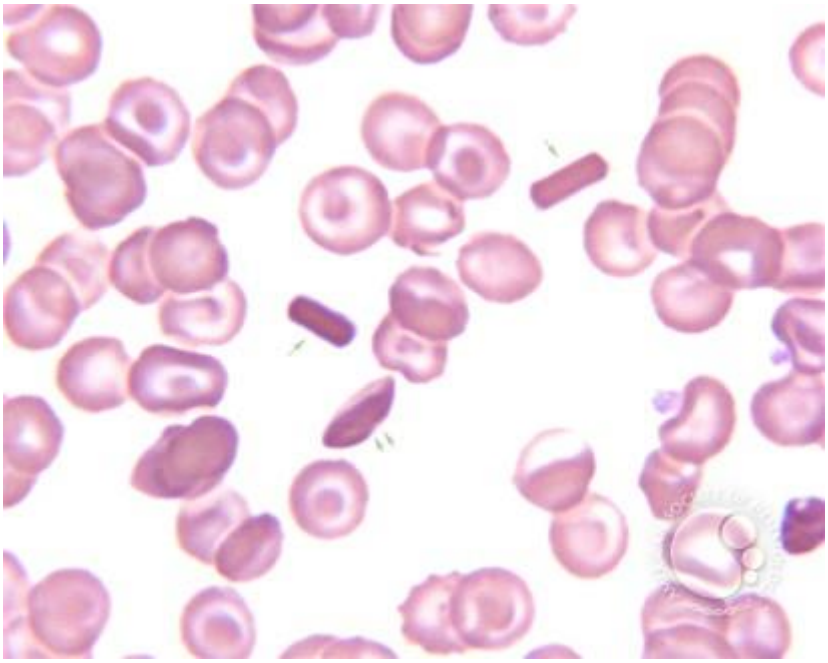
- Autosomal recessive mutation in β chain of hemoglobin.

- Normal glutamic acid is replaced by lysine → Hb C is less soluble → aggregates in crystals → RBC's rigidity → extravascular hemolysis.

- Less common than sickle cell daisese.

- Presents with mild anemia due to extravascular hemolysis.

- Characteristic HbC crystals are seen in RBCs on blood smear.



B. Normocytic anemia with predominant intravascular hemolysis:

- Intravascular hemolysis involves destruction of RBCs within vessels.

- Clinical and laboratory findings include:

- Hemoglobinemia.

- Hemoglobinuria.

- Hemosiderinuria: Renal tubular cells pick up some of the hemoglobin that is filtered into the urine and break it down into iron, which accumulates as hemosiderin; tubular cells are eventually shed resulting in hemosiderinuria.

- Decreased serum haptoglobin (Haptoglobin binds circulating hemoglobin and reduces renal excretion of free hemoglobin, preventing tubular injury).

i. Paroxysmal nocturnal hemoglobinuria (Intrinsic):

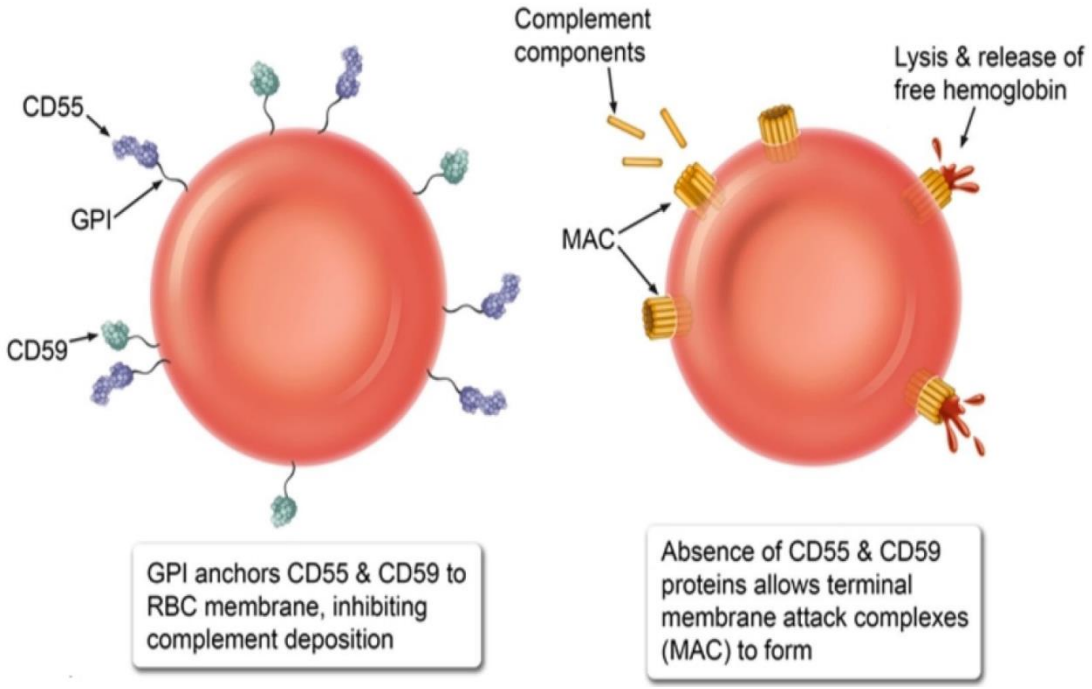
- Acquired defect in myeloid stem cells resulting in absent glycosyl phosphatidyl inositol (GPI); a glycolipid necessary for the attachment of several cell-surface proteins, including CD55 (decay-accelerating factor) and CD59 (MAC inhibitory protein).

- Blood cells coexist with complement.

- Decay accelerating factor (DAF) on the surface of blood cells protects against complement-mediated damage by inhibiting C3 convertase.

- DAF is secured to the cell membrane by GPI (an anchoring protein).

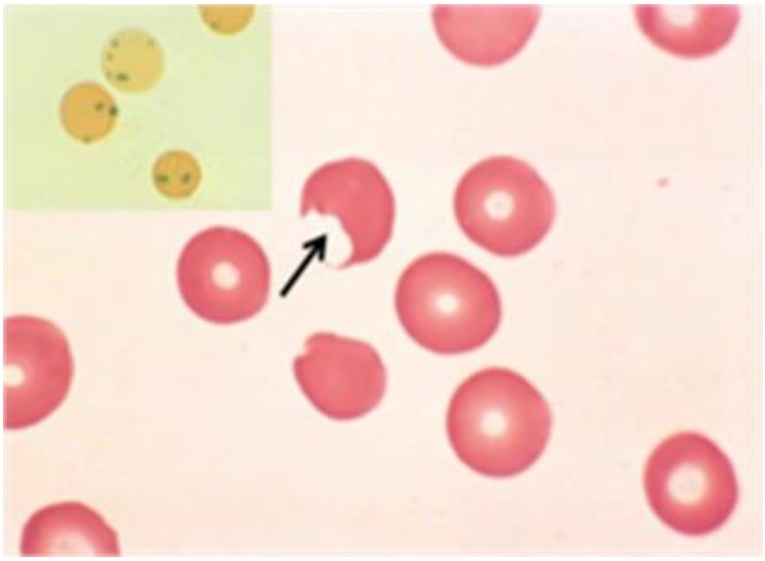
- Absence of GPI leads to absence of DAF, rendering cells susceptible to complement-mediated damage.



- Intravascular hemolysis occurs episodically, often at night during sleep. Mild respiratory acidosis develops with shallow breathing during sleep and activates complement.
- RBCs, WBCs, and platelets are also lysed → Pancytopenia.
- Intravascular hemolysis leads to hemoglobinemia and hemoglobinuria (especially in the morning); hemosiderinuria is seen days after hemolysis.
- Confirmatory test is the acidified serum test or flow cytometry to detect lack of CD55 (DAF) on blood cells.
- Main cause of death is thrombosis of the hepatic, portal, or cerebral veins. Destroyed platelets release cytoplasmic contents into circulation, inducing thrombosis.
- Suspect PNH in patients with hemolytic anemia, a hypercoagulable state, and pancytopenia.
- Complications include iron deficiency anemia (due to chronic loss of hemoglobin in the urine) and acute myeloid leukemia (AML), which develops in 10% of patients.

ii. Glucose 6 phosphate dehydrogenase deficiency (Intrinsic):

- X-linked recessive disorder (almost exclusive to males) resulting in reduced half-life of G6PD; renders cells susceptible to oxidative stress:
  - RBCs are normally exposed to oxidative stress, in particular H_2O_2.
  - Glutathione (an antioxidant) neutralizes H_2O_2 but becomes oxidized in the process.
  - NADPH, a by-product of G6PD, is needed to regenerate reduced glutathione.
  - ↓ G6PD → ↓ NADPH → ↓ reduced glutathione → oxidative injury by H_2O_2 → intravascular hemolysis.
- G6PD deficiency has two major variants:
  - African variant: mildly reduced half-life of G6PD leading to mild intravascular hemolysis with oxidative stress
  - Mediterranean variant: markedly reduced half-life of G6PD leading to marked intravascular hemolysis with oxidative stress.
- Causes of oxidative stress include infections, drugs (primaquine, sulfa drugs, and dapsone), and fava beans.
- Oxidative stress precipitates Hb as Heinz bodies.
- Heinz bodies are removed from RBCs by splenic macrophages, resulting in bite cells. Leads to predominantly intravascular hemolysis.
- Presents with hemoglobinuria and back pain hours after exposure to oxidative stress.



iii. Immunohemolytic anemia (Extrinsic):

- Antibody-mediated (IgG or IgM) destruction of RBCs.

A. IgG-mediated disease usually involves extravascular hemolysis:

- IgG binds RBCs in the relatively warm temperature of the central body (warm agglutinin); membrane of antibody-coated RBC is consumed by splenic macrophages, resulting in spherocytes.

- Associated with SLE (most common cause), CLL, and certain drugs (classically, penicillin and cephalosporins):
  - Drug may attach to RBC membrane (penicillin) with subsequent binding of antibody to drug-membrane complex.
  - Drug may induce production of autoantibodies (α-methyldopa) that bind self-antigens on RBCs.

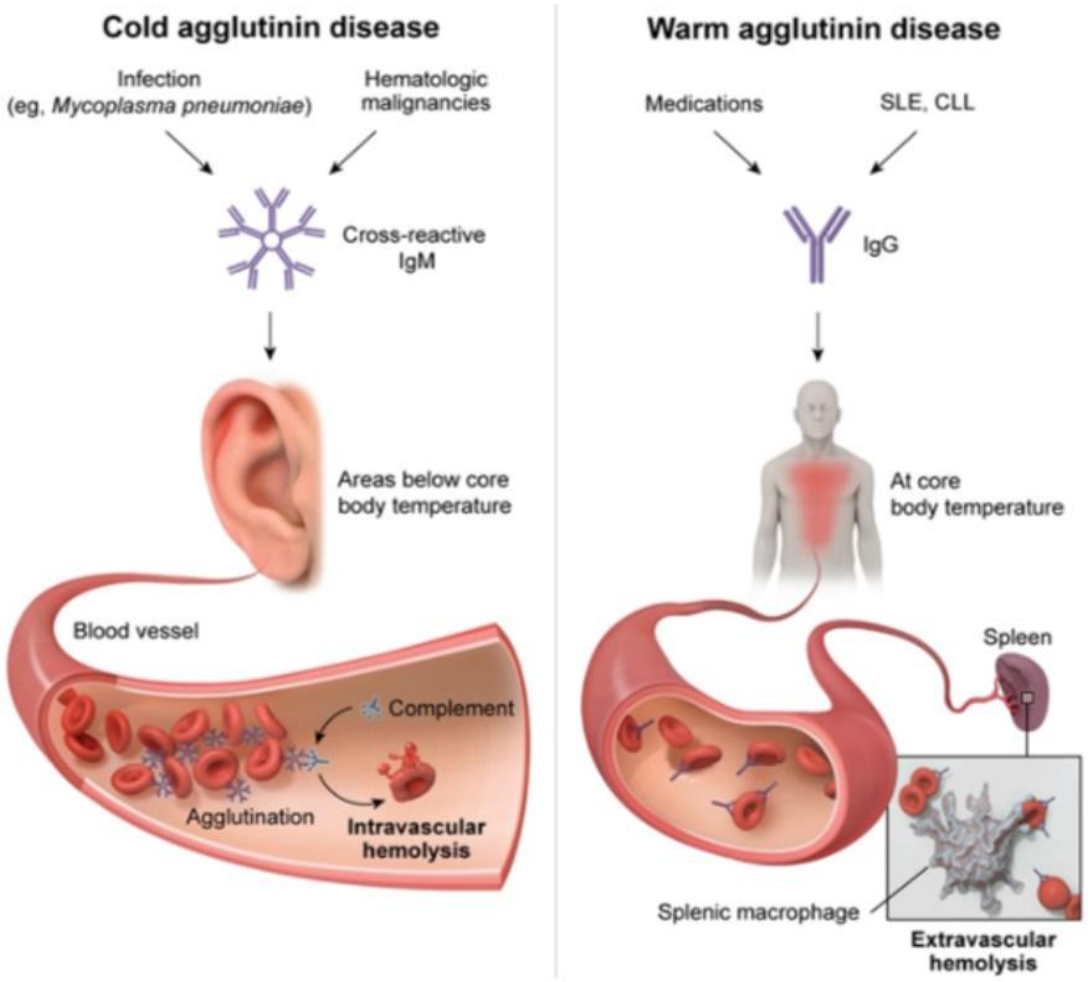
- Treatment involves cessation of the offending drug, steroids, IVIG, and, if necessary, splenectomy.

B. IgM-mediared disease usually involves intravascular hemolysis:

- IgM binds RBCs and fixes complement in the relatively cold temperature of the extremities (cold agglutinin).
- Associated with Mycoplasma pneumoniae and infectious mononucleosis.

- Coombs test is used to diagnose IHA; testing can be direct or indirect:
- Direct Coombs test: confirms the presence of antibody-coated RBCs. Anti-IgG is added to patient RBCs; agglutination occurs if RBCs are already coated with antibody. This is the most important test for IHA.

- Indirect Coombs test: confirms the presence of antibodies in patient serum. Anti-IgG and test RBCs are mixed with the patient serum; agglutination occurs if serum antibodies are present.



CLL = chronic lymphocytic leukemia; **SLE** = systemic lupus erythematosus.

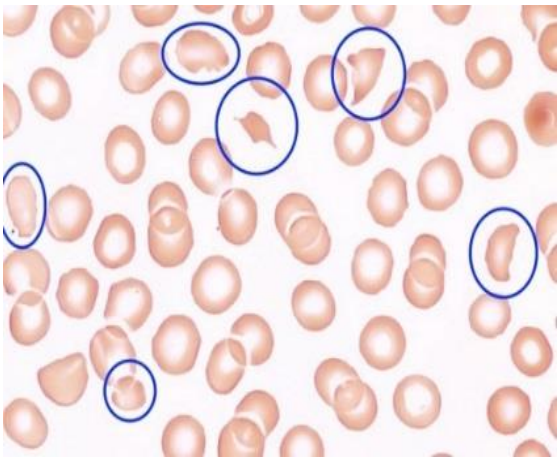
	Patient component	Reagent(s)	⊕ Result (agglutination)	⊖ Result (no agglutination)
Direct Coombs	RBCs +/– anti-RBC Ab	Anti-human globulin (Coombs reagent)	⊕ Result Anti-RBC Ab present	⊖ Result Anti-RBC Ab absent
Indirect Coombs	Patient serum +/– anti-donor RBC Ab	Donor blood Anti-human globulin (Coombs reagent)	⊕ Result Anti–donor RBC Ab present	⊖ Result Anti–donor RBC Ab absent

C. Microangiopathic hemolytic anemia (Extrinic):

- Intravascular hemolysis that results from vascular pathology; RBCs are destroyed as they pass through the circulation.
- Iron deficiency anemia occurs with chronic hemolysis.
- Occurs with microthrombi (TTP-HUS, DIC, HELLP, SLE, and malignant hypertension); microthrombi produce schistocytes (helmet cells) on blood smear.

D. Macroangiopathic hemolytic anemia (Extrinsic):

- Prosthetic heart valves and aortic stenosis may also cause hemolytic anemia 2° to mechanical destruction of RBCs.
- Schistocytes on peripheral blood smear.



E. Infections (Extrinsic):

- ↑ destruction of RBCs (malaria, Babesia).
- RBCs rupture as a part of the Plasmodium life cycle, resulting in intravascular hemolysis and cyclical fever.

2. Nonhemolytic, normocytic anemia:

- Decreased production of RBCs by bone marrow; characterized by low corrected reticulocyte count.

- Etiologies include:

- Renal failure: decreased production of EPO by peritubular interstitial cells.
- Damage to bone marrow precursor cells (may result in anemia or pancytopenia).

A. Anemia of chronic disease:

- Anemia associated with chronic inflammation (endocarditis or autoimmune conditions) or cancer; most common type of anemia in hospitalized patients.

- Associated with conditions such as rheumatoid arthritis, SLE, neoplastic disorders, and chronic kidney disease.

- Chronic disease results in production of acute phase reactants from the liver, including hepcidin.

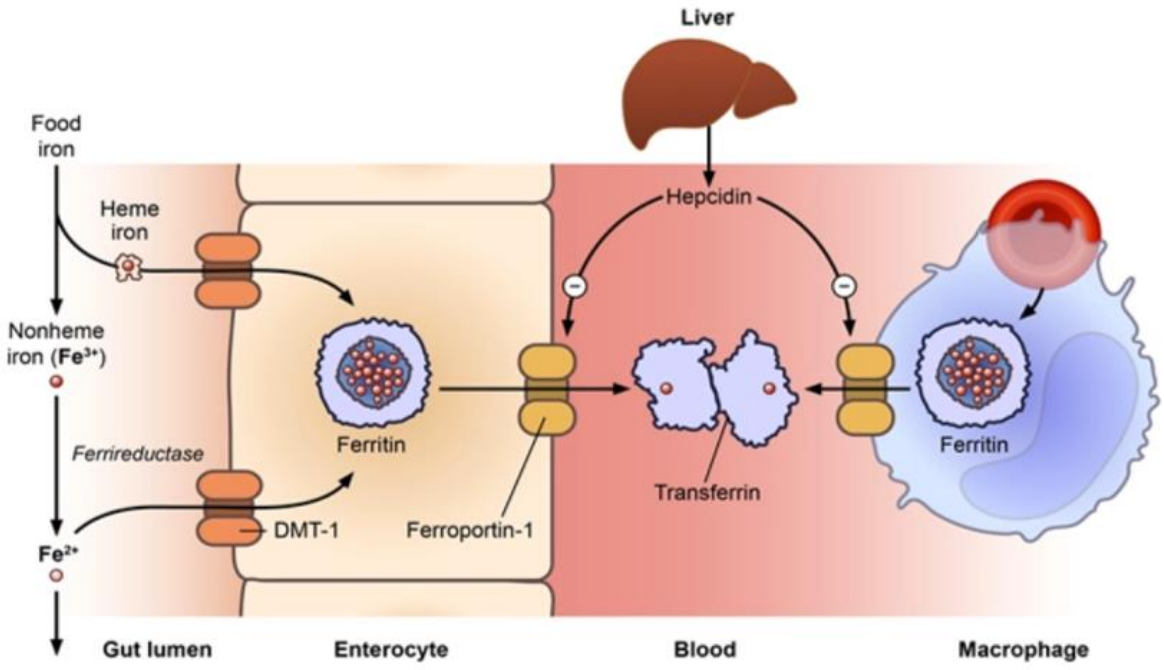
- Hepcidin sequesters iron in storage sites by:

- Binding ferroportin on intestinal mucosal cells and macrophages, thus inhibiting iron transport) → ↓ release of iron from macrophages and ↓ iron absorption from gut. Aim is to prevent bacteria from accessing iron, which is necessary for their survival.

- Laboratory findings include:

- ↑ ferritin, ↓ TIBC, ↓ serum iron, and ↓ % saturation.
- ↑ Free erythrocyte protoporphyrin (FEP).

- Treatment involves addressing the underlying cause; exogenous EPO is useful in a subset of patients, especially those with cancer.



B. Aplastic anemia:

- Damage to hematopoietic stem cells, resulting in pancytopenia (anemia, thrombocytopenia, and leukopenia) with low reticulocyte count (not to be confused with aplastic crisis, which causes anemia only).

- Etiologies include:

- Idiopathic (immune mediated, 1° stem cell defect); may follow acute hepatitis.

- Radiation and drugs (benzene, chloramphenicol, alkylating agents, antimetabolites).

- Viral agents (parvovirus B19, EBV, HIV, hepatitis viruses).

- Fanconi anemia (DNA repair defect causing bone marrow failure); also, short stature, ↑ incidence of tumors/leukemia, café-au-lait spots, thumb/radial defects.

- ↓ reticulocyte count, ↑ EPO (A compensatory increase in circulating erythropoietin levels would be expected in individuals with aplastic anemia and normal renal function).

- Bone marrow aspiration is usually "dry" and histopathology shows marrow replacement with fat cells and fibrous stroma.

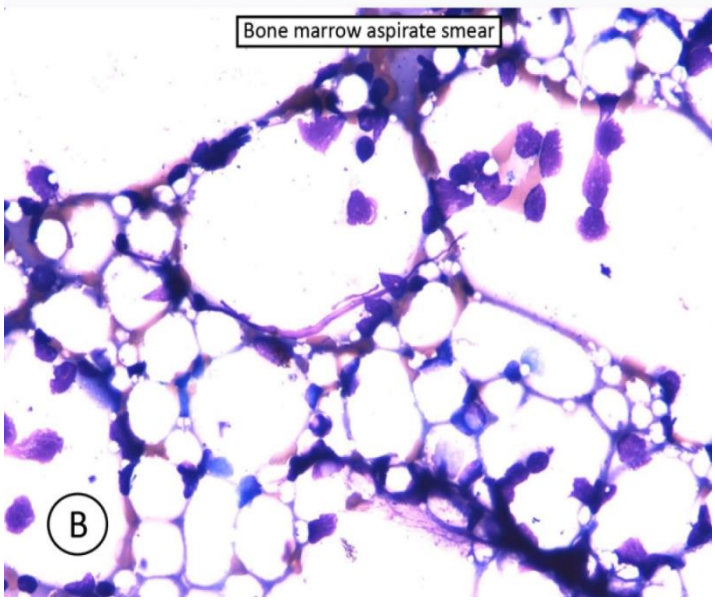
- Symptoms: fatigue, malaise, pallor, purpura, mucosal bleeding, petechiae, infection.

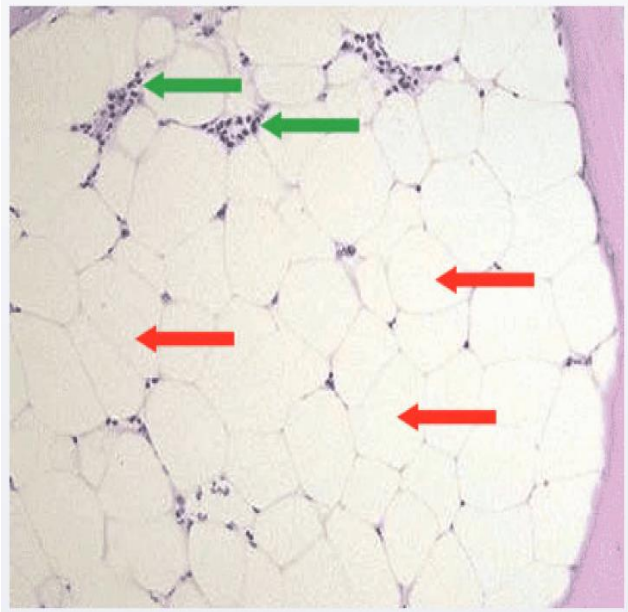
- Treatment:

- Cessation of any causative drugs and supportive care with transfusions and marrow-stimulating factors (erythropoietin, GM-CSF, and G-CSF).

- Immunosuppression may be helpful as some idiopathic cases are due to abnormal T-cell activation with release of cytokines.

- May require bone marrow transplantation as a last resort





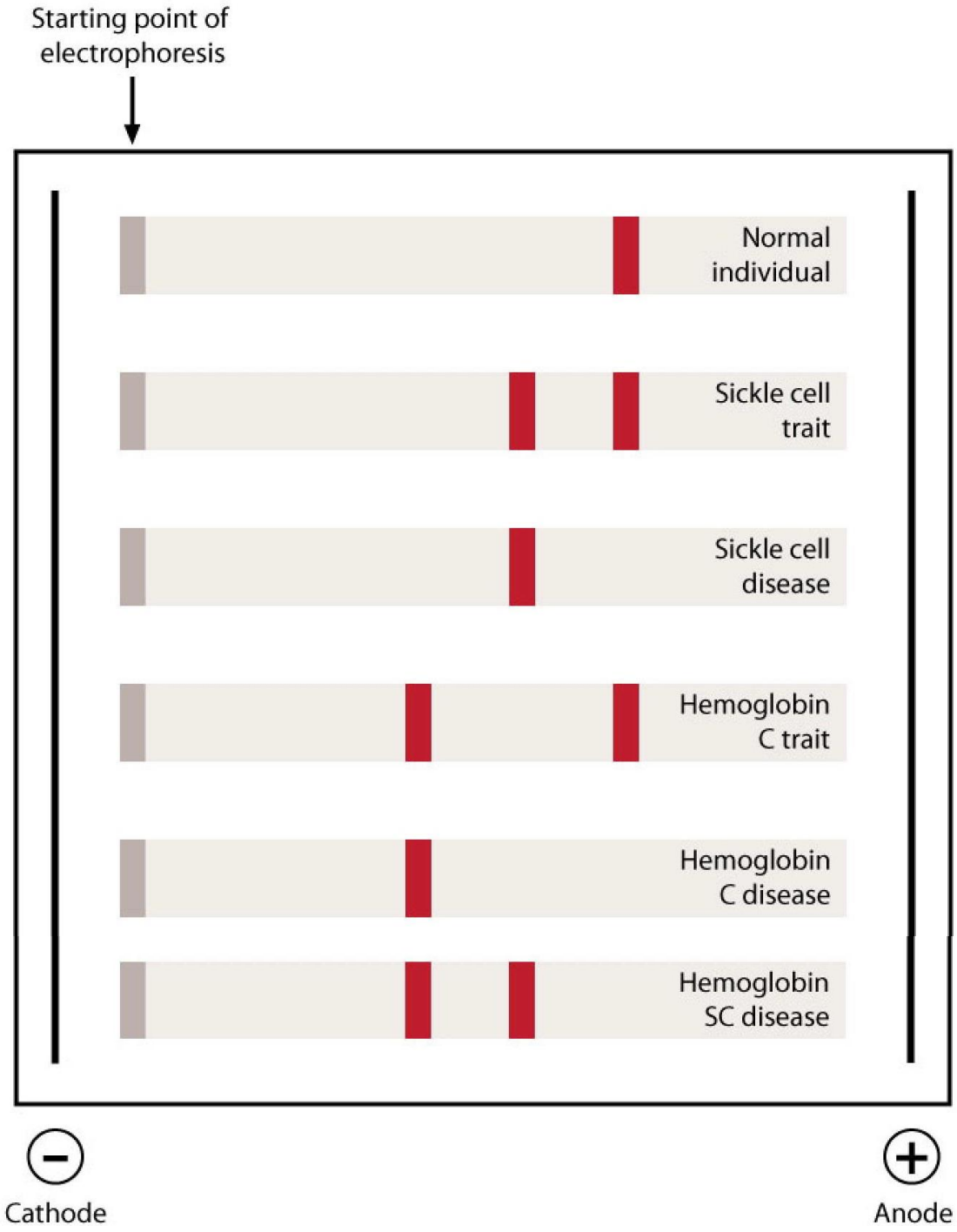
❖ Pure red cell aplasia (PRCA):

- Pure red cell aplasia (PRCA) is a rare form of marrow failure characterized by severe hypoplasia of marrow erythroid elements in the setting of normal granulopoiesis and thrombopoiesis.
- The pathogenesis of PRCA often involves the inhibition of erythropoietic precursors and progenitors by IgG autoantibodies or cytotoxic T lymphocytes.
- It has been associated with immune system diseases such as thymomas and lymphocytic leukemias.
- When a thymoma is present, removal can occasionally cure PRCA. Thus, all patients with PRCA should undergo a chest CT scan.
- PRCA can also result from parvovirus B19 infection. This virus preferentially attacks and destroys proerythroblasts. Recent parvovirus infection can be confirmed via the detection of anti-B19 IgM antibodies in the serum.

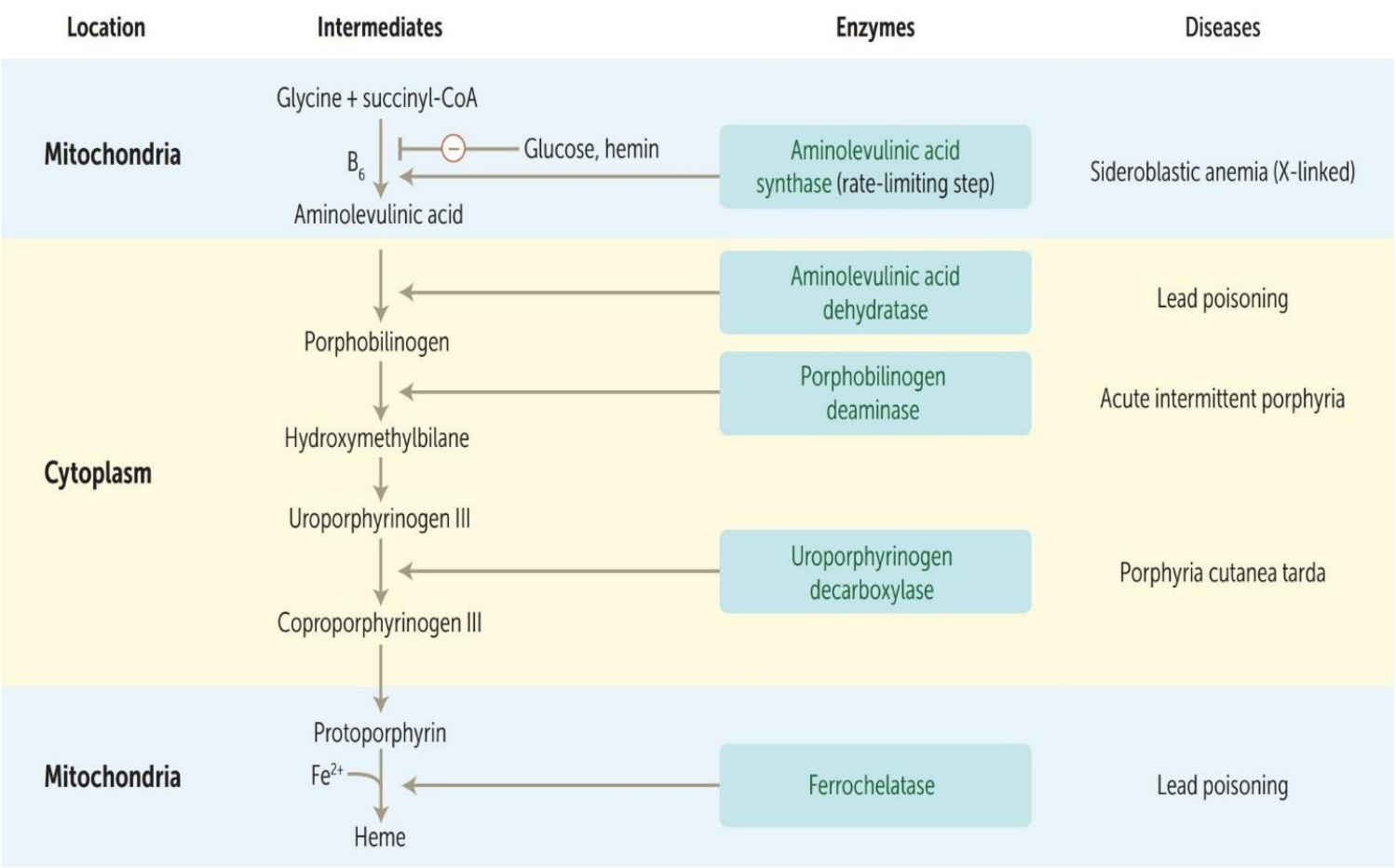
❖ Hemoglobin electrophoresis:

- Hemoglobin electrophoresis is used to assess for different forms of hemoglobin in patients with suspected hemoglobinopathy.
- Normal hemoglobin consists primarily of hemoglobin A (HbA), which migrates rapidly toward the positive electrode (anode) because of its negative charge.
- Both HbC and HbS result from missense mutations, a type of mutation in which a single base substitution results in a codon that codes for a different amino acid.
- Hemoglobin S (HbS) is an abnormal type of hemoglobin in which a nonpolar amino acid (valine) replaces a negatively charged amino acid (glutamate) in the beta globin chain. This amino acid replacement decreases the negative charge on the HbS molecule, which causes HbS to move more slowly toward the anode.
- Similarly, hemoglobin C (HbC) has a glutamate residue replaced by lysine in the beta globin chain. Because lysine is a positively charged amino aqjd, HbC has even less total negative charge than HbS and moves even more slowly toward the anode.
- Patients with sickle cell disease have HbS mutations in both beta chains; those with HbC disease have HbC mutations involving both beta chains. Patients with hemoglobin SC disease have 1 HbS allele and 1 HbC allele and will have 2 hemoglobin bands on electrophoresis.
- The speed of hemoglobin movement during gel electrophoresis is hemoglobin A > hemoglobin S > hemoglobin C.

Hemoglobinopathy electrophoresis patterns



Porphyria



↓ heme → ↑ ALA synthase activity
↑ heme → ↓ ALA synthase activity

Acute intermittent porphyria

- Enzyme deficiency: Porphobilinogen deaminase, previously called uroporphyrinogen I synthase (autosomal dominant mutation).

- Acculamted substrate: Porphobilinogen, ALA.

- Symptoms (5 P's):

- Painful abdomen.
- Port wine-colored Pee.
- Polyneuropathy.
- Psychological disturbances.
- Precipitated by drugs (cytochrome P-450 inducers), alcohol, starvation

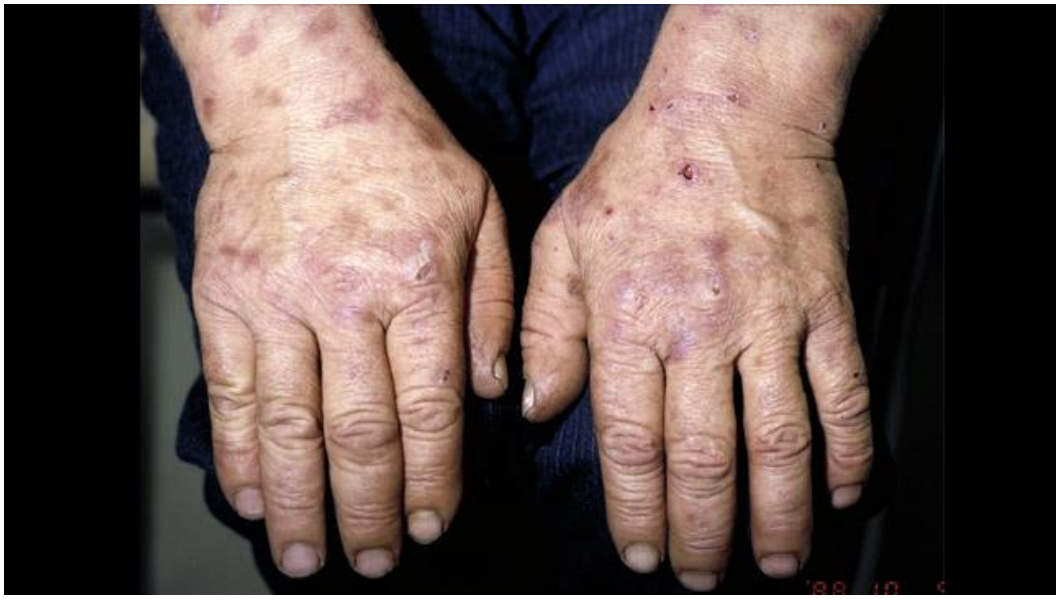
- Treatment: hemin and glucose.

❖ N.B:

- ALA synthase is upregulated by CYP450 inducers (most antiepileptics, griseofulvin, rifampin) and downregulated by heme and glucose.
- As such, avoidance of alcohol, smoking, and other CYP450-inducing drugs is important for preventing acute attacks. Likewise, intravenous heme administration and carbohydrate loading (such as with dextrose infusion) are useful for ameliorating acute symptoms.

Porphyria cutanea tarda

- Most common porphyria.
- Enzyme deficiency: Uroporphyrinogen decarboxylase.
- Acculamted substrate: Uroporphyrin (tea-colored urine)
- Symptoms: Blistering cutaneous photosensitivity and hyperpigmentation (Uroporphyrin has aromatic chain which absorp light → photosensitivity) .
- Exacerbated with alcohol consumption.
- Associated with hepatitis C.
- Treatment: phlebotomy, sun avoidance, antimalarials (hydroxychloroquine).



❖ N.B:

- In general, enzyme deficiencies in the early steps of porphyrin synthesis cause neurovisceral symptoms (acute porphyrias); deficiencies in the latter steps (after condensation of PBG to HMB) result in photosensitivity (cutaneous porphyrias).

❖ Iron poisoning:

	Acute	Chronic
Findings	High mortality rate with accidental ingestion by children (adult iron tablets may look like candy)	Seen in patients with 1° (hereditary) or 2° (chronic blood transfusions for thalassemia or sickle cell disease) hemochromatosis.
Mechanism	Cell death due to peroxidation of membrane lipids	
Symptoms/signs	Nausea, vomiting, gastric bleeding, lethargy, scarring leading to GI obstruction	Arthropathy, cirrhosis, cardiomyopathy, diabetes mellitus, hypogonadism.
Treatment	Chelation (IV deferoxamine, oral deferasirox) and gastric lavage.	Phlebotomy (patients without anemia) or chelation.

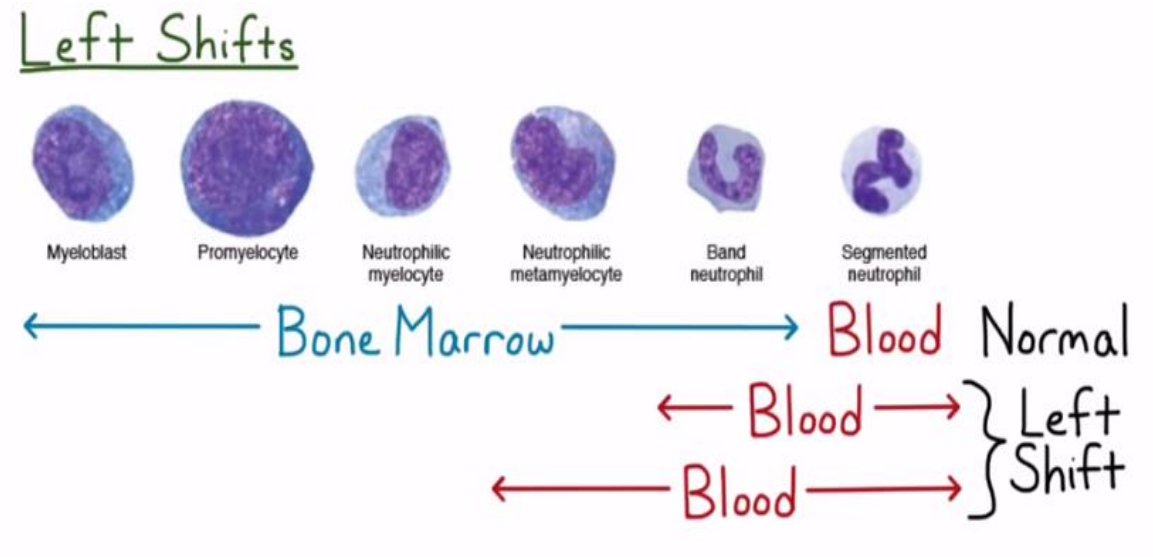
Leukopenias

CELL TYPE	CELL COUNT	CAUSES
Neutropenia	Absolute neutrophil count < 1500 cells/mm^3 Severe infections typical when < 500 cells/mm^3	Sepsis/postinfection, drugs (including chemotherapy), aplastic anemia, SLE, radiation
Lymphopenia	Absolute lymphocyte count < 1500 cells/mm^3 (< 3000 cells/mm^3 in children)	HIV, DiGeorge syndrome, SCID, SLE, corticosteroids[a], radiation, sepsis, postoperative
Eosinopenia	Absolute eosinophil count < 30 cells/mm^3	Cushing syndrome, corticosteroids[a]

[a]Corticosteroids cause neutrophilia, despite causing eosinopenia and lymphopenia. Corticosteroids ↓ activation of neutrophil adhesion molecules, impairing migration out of the vasculature to sites of inflammation. In contrast, corticosteroids sequester eosinophils in lymph nodes and cause apoptosis of lymphocytes.

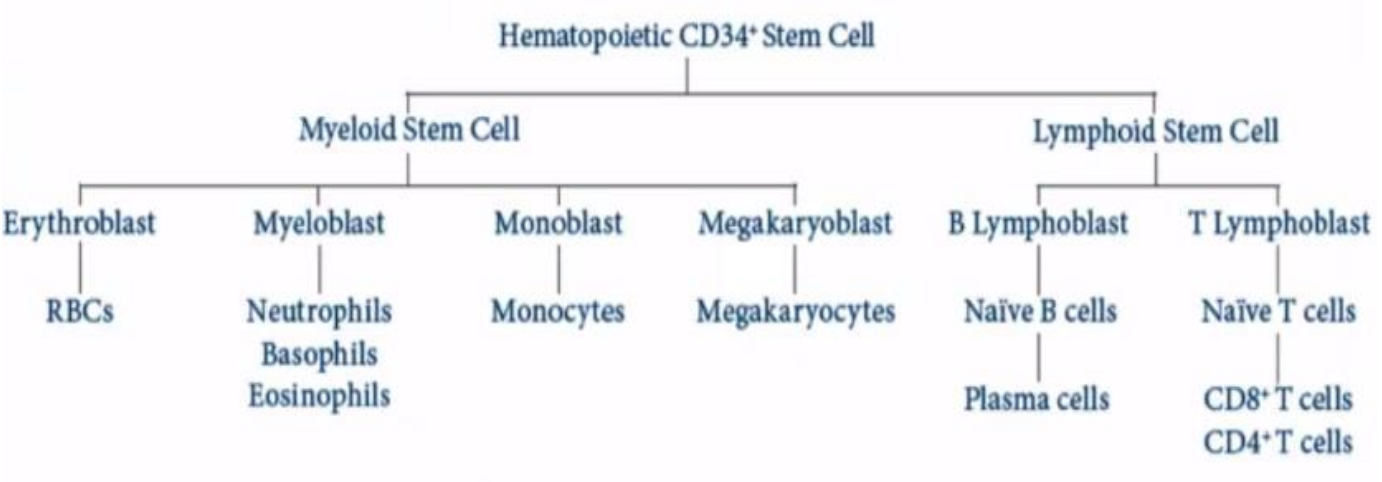
▪ Left shift:

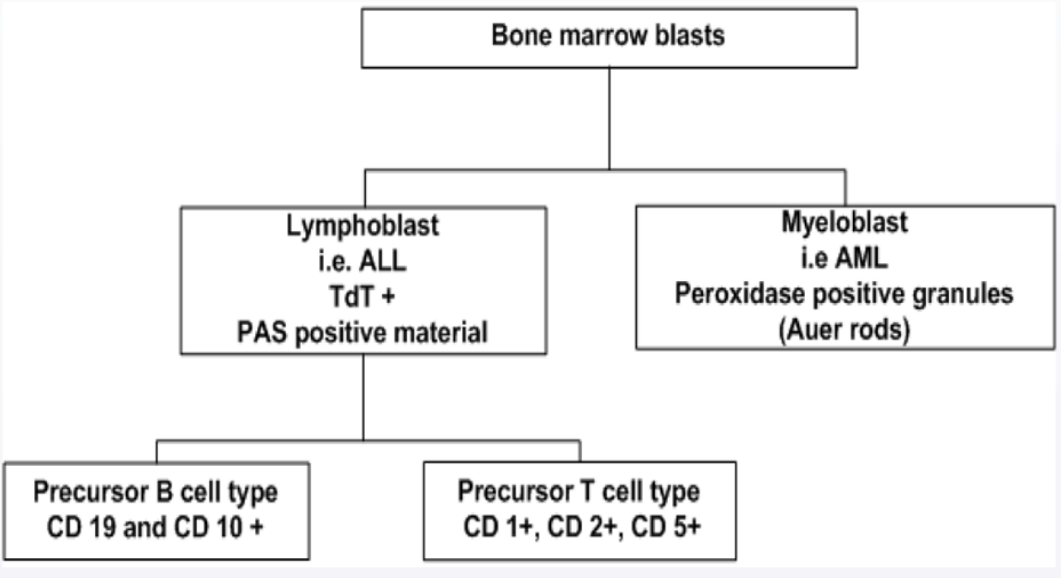
- A left shift is a shift to a more immature cell in the maturation process.
- ↑ neutrophil precursors, such as band cells and metamyelocytes, in peripheral blood.
- Usually seen with neutrophilia in the acute response to infection or inflammation.
- Called leukoerythroblastic reaction when left shift is seen with immature RBCs; occurs with severe anemia (physiologic response) or marrow response (fibrosis, tumor taking up space in marrow).

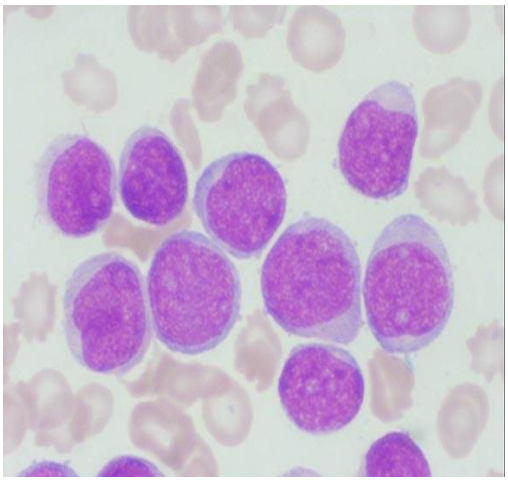

Left Shifts
Myeloblast
Promyelocyte
Neutrophilic myelocyte
Neutrophilic metamyelocyte
Band neutrophil
Segmented neutrophil
Bone Marrow
Blood
Normal
Blood
Blood
Left Shift

Acute leukemia

- Neoplastic proliferation of blasts; defined as the accumulation of > 20% blasts in the bone marrow.
- Increased blasts "crowd-out" normal hematopoiesis, resulting in an "acute" presentation with anemia (fatigue), thrombocytopenia (bleeding), or neutropenia (infection).
- Blasts usually enter the blood stream, resulting in a high WBC count.
- Blasts are large, immature cells, often with punched out nucleoli.
- Leukemic cell infiltration of liver, spleen, lymph nodes, and skin (leukemia cutis) possible.
- Acute leukemia is subdivided into acute lymphoblastic leukemia (ALL) or acute myelogenous leukemia (AML) based on the phenotype of the blasts.







Acute lymphoblastic leukemia

- Neoplastic accumulation of lymphoblasts (> 20%) in the bone marrow:
  - Lymphoblasts are characterized by positive nuclear staining for TdT, a DNA polymerase.
  - TdT is absent in myeloid blasts and mature lymphocytes.

- The most common pediatric malignancy is acute lymphoblastic leukemia (ALL).

- Most commonly arises in children; B-cell ALL is responsible for approximately 70-80% of all cases of ALL, whereas T-cell ALL accounts for 15-17% of all cases of ALL.

- Associated with Down syndrome (usually arises after the age of 5 years).

- Subclassified into B-ALL and T-ALL based-on surface markers. Precursor B-ALL and precursor T-ALL can only be distinguished by immunophenotyping.

- B-ALL is the most common type of ALL:
  - Usually characterized by lymphoblasts (TdT+) that express CD10, CD19, and CD20.

  - Excellent response to chemotherapy; requires prophylaxis to scrotum and CSF.

  - Prognosis is based on cytogenetic abnormalities:
    - t (12;21) has a good prognosis; more commonly seen in children.
    - t (9;22) has a poor prognosis; more commonly seen in adults (Philadelphia+ ALL).

- T-ALL is characterized by lymphoblasts (TdT+) that express markers ranging from CD2 to CD8 (CD3, CD4, CD7). The blasts do not express CD10. Usually presents in teenagers as a mediastinal (thymic) mass (called acute lymphoblastic lymphoma because the malignant cells form a mass) that can compress the great vessels, causing superior vena cava syndrome. It can also compress the esophagus causing dysphagia, while compression of the trachea may lead to dyspnea and stridor.

Acute myeloid leukemia

- Neoplastic accumulation of myeloblasts (> 20%) in the bone marrow.

- Myeloblasts are usually characterized by positive cytoplasmic staining for myeloperoxidase (MPO). Crystal aggregates of MPO may be seen as Auer rods.

- Most commonly arises in older adults (average age is 50-60 years).

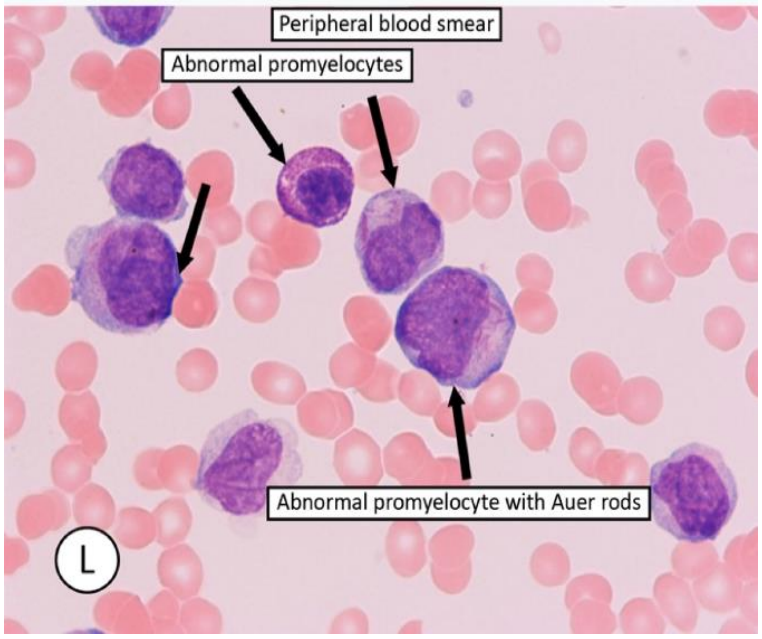
- Subclassified based on cytogenetic abnormalities, lineage of myeloblasts, and surface markers. High-yield subtypes include:

A. Acute promyelocytic leukemia (APL):
  - Characterized by t (15;17), which involves translocation of the retinoic acid receptor (RAR) on chromosome 17 to chromosome 15; RAR disruption blocks maturation and promyelocytes (blasts) accumulate.

- APL is referred to as M3 in the FAB classification of leukemia.

- Abnormal promyelocytes contain numerous primary granules (Auer rods) that increase the risk for DIC.

- Treatment is with all-trans-retinoic acid (ATRA, a vitamin A derivative), which binds the altered receptor and causes the blasts to mature and eventually die.



B. Acute monocytic leukemia:

- Proliferation of monoblasts; usually lack MPO.

- Blasts characteristically infiltrate gums.

C. Acute megakaryoblastic leukemia:

- Proliferation of megakaryoblasts; lack MPO.

- Associated with Down syndrome (usually arises before the age of 5).

❖ Myelodysplastic syndrome:

- Myelodysplastic syndromes usually present with cytopenia, hypercellular bone marrow, abnormal maturation of cells, and increased blasts (< 20%).

- AML may also arise from pre-existing dysplasia (myelodysplastic syndromes), especially with prior exposure to alkylating agents or radiotherapy.

- Most patients die from infection or bleeding, though some progress to acute leukemia.

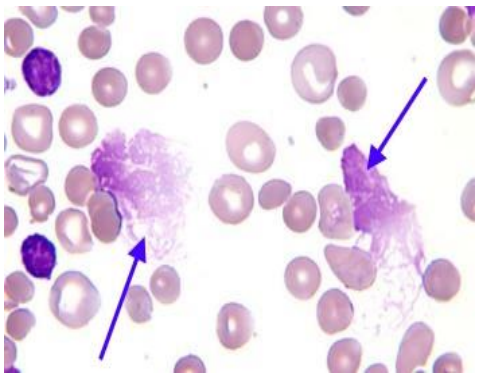
Chronic leukemia

- Neoplastic proliferation of mature circulating lymphocytes; characterized by a high WBC count.
- Usually insidious in onset and seen in older adults.

Chronic lymphocytic leukemia/Small lymphocytic lymphoma

- Neoplastic proliferation of naive B cells that co-express CD5 and CD20; most common leukemia overall.
- Age: > 60 years, often asymptomatic, progresses slowly.
- Increased lymphocytes and smudge cells (CLL = Crushed Little Lymphocytes) are seen on blood smear.
- Involvement of lymph nodes leads to generalized lymphadenopathy and is called small lymphocytic lymphoma (SLL).
- Complications include:

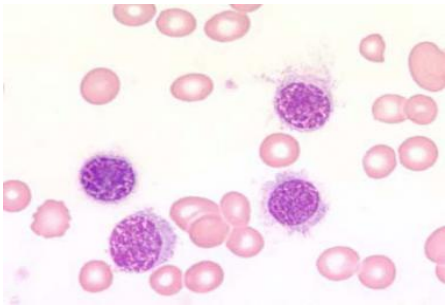
- Hypogammaglobinemia: Infection is the most common cause of death in CLL.
- Autoimmune hemolytic anemia.
- Transformation to diffuse large B-cell lymphoma (Richter transformation): marked clinically by an enlarging lymph node or spleen.



Hairy cell leukemia

- Neoplastic proliferation of mature B cells characterized by filamentous, hair-like projections (fuzzy appearing on LM).
- Cells are positive for tartrate-resistant acid phosphatase (trapped in a hairy situation).
- Clinical features include splenomegaly (due to accumulation of hairy cells in red pulp) and "dry tap" on bone marrow aspiration (due to marrow fibrosis).

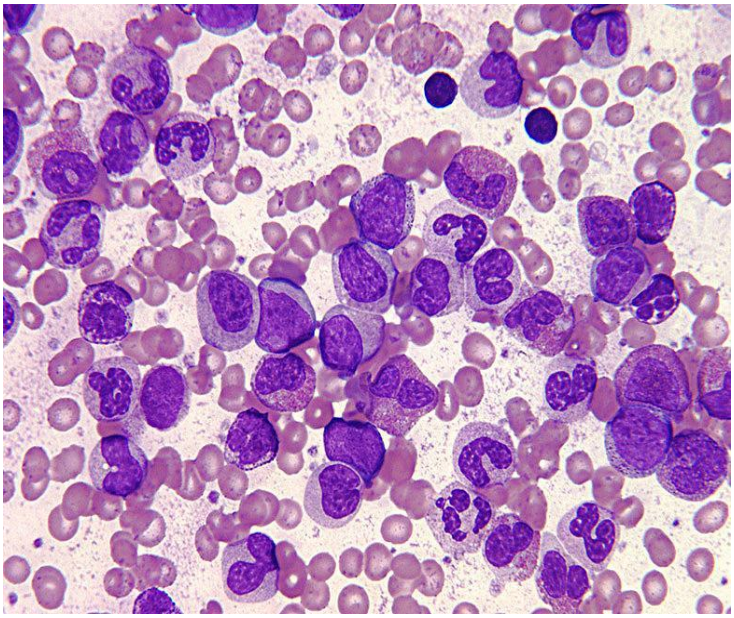
- Lymphadenopathy is usually absent.
- Excellent response to 2-CDA (cladribine), an adenosine deaminase inhibitor; adenosine accumulates to toxic levels in neoplastic B cells.

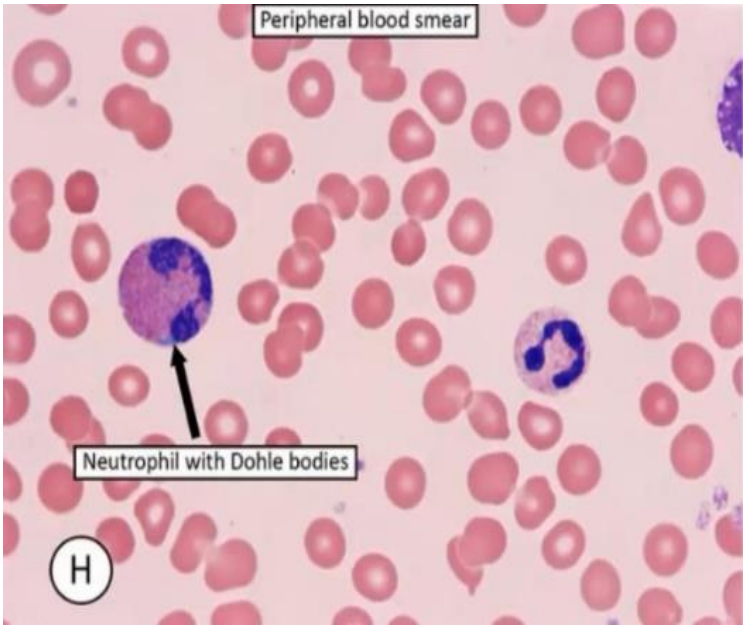


Chronic myeloid leukemia

- Neoplastic proliferation of mature myeloid cells, especially granulocytes and their precursors; basophils are characteristically increased.
- Driven by t(9;22) (Philadelphia chromosome) which generates a BCR-ABL fusion protein with increased tyrosine kinase activity.
- Occurs across the age spectrum with peak incidence 45-85 years, median age at diagnosis 64 years.
- First line treatment is imatinib, which blocks tyrosine kinase activity.
- Splenomegaly is common. Enlarging spleen suggests accelerated phase of disease; transformation to acute leukemia usually follows shortly thereafter.
- Can transform to AML (2/3 of cases) or ALL (1/3 of cases) since mutation is in a pluripotent stem cell ("blast crisis").
- CML is distinguished from a leukemoid reaction (reactive neutrophilic leukocytosis) by:

1. Negative leukocyte alkaline phosphatase (LAP) stain (granulocytes in a leukemoid reaction are LAP positive).
2. Increased basophils (absent with leukemoid reaction).
3. t(9;22) (absent in leukemoid reaction).
4. The peripheral smear on leukemoid reaction can show Dohle bodies, which are light blue (basophilic) peripheral granules in neutrophils. The blue color is likely due to ribosomes bound with rough endoplasmic reticulum.




Peripheral blood smear
Neutrophil with Dohle bodies
H

Myeloproliferative disorders

- Neoplastic proliferation of mature cells of myeloid lineage; disease of late adulthood average age is 50 - 60 years.
- Results in high WBC count with hypercellular bone marrow.
- Cells of all myeloid lineages are increased; classified based on the dominant myeloid cell produced.
- Associated with a mutation in Janus kinase 2 (JAK2), a non-receptor tyrosine kinase except CML (associated with Philadelphia chromosome).
- Complications include:

1. Increased risk for hyperuricemia and gout due to high turnover of cells.
2. Progression to marrow fibrosis.
3. Transformation to acute leukemia.

Polycythemia Vera

- Neoplastic proliferation of mature myeloid cells, especially RBCs.
- Granulocytes and platelets are also increased.
- Disorder of ↑ hematocrit.
- In polycythemia vera, mutations in JAK2 cause constitutive kinase activation, rendering hematopoietic cells more sensitive to growth factors such as erythropoietin and thrombopoietin.
- JAK2 mutations have also been implicated in essential thrombocythemia, primary myelofibrosis, and other myeloproliferative disorders.
- Clinical symptoms are mostly due to hyperviscosity of blood:

- Blurry vision and headache.
- Increased risk of venous thrombosis (hepatic vein, portal vein, and dural sinus).
- Rare but classic symptom is erythromelalgia (severe, burning pain and red-blue coloration) due to episodic blood clots in vessels of the extremities. Responds to aspirin.
- Flushed face due to congestion (plethora).
- Itching, especially after bathing due to histamine release from increased mast cells.
- In addition, reversible, moderate hypertension frequently occurs as a result of the expanded blood volume.

- Treatment is phlebotomy; second-line therapy is hydroxyurea, ruxolitinib (JAK1/2 inhibitor).

- Without treatment, death usually occurs within one year.

- <u>PV must be distinguished from reactive polycythemia:</u>

- In PV, erythropoietin (EPO) levels are decreased (vs 2° polycythemia, which presents with endogenous or artificially ↑ EPO), and Sao_2 is normal.

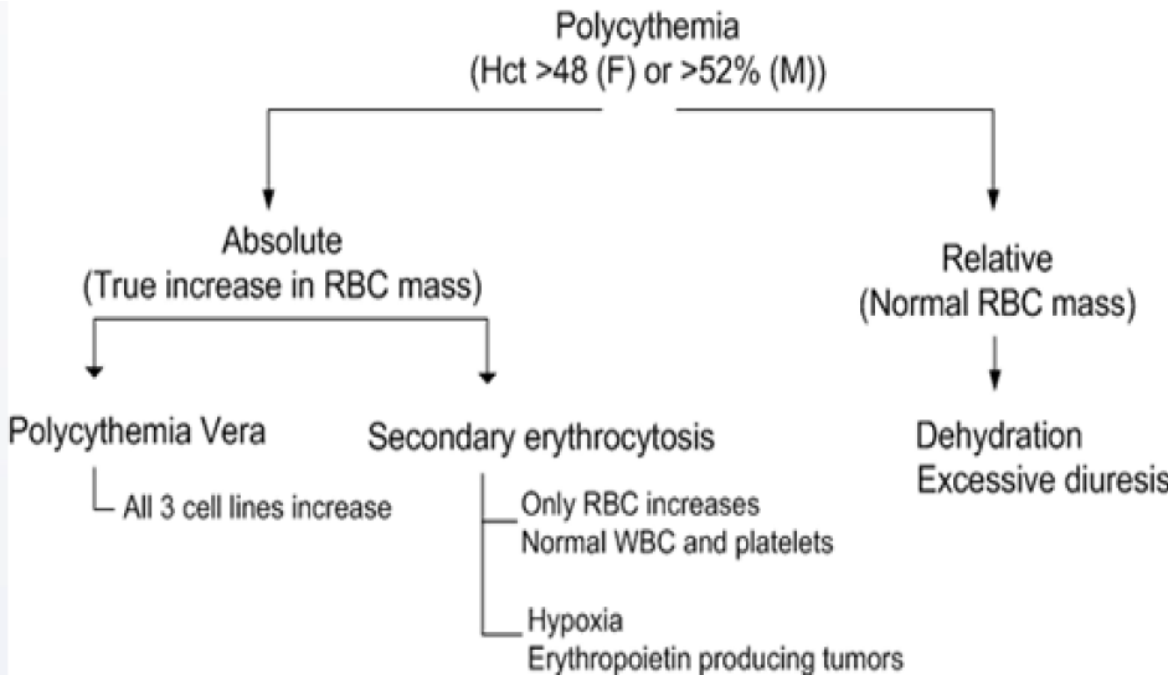
- In reactive polycythemia due to high altitude or lung disease, Sao_2 is low, and EPO is increased.

- In reactive polycythemia due to ectopic EPO production from renal cell carcinoma, EPO is high, and Sao_2, is normal.

Polycythemia

	PLASMA VOLUME	RBC MASS	O_2 SATURATION	EPO LEVELS	ASSOCIATIONS
Relative	↓	–	–	–	Dehydration, burns.
Appropriate absolute	–	↑	↓	↑	Lung disease, congenital heart disease, high altitude.
Inappropriate absolute	–	↑	–	↑	Exogenous EPO: athlete abuse ("blood doping"). Inappropriate EPO secretion: malignancy (eg, renal cell carcinoma, hepatocellular carcinoma).
Polycythemia vera	↑	↑↑	–	↓	EPO ↓ in PCV due to negative feedback suppressing renal EPO production.

↑↓ = 1° disturbance



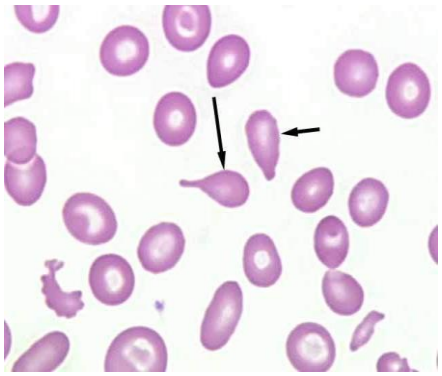
Essential thrombocythemia

- Neoplastic proliferation of mature myeloid cells, especially platelets.
- RBCs and granulocytes are also increased.
- Associated with JAK2 kinase mutation (30-50% of cases).
- Symptoms are related to an increased risk of bleeding and/or thrombosis.
- Rarely progresses to marrow fibrosis or acute leukemia.
- No significant risk for hyperuricemia or gout.

Myelofibrosis

- Neoplastic proliferation of mature myeloid cells, especially megakaryocytes.
- Associated with JAK2 kinase mutation (30-50% of cases).
- Megakaryocytes produce excess platelet-derived growth factor (PDGF) causing marrow fibrosis.
- Clinical features include:

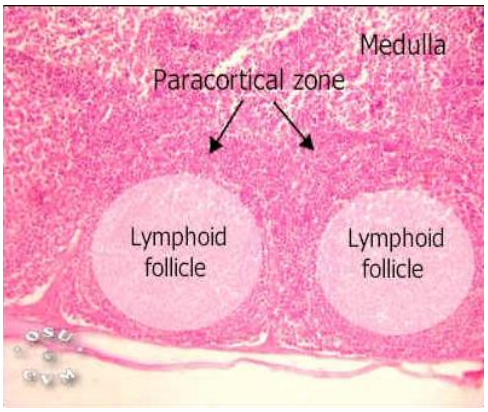
- Splenomegaly due to extramedullary hematopoiesis.
- Often associated with "teardrop" RBCs. "Bone marrow is crying because it's fibrosed and is a dry tap."
- Increased risk of infection, thrombosis, and bleeding.



	RBCs	WBCs	PLATELETS	PHILADELPHIA CHROMOSOME	*JAK2* MUTATIONS
Polycythemia vera	↑	↑	↑	⊖	⊕
Essential thrombocythemia	–	–	↑	⊖	⊕ (30–50%)
Myelofibrosis	↓	Variable	Variable	⊖	⊕ (30–50%)
CML	↓	↑	↑	⊕	⊖

Lymphadenopathy

- LAD refers to enlarged lymph nodes:
  - Painful LAD is usually seen in lymph nodes that are draining a region of acute infection (acute lymphadenitis).
  - Painless LAD can be seen with chronic inflammation (chronic lymphadenitis), metastatic carcinoma, or lymphoma.
- In inflammation, lymph node enlargement is due to hyperplasia of particular regions of the lymph node:
  - Follicular hyperplasia (B-cell region) is seen with rheumatoid arthritis, for example.
  - Paracortex hyperplasia (T-cell region) is seen with viral infections (infectious mononucleosis).



Lymphoma

- Neoplastic proliferation of lymphoid cells that forms a mass; may arise in a lymph node or in extranodal tissue.
- Divided into non-Hodgkin lymphoma (NHL, 60%) and Hodgkin lymphoma (HL, 40%):

Hodgkin lymphoma	Non-Hodgkin lymphoma
Both may present with constitutional ("B") signs/symptoms: low-grade fever, night sweats, weight loss.	
Localized, single group of nodes with contiguous spread (stage is strongest predictor of prognosis). **Better prognosis.**	Multiple lymph nodes involved; extranodal involvement common; noncontiguous spread. Worse prognosis.
Characterized by Reed-Sternberg cells.	Majority involve B cells; a few are of T-cell lineage.
Bimodal distribution: young adulthood and >55 years; more common in men except for nodular sclerosing type.	Can occur in children and adults.
Associated with EBV.	May be associated with autoimmune diseases and viral infections (HIV, EBV, HTLV).

Non-Hodgkin lymphoma

- NHL is further classified based on cell type (B versus T), cell size, pattern of cell growth, expression of surface markers, and cytogenetic translocations:

- Small B cells: follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, and small lymphocytic lymphoma (CLL cells that involve tissue).

- Intermediate-sized B cells: Burkitt lymphoma.

- Large B cells: diffuse large B-cell lymphoma.

A. Neoplasms of mature B cells:

1. Follicular lymphoma:

- Neoplastic proliferation of small B cells (CD20) that form follicle-like nodules.

- Clinically presents in late adulthood with generalized painless lymphadenopathy.

- Indolent course "waxing and waning".

- Driven by t(14,18):

- BCL_2 on chromosome 18 translocates to the Ig heavy chain locus on chromosome 14 → Results in overexpression of Bcl_2, which inhibits apoptosis.

- Treatment is reserved to patients who are symptomatic and involves low-dose chemotherapy or rituximab (anti-CD20 antibody).

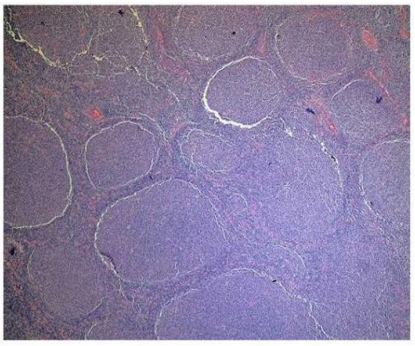
- Progression to diffuse large B-cell lymphoma is an important complication; presents as an enlarging lymph node.

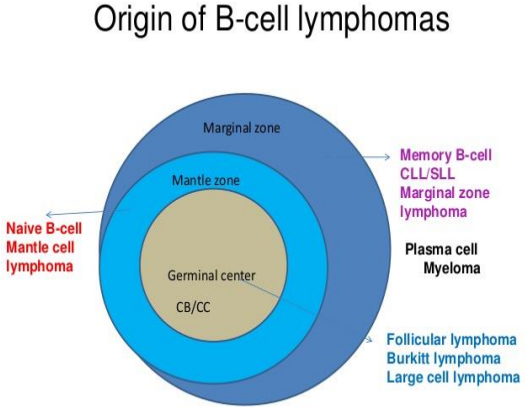
- Follicular lymphoma is distinguished from reactive follicular hyperplasia by:

- Disruption of normal lymph node architecture (maintained in follicular hyperplasia).

- Bcl_2 expression in follicles (not expressed in follicular hyperplasia).

- Monoclonality (follicular hyperplasia is polyclonal).





2. Mantle cell lymphoma:

- Neoplastic proliferation of small B cells (CD20) that expands the mantle zone.
- Clinically presents in late adulthood with painless lymphadenopathy.
- Driven by t(11;14):

- Cyclin D1 gene on chromosome 11 translocates to Ig heavy chain locus on chromosome 14.
- Overexpression of cyclin D1 promotes G_1/S transition in the cell cycle, facilitating neoplastic proliferation.

3. Marginal zone lymphoma:

- Neoplastic proliferation of small B cells that expands the marginal zone; t(11;18).
- Associated with chronic inflammatory states such as Hashimoto thyroiditis, Sjogren syndrome, and H. pylori gastritis.
- The marginal zone is formed by post-germinal center B cells.
- MALToma is marginal zone lymphoma in mucosal sites. Gastric MALToma may regress with treatment of H Pylori.

4. Burkitt lymphoma:

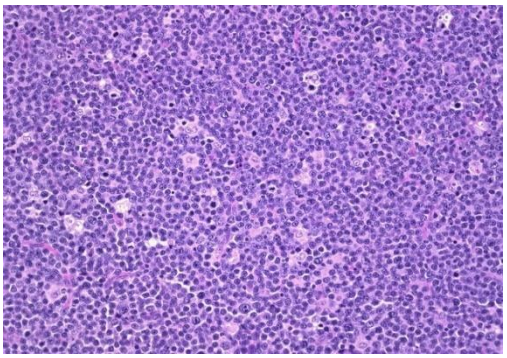
- Neoplastic proliferation of intermediate-sized B cells (CD20+).
- Almost all cases of endemic Burkitt lymphoma are associated with Epstein-Barr virus (EBV) infection.
- Classically presents as an extranodal mass in a child or young adult:

- African form usually involves the jaw "endemic".
- Sporadic form usually involves the abdomen.

- Driven by translocations of c-myc (chromosome 8):

- t(8;14) is most common, resulting in translocation of c-myc on chromosome 8 to the Ig heavy chain locus on chromosome 14 (Burk 8).
- Overexpression of c-myc oncogene promotes cell growth.

- A high mitotic index and high cell death rate are typically seen. Benign macrophages that phagocytize the resulting cellular debris ("tingible body macrophages") are diffusely distributed throughout the malignant tissue. The clear spaces that surround these macrophages contribute to the characteristic "starry sky" appearance of Burkitt lymphoma.



5. Diffuse large B-cell lymphoma:

- Neoplastic proliferation of large B cells (CD20+) that grow diffusely in sheets.
- Most common form of NHL.
- Clinically aggressive (high-grade).
- Arises sporadically or from transformation of a low-grade lymphoma (follicular lymphoma).
- Associated with mutations in BCL-2, BCL-6.
- Presents in late adulthood as an enlarging lymph node or an extranodal mass.

6. Primary central nervous system lymphoma:

- Most commonly associated with HIV/AIDS.
- Considered an AIDS-defining illness.
- Variable presentation: confusion, memory loss, seizures.
- Mass lesion(s) on MRI, needs to be distinguished from toxoplasmosis via CSF analysis or other lab tests.

B. Neoplasms of mature T cells:

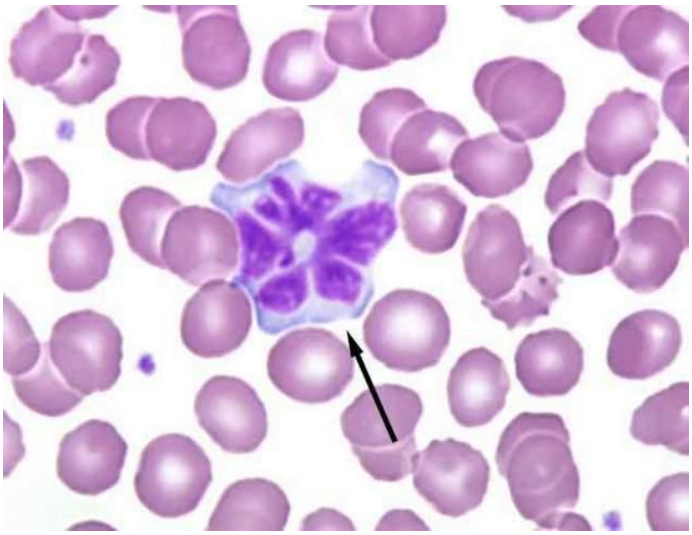
1. Adult T-cell leukemia/lymphoma:

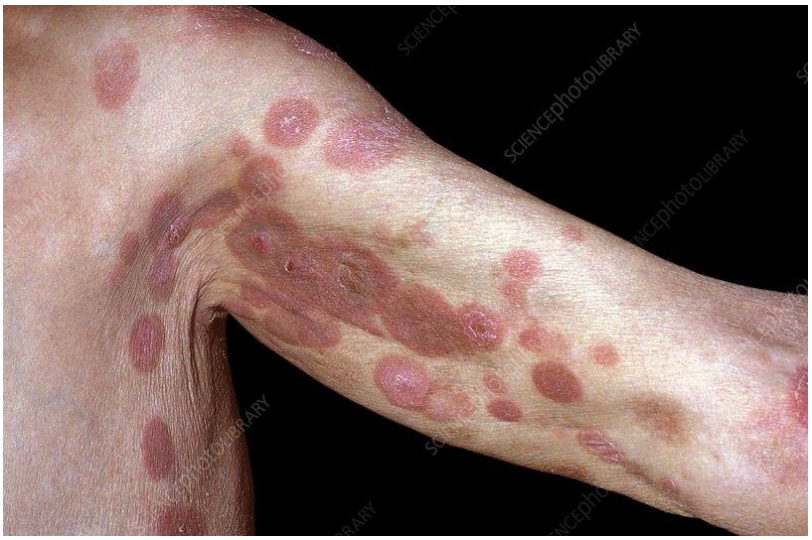
- Neoplastic proliferation of mature CD4 T cells.
- Associated with HTLV-L; most commonly seen in Japan and the Caribbean.
- Clinical features include rash (skin infiltration), generalized lymphadenopathy with hepatosplenomegaly, and lytic (punched-out) bone lesions with hypercalcemia.

2. Mycosis fungoides:

- Neoplastic proliferation of mature CD4 T cells that infiltrate the skin, producing localized skin rash, plaques, and nodules.
- Aggregates of neoplastic cells in the epidermis are called Patitrier microabscesses.

- Cells can spread to involve the blood, producing Sezary syndrome.
- Characteristic lymphocytes with cerebriform nuclei (Sezary cells) are seen on blood smear.



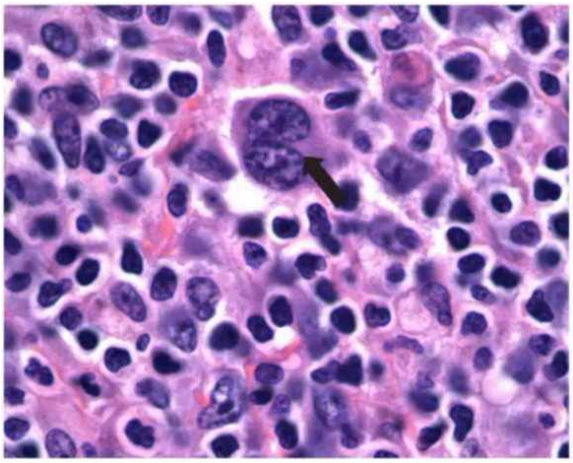


Chromosomal translocations

TRANSLOCATION	ASSOCIATED DISORDER	NOTES
t(8;14)	Burkitt (Burk-8) lymphoma (c-*myc* activation)	The Ig heavy chain genes on chromosome 14 are constitutively expressed. When other genes (eg, *c-myc* and *BCL-2*) are translocated next to this heavy chain gene region, they are overexpressed.
t(11;14)	Mantle cell lymphoma (cyclin D1 activation)	
t(11;18)	Marginal zone lymphoma	
t(14;18)	Follicular lymphoma (*BCL-2* activation)	
t(15;17)	APL (M3 type of AML; responds to all-trans retinoic acid)	
t(9;22) (Philadelphia chromosome)	CML (*BCR-ABL* hybrid), ALL (less common, poor prognostic factor); Philadelphia CreaML cheese	

Hodgkin lymphoma

- Neoplastic proliferation of Reed-Sternberg (RS) cells, which are large B cells with binucleate or bilobed with the 2 halves as mirror images and prominent nucleoli ('owl-eyed nuclei'); classically positive for CD15 and CD30.



- RS cells secrete cytokines:
  - Occasionally results in constitutional symptoms (fever, chills, and night sweats).
  - Attract reactive lymphocytes, plasma cells, macrophages, and eosinophils.
  - May lead to fibrosis.
- Reactive inflammatory cells make up a bulk of the tumor and form the basis for classification of HL, Subtypes include:
  - Nodular sclerosis.
  - Lymphocyte-rich.
  - Mixed cellularity.
  - Lymphocyte-depleted.
- Nodular sclerosis is the most common subtype of HL (70% of all cases):
  - Classic presentation is an enlarging cervical or mediastinal lymph node in a young adult, usually female.
  - Lymph node is divided by bands of sclerosis; RS cells are present in lake-like spaces (lacunar cells).
- Important considerations regarding other subtypes of HL:
  - Lymphocyte-rich has the best prognosis of all types.
  - Mixed cellularity is often associated with abundant eosinophils (RS cells produce IL-5).
  - Lymphocyte-depleted is the most aggressive at all types; usually seen in the elderly and HIV-positive individuals.

❖ N.B:

1. EBV infection is associated with increased incidence of both Hodgkin's and non-Hodgkin's lymphomas as well as nasopharyngeal carcinoma.

2. Benign lymph node enlargement in response to antigenic stimulation is associated with a polyclonal proliferation of lymphocytes.

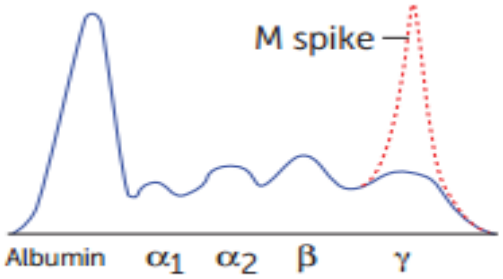
- A monoclonal lymphocytic proliferation is strong evidence of malignancy.

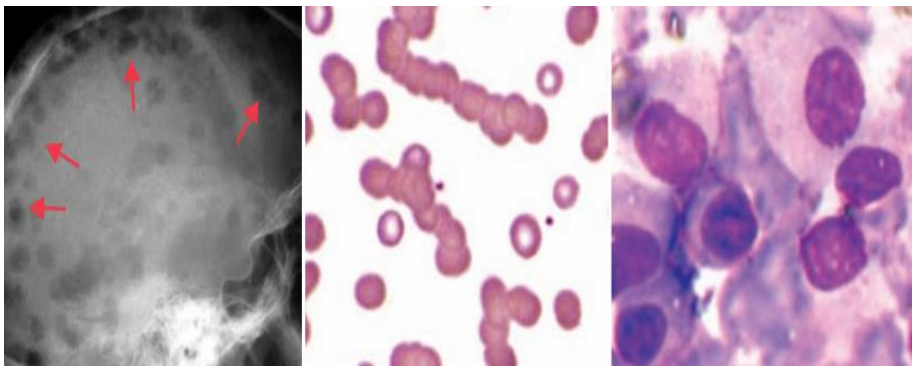
Plasma cell disorders (dyscrasis)

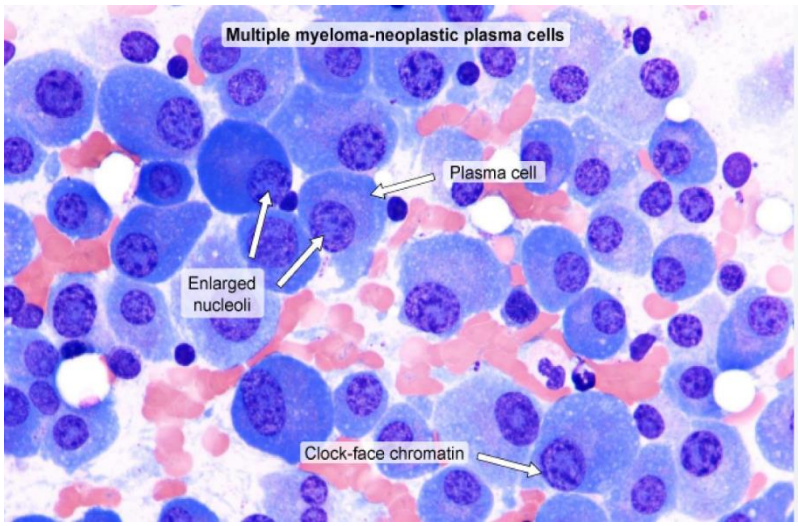
Multiple myeloma

- Malignant proliferation of plasma cells in the bone marrow.
- Most common primary malignancy of bone; metastatic cancer, however, is the most common malignant lesion of bone overall.
- High serum IL-6 is sometimes present; stimulates plasma cell growth and immunoglobulin production.
- Clinical features include:

- Bone pain with hypercalcemia:
  - Neoplastic plasma cells activate the RANK receptor on osteoclasts, leading to bone destruction.
  - Lytic, 'punched-out' skeletal lesions are seen on x-ray, especially in the vertebrae (Back pain) and skull; increased risk for fracture.
- Normocytic anemia: due to ineffective erythropoiesis.
- Elevated serum protein: Neoplastic plasma cells produce immunoglobulin; M spike is present on serum protein electrophoresis (SPEP), most commonly due to monoclonal IgG or IgA. Multiple Myeloma → Monoclonal M protein spike.
- Numerous plasma cells with "clock-face" chromatin and intracytoplasmic inclusions containing immunoglobulin.
- Increased risk of infection: Monoclonal antibody lacks antigenic diversity; infection is the most common cause of death in multiple myeloma.
- Rouleaux formation of RBCs on blood smear (RBCs stacked like poker chips in blood smear). Increased serum paraproteins (immunoglobulin) decreases charge between RBCs.
- Primary AL amyloidosis: Free light chains circulate in serum and deposit in tissues.
- Proteinuria: Free light chain is excreted in the urine as Bence Jones protein; deposition in kidney tubules leads to risk for renal failure (myeloma kidney).
- Think CRAB:
  - HyperCalcemia.
  - Renal involvement.
  - Anemia.
  - Bone lytic lesions/Back pain.







Monoclonal gammopathy of undetermined significance (MGUS)

- Increased serum protein with M spike on SPEP; other features of multiple myeloma are absent (no lytic bone lesions, hypercalcemia, AL amyloid, or Bence jones proteinuria).
- Common in elderly (seen in 5% of 70-year-old individuals); 1% of patients with MGUS develop multiple myeloma each year.

Waldenstorm macroglobulinemia

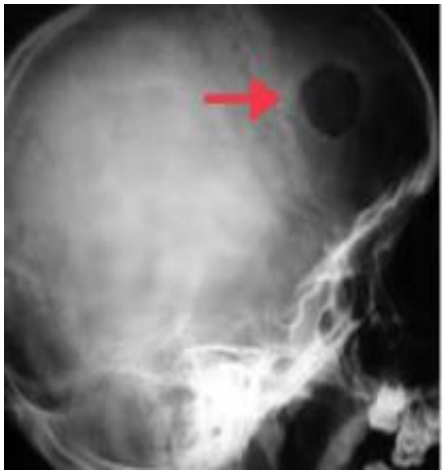
- B-cell lymphoma with monoclonal IgM production.
- Clinical features include:

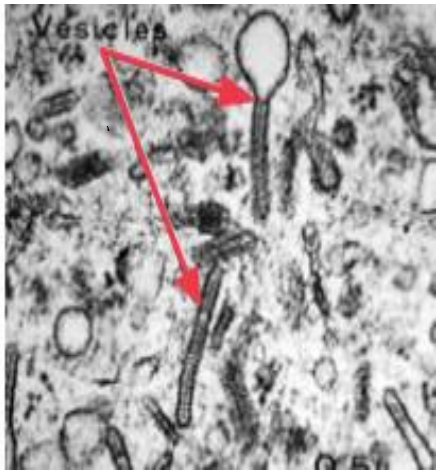
- Generalized lymphadenopathy, lytic bone lesions are absent.
- Increased serum protein with M spike (comprised of IgM).
- Visual and neurologic deficits (retinal hemorrhage or stroke): IgM (large pentamer) causes serum hyperviscosity.
- Bleeding: Viscous serum results in defective platelet aggregation.

- Acute complications are treated with plasmapheresis, which removes IgM from the serum.

Langerhans cell histiocytosis

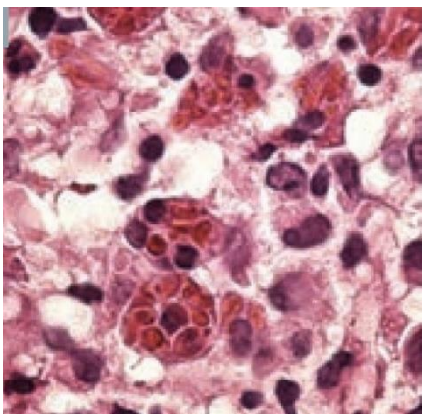
- Collective group of proliferative disorders of dendritic (Langerhans) cells.
- Presents in a child as lytic bone lesions and skin rash or as recurrent otitis media with a mass involving the mastoid bone.
- Cells are functionally immature and do not effectively stimulate primary T cells via antigen presentation.
- Cells express S-100 (mesodermal origin) and CD1a.
- Birbeck granules ("tennis rackets" or rod shaped on EM) are characteristic.





Hemophagocytic lymphohistiocytosis

- Systemic overactivation of macrophages and cytotoxic T cells → fever, pancytopenia, hepatosplenomegaly, ↑↑↑ serum ferritin levels.
- Can be inherited or 2° to strong immunologic activation (after EBV infection, malignancy).
- Bone marrow biopsy shows macrophages phagocytosing marrow elements.



Chronic venous insufficiency (CVI)

- Chronic venous insufficiency is a common cause of lower extremity edema that may be accompanied by varicose veins, skin discoloration, and medial skin ulceration.

- Chronic venous insufficiency is most commonly caused by incompetence of venous valves leading to venous hypertension in the deep venous system of the legs.

- Risk factors for CVI include advancing age, obesity, family history, pregnancy, sedentary lifestyle, previous LE trauma, and previous LE venous thrombosis.

- Pathogenesis:

- Venous blood normally flows from superficial veins through perforating veins into the deep veins of the extremities.

- Blood from the deep veins then travels upward and eventually drains into the inferior vena cava.

- Valves located in the superficial, perforating, and deep veins prevent backward flow from deep to superficial veins.

- Chronically elevated intraluminal pressure can lead to dilation of the veins (varicose veins) and incompetence of the valves.

- This allows retrograde flow into superficial veins and results in a further increase in venous pressure.

- Presentation:

- In relatively severe cases, redirection of blood from the deep venous system to the superficial venous system may lead to other physical examination findings, including abnormal venous dilation (varicose veins), skin discoloration, lipodermatosclerosis, or skin ulceration (characteristically on the medial aspect of the lower leg).

- This increased pressure damages capillaries causing loss of fluid, plasma proteins and erythrocytes into the tissue.

- Erythrocyte extravasation causes hemosiderin deposition and the classic coloration of stasis dermatitis.

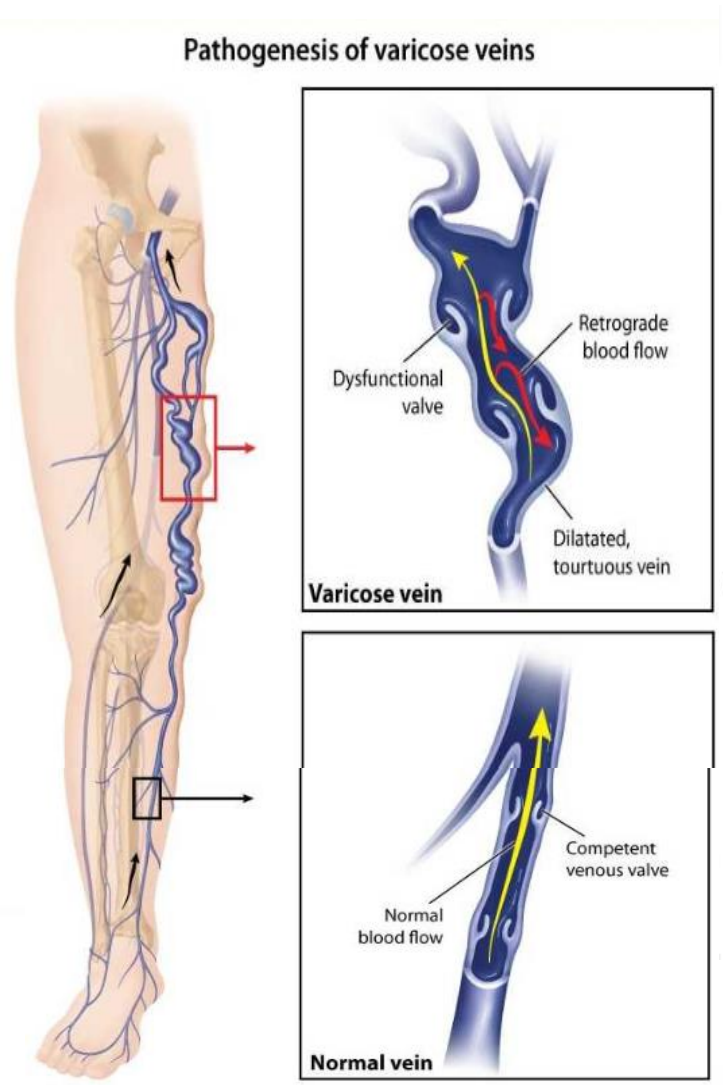
- Stasis dermatitis most classically involves the medial leg below the knee and above the medial malleolus.

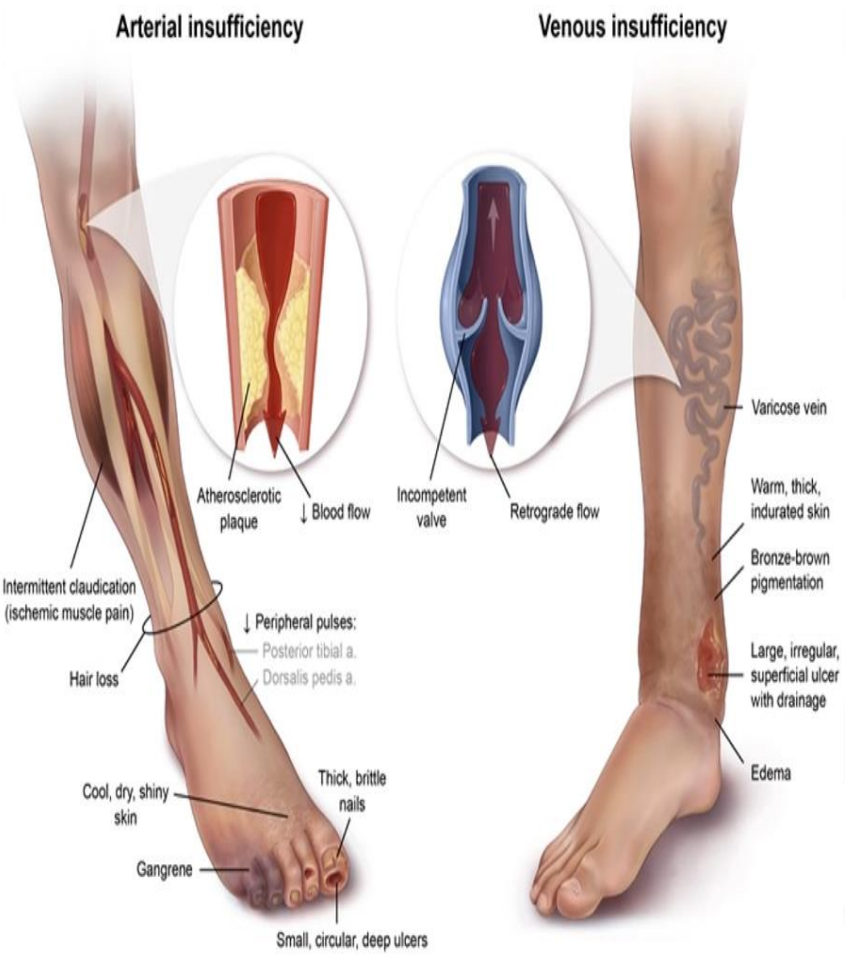
- Pitting edema is the most common physical examination finding. Patients may present with leg discomfort, pain, or swelling that is typically worse in the evening or following prolonged standing and improves after walking or leg elevation.

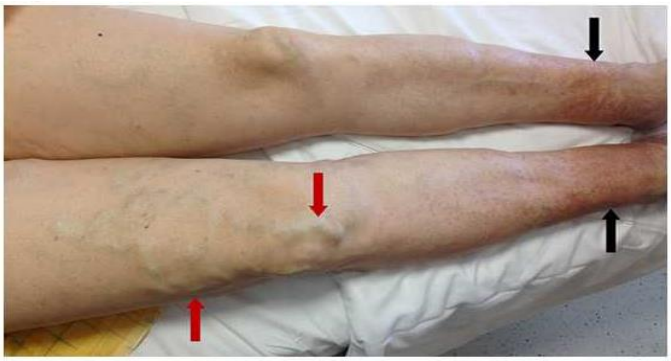
- Inflammation of venules and capillaries as well as fibrin deposition and platelet aggregation cause microvascular disease and ultimately ulcerations will occur.

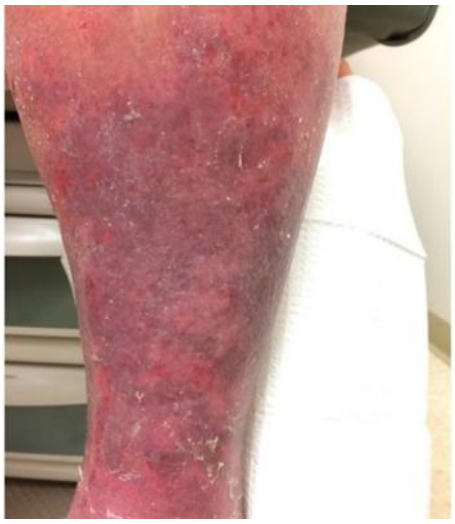
- **Treatment:**

- Initial treatment includes conservative measures with leg elevation, exercise, and compression therapy with compression stockings.









Blood transfusion therapy

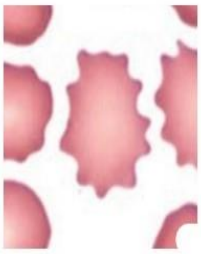
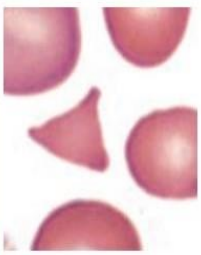
COMPONENT	DOSAGE EFFECT	CLINICAL USE
Packed RBCs	↑ Hb and O_2 carrying capacity	Acute blood loss, severe anemia
Platelets	↑ platelet count (↑ ~ 5000/mm^3/unit)	Stop significant bleeding (thrombocytopenia, qualitative platelet defects)
Fresh frozen plasma/prothrombin complex concentrate	↑ coagulation factor levels; FFP contains all coagulation factors and plasma proteins; PCC generally contains factors II, VII, IX, and X, as well as protein C and S	Cirrhosis, immediate anticoagulation reversal
Cryoprecipitate	Contains fibrinogen, factor VIII, factor XIII, vWF, and fibronectin	Coagulation factor deficiencies involving fibrinogen and factor VIII

Blood transfusion risks include infection transmission (low), transfusion reactions, iron overload (may lead to 2° hemochromatosis), hypocalcemia (citrate is a Ca^{2+} chelator), and hyperkalemia (RBCs may lyse in old blood units).

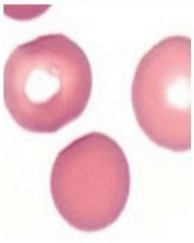
❖ N.B:

- Patients who receive the equivalent of more than one body blood volume (5-6 liters) of whole blood transfusions or packed red blood cells over a period of 24 hours may develop elevated plasma levels of citrate (a substance added to stored blood).
- Citrate chelates calcium and magnesium and may reduce their plasma levels, causing paresthesias due to hypocalcemia.

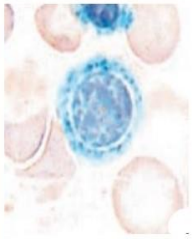
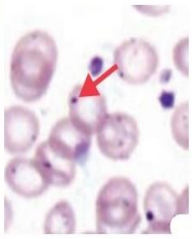
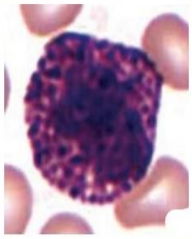
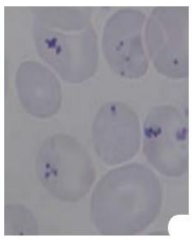
RBC morphology

TYPE	EXAMPLE	ASSOCIATED PATHOLOGY	NOTES
Acanthocytes ("spur cells")		Liver disease, abetalipoproteinemia	Projections of varying size at irregular intervals.
Echinocytes ("burr cells")		Liver disease, ESRD, pyruvate kinase deficiency	Smaller and more uniform projections than acanthocytes
Dacrocytes ("teardrop cells")		Bone marrow infiltration (eg, myelofibrosis)	RBC "sheds a tear" because it's mechanically squeezed out of its home in the bone marrow
Schistocytes (eg, "helmet" cells)		MAHAs (eg, DIC, TTP/HUS, HELLP syndrome), mechanical hemolysis (eg, heart valve prosthesis)	Fragmented RBCs
Degmacytes ("bite cells")		G6PD deficiency	Due to removal of Heinz bodies by splenic macrophages
Elliptocytes		Hereditary elliptocytosis	Caused by mutation in genes encoding RBC membrane proteins (eg, spectrin)

RBC morphology *(continued)*

TYPE	EXAMPLE	ASSOCIATED PATHOLOGY	NOTES
Spherocytes		Hereditary spherocytosis, autoimmune hemolytic anemia	Small, spherical cells without central pallor
Macro-ovalocytes		Megaloblastic anemia (also hypersegmented PMNs)	
Target cells		HbC disease, Asplenia, Liver disease, Thalassemia	"HALT," said the hunter to his target
Sickle cells		Sickle cell anemia	Sickling occurs with low O_2 conditions (eg, high altitude, acidosis)

RBC inclusions

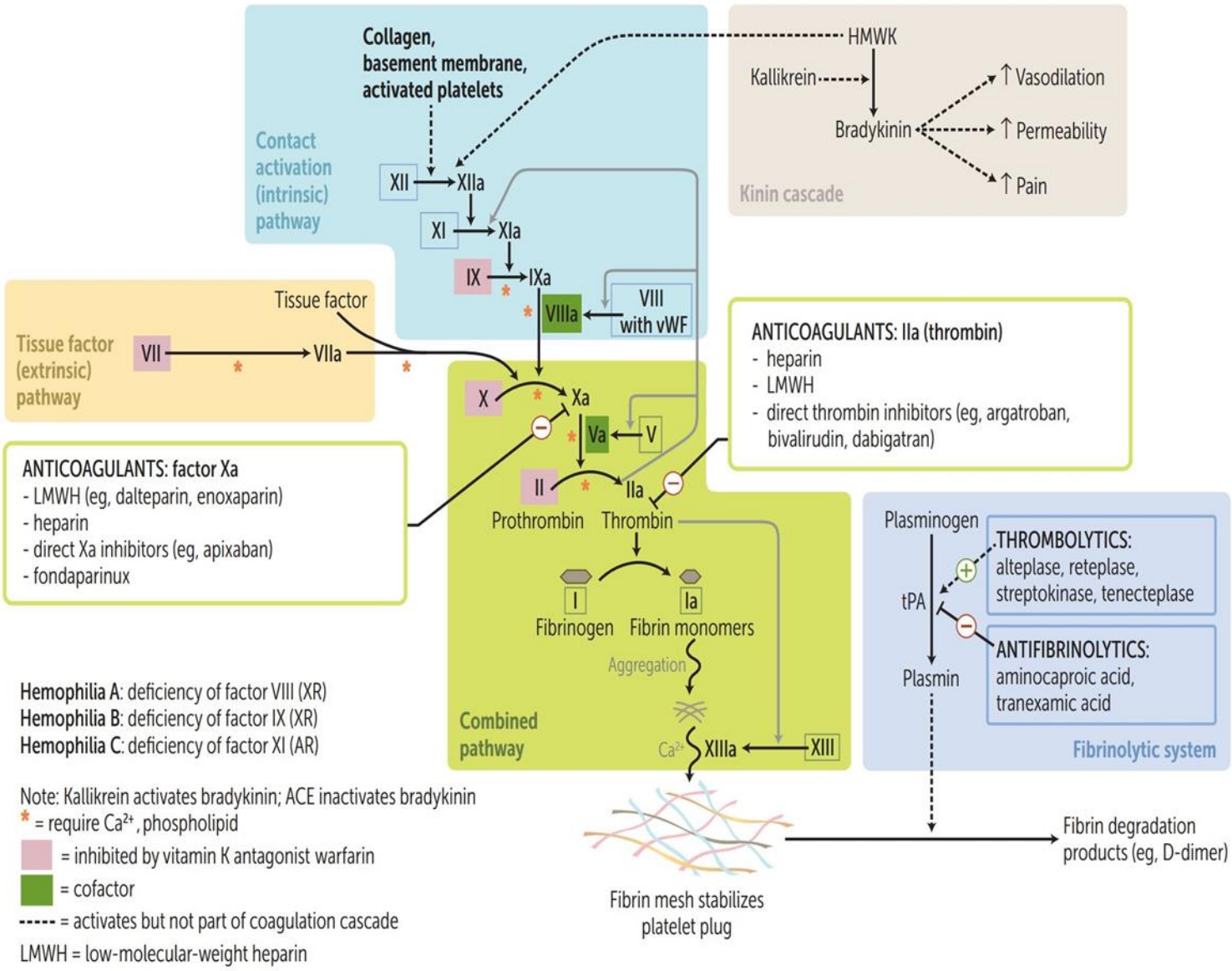
TYPE	EXAMPLE	ASSOCIATED PATHOLOGY	NOTES
Bone marrow			
Iron granules (eg, in ringed sideroblasts)		Sideroblastic anemias (eg, lead poisoning, myelodysplastic syndromes, alcoholism)	Perinuclear mitochondria with excess iron (forming ring in ringed sideroblasts) Require Prussian blue stain to be visualized
Peripheral smear			
Howell-Jolly bodies		Functional hyposplenia (eg, sickle cell disease), asplenia	Basophilic nuclear remnants (do not contain iron) Usually removed by splenic macrophages
Basophilic stippling		Sideroblastic anemias, thalassemias	Basophilic ribosomal precipitates (do not contain iron)
Pappenheimer bodies		Sideroblastic anemia	Basophilic granules (contain iron)
Heinz bodies		G6PD deficiency	Denatured and precipitated hemoglobin (contain iron) Phagocytic removal of Heinz bodies → bite cells Requires supravital stain (eg, crystal violet) to be visualized

CHAPTER 2

Pharmacology

Anticoagulants

Coagulation and kinin pathways



Warfarin

- Mechanism of action:

- Inhibits epoxide reductase which interferes with γ-carboxylation of vitamin K-dependent clotting factors II, VII, IX, and X, and proteins C and S.

- Metabolism affected by polymorphisms in the gene for vitamin K epoxide reductase complex (VKORC1).

- Monitoring:

- Decreased levels of vitamin K-dependent clotting factors, especially factor VII → prolong the prothrombin time (PT).

- Another useful lab value is the ratio of the patient's PT to a control, also called the international normalized ratio (INR).

- The target INR for therapeutic warfarin anticoagulation is 2-3. Bleeding is a common complication of warfarin therapy and the risk is increased with INRs above 3.0.

▪ Clinical Use:

- Chronic anticoagulation (venous thromboembolism prophylaxis, and prevention of stroke in atrial fibrillation).

- Not used in pregnant women (because warfarin, unlike heparin, crosses placenta).

- Follow PT/INR.

▪ Adverse effects:

- Bleeding, teratogenic, skin/tissue necrosis, drug-drug interactions.

- Proteins C and S have shorter half-lives than clotting factors II, VII, IX, and X, resulting in early transient hypercoagulability with warfarin use. Skin/ tissue necrosis within first few days of large doses believed to be due to small vessel microthromboses.

Drugs/supplements that affect warfarin metabolism (selected)	
CYP450 Inhibitors **↑ Warfarin effect** (↑ bleeding risk)	• Acetaminophen, NSAIDs • Antibiotics/antifungals (eg, metronidazole) • Amiodarone • Cimetidine • Cranberry juice, *Ginkgo biloba*, vitamin E • Omeprazole • Thyroid hormone • SSRIs (eg, fluoxetine)
CYP450 Inducers **↓ Warfarin effect** (↓ in efficacy)	• Carbamazepine • Ginseng • Green vegetables (eg, spinach) • Oral contraceptives • Phenobarbital • Rifampin • St. John's wort

NSAIDs = nonsteroidal anti-inflammatory drugs; **SSRIs** = selective serotonin reuptake inhibitors.

- Antagonist:

- For reversal of warfarin, give vitamin K.

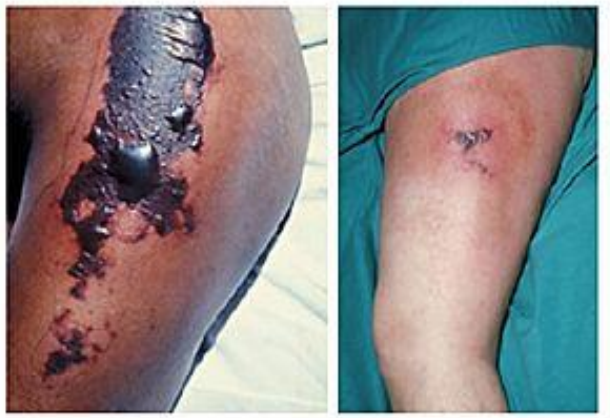
- For rapid reversal, give fresh frozen plasma.

- Heparin "bridging":

- Heparin frequently used when starting warfarin. Heparin's activation of antithrombin enables anticoagulation during initial, transient hypercoagulable state caused by warfarin. Initial heparin therapy reduces risk of recurrent venous thromboembolism and skin/tissue necrosis.

❖ N.B:

1. The photo below depicts warfarin-induced skin necrosis which happens in patients with a protein C or S deficiency who are started on warfarin.
- Protein C is natural anticoagulant (as is protein S).
- Following the initiation of warfarin, a rapid drop in factor VII (shortest half-life) and protein C (2nd shortest half-life) levels occurs, but the other procoagulant vitamin K dependent factors (II, IX, and X) decline at a slower rate. This results in a transient hypercoagulable state.
- If a protein C deficiency is present, the transient procoagulant/anticoagulant imbalance is further exaggerated, causing a relative hypercoagulable state with thrombotic occlusion of the microvasculature and skin necrosis.
- Treatment of warfarin-induced skin necrosis includes discontinuing warfarin and administering fresh frozen plasma to replenish protein C content.



2. Most available rodenticides contain brodifacoum, a long-acting 4-hydroxycoumarin derivative.
- A patient who has ingested a quantity of rodenticide sufficient to cause coagulopathy and abnormal bleeding (similar to warfarin toxicity) requires immediate treatment with fresh frozen plasma in addition to vitamin K.

Heparin

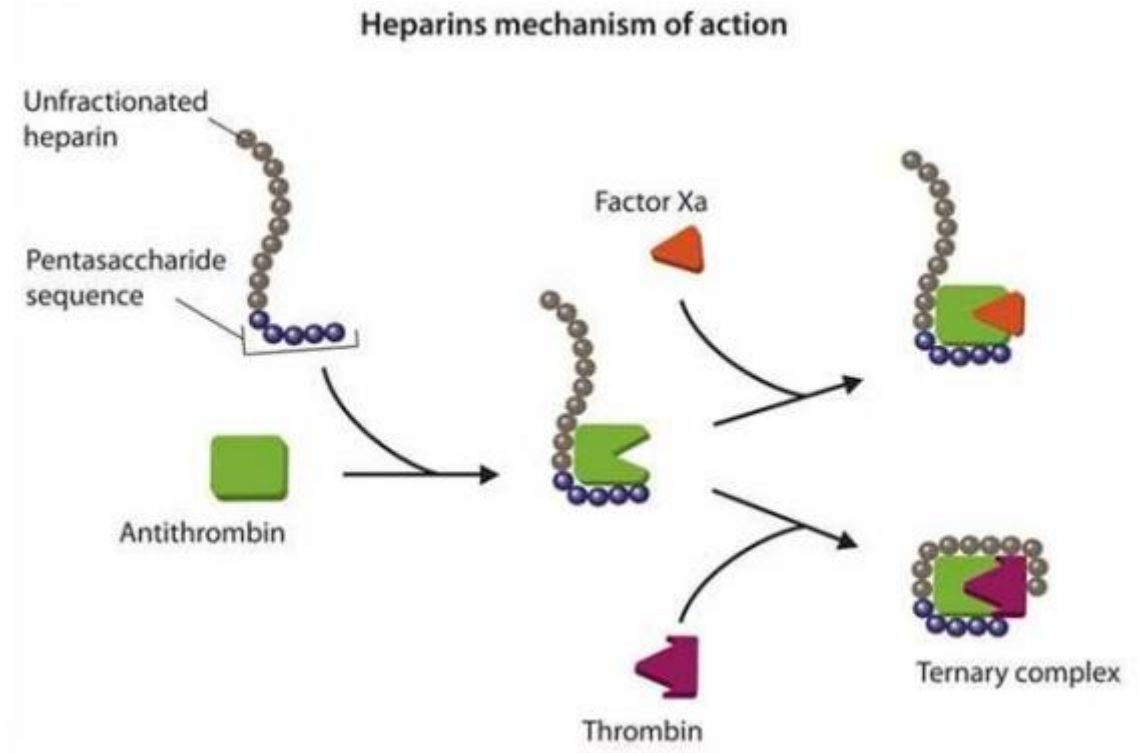
- Mechanism of action:

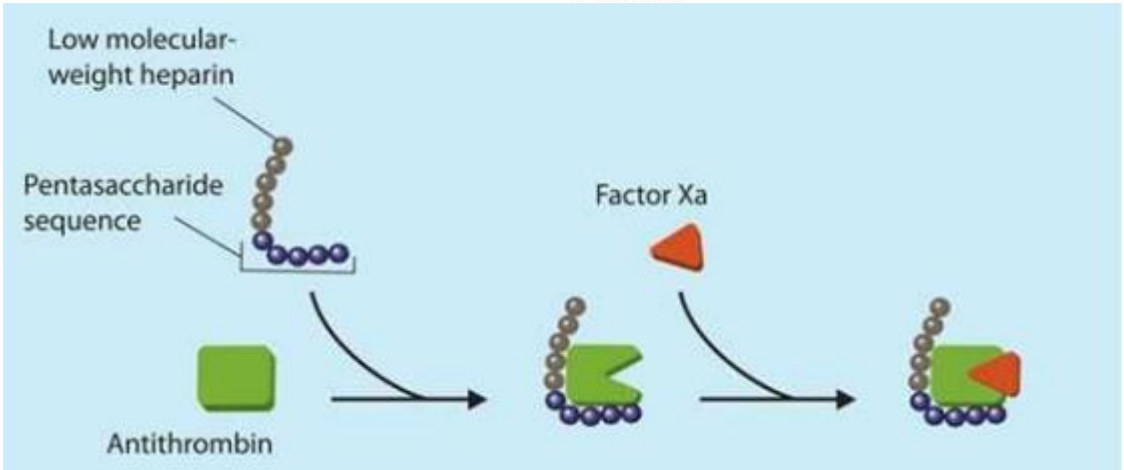
- Heparin enhances the activity of antithrombin → Lowers the activity of thrombin and factor Xa.

- Short half-life.

- Both unfractionated heparin and low molecular weight heparin (LMWH) contain a pentasaccharide sequence that binds to antithrombin and causes a conformational change that increases its ability to inactivate Factor Xa.

- Only unfractionated heparin (not LMWH) has a pentasaccharide chain long enough (>18 saccharide units) to bind to both antithrombin and thrombin. As a result, unfractionated heparin has equal activity against Factor Xa and thrombin, while LMWH has greater activity against Factor Xa than thrombin.





- Clinical Use:

- Immediate anticoagulation for pulmonary embolism (PE), acute coronary syndrome, MI, deep venous thrombosis (DVT).

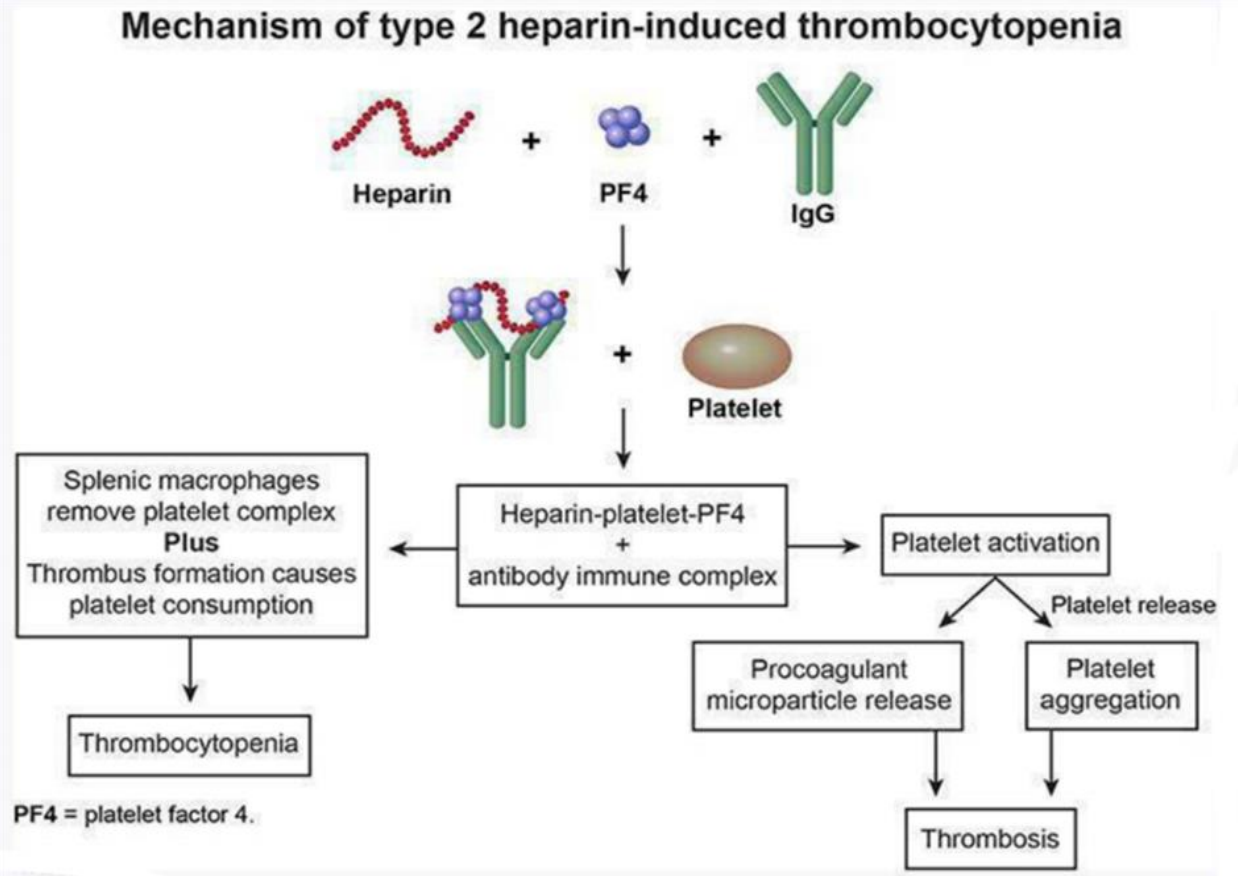
- Used during pregnancy (does not cross placenta).

- Follow PTT.

- Adverse effects:

- Bleeding, thrombocytopenia (HIT), osteoporosis, drug-drug interactions.

- Heparin-induced thrombocytopenia (HIT): development of IgG antibodies against heparin-bound platelet factor 4 (PF4). Antibody-heparin-PF4 complex activates platelets → thrombosis and thrombocytopenia.



- Antidote:

- Protamine sulfate is a peptide that binds to heparin forming a complex that has no anticoagulant activity (chemical inactivation).

Low-molecular-weight heparins

- Drugs: Enoxaparin, dalteparin.
- Enoxaparin is a form of low molecular weight heparin that, like heparin, functions by binding and activating antithrombin III.
- LMWH acts primarily on factor Xa, not thrombin. When active, antithrombin III binds to factor Xa and stops factor Xa from converting prothrombin to thrombin. Less thrombin is produced, resulting in an anticoagulant effect.
- LMWH have better bioavailability, and 2-4 times longer half-life; can be administered subcutaneously and without laboratory monitoring.
- Enoxaparin has been shown to cause a statistically significant reduction in death and recurrent myocardial infarction when used in the acute treatment of myocardial infarction as compared with unfractionated heparin (ESSENCE, TIMM 1 trials).
- Occurrence of heparin- induced thrombocytopenia (HIT) is much more common with the use of unfractionated heparin compared to low molecular weight heparin.
- Not easily reversible: Protamine sulfate is not very effective in treating toxicity caused by low molecular weight heparin.

Heparin vs warfarin

	Heparin	Warfarin
ROUTE OF ADMINISTRATION	Parenteral (IV, SC)	Oral
SITE OF ACTION	Blood	Liver
ONSET OF ACTION	Rapid (seconds)	Slow, limited by half-lives of normal clotting factors
MECHANISM OF ACTION	Activates antithrombin, which ↓ the action of IIa (thrombin) and factor Xa	Impairs synthesis of vitamin K–dependent clotting factors II, VII, IX, and X, and anti-clotting proteins C and S
DURATION OF ACTION	Hours	Days
AGENTS FOR REVERSAL	Protamine sulfate	Vitamin K, FFP, PCC
MONITORING	PTT (intrinsic pathway)	PT/INR (extrinsic pathway)
CROSSES PLACENTA	No	Yes (teratogenic)

❖ N.B:

1. Heparin is the most important cause of thrombocytopenia in hospitalized patients.

- Occurrence of heparin- induced thrombocytopenia (HIT) is much more common with the use of unfractionated heparin compared to low molecular weight heparin.
- HIT more commonly leads to paradoxical thrombosis rather than bleeding.
- HIT is a serious disorder caused by antibodies to heparin and platelet factor IV.
- Direct thrombin inhibitors (hirudin, lepirudin and argatroban, dabigatran) do not require antithrombin-III for their action and are drugs of choice in the treatment of HIT.
- Patients with HIT need ongoing anticoagulation due to the presence of or possibility of thrombosis. Upon clinical suspicion of HIT, the most important initial step in treatment is to stop all forms of heparin.

2. Pregnancy itself is an independent risk factor for deep venous thrombosis (DVT) due to several reasons.

- During pregnancy, there are increased levels of clotting factors, decreased fibrinolysis, and reduced levels of the natural anticoagulant protein S.
- Additionally, decreased venous tone and the pressure of the gravid uterus on the inferior vena cava predispose pregnant women to venous stasis.
- DVT occurs with equal frequency during all trimesters of pregnancy.
- The mainstay of DVT treatment in pregnancy is heparin (Coumadin is not used because it is teratogenic).

Direct thrombin inhibitors

- Drugs:
- Bivalirudin (related to hirudin, the anticoagulant used by leeches), Argatroban, Dabigatran (only oral agent in class).

- Mechanism of action:
- Directly inhibits activity of free and clot-associated thrombin.

- Clinical Use:
- Venous thromboembolism, atrial fibrillation.

- Can be used in HIT, when heparin is BAD for the patient.

- Does not require lab monitoring.

- Adverse effects:
- Bleeding; can reverse dabigatran with idarucizumab. Can attempt to use activated prothrombin complex concentrates (PCC) and/or antifibrinolytics (tranexamic acid).

Direct factor Xa inhibitors

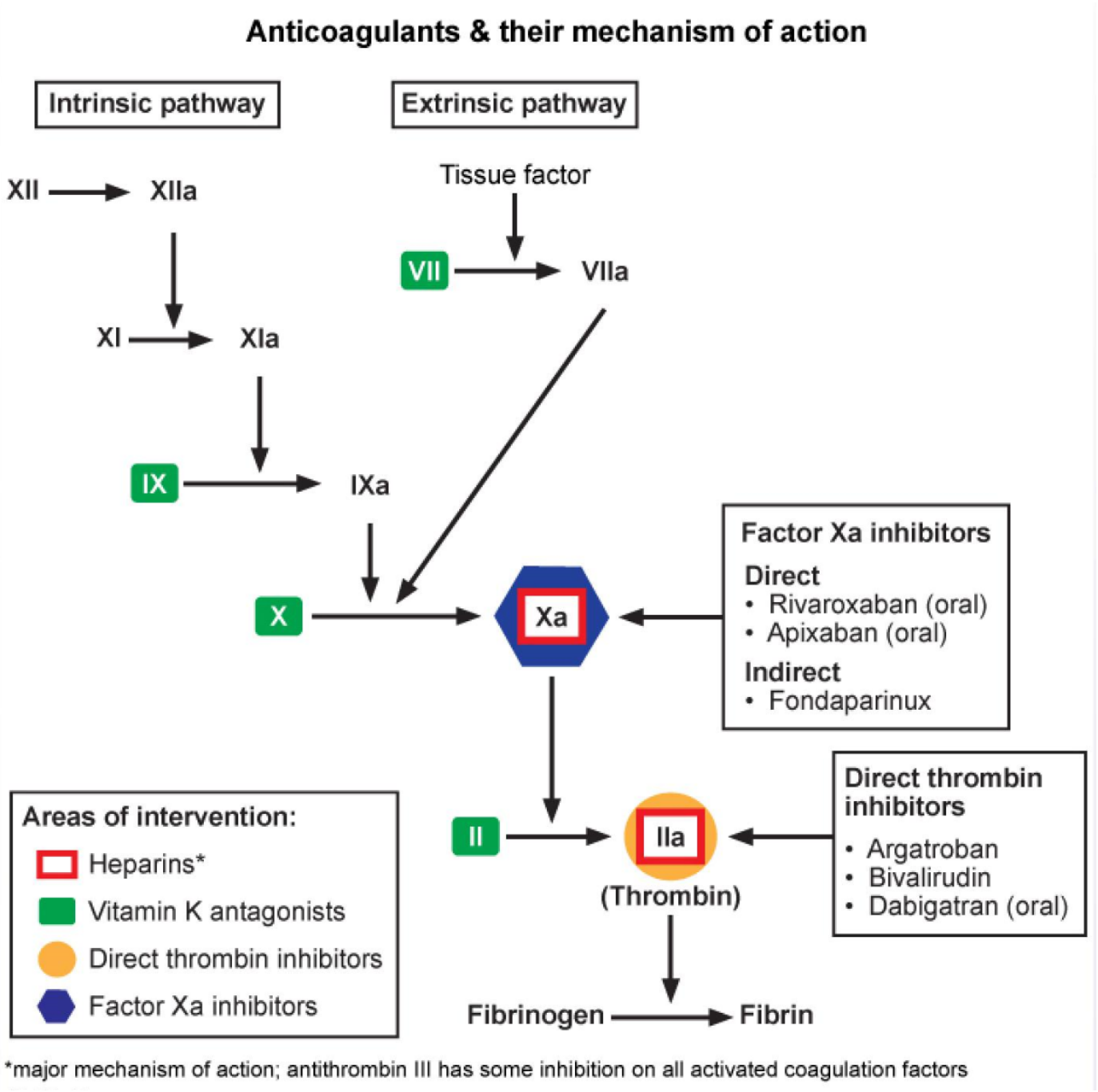
- Drugs:
- ApiXaban, rivaroXaban.

- Mechanism of action:
- Bind to the active site of factor Xa and prevent thrombin formation.

- Clinical Use:
- Treatment and prophylaxis for DVT and PE (rivaroxaban); stroke prophylaxis in patients with atrial fibrillation.

- These drugs can be administered orally as monotherapy and do not require laboratory monitoring.

- Adverse effects:
- Bleeding. Reverse with andeXanet alfa.



❖ N.B:

- Fondaparinux is a synthetic pentasaccharide Factor Xa inhibitor (indirect Xa inhibitor).
- It has none of the required long saccharide units that bind to thrombin and much lower antithrombin activity compared to unfractionated heparin.

Thrombolytics

- Drugs: Alteplase (tPA), reteplase (rPA), tenecteplase (TNK-tPA), streptokinase.

- Mechanism of action:
- Directly or indirectly aid conversion of plasminogen to plasmin, which cleaves thrombin and fibrin clots.

- Fibrinolytic drugs can be non-fibrin-specific (such as streptokinase) or fibrin-specific drugs (such as tPA, reteplase, and tenecteplase) act only on fibrin attached to recently formed clot without systemic activation.

- ↑ PT, ↑ PTT, no change in platelet count.

- Clinical Use: Early MI within 6 hours of the onset of symptoms, early ischemic stroke, direct thrombolysis of severe PE.

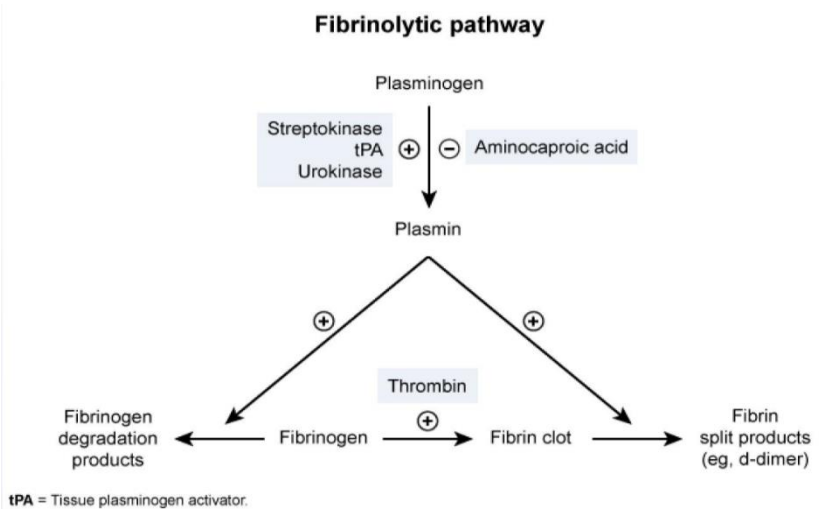
- Adverse effects:
- Bleeding.

- Theoretically, the fibrin-specific fibrinolytics are associated with less systemic activation of plasmin and a decreased risk of bleeding.

- Absolute Contraindications to Thrombolytics:
  - Major bleeding into the bowel (melena) or brain (any type of CNS bleeding).
  - Recent surgery (within the last 2 weeks).
  - Severe hypertension (above 180/110).
  - Non-hemorrhagic stroke within the last 6 months.
  - Heme-positive brown stool is not an absolute contraindication to the use of thrombolytics.

- Restoration of blood flow after clot lysis in MI can lead to arrhythmias, also referred to as reperfusion arrhythmia. Typically, these arrhythmias are benign and not associated with increased mortality.

- Treat toxicity with aminocaproic acid and tranexemic acid (anti-fibrinolytics), an inhibitor of fibrinolysis. Fresh frozen plasma and cryoprecipitate can also be used to correct factor deficiencies.



Antiplatelet drugs

- Antiplatelet drugs work by one of three basic mechanisms:

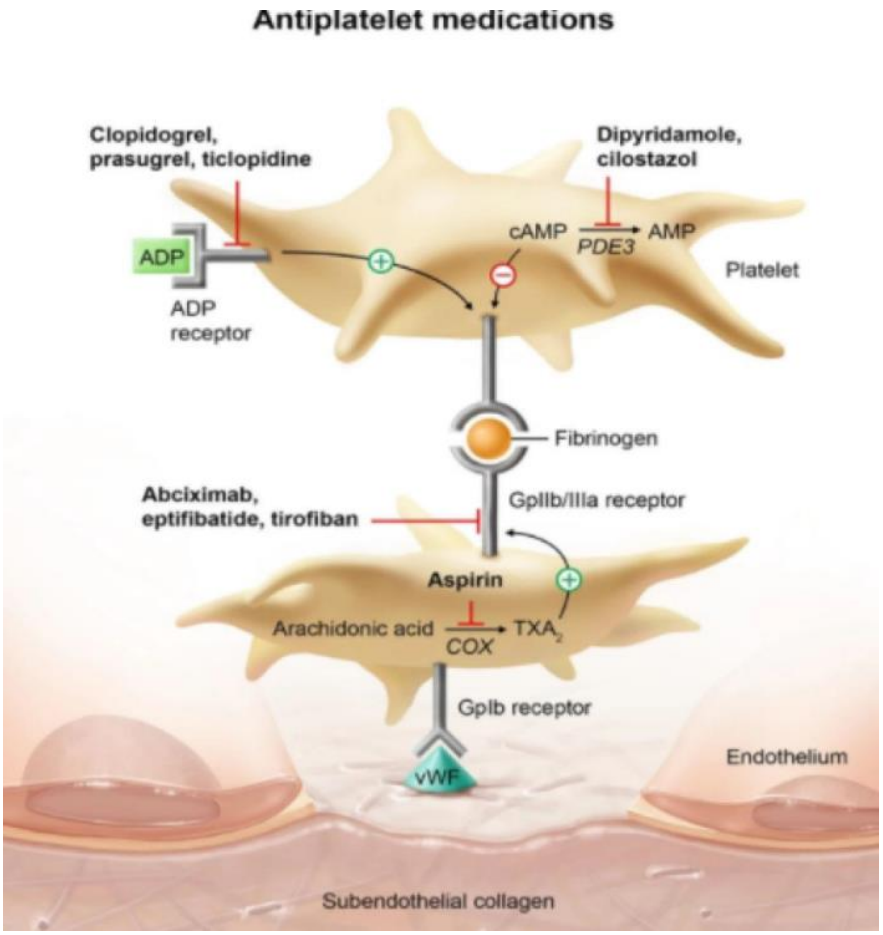
1. Blocking the formation of ligands (aspirin decreases thromboxane A2 formation).

2. Blocking interaction of ligands with receptors on platelets (clopidogrel and ticlopidine work as ADP antagonists).

3. Binding to the glycoprotein receptor IIb/IIIa on activated platelets, preventing aggregation (Abciximab, eptifibatide, tirofiban).

4. Interfering with intracellular signaling (cilostazol and dipyridamole increase cAMP by decreasing phosphodiesterase activity).

- Ticlopidine and clopidogrel are useful in the treatment and prevention of ischemic strokes, acute coronary syndrome and peripheral vascular disease. These agents can be combined with aspirin to get an additive antiplatelet effect, as the mechanism of action of ticlopidine and clopidogrel is different from aspirin.



ADP receptor inhibitors

- Drugs:
- Clopidogrel, prasugrel, ticagrelor (reversible), ticlopidine (irreversible).

- Mechanism of action:
- Inhibit platelet aggregation by blocking ADP (P2Y12) receptors → Prevent expression of glycoproteins IIb/IIIa on platelet surface.

- Clinical Use:
- Acute coronary syndrome; coronary stenting, ↓ incidence or recurrence of thrombotic stroke.

- Clopidogrel and aspirin are equally efficacious in the prevention of thromboembolic disease, and have a synergistic effect when used together because of their unique mechanisms of action.

- In patients with an aspirin allergy, aspirin can cause bronchoconstriction with shortness of breath and wheezing. In such patients, the best alternative antiplatelet agent is clopidogrel.

- Adverse effects:
- Ticlopidine is rarely used due to the occurrence of serious side effects. Neutropenia is seen in about 1 percent of patients on ticlopidine and typically presents with fever and mouth ulcers. Though this is rare, it is a serious complication and complete blood count should be monitored biweekly for the first three months.

- TTP may be seen.

Glycoprotein IIb/IIIa inhibitors

- Drugs:
- Abciximab, eptifibatide, tirofiban. Abciximab is made from monoclonal antibody Fab fragments.

- Mechanism of action:
- Bind to the glycoprotein receptor IIb/IIIa (fibrinogen receptor) on activated platelets, preventing aggregation.

- Clinical Use:
- Unstable angina, percutaneous transluminal coronary angioplasty.

- Adverse effects:
- Bleeding, thrombocytopenia.

Cilostazol, dipyridamole

- Mechanism of action:

- Phosphodiesterase III inhibitor → ↑ cAMP in platelets → resulting in inhibition of platelet aggregation; vasodilators.

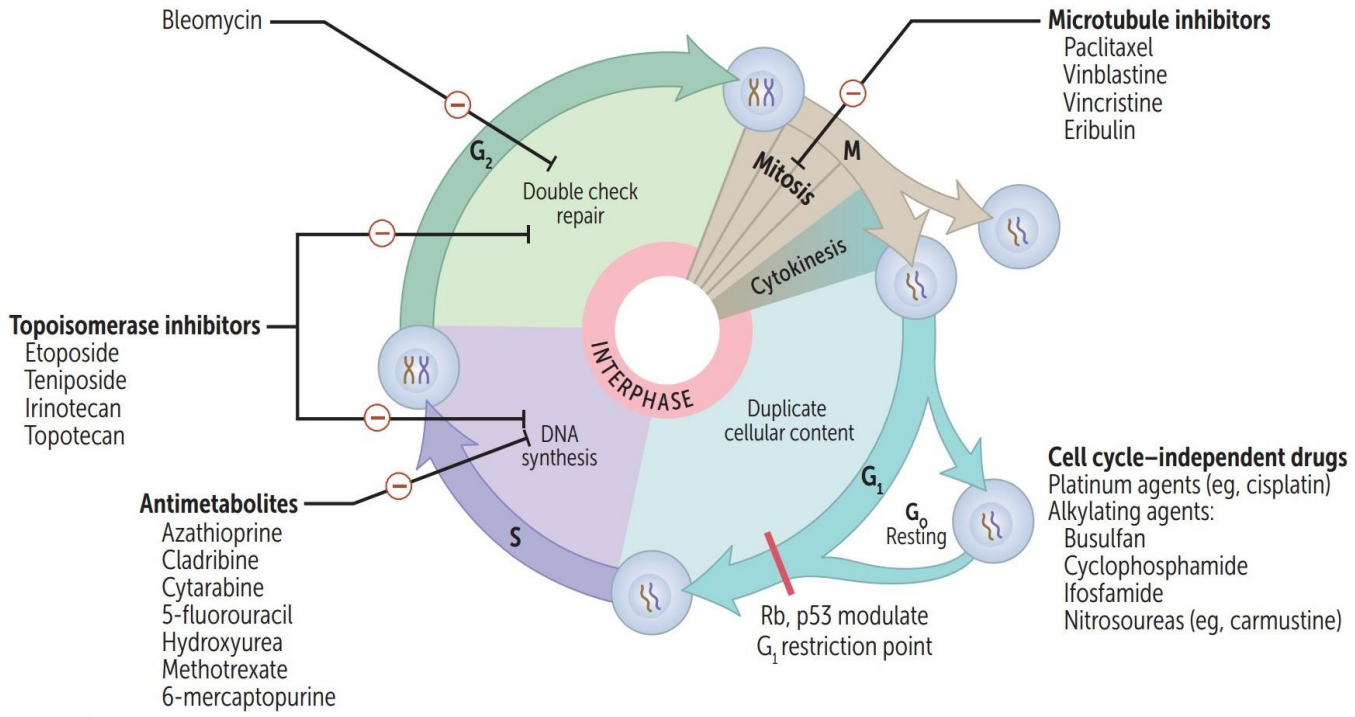
- Clinical Use:

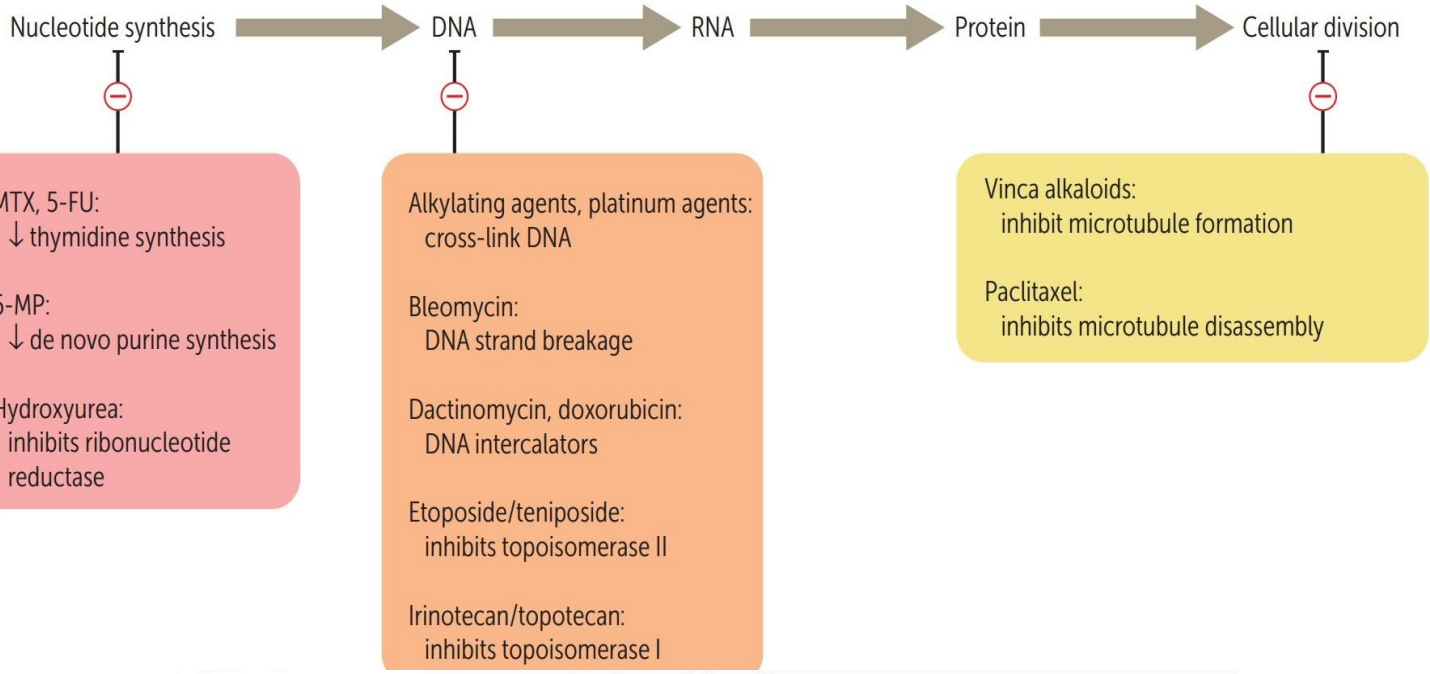
- Intermittent claudication, coronary vasodilation, prevention of stroke or TIAs (combined with aspirin), angina prophylaxis.

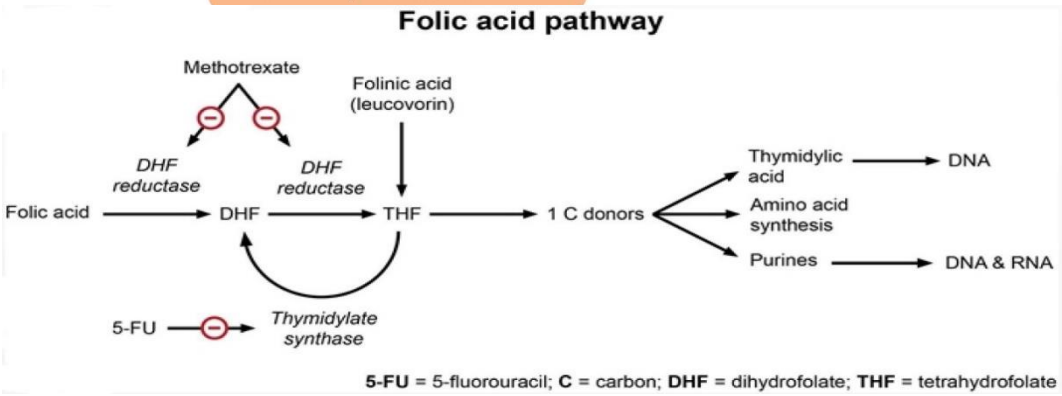
- Adverse effects:

- Nausea, headache, facial flushing, hypotension, abdominal pain.

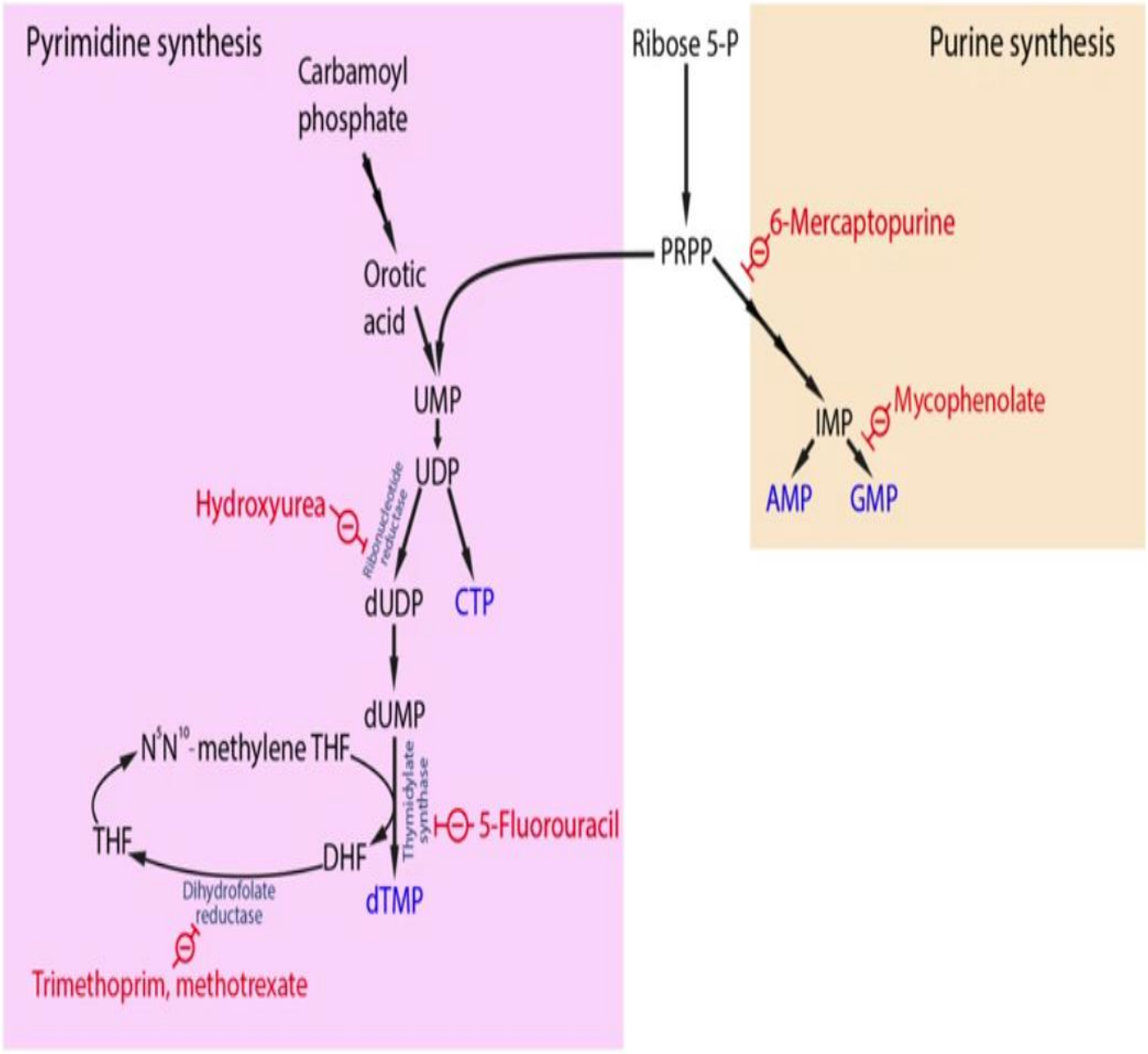
Cancer drugs







Antimetabolites



A. Methotrexate:

- Mechanism of action:

- Folic acid analog that competitively inhibits dihydrofolate reductase → ↓ dTMP → ↓ DNA synthesis.

- Clinical uses:

- Cancers: leukemias (ALL), lymphomas, choriocarcinoma, sarcomas.

- Non-neoplastic: ectopic pregnancy, medical abortion (with misoprostol), rheumatoid arthritis, psoriasis, IBD, vasculitis.

- Side effects:

- Myelosuppression, which is reversible with leucovorin "rescue".

- Toxic to 2L (Lung, Liver) → Pulmonary fibrosis, Hepatotoxicity.

- Mucositis (mouth ulcers).

B. 5-fluorouracil:

- Mechanism of action:

- Pyrimidine analog bioactivated to 5-FdUMP, which covalently complexes folic acid. This complex inhibits thymidylate synthase → ↓ dTMP → ↓ DNA synthesis.

- Clinical uses:

- Colon cancer, pancreatic cancer, basal cell carcinoma (topical).

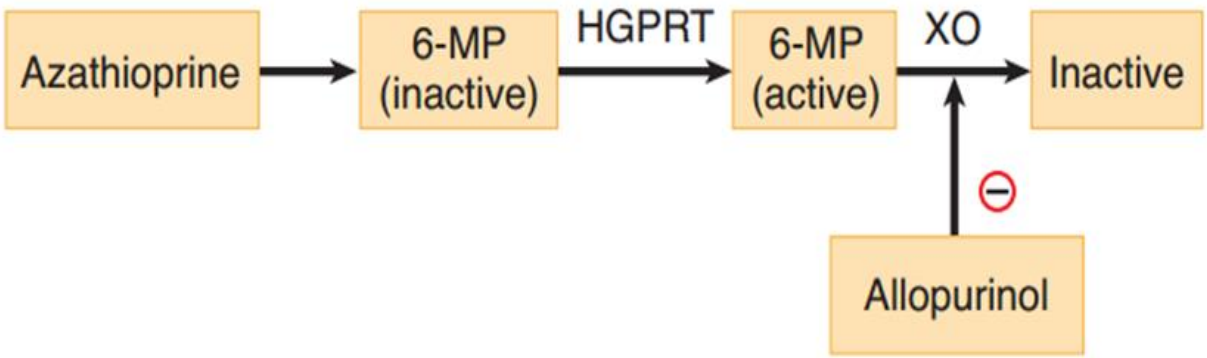
- Effects enhanced with the addition of leucovorin.

- Side effects: Myelosuppression, worsened with the addition of leucovorin (folinic acid).

❖ N.B:

- The key metabolic difference between methotrexate and 5-fluorouracil is that methotrexate prevents the reduction of folic acid to tetrahydrofolate, while 5-FU binds tetrahydrofolate and thymidylate synthetase in a stable-reaction intermediate form, thereby effectively decreasing the amount of thymidylate synthetase available for thymidine synthesis.
- Leucovorin is a tetrahydrofolate derivative that does not require reduction by dihydrofolate reductase before it can function as a cofactor for thymidylate synthase and other enzymes involved with purine and amino acid synthesis.
- Because leucovorin bypasses the dihydrofolate reductase step that is inhibited by methotrexate, it can be used to "rescue" normal cells from the toxicity of methotrexate.
- 5-FU, on the other hand, requires the presence of reduced folate in order to form complexes with thymidylate synthetase. 5-FU has a reduced cytotoxic effect in cells that are deficient in tetrahydrofolate. For this reason, leucovorin can be utilized to potentiate the toxicity of fluoropyrimidines such as fluorouracil by strengthening the association of the drug with thymidylate synthase.

C. Azathioprine, 6-mercaptopurine:



- Mechanism of action:
- Purine (thiol) analogs → ↓ de novo purine synthesis.
- Activated by HGPRT.
- Azathioprine is metabolized into 6-MP.
- Clinical uses: Preventing organ rejection, rheumatoid arthritis, IBD, SLE; used to wean patients off steroids in chronic disease and to treat steroid-refractory chronic disease.
- Side effects:
- Myelosuppression, GI and liver toxicity.
- Azathioprine and 6-MP are metabolized by xanthine oxidase; thus, both have ↑ toxicity with allopurinol or febuxostat.

❖ N.B:

- 6-mercaptopurine and 6-thioguanine are cytotoxic purine analogs that inhibit de novo purine synthesis after being converted to active metabolites by hypoxanthine-guanine phosphoribosyl transferase (HGPRT).
- Degradation of these agents to inactive metabolites is primarily accomplished by xanthine oxidase (XO) and thiopurine methyltransferase (TPMT) in the liver.
- The effect of xanthine oxidase on the metabolism of these agents is so significant that chemotherapy doses must be reduced by 75% if used with the xanthine oxidase inhibitor allopurinol.

D. Cladribine:

- Mechanism of action:
- Purine analog → multiple mechanisms (inhibition of DNA polymerase, DNA strand breaks).
- Clinical uses: Cladribine is the drug of choice for hairy cell leukemia.
- Side effects: Myelosuppression, nephrotoxicity, and neurotoxicity.

E. Cytarabine (arabinofuranosyl cytidine):

- Mechanism of action:
- Pyrimidine analog → inhibition of DNA polymerase.
- Clinical uses:
- Leukemias (AML), lymphomas.
- Side effects:
- Myelosuppression with megaloblastic anemia.
- CYTarabine causes panCYTopenia.

Alkylating agents

A. Busulfan:

- Mechanism of action:

- Cross-links DNA.

- Clinical uses:

- CML. Also used to ablate patient's bone marrow before bone marrow transplantation.

- Side effects:

- Severe myelosuppression (in almost all cases), pulmonary fibrosis, hyperpigmentation.

B. Cyclophosphamide, ifosfamide:

- Mechanism of action:

- Cross-link DNA at guanine N-7.

- Require bioactivation by liver.

- Clinical uses:

- Solid tumors, leukemia, lymphomas.

- Side effects:

- Myelosuppression; hemorrhagic cystitis (due to toxic metabolite acrolein), prevented with mesna (thiol group of mesna binds toxic metabolites) or N-acetylcysteine.

C. Nitrosoureas (Carmustine, lomustine, semustine, streptozocin):

- Mechanism of action

- Require bioactivation.

- Cross blood-brain barrier → CNS.

- Clinical uses:

- Brain tumors (including glioblastoma multiforme).

- Side effects:

- CNS toxicity (convulsions, dizziness, ataxia).

Antitumor antibiotics

A. Bleomycin:

- Mechanism of action:

- Induces free radical formation → breaks in DNA strands.

- Clinical uses: Testicular cancer, Hodgkin lymphoma.

- Side effects:

- Pulmonary fibrosis, skin hyperpigmentation.

- Minimal myelosuppression.

B. Dactinomycin (actinomycin D):

- Mechanism of action:

- Intercalates in DNA.

- Clinical uses: Wilms tumor, Ewing sarcoma, rhabdomyosarcoma. Used for childhood tumors ("children act out").

- Side effects: Myelosuppression.

C. Doxorubicin, daunorubicin:

- Mechanism of action:

- Generate free radicals.

- Intercalate in DNA → breaks in DNA → ↓ replication.

- Clinical uses: Solid tumors, leukemias, lymphomas.

- Side effects:

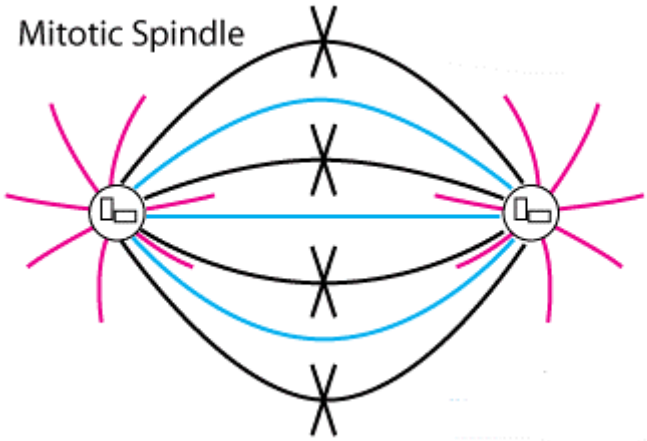
- Cardiotoxicity (dilated cardiomyopathy), myelosuppression, alopecia.

- Dexrazoxane (iron chelating agent), used to prevent cardiotoxicity.

❖ N.B:

- The anthracyclines (daunorubicin, doxorubicin, epirubicin and idarubicin) are chemotherapeutic agents associated with severe cardiotoxicity.
- The generation of free radicals is implicated in the unique ability of these agents to cause cardiotoxicity.
- Dilated cardiomyopathy is cumulative dose-dependent and may present many months after discontinuation of the drug.
- The most effective method of preventing doxorubicin cardiomyopathy is dexrazoxane. It is an iron-chelating agent that decreases formation of oxygen free radicals by doxorubicin and other anthracyclines.

Microtubule inhibitors



A. Vincristine, vinblastine:

- Mechanism of action:

- Vinca alkaloids that bind β-tubulin and inhibit its polymerization into microtubules → prevent mitotic spindle formation (M-phase arrest).

- Clinical uses:

- Solid tumors, leukemias, Hodgkin (vinblastine) and non-Hodgkin (vincristine) lymphomas.

- Side effects:

- Vincristine: neurotoxicity (areflexia, peripheral neuritis), constipation (including paralytic ileus). Vincristine cause s neurotoxicity by interfering with microtubule formation in nerve axons.

- Vinblastine: myelosupression (vinblastine blast your bone marrow)

B. Paclitaxel, other taxols:

- Mechanism of action:

- Hyperstabilize polymerized microtubules in M phase so that mitotic spindle cannot break down (anaphase cannot occur).

- Clinical uses:

- Ovarian and breast carcinomas.

- Side effects:

- Myelosuppression, neuropathy, hypersensitivity.

Cisplatin, carboplatin

- Mechanism of action:
  - Cisplatin is a platinum-containing compound that exerts its chemotherapeutic effect by forming a reactive oxygen species that can form DNA crosslinks.

- Clinical Use:
  - Testicular, bladder, ovary, and lung carcinomas.

- Adverse effects:
  - Nephrotoxicity, peripheral neuropathy, ototoxicity.

  - Prevent nephrotoxicity with amifostine (free radical scavenger) and chloride (saline) diuresis.

❖ N.B:

- The most prominent adverse effect associated with use of cisplatin is nephrotoxicity. This drug causes acute tubular injury, and a significant percentage of patients will develop mild renal insufficiency after the first course of therapy if preventative measures are not taken.
- Amifostine is a thiol-based cytoprotective free-radical scavenging agent used to decrease the cumulative nephrotoxicity associated with platinum-containing agents, thereby disallowing reaction with the renal tubules.
- Another preventative measure is establishing a chloride diuresis (via intravenous normal saline) because cisplatin stays in a nonreactive state when in a higher chloride concentration.

Etoposide, teniposide

- Mechanism of action:
  - Etoposide inhibits topoisomerase II.

- Clinical Use:
  - Solid tumors (particularly testicular and small cell lung cancer), leukemias, lymphomas.

- Adverse effects:
  - Myelosuppression, alopecia.

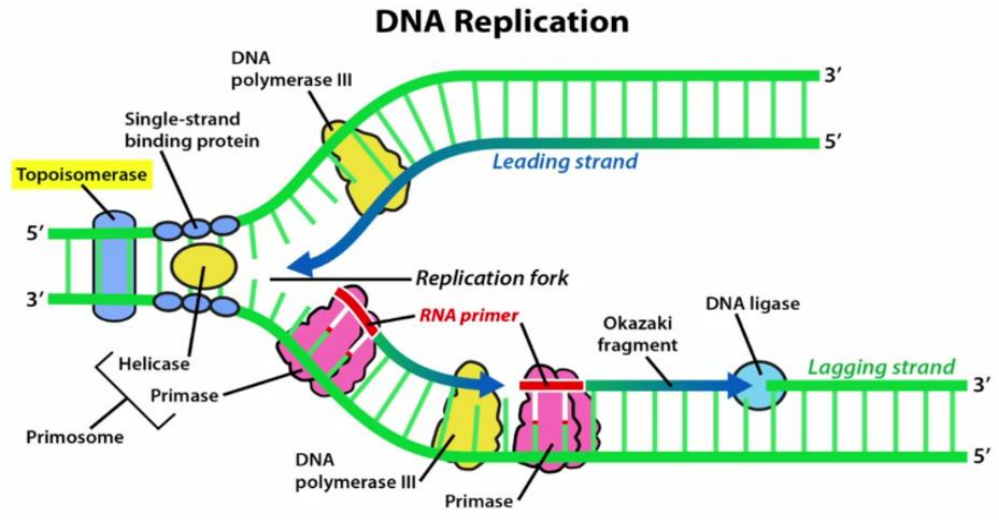
Irinotecan, topotecan

- Mechanism of action:
  - Inhibit topoisomerase I and prevent DNA unwinding and replication.

- Clinical Use:
  - Colon cancer (irinotecan); ovarian and small cell lung cancers (topotecan).

- Adverse effects:

- Severe myelosuppression, diarrhea.



Hydroxyurea

- Mechanism of action:

- Inhibits ribonucleotide reductase →↓ DNA Synthesis (S-phase specific).

- Clinical Use:

- Melanoma, CML, sickle cell disease (↑ HbF).

- Adverse effects:

- Severe myelosuppression.

BeVacizumab

- Mechanism of action:

- Monoclonal antibody against VEGF.

- Inhibits angiogenesis. (BeVacizumab inhibits Blood Vessel).

- Clinical Use:

- Solid tumors (colorectal cancer, renal cell carcinoma).

- Adverse effects:

- Hemorrhage, blood clots, and impaired wound healing.

Erlotinib

- Mechanism of action:
  - EGFR tyrosine kinase inhibitor.
- Clinical Use:
  - Non-small cell lung carcinoma.
- Adverse effects:
  - Rash.

Cetuximab, Panitumumab

- Mechanism of action:
  - Monoclonal antibody against EGFR.
- Clinical Use:
  - Stage IV colorectal cancer (wild-type KRAS), head and neck cancer.
- Adverse effects:
  - Rash, elevated LFTs, diarrhea.

Imatinib, Dasatinib

- Mechanism of action:
  - Tyrosine kinase inhibitor of BCR-ABL (Philadelphia chromosome fusion gene in CML) and c-kit (common in GI stromal tumors).
- Clinical Use:
  - CML, GI stromal tumors.
- Adverse effects:
  - Fluid retention.

Rituximab

- Mechanism of action:
  - Monoclonal antibody against CD20, which is found on most B-cell neoplasms.
- Clinical Use:
  - Non-Hodgkin lymphoma, CLL, ITP, rheumatoid arthritis.
- Adverse effects:
  - ↑ risk of progressive multifocal leukoencephalopathy.

Vemurafenib, Dabrafenib

- Mechanism of action:

- Small molecule inhibitor of BRAF oncogene ⊕ melanoma.

- VEmuRAF-enib is for V600E mutated BRAF inhibition.

- Clinical Use:

- Metastatic melanoma.

❖ Common chemotoxicities:

- Cisplatin/Carboplatin → ototoxicity (and nephrotoxicity).
- Vincristine → peripheral neuropathy.
- Bleomycin, Busulfan → pulmonary fibrosis.
- Doxorubicin → cardiotoxicity.
- Trastuzumab → cardiotoxicity.
- Cisplatin/Carboplatin → nephrotoxic (and acoustic nerve damage).
- CYclophosphamide → hemorrhagic cystitis.
- 5-FU → myelosuppression.
- 6-MP → myelosuppression.
- Methotrexate → myelosuppression.