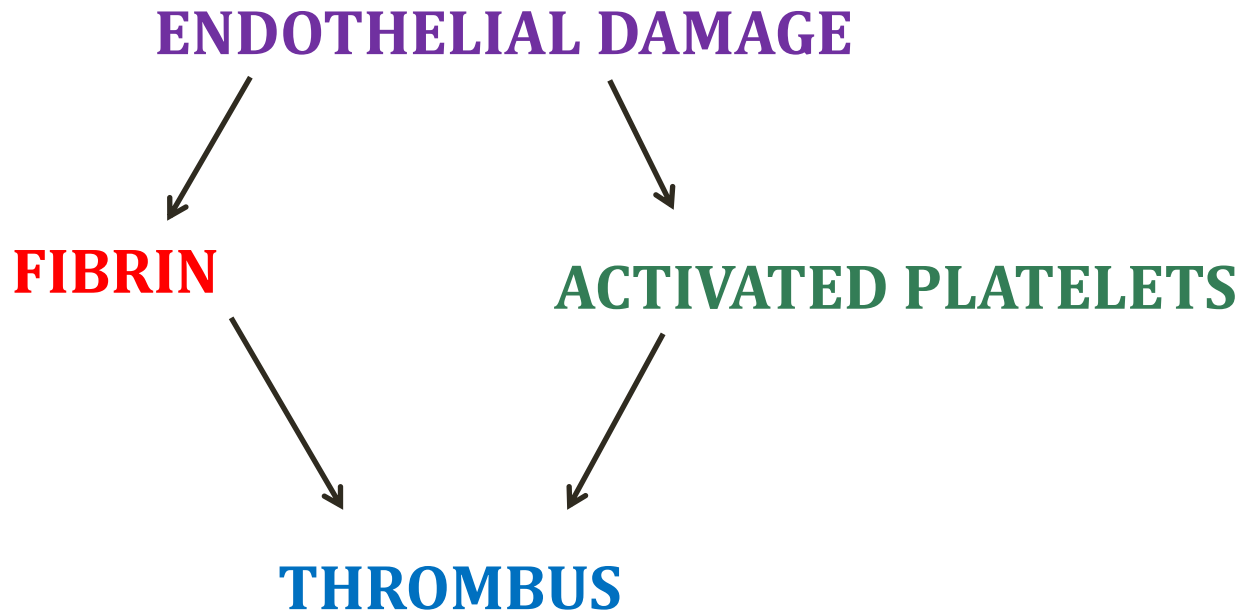


Coagulation

Jason Ryan, MD, MPH

Thrombus Formation



Vasoconstriction

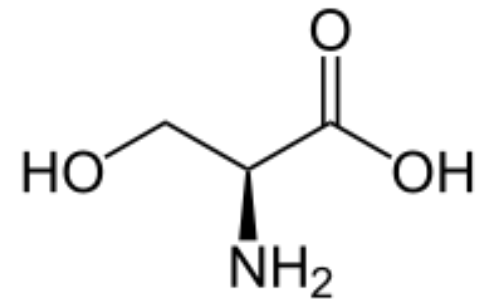
- 1st line of defense against bleeding
- Occurs in response to endothelial damage
- Key mediator: **endothelins**
 - Proteins
 - Potent vasoconstrictors
 - Released by **endothelial cells** near site of damage
 - Endothelin receptor blockers used in pulmonary hypertension

Coagulation Factors

- Proteins synthesized in **liver**
- Soluble in plasma
- Activate when triggered by endothelial damage
- Form an insoluble protein: Fibrin
- Fibrin mesh prevents blood loss

Coagulation Factors

- Most circulate as inactive enzymes (zymogens)
- Many activate to become **serine proteases**
 - Serine: amino acid
 - Protease: cleaves proteins
 - Serine protease: protein cleavage enzyme, contains serine

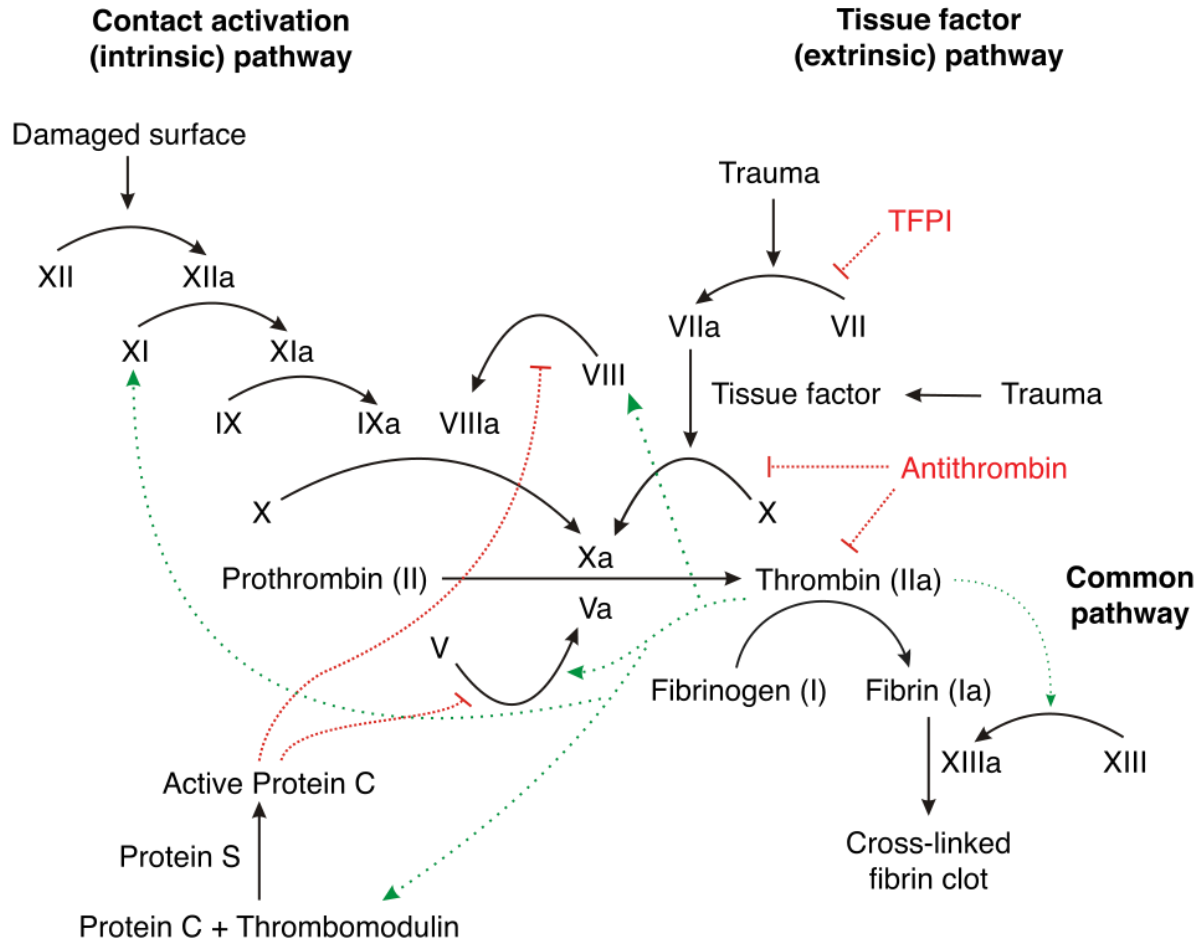


Serine

Coagulation Cascade

- **Sequential** activation of clotting factor zymogens
- Constant low level of activation in serum
- **Amplification** occurs with endothelial damage
- Leads to **fibrin generation**

Coagulation Cascade



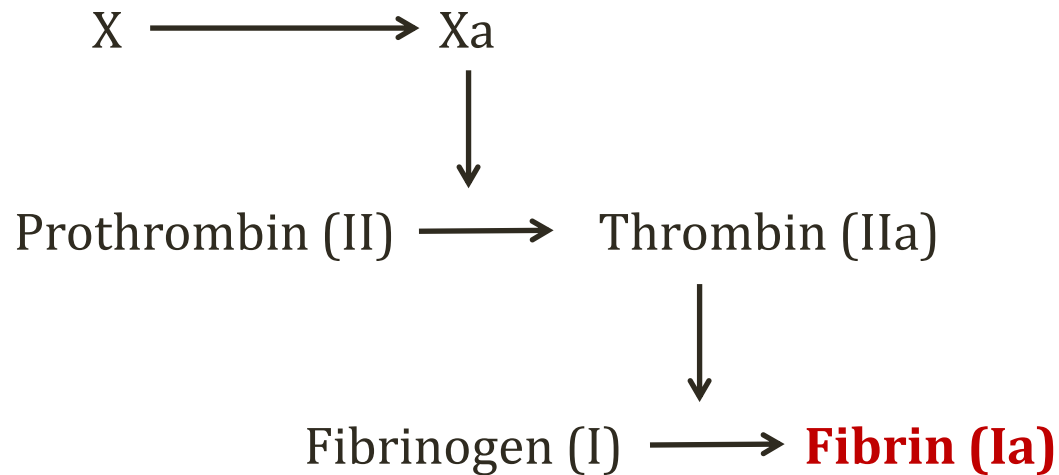
Joe D/Wikipedia

Coagulation Cascade

- Center of cascade is activation of $X \rightarrow Xa$
- Xa converts prothrombin (II) \rightarrow thrombin (IIa)
- Thrombin (IIa): Fibrinogen (I) \rightarrow fibrin (Ia)
- Fibrin forms plug to stop bleeding
- **Activation $X \rightarrow Xa$ makes fibrin**



Coagulation Cascade



Coagulation Cascade

Xa inhibitors

Rivaroxaban

Apixaban

↓ **Thrombin**

Direct Thrombin Inhibitors (DTIs)

Hirudin

Lepirudin

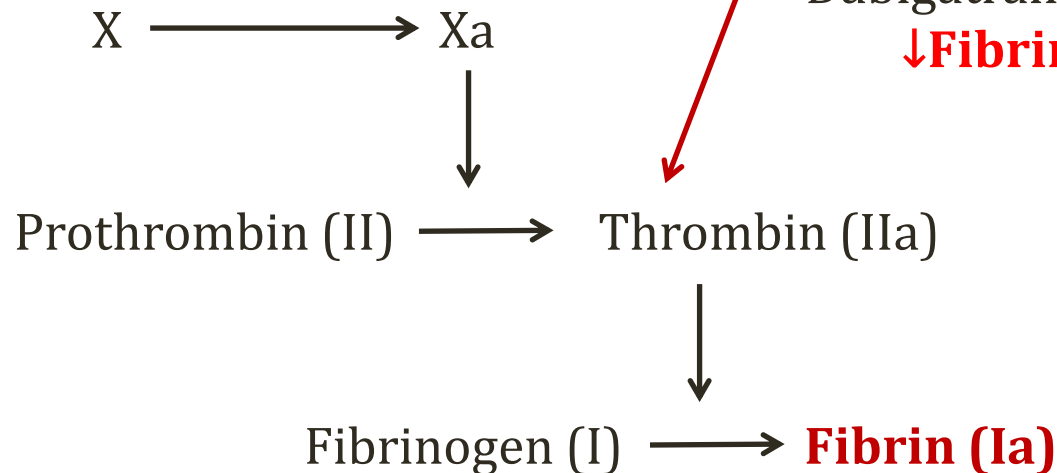
Bivalirudin

Desirudin

Argatroban

Dabigatran (PO)

↓ **Fibrin**



Tissue Factor

Thromboplastin

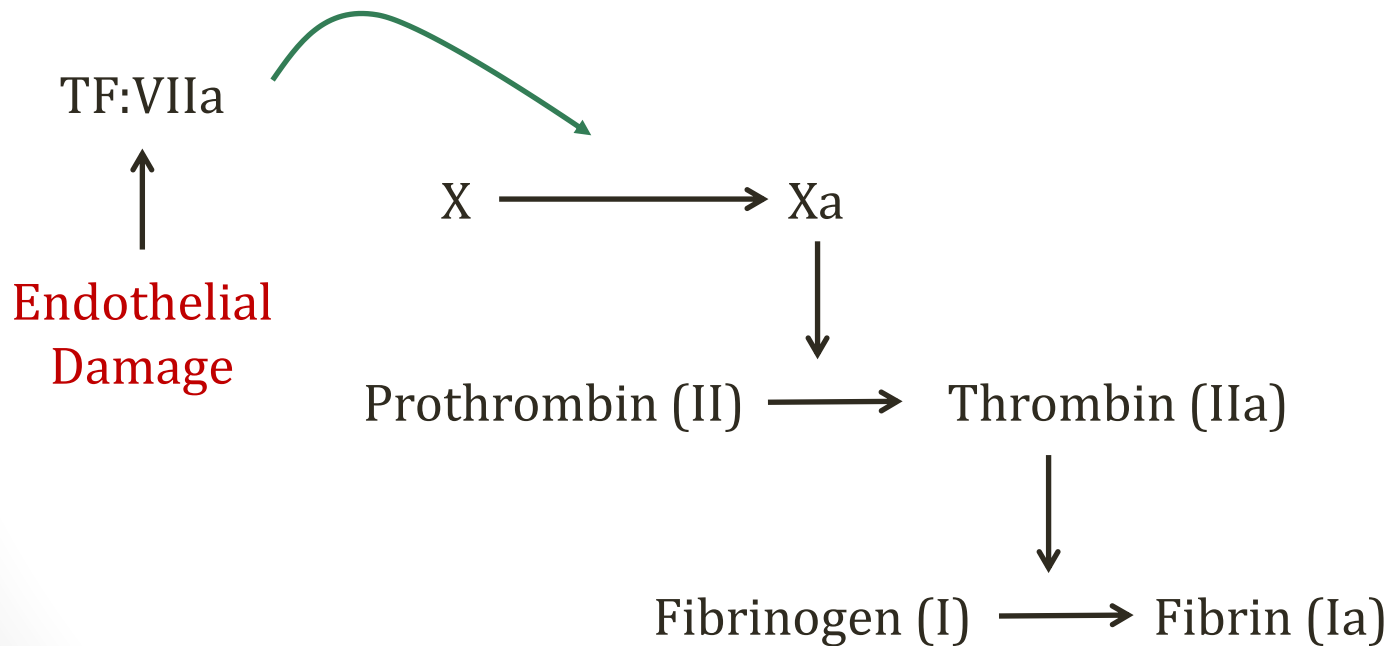
- **Glycoprotein**
- Constitutively expressed in **sub-endothelial** cells
- Not expressed by endothelial cells
- No significant contact of with circulating blood
- Exposed by endothelial damage
- **Major activator of coagulation system**
- Basis for **Prothrombin Time and INR**
 - Tissue factor added to blood sample
 - Time to form clot = PT

Coagulation Cascade

- Primary event: Exposure of tissue factor
- Interacts with factor VII → VIIa
- **TF:VIIa activates Xa**

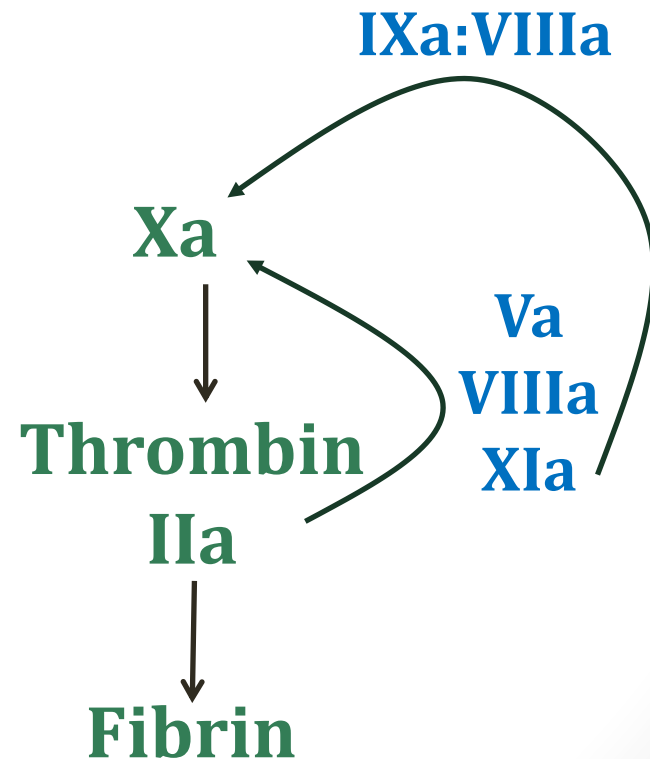
TF:VIIa

Coagulation Cascade

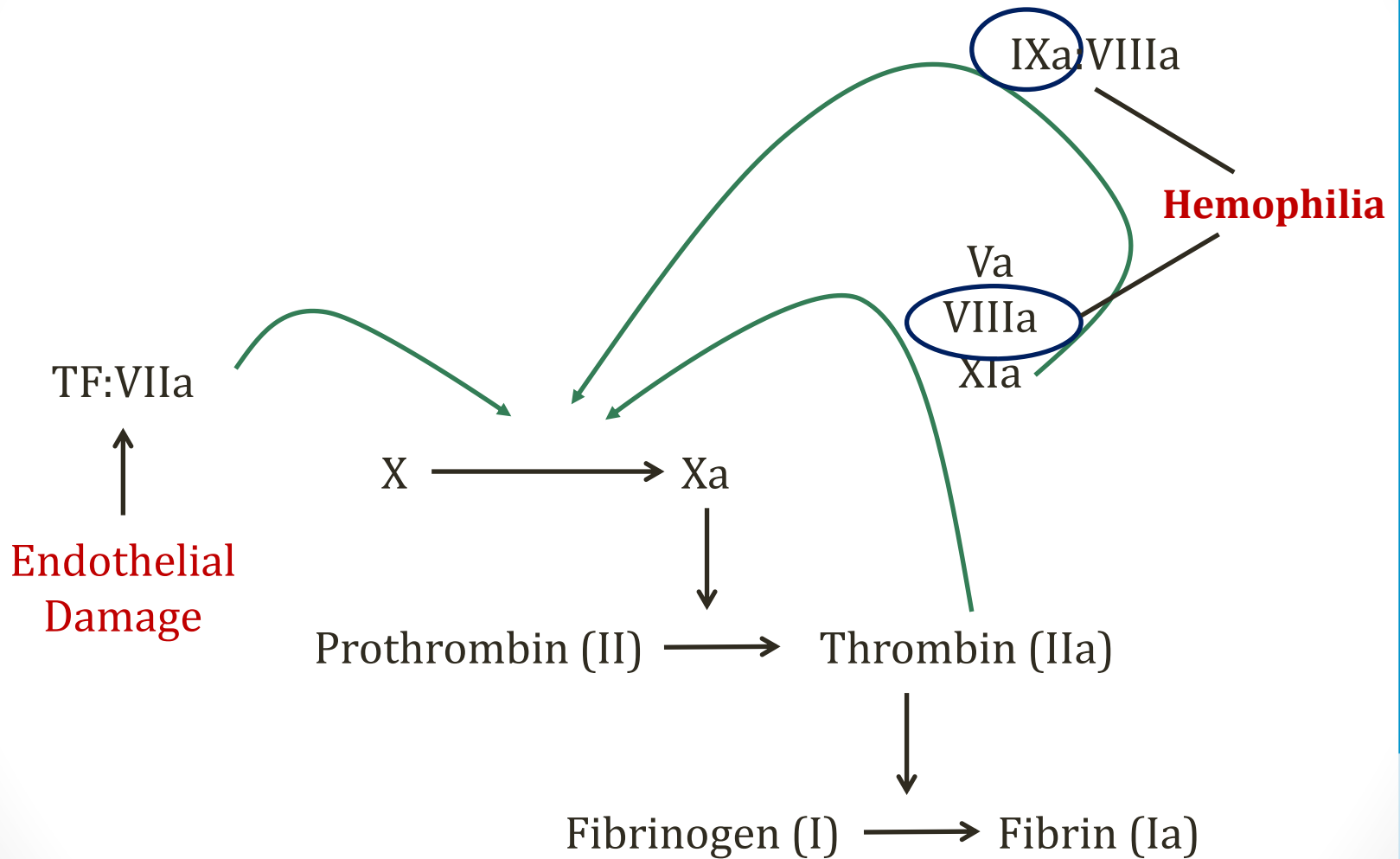


Thrombin

- **Thrombin (IIa) makes more thrombin**
- Can activate cascade (positive feedback)
 - **Factor V** → Va
 - **Factor XI** → XIa
 - **Factor VIII** → VIIIa
- Factor XIa activates **IX → IXa**
 - IX uses VIIIa as a co-factor
- IXa can also activate Xa
 - More amplification



Coagulation Cascade



Factor VIII

- Produced in endothelial cells (not the liver)
- Circulates bound to **von Willebrand Factor**
 - vWF critical for platelet aggregation
 - vWF produced by endothelial cells and megakaryocytes
 - Binding to vWF increases VIII plasma half life
- Released from vWF in response to vascular injury
 - Vascular injury → ↑ thrombin → becomes VIIIa

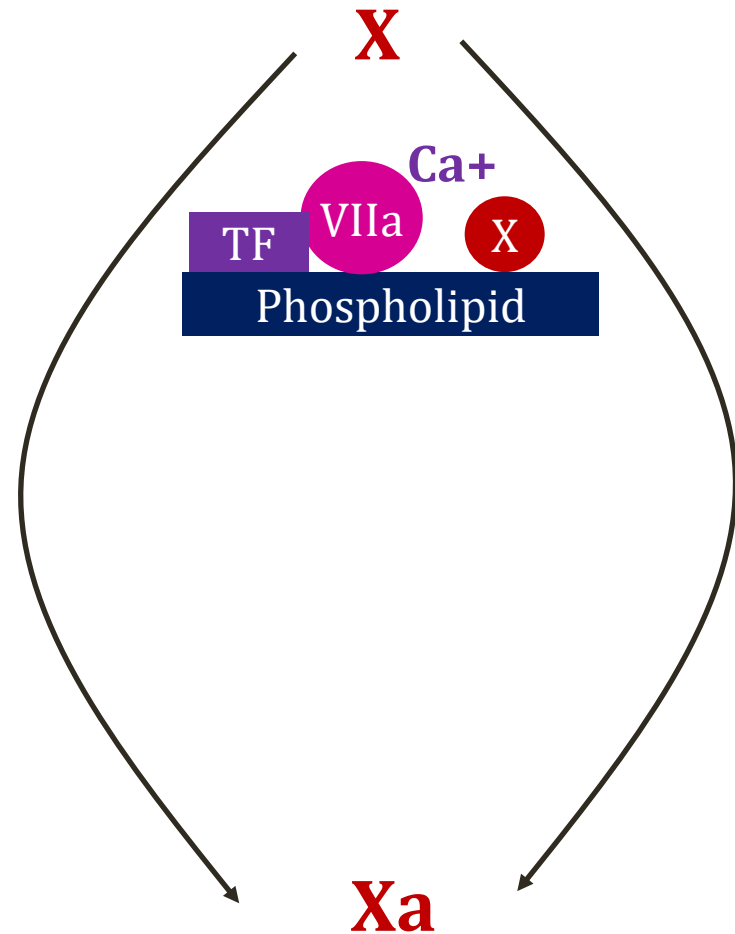
VIII—vWF

Multicomponent Complexes

- Two complexes for conversion $X \rightarrow X_a$
- Three components bound together:
 - Active clotting factor functioning as enzyme
 - Co-factor
 - Substrate
- Require **phospholipids and calcium**
 - Phospholipid: Occur on surfaces of cells
 - TF-bearing cells or platelets
 - Calcium: Co-factor

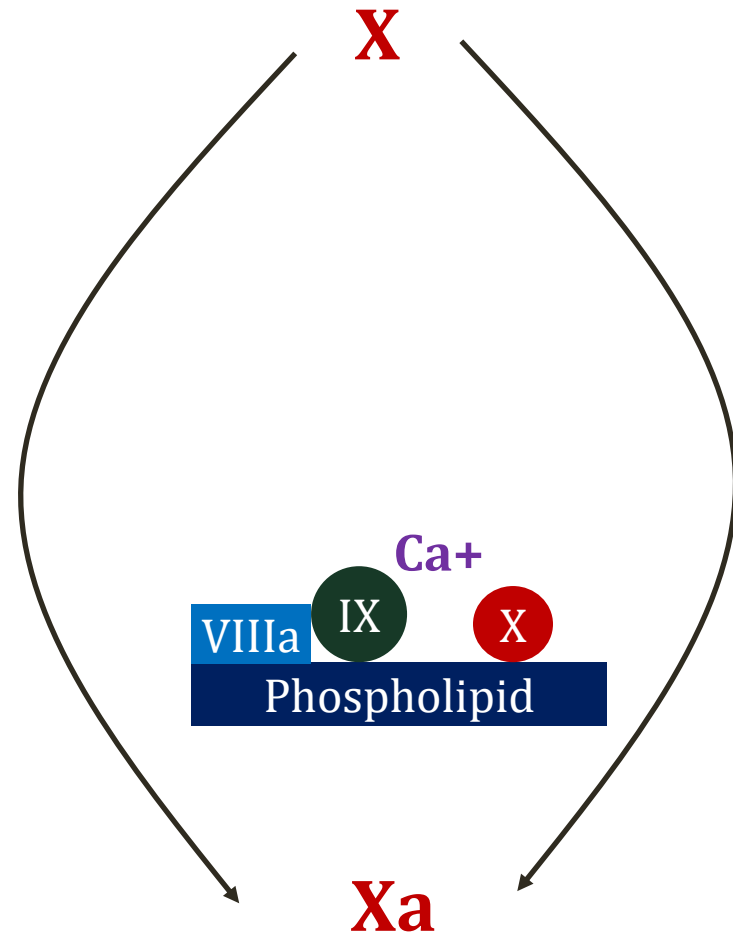
Multicomponent Complexes

- Extrinsic Xase
 - Phospholipid: TF-bearing cells
 - Enzyme: **Factor VIIa**
 - Co-factor: Tissue factor
 - Substrate: Factor X

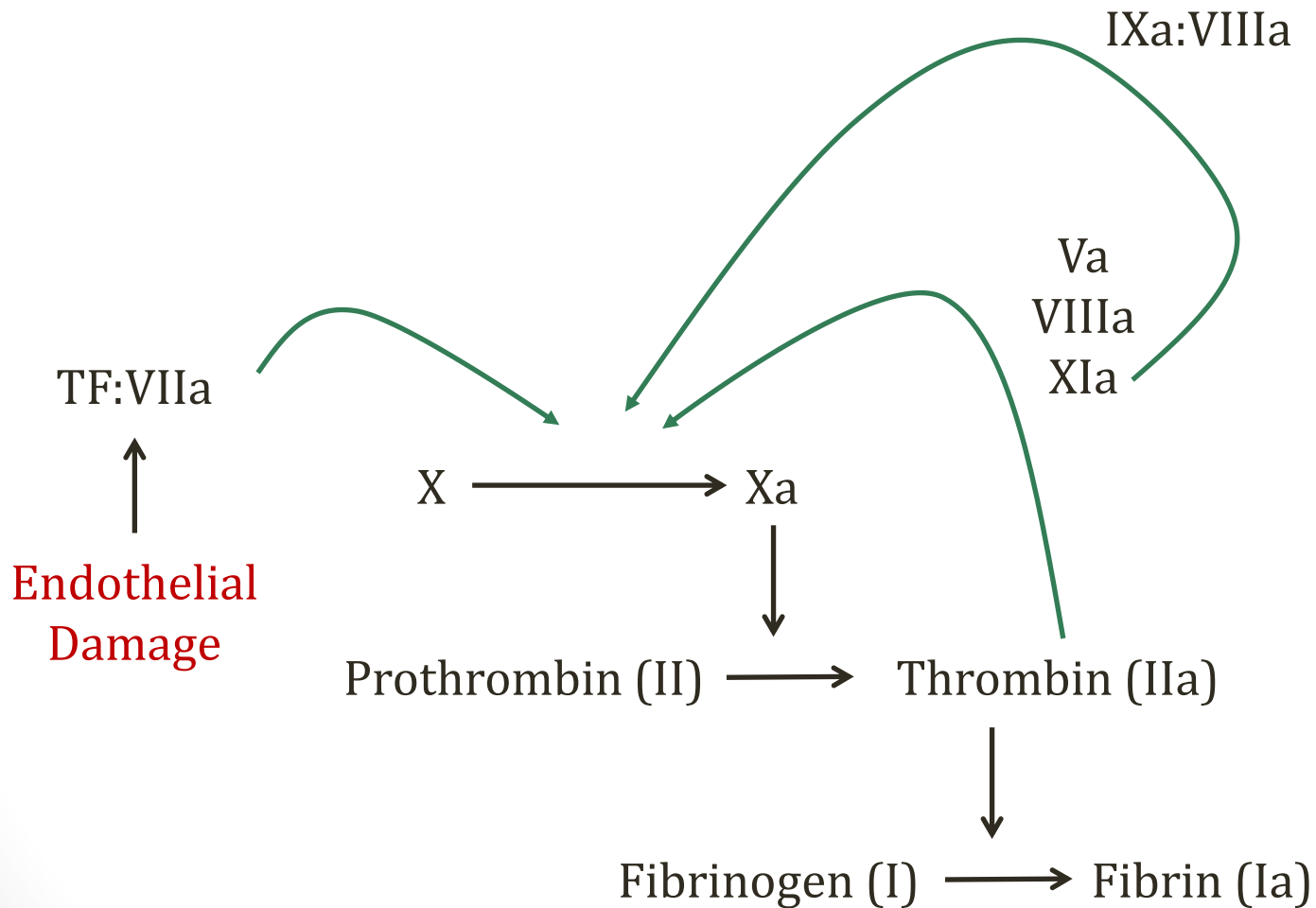


Multicomponent Complexes

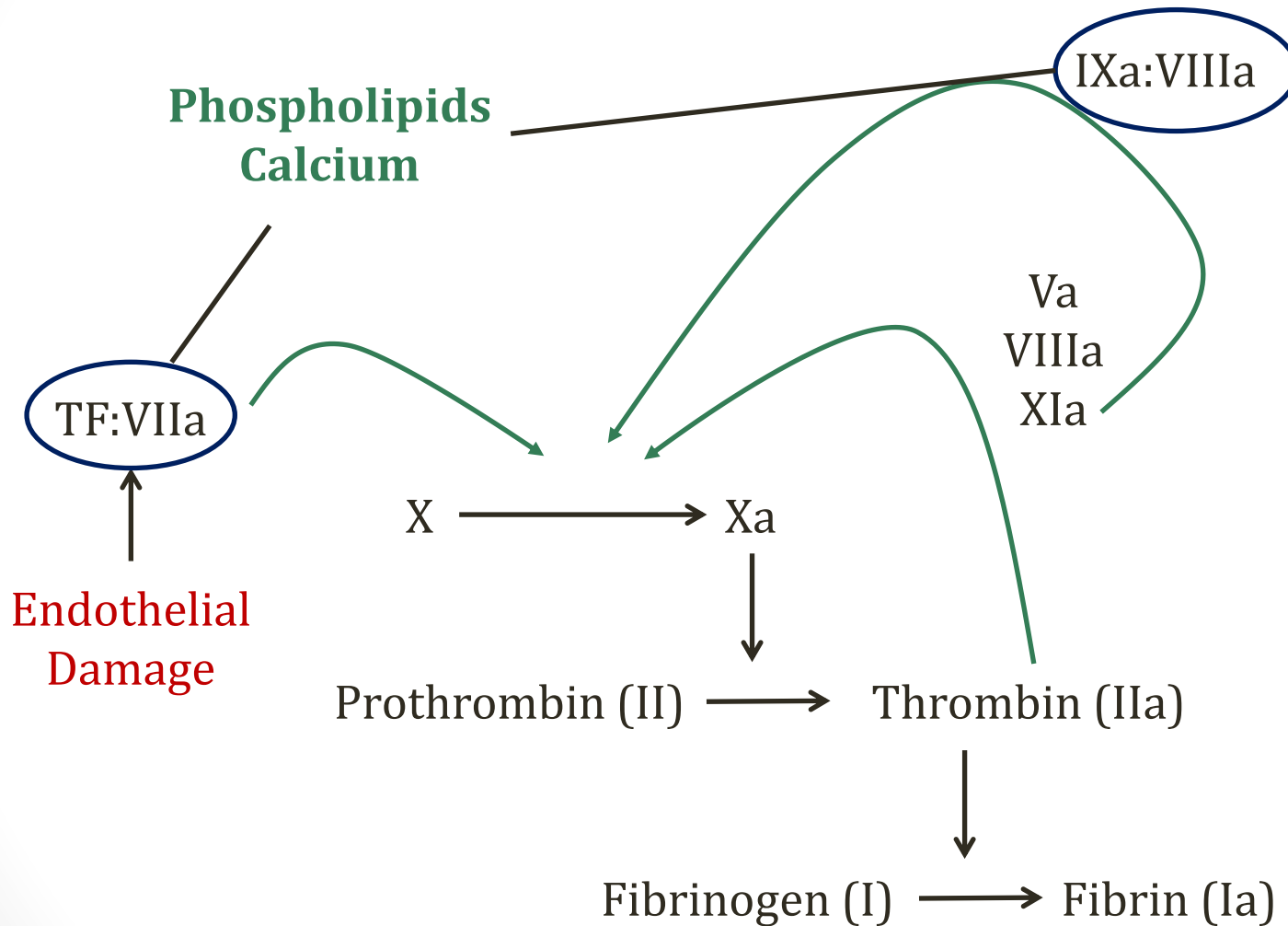
- Intrinsic Xase
 - Phospholipid: Platelets
 - Enzyme: **Factor IXa**
 - Co-factor: Factor VIII (VIIIa)
 - Substrate: Factor X



Coagulation Cascade



Coagulation Cascade



Calcium

- Factor IV
- Required for clot formation
- Activated platelets release calcium
- EDTA binds calcium in blood samples
- Prevents clotting

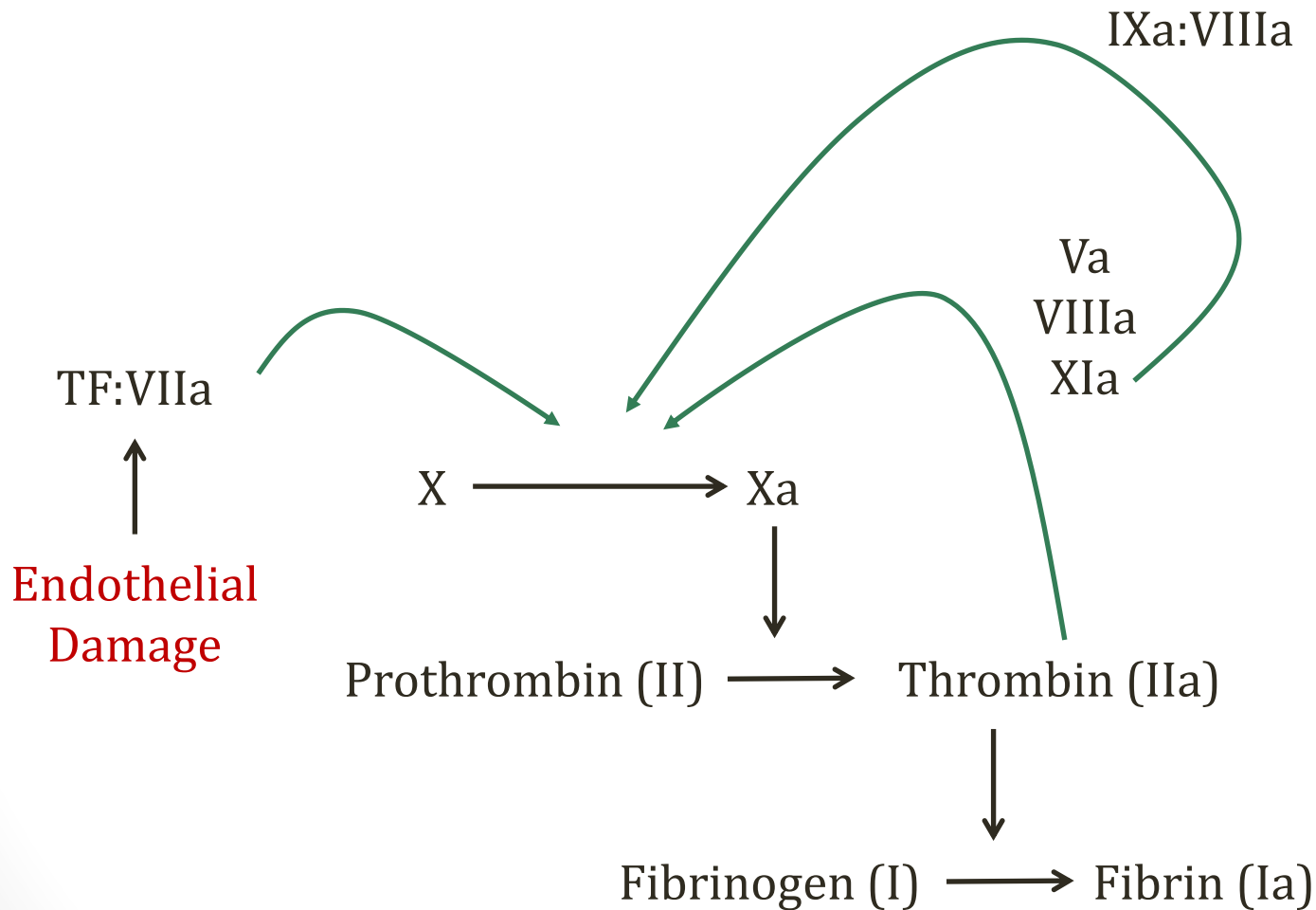


Tannim101/Wikipedia-

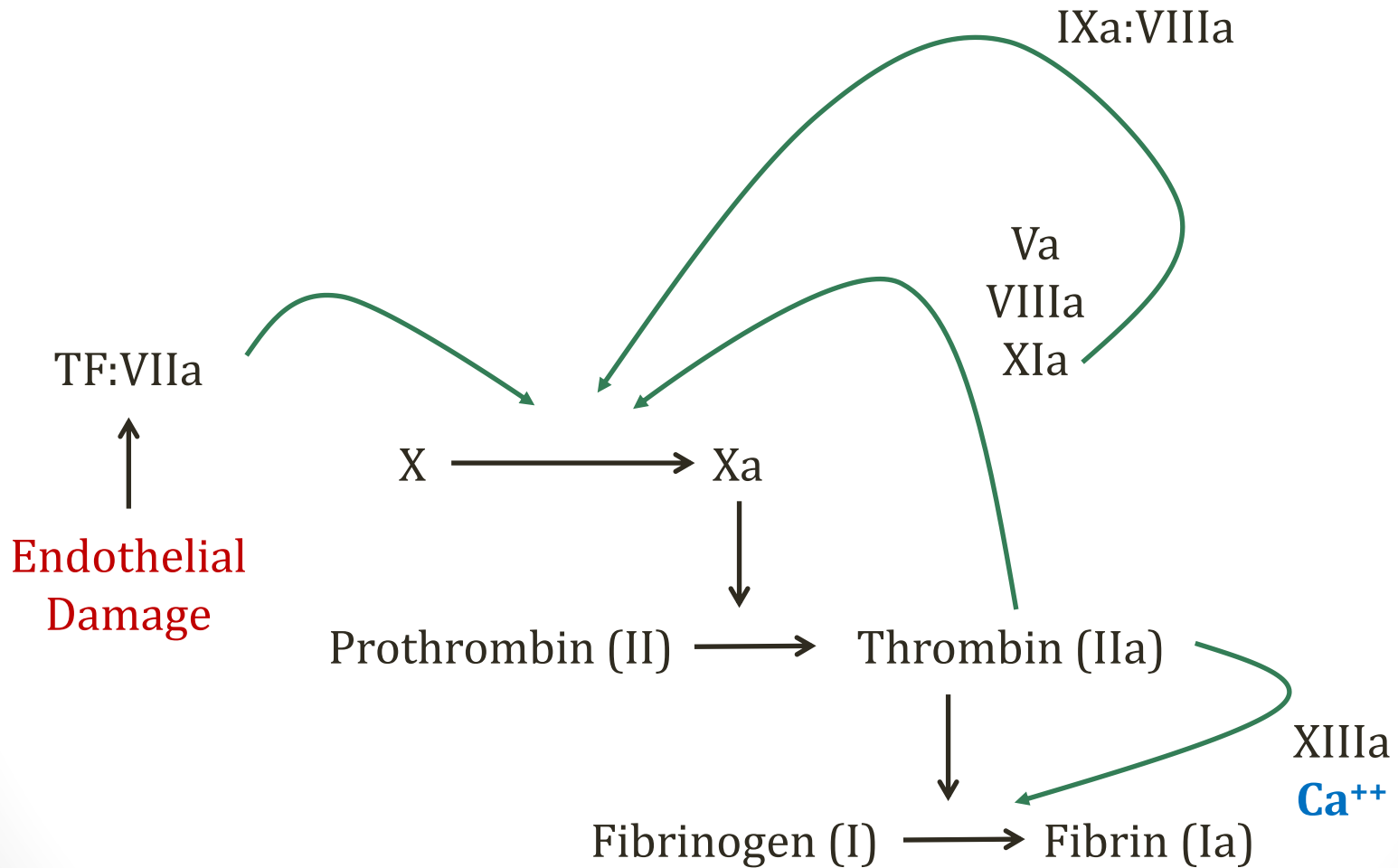
Factor XIII

- **Crosslinks** fibrin
- Stabilizes fibrin plug
- Absence of XIII → inadequate clot formation
- Requires **calcium** as co-factor
- Activated by thrombin (IIa) formation

Coagulation Cascade



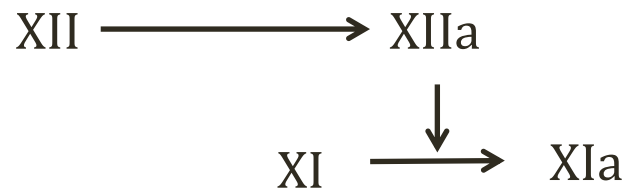
Coagulation Cascade



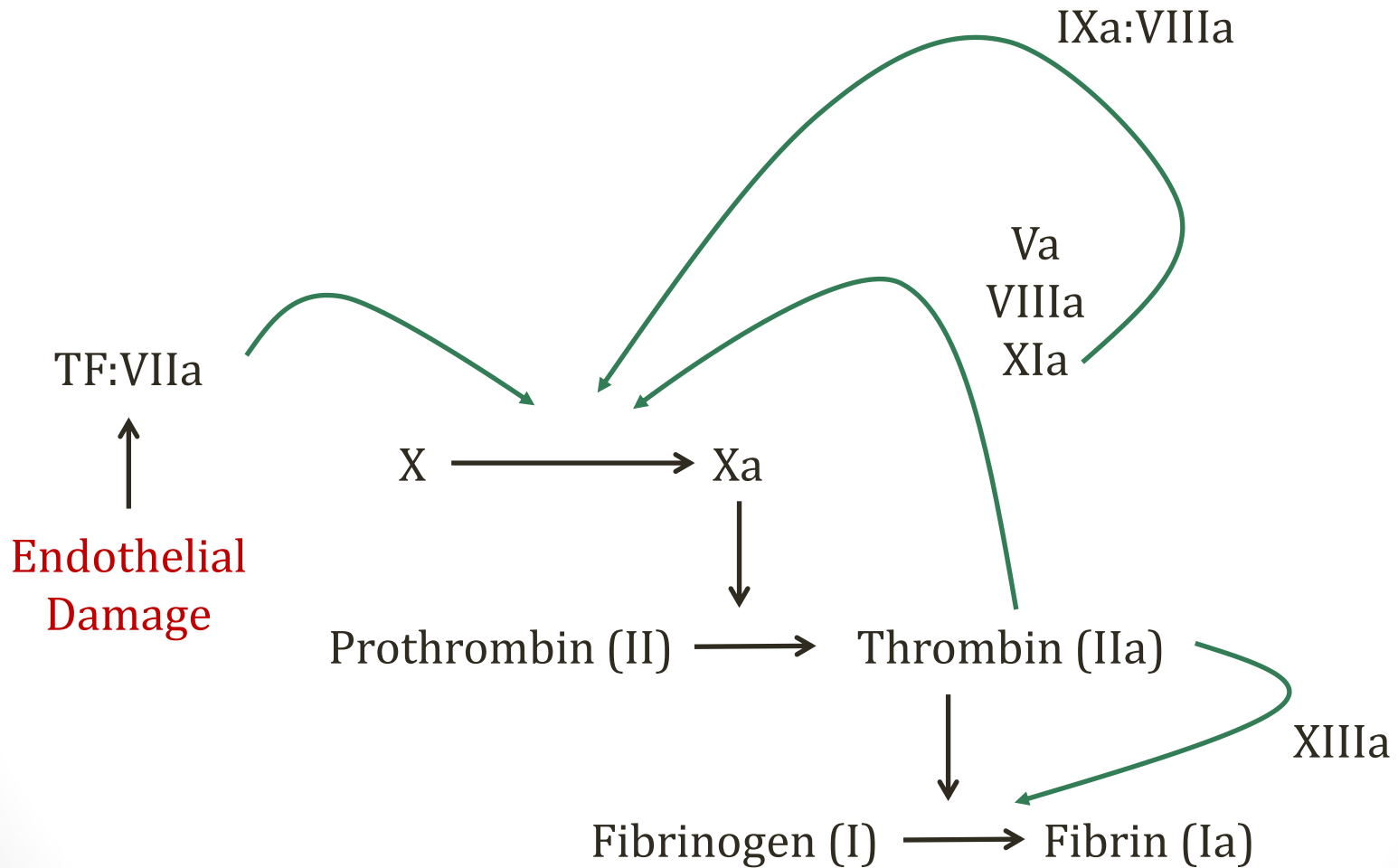
Factor XII

Hageman factor

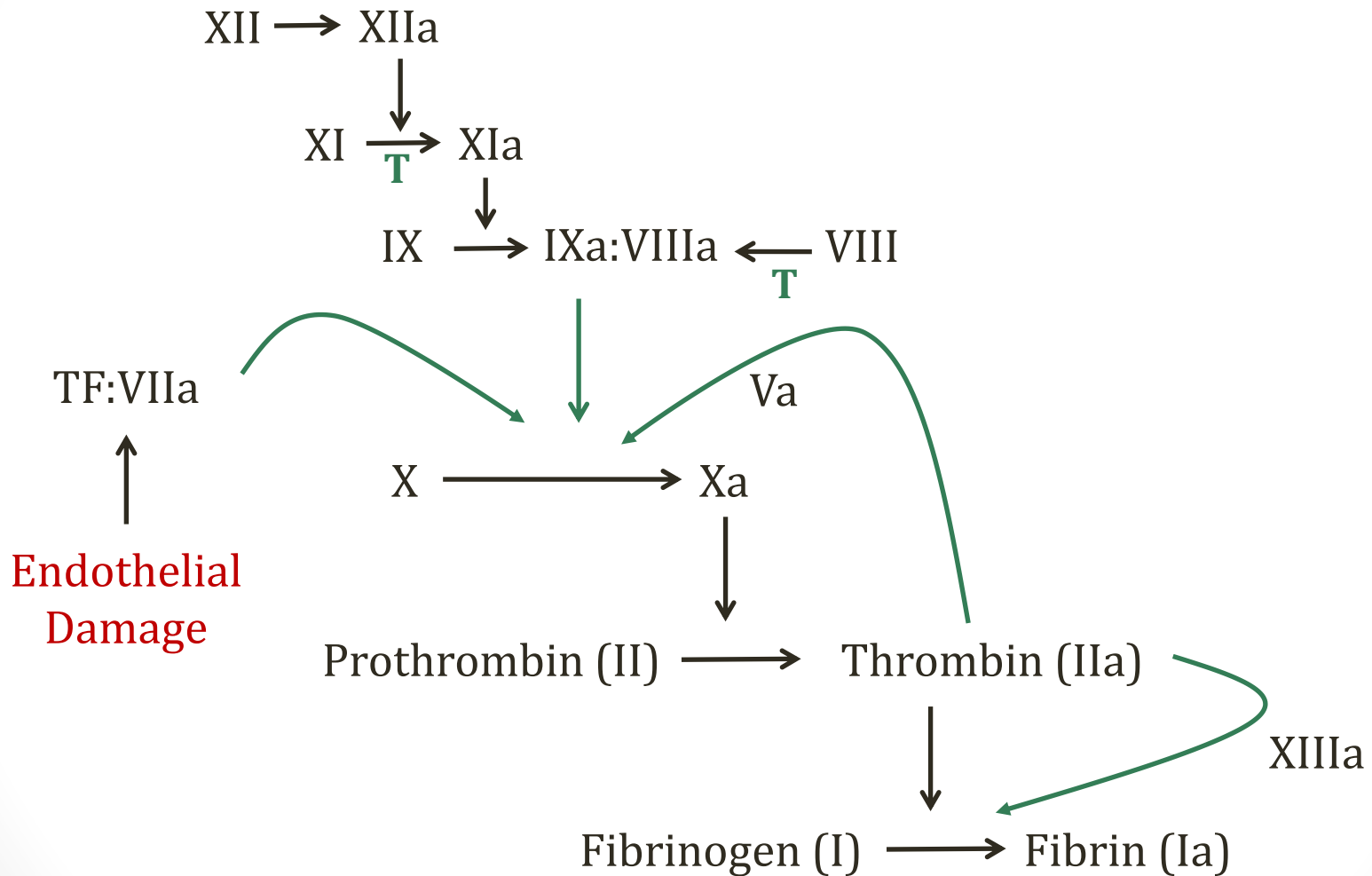
- Can activate factor XI (XIa)
- Physiologic significance unclear
- Important for testing of coagulation system
- Activated by **contact** with negatively charges
- Factor XII → XIIa via contact with silica
- Basis for **partial thromboplastin time (PTT)**



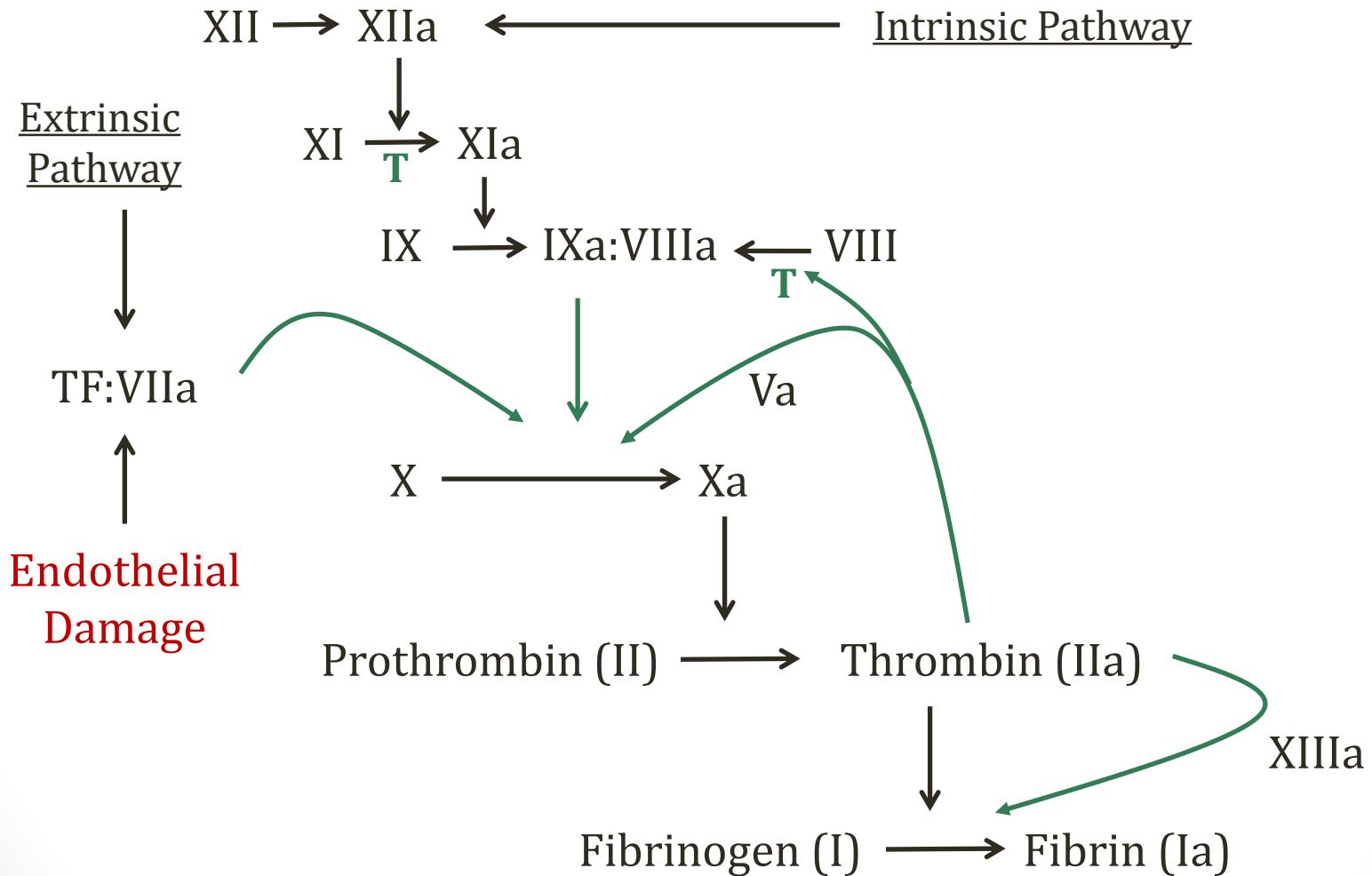
Coagulation Cascade



Coagulation Cascade



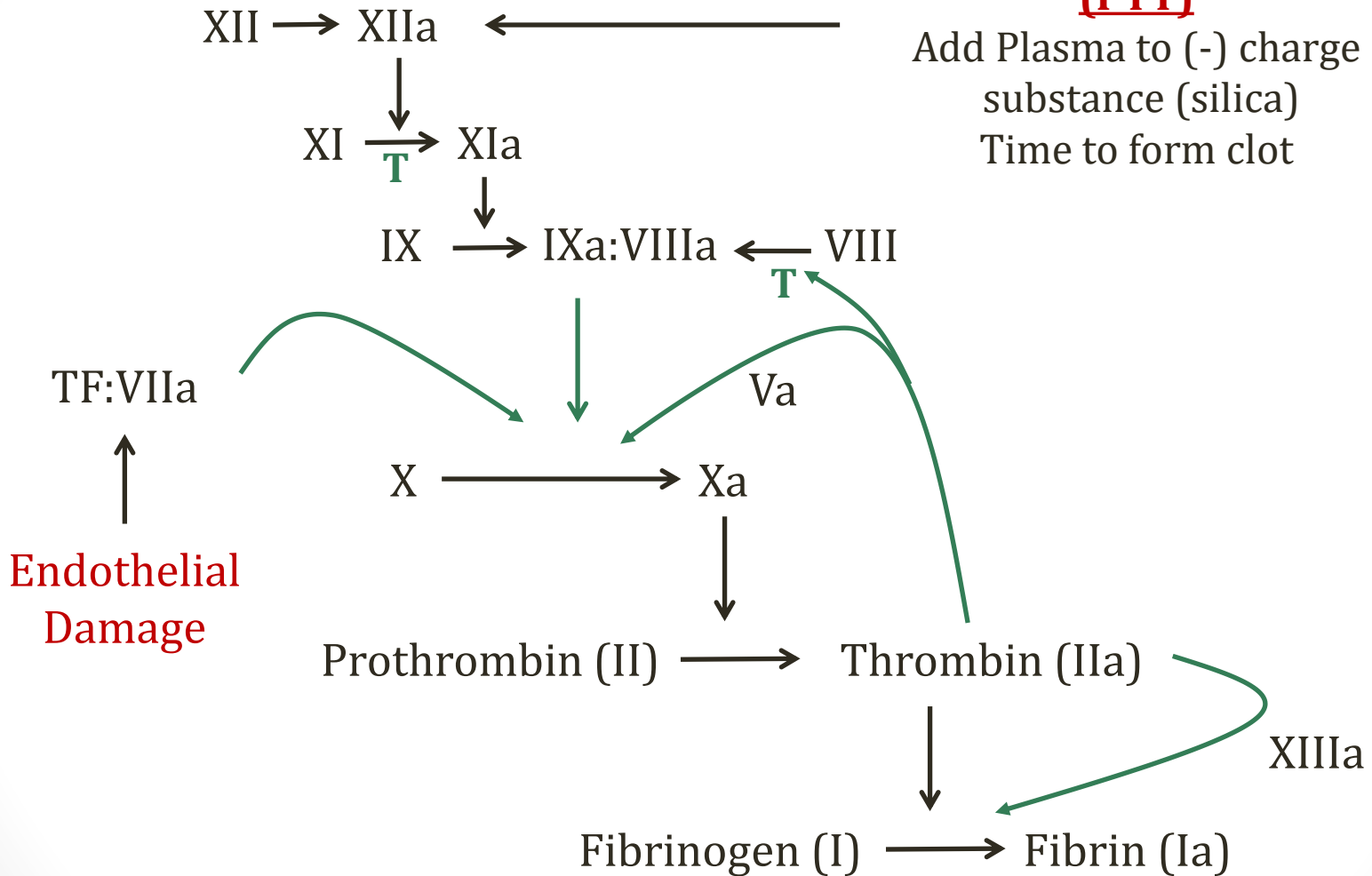
Coagulation Cascade



Coagulation Cascade

Activated Partial Thromboplastin Time (PTT)

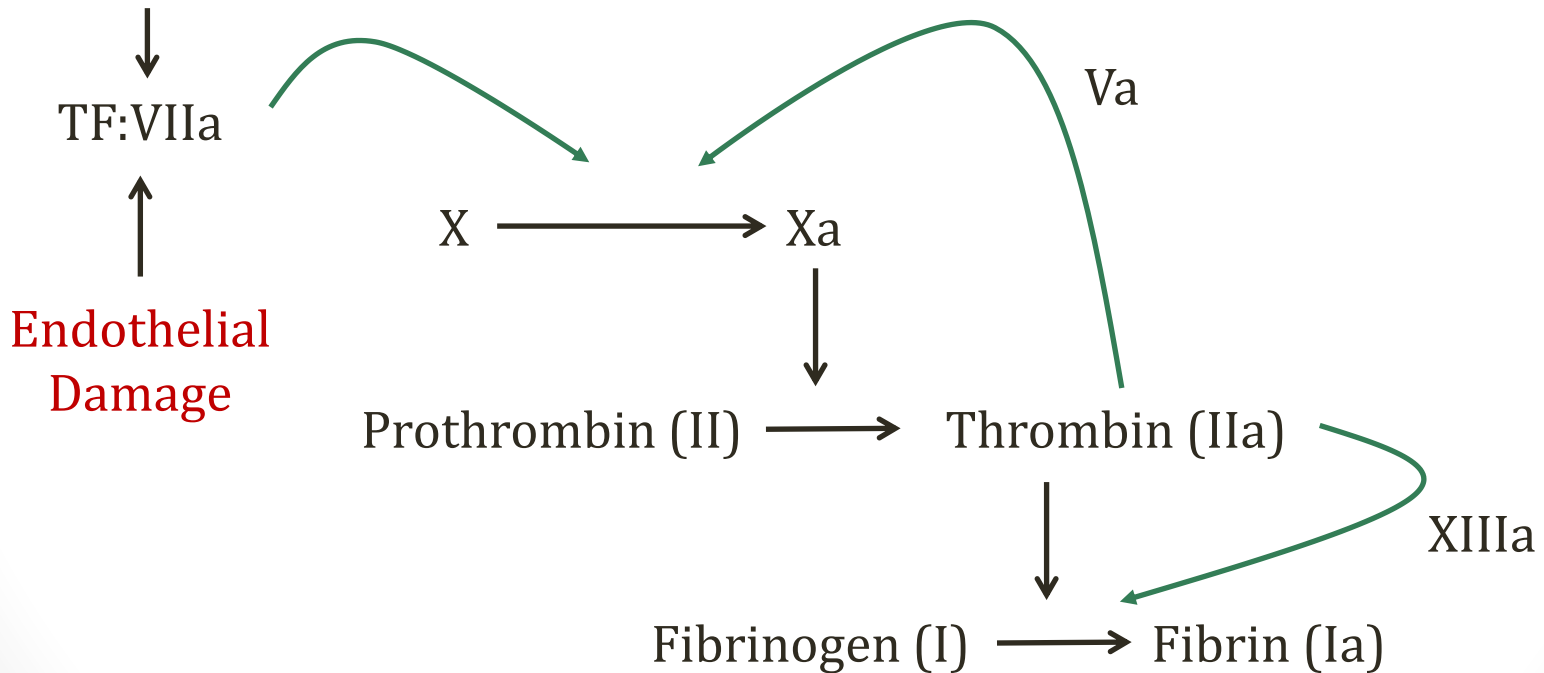
Add Plasma to (-) charge substance (silica)
Time to form clot



Coagulation Cascade

Prothrombin Time (PT)

Add Plasma to TF
Time to form clot



Intrinsic Pathway

Contact Pathway

- Requires **kinins** for normal function
- Kinins = **peptide** hormones/signaling molecules
- Short half lives
- Circulate as inactive precursors: kininogens
- Activated by kallikreins
- Kinins **link coagulation with inflammation**

Intrinsic Pathway

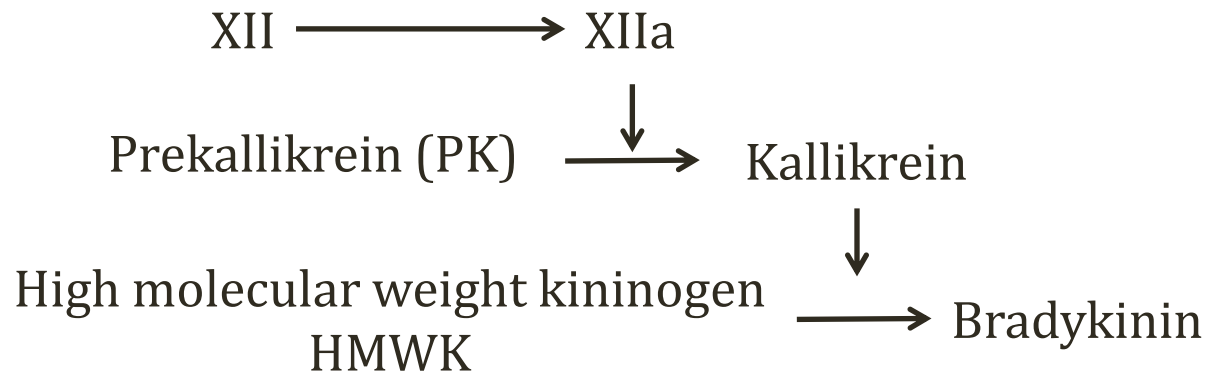
Kinin System

- **Bradykinin**
 - Vasodilator
 - Increases vascular permeability
 - Pain
- Degraded by angiotensin converting enzyme (ACE)
 - **ACE inhibitors** can raise bradykinin levels
 - Dangerous side effect: **angioedema**
- Also degraded by C1 inhibitor (complement system)
 - C1 inhibitor deficiency → **hereditary angioedema**

Intrinsic Pathway

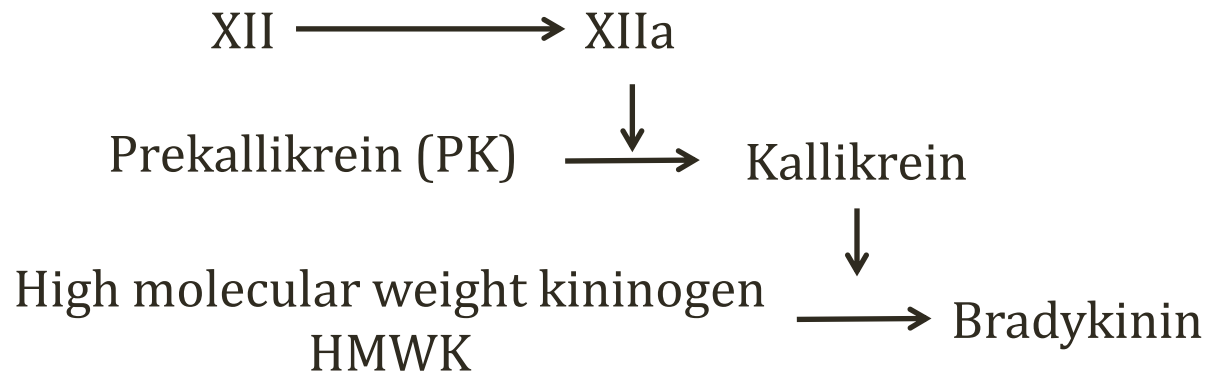
Factor XII

- **Activates clotting and produces bradykinin**
- Requires PK, HMWK for normal function



Prekallikrein Deficiency

- Rare condition
- Results in **markedly prolonged PTT**
- **XII cannot activate normally**
- No bleeding problems



Kinin System

Key Points

- Activated by factor XII
- Link between coagulation and inflammation
- Bradykinin
 - ACE inhibitors
 - Hereditary angioedema
- Prekallikrein Deficiency: \uparrow PTT

Coagulation Inhibitors

- Important **deactivators** of coagulation
 - Antithrombin III
 - Proteins C and S
 - Tissue factor pathway inhibitor

Antithrombin III

- Serpin (inhibitor of serine proteases)
- Inhibits serine proteases: factors II, VII, IX, X, XI, XII
- Produced by liver
- Activated by endothelium
 - Endothelium makes heparan sulfate molecules
 - Activate antithrombin
 - Basis for role of **heparin drug therapy**
- Deficiency: Hypercoagulable state

Proteins C and S

- Glycoproteins synthesized in liver
- Protein C: zymogen
- Active form: activated protein C (APC)
- APC primarily inactivates factors **Va and VIIIa**

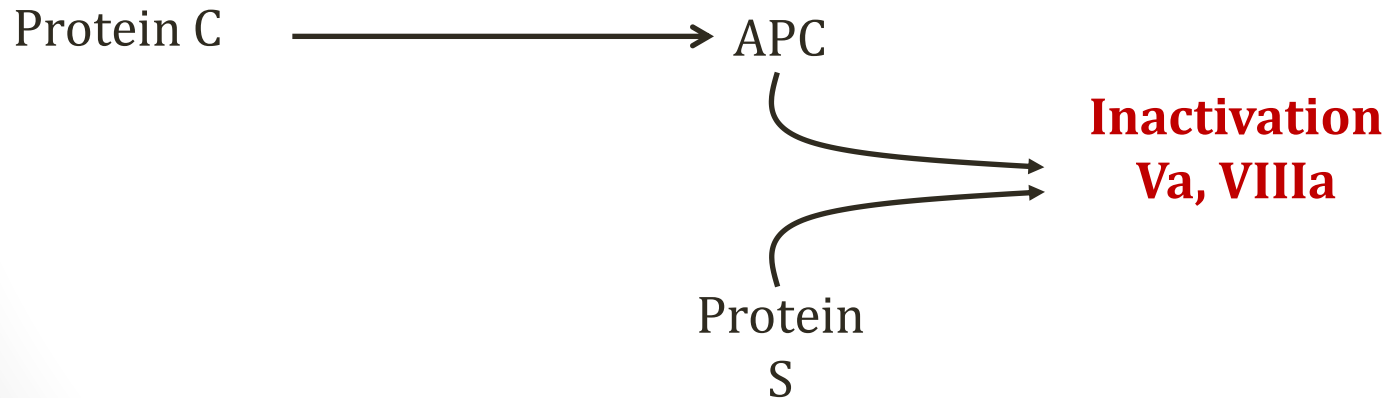
Proteins C and S

- Protein C activated by **thrombomodulin**
 - Cell membrane protein
 - Found on **endothelial cells**
- Thrombomodulin binds thrombin
 - Complex activates protein C to APC

Proteins C and S

- APC requires **protein S** as co-factor
- Protein S circulates in active form (not a zymogen)

Thrombomodulin: Thrombin



TFPI

Tissue factor pathway inhibitor

- **Inactivates Xa** via two mechanisms
 - Directly binds Xa
 - Binds TF/FVIIa complex → prevents X activation
- Plasma levels increased with heparin administration
 - May contribute to antithrombotic effect

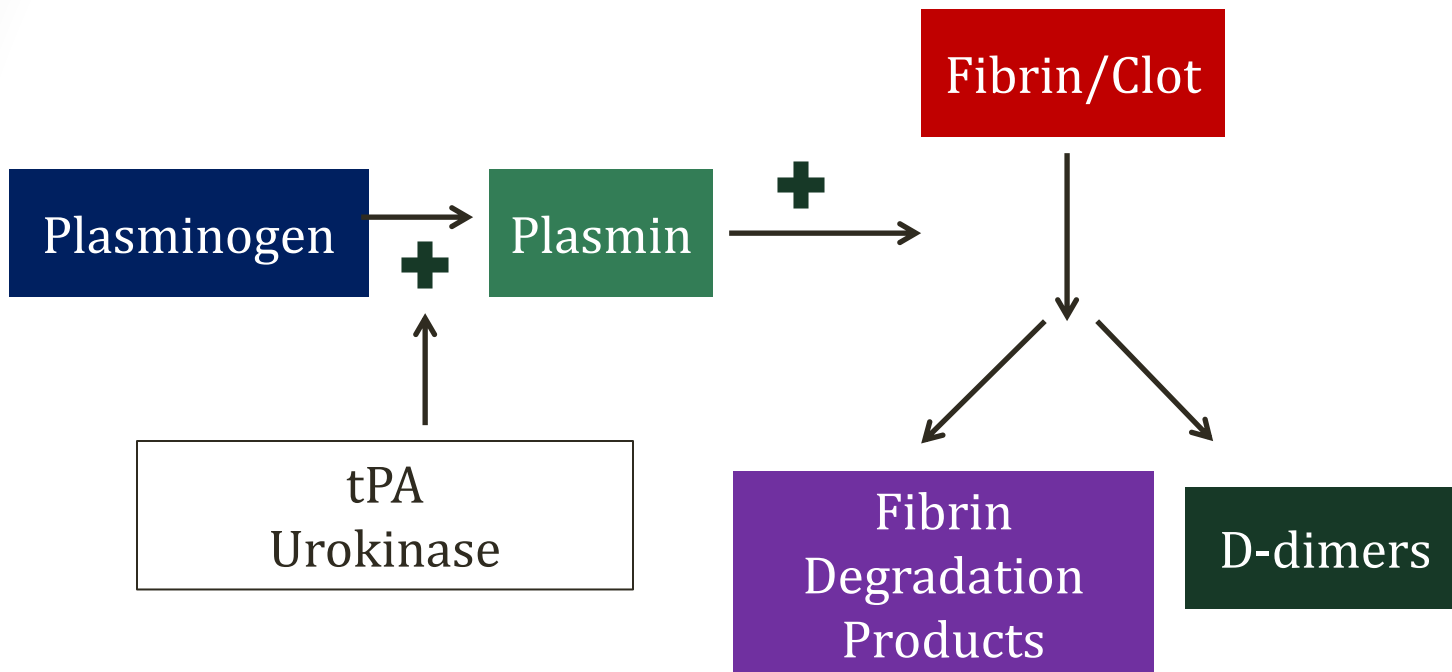
Plasminogen and Plasmin

- Plasminogen synthesized by liver (zymogen)
- Converted to active enzyme: **plasmin**
- Main role of plasmin is breakdown of fibrin
 - Broad substrate specificity
 - Also degrades clotting factors, fibrinogen

Plasminogen Activators

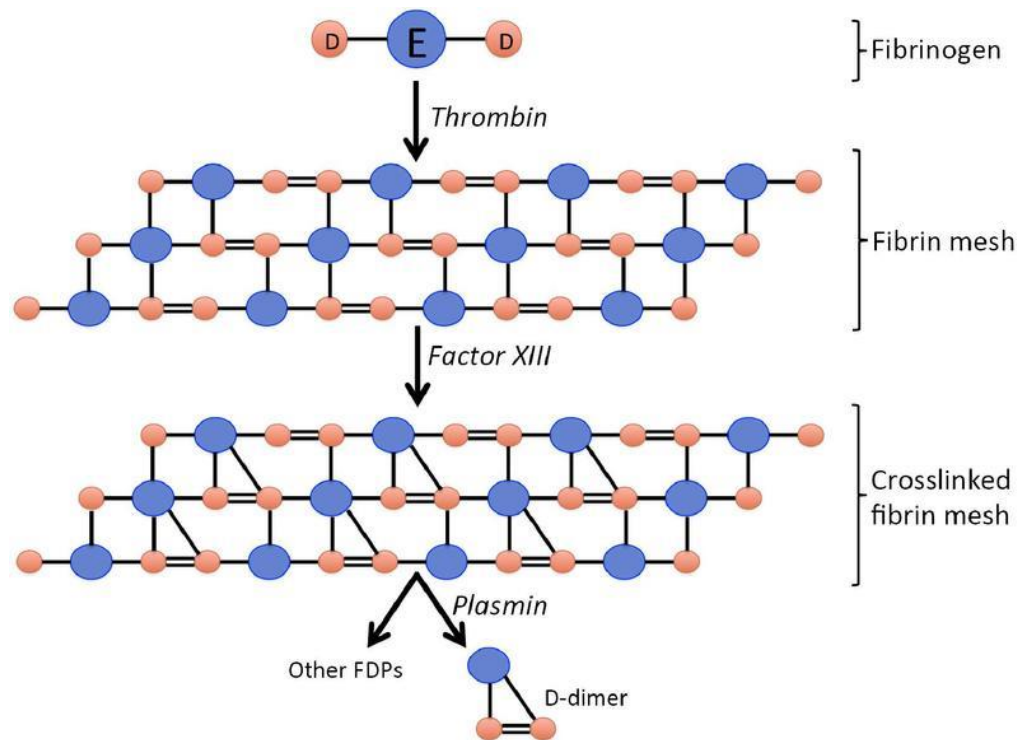
- **Tissue plasminogen activator (tPA) and urokinase**
 - Synthesized by endothelial and other cells
 - Used as drug therapy for **acute MI and stroke**
 - **Streptokinase**: Streptococcal protein; activates plasminogen

FDPs and D-dimer



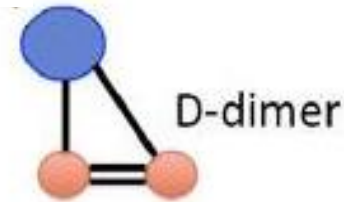
FDPs and D-dimer

- Fibrinogen has two domains: E (central) and D (side)
- Crosslinking of fibrin (XIII) creates E linked two Ds



Mpt-matthew/Wikipedia

FDPs and D-dimer



- D-dimer is a special type of FDP
- Presence of D-dimers indicates **clot breakdown**
 - Breakdown of *crosslinked* fibrin from XIII
 - Elevated D-dimer used for diagnosis of DVT/PE

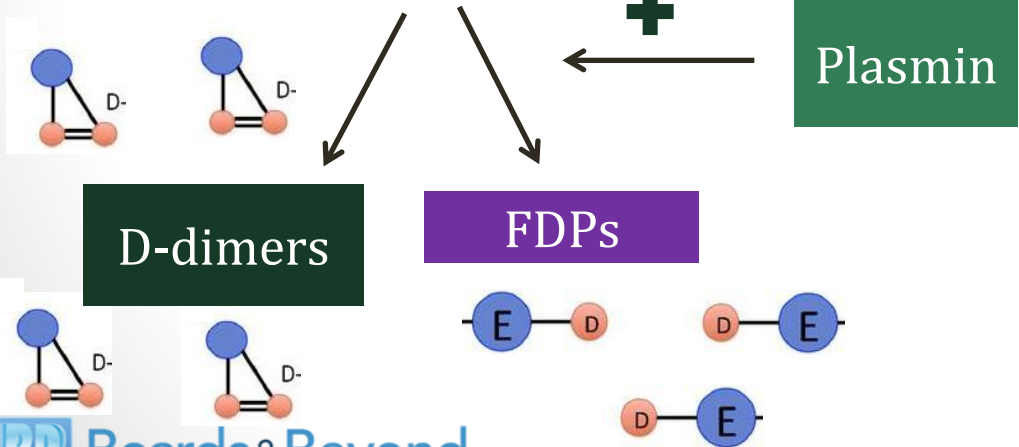
Fibrin/Clot

+

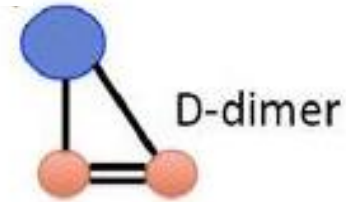
Plasmin

D-dimers

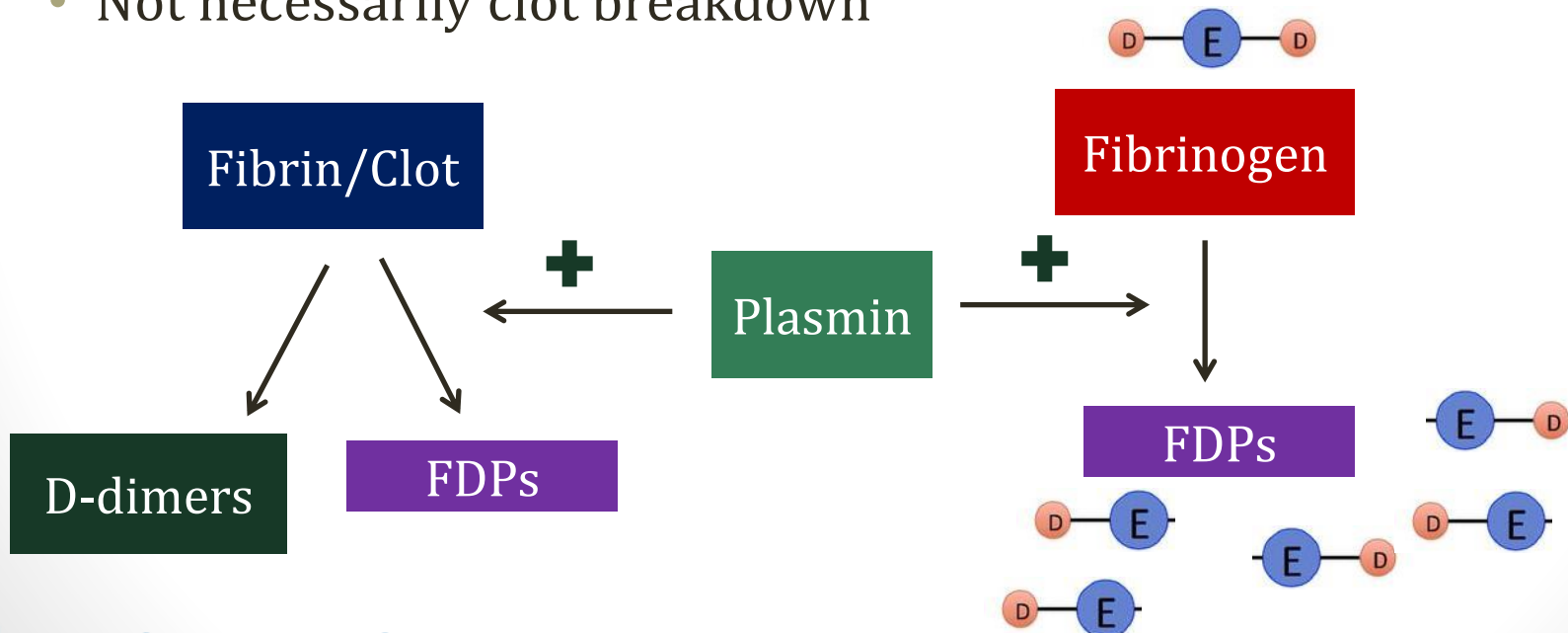
FDPs



FDPs and D-dimer



- ↑ FDPs seen in breakdown of **clot**
- Also seen in absence of clot from **fibrinogen breakdown**
- Plasmin can convert fibrinogen → FDPs
- FDPs indicate plasmin activity only
- Not necessarily clot breakdown



Primary Fibrinolysis

- Rarely phenomena: **Plasmin overactive**
- Causes **↑ FDP** with *normal D-dimer*
 - “Hyperfibrinolysis”
 - Plasmin breakdown of fibrinogen (not fibrin) → FDPs
 - No clot or crosslinked fibrin → No d-dimers
 - **Plasmin can deplete clotting factors**
 - Increased PT/PTT with bleeding (like DIC)
 - Prostate cancer: release of urokinase
 - Cirrhosis: Loss of alpha2 antiplasmin from liver

FDPs and D-dimer

Key Points

- **Clot breakdown:** FDPs and D-dimers
- **Hyperfibrinolysis:** FDPs with normal D-dimer levels
- ↑ D-dimer used to diagnosis thrombotic disorders
- Elevated levels seen in DVT/PE
 - Sensitive but not specific
 - Elevated in many other disorders

Vitamin K

- Required for synthesis of many clotting factors
 - “Vitamin K dependent clotting factors”
- Vitamin K dependent factors: II, VII, IX, X, C, S
- Vitamin K deficiency: **bleeding**
- **Warfarin**: Vitamin K antagonist

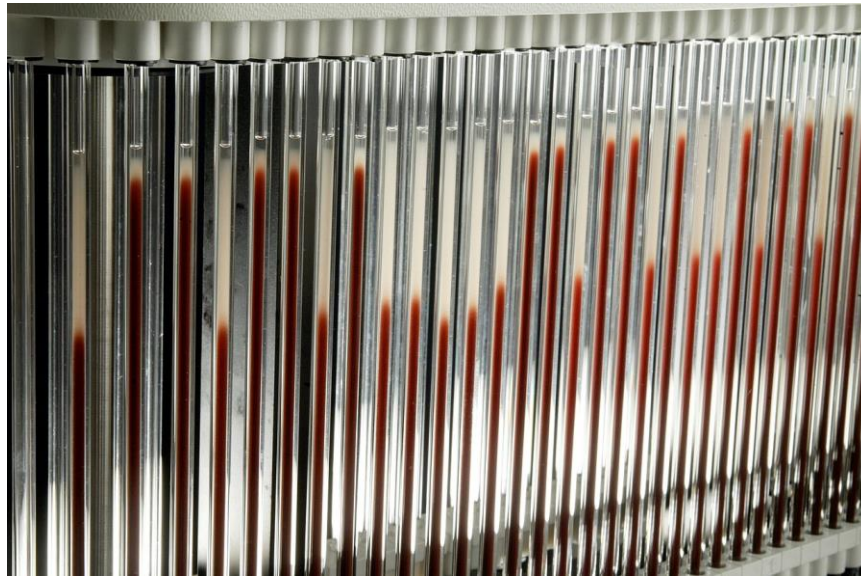


Gonegonegone /Wikipedia

ESR

Erythrocyte Sedimentation Rate

- Rate of RBC sedimentation in test tube
 - Normal 0-22 mm/hr for men; 0-29 mm/hr for women
- Increased in **inflammatory conditions**



MechESR/Wikipedia

ESR

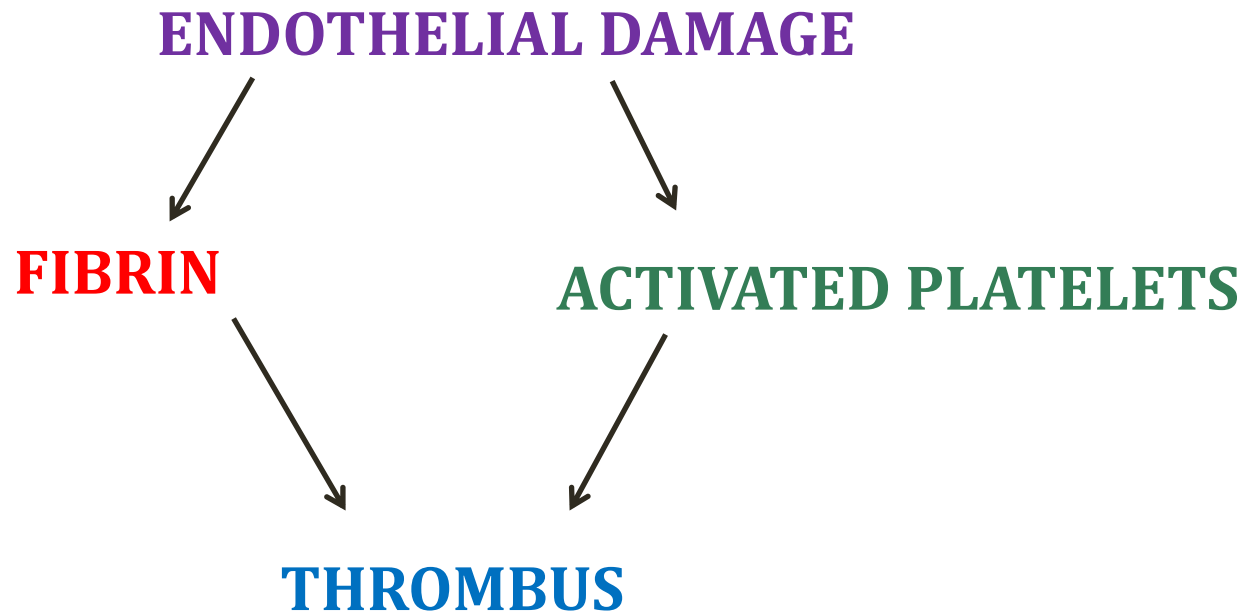
Erythrocyte Sedimentation Rate

- ESR increased by **“acute phase reactants”** in plasma
 - Serum proteins that rise in inflammation or tissue injury
 - Driven by cytokines
 - Most come from liver
- Key acute phase reactants
 - **Fibrinogen**
 - Ferritin
 - C-reactive protein (binds bacteria; activates complement)

Platelet Activation

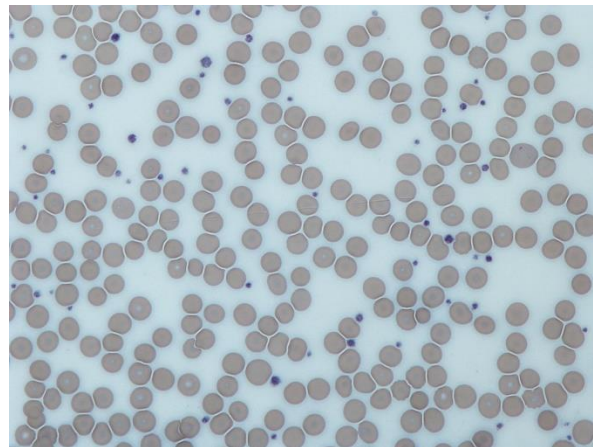
Jason Ryan, MD, MPH

Thrombus Formation



Platelets

- Small cells derived from **megakaryocytes**
- Do not contain a nucleus
- Short lifespan: about 8-10 days
- Production regulated by **thrombopoietin** (TPO)
 - Glycoprotein produced mostly in liver

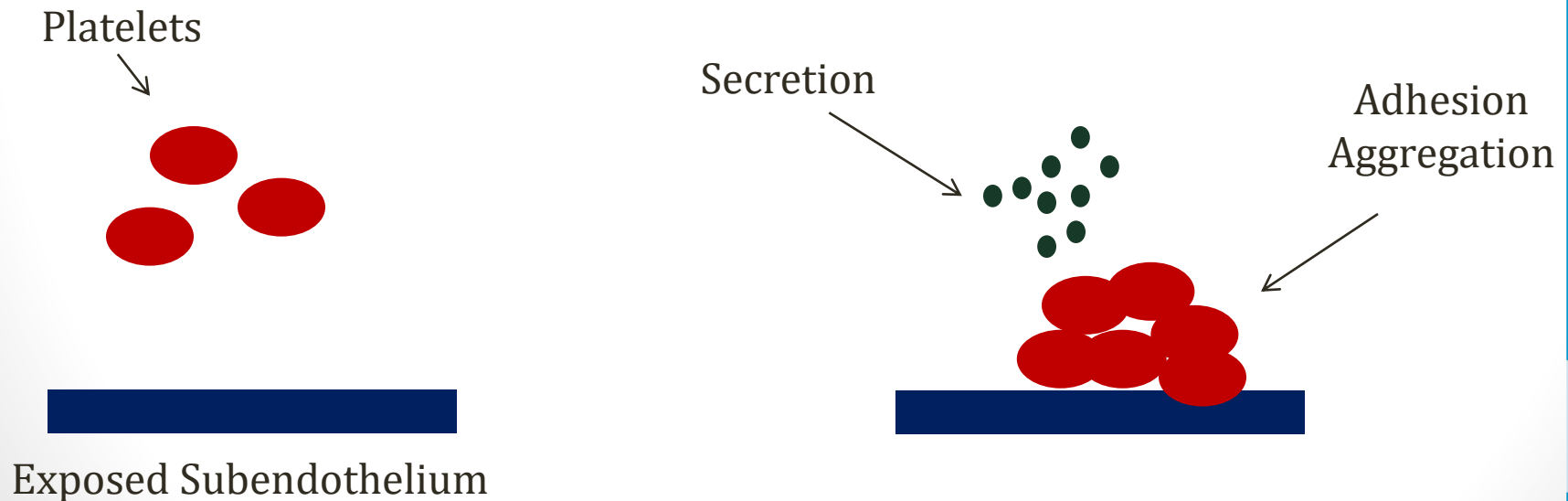


Platelets

- Aid in hemostasis after vascular injury
- Circulate in “inactive” form
- Can “activate” due to:
 - Endothelial injury
 - Stimuli from other activated platelets
- Activated platelets **seal damaged vessels**

Platelets Actions

- **Adhesion** to sub-endothelium
- **Aggregation**: Platelet-platelet binding
- **Secretion**: Release of granule contents
- Net result: Seal openings in vascular tree



Von Willebrand Factor

- Large **glycoprotein**
- Synthesized by endothelial cells and **megakaryocytes**
 - Stored in **Weibel–Palade bodies** in endothelial cells
- Present in platelets (stored in **alpha granules**)
- Some found in plasma
- Released on vascular injury
 - Activated platelets degranulate
 - Endothelial cells release vWF

Von Willebrand Factor

- Several roles in hemostasis
- #1: Carrier protein for **factor VIII**
 - Factor VIII released in presence of thrombin (VIIIa)
- #2: **Binds platelets** to damaged endothelium
- #3: Binds activated platelets together (aggregation)

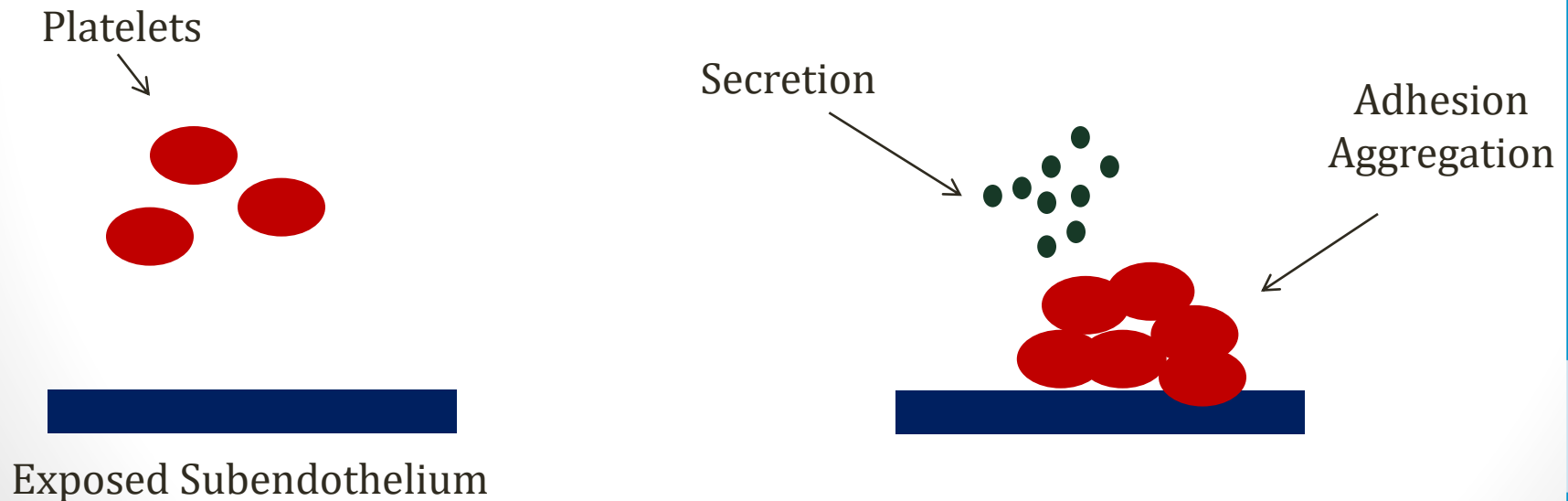
VIII—vWF

Membrane Glycoproteins

- Glycoproteins (amino acids and glucose molecules)
- Found on surface of platelets
- Interact with other structures/molecules
- Important for hemostasis
- GPIb, GPIIb/IIIa

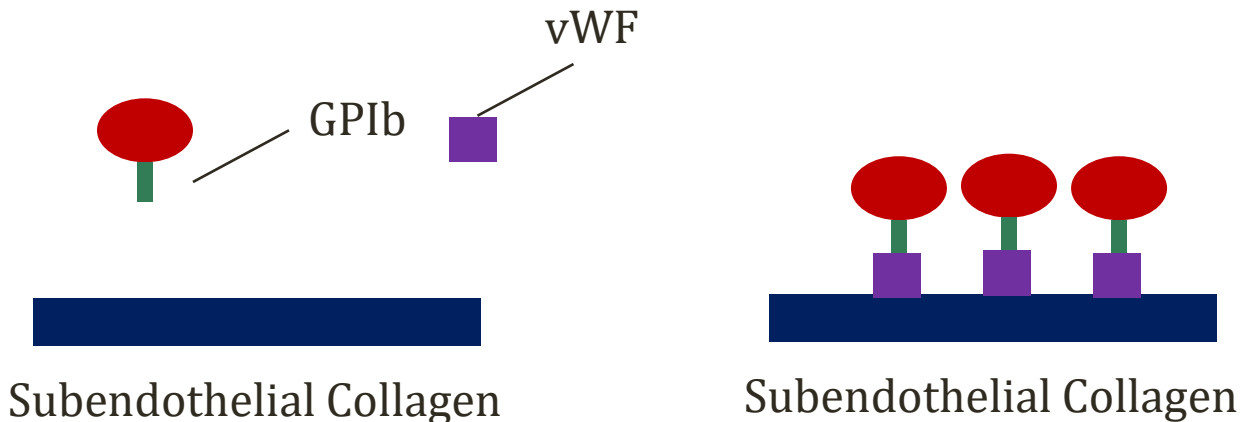
Platelets Actions

- **Adhesion** to sub-endothelium
- **Aggregation**: Platelet-platelet binding
- **Secretion**: Release of granule contents



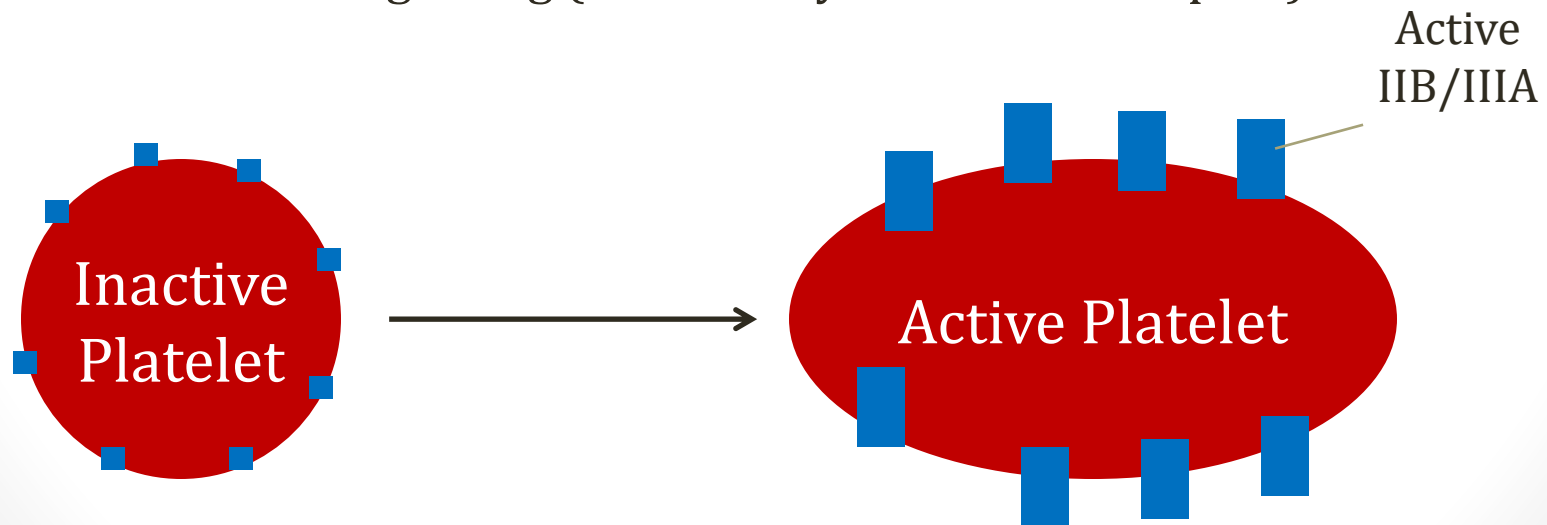
Platelet Adhesion

- Vascular damage: **exposure of collagen**
- Subendothelial collagen **binds vWF**
- vWF **binds GPIb** on platelets



Platelet Aggregation

- Mediated by **GPIIb/IIIa receptor**
 - Most abundant surface receptor on platelets
- Platelet activation → GPIIb/IIIa changes conformation
 - Becomes capable of binding
 - Will not bind when platelets are inactive
 - “Inside-out” signaling (cell activity → altered receptor)

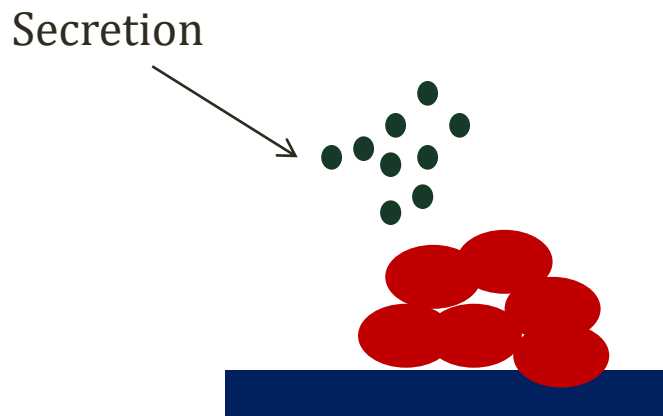


Platelet Aggregation

- GPIIb/IIIa binds **fibrinogen or vWF**
- Links platelets together (aggregation)
- Basis for **IIB/IIIA receptor blocking drugs**

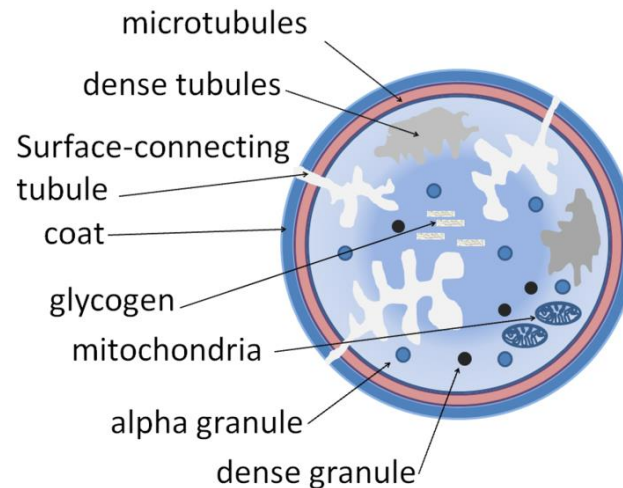
Platelet Secretion

- Platelets activated by:
 - Binding to subendothelial collagen
 - Stimulation by activating substances
- Secretion of stored activators → more activation



Platelet Granules

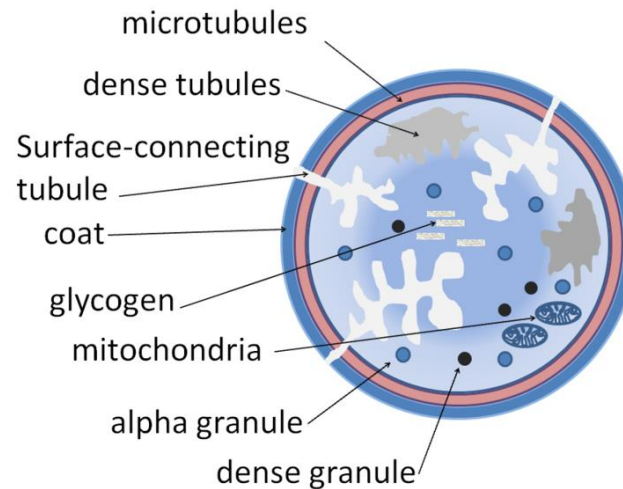
- Two types of platelet granules: **alpha** and **dense**
- Contents promote hemostasis
- **Released on activation** by:
 - Platelet binding to collagen
 - Granule contents from other platelets



Dr Graham Beards/Wikipedia

Platelet Granules

- **Alpha** granules (most abundant)
 - Fibrinogen
 - von Willebrand factor
 - platelet factor 4
- **Dense** granules
 - ADP
 - Calcium
 - Serotonin



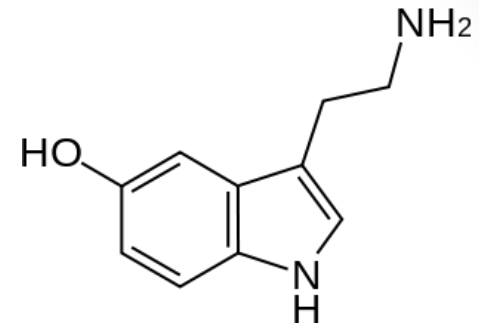
Dr Graham Beards/Wikipedia

Platelet Factor 4

PF4

- Released from **alpha** granules
- Binds to endothelial cells
- Numerous biologic effects described
- **Heparin induced thrombocytopenia**
 - Rare, life-threatening effect of heparin administration
 - Antibodies formed to PF4 complexed with heparin
 - Antibodies bind PF4-heparin → platelet activation
 - Diffuse thrombosis
 - Low platelets from consumption

Serotonin



- Stored in **dense** granules
- Released on platelet activation
- Basis for **serotonin release assay**
 - Diagnostic test for HIT
 - Donor platelets radiolabeled with ¹⁴C-serotonin
 - Patient serum and heparin added
 - HIT antibodies → excessive serotonin release

Adenosine Diphosphate

ADP

- Released from **dense** granules
- Also released by red blood cells when damaged
- Binds to two G-protein receptors: P2Y₁ and P2Y₁₂
- Binding leads to ↓ cAMP formation
 - ↑ cAMP blocks platelet activation
 - **Phosphodiesterase inhibitors** → ↑ cAMP

Adenosine Diphosphate

ADP

- P2Y₁
 - Calcium release, change in platelet shape
- P2Y₁₂
 - Platelet degranulation, ↑ aggregation
- Many **P2Y₁₂ receptor blocking drugs**
 - “ADP receptor blockers”
 - Inhibit platelet activity
 - Clopidogrel, prasugrel, ticlopidine, ticagrelor

Thromboxane A2

TXA2

- Powerful **platelet activator**
- TXA2 receptors found on platelets
- Basis for aspirin therapy



Shizhao/Wikipedia

Thromboxane A2

TXA2

- Lipids in cell membranes → arachidonic acid (AA)
 - Enzyme: phospholipase A2
 - Occurs in endothelial cells near damaged endothelium
 - AA released at sites of vascular injury
 - Also stored in platelets
- AA converted by platelets to TXA2
 - Enzyme: Cyclooxygenase (COX)
- **Aspirin**: Inhibits COX → ↓ TXA2 → platelet activation

Bleeding Time

- Test of **platelet function**
- Small cut to patient's arm
- Filter paper applied/removed until bleeding stops
- Rarely done in modern era



Crystal/Flikr

Hypercoagulable States

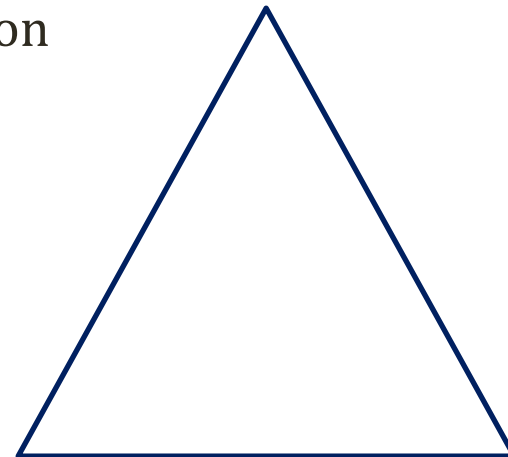
Jason Ryan, MD, MPH

Hypercoagulable States

- Predisposition to venous or arterial thrombi
- Often DVT/PEs (“Venous thromboembolism”)
- Sometimes arterial thrombosis
 - Stroke
 - Myocardial infarction
 - Ischemic limb

Virchow's Triad

- **Endothelial damage**
 - Endothelium makes numerous natural anticoagulants
 - Nitric oxide, prostaglandins, antithrombin, tPA, APC
- **Stasis of blood**
 - Normal blood flow prevents pooling of clotting factors
- **Hypercoagulability**
 - Conditions that increase clot formation



Hypercoagulable States

- **Post-op**
 - Hypercoagulable (inflammation from surgery)
 - Stasis (immobile)
 - Endothelial damage (surgery)
- **Fall/Hip Fracture/Trauma**
 - Hypercoagulable (inflammation from trauma)
 - Stasis (immobility)
 - Endothelial damage (trauma)
- **Long plane flights**
 - Stasis (immobility)



Wikipedia/Public Domain

Hypercoagulable States

- **Malignancy**
 - Some tumors produce pro-coagulants (i.e. tissue factor)
 - Adenocarcinomas: some data that mucin is thrombogenic
- Normal cells may produce pro-coagulants
 - Reaction to presence/growth of tumor
- Decreased activity, surgery, bed rest



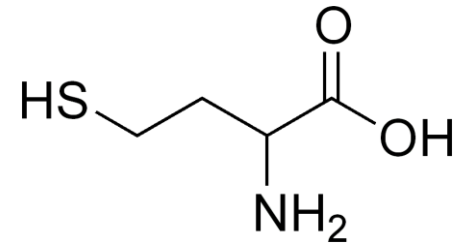
Public Domain

Hypercoagulable States

- **Pregnancy**
 - Probably evolved to protect against blood loss at delivery
 - Many clotting factor levels change
 - Increased fibrinogen
 - Decreased protein S
 - Fetus also obstructs venous return → DVTs common
- Oral contraceptive pills (OCPs)
 - Estrogen increases production coagulation factors

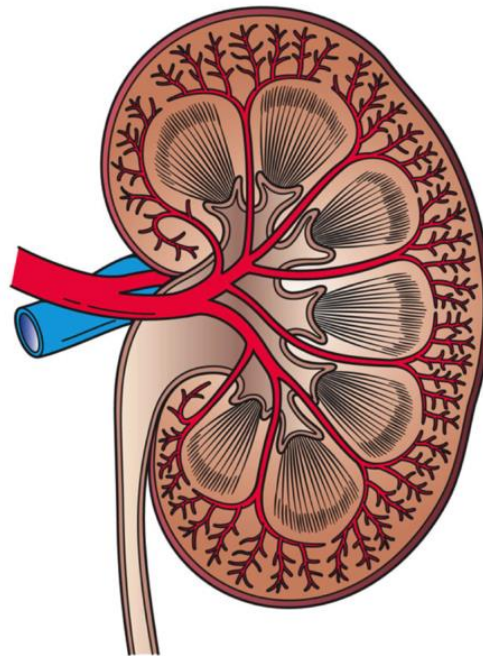
Hypercoagulable States

- **Elevated homocysteine** (amino acid)
- Associated with **arterial and venous** clots
- High levels may cause:
 - Endothelial injury
 - Activation of some clotting factors
- Elevated levels caused by:
 - Folate/B12/B6 deficiency
 - Homocystinuria (cystathionine beta synthase deficiency)
- Levels lowered by **folate**
 - Most clinical trials of folate did not show benefit



Hypercoagulable States

- **Nephrotic syndrome**
 - Multiple mechanisms
 - Loss of anti-clotting factors in urine (ATIII)



Holly Fischer/Wikipedia

Hypercoagulable States

- **Smoking**

- Associated with atherosclerosis and MI/Stroke
- Some data linking smoking to DVT/PE
- Evidence that smoking increases fibrinogen levels



Pixabay/Public Domain

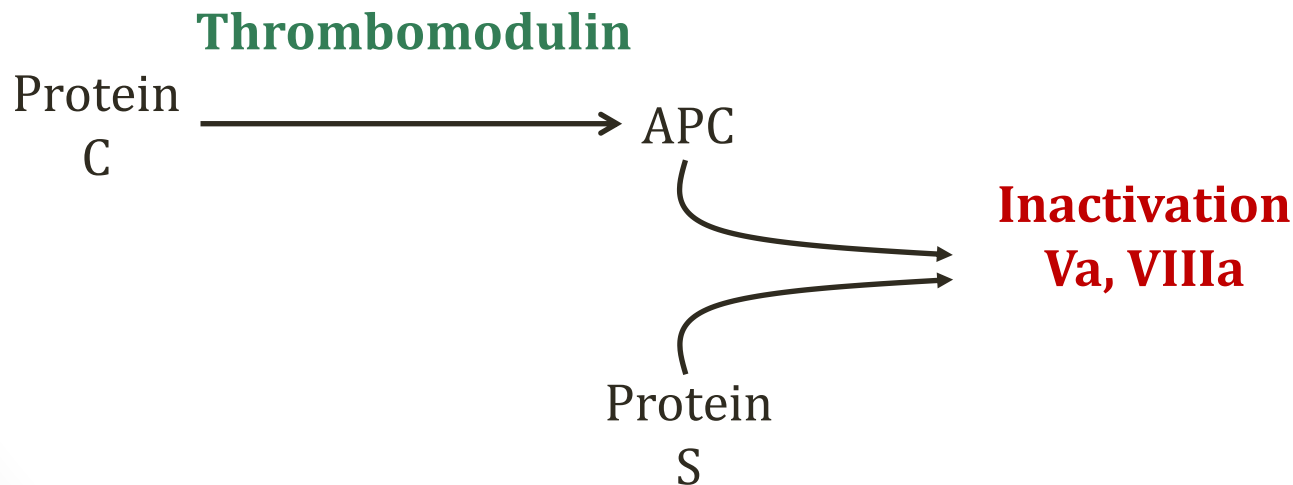
Inherited Thrombophilia

- Inherited hypercoagulable states
- Genetic tendencies to **VTE**
- Most involve coagulation pathway defects
- All associated with venous clots (DVT/PE)

Hypercoagulable Condition
Factor V Leiden Mutation
Prothrombin gene mutation
Antithrombin deficiency
Protein C/S deficiency

Factor V Leiden Mutation

- Named for Leiden, Netherlands
- Abnormal **factor V**
- Not inactivated by **activated protein C (APC)**
- Factor V remains active longer → hypercoagulability

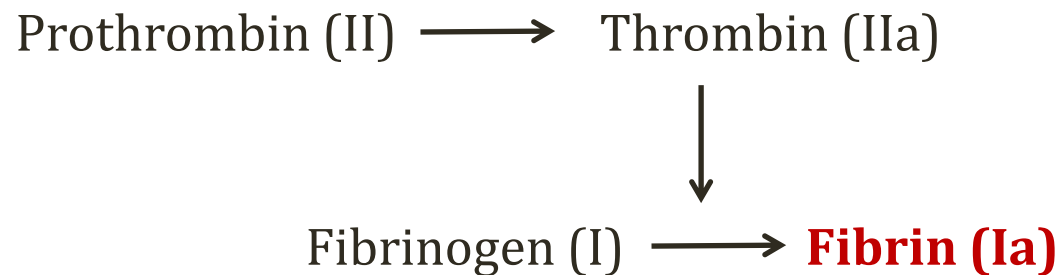


Factor V Leiden Mutation

- **Point mutation in factor V gene**
 - Guanine to adenine change
- Result: Single amino acid change
 - Arginine to glutamine substitution
 - Position 506 in factor V

Prothrombin Gene Mutation

- **Prothrombin 20210** gene mutation
 - Guanine to adenine change in prothrombin gene
 - Occurs at nucleotide 20210
- Heterozygous carriers: 30% ↑ prothrombin levels

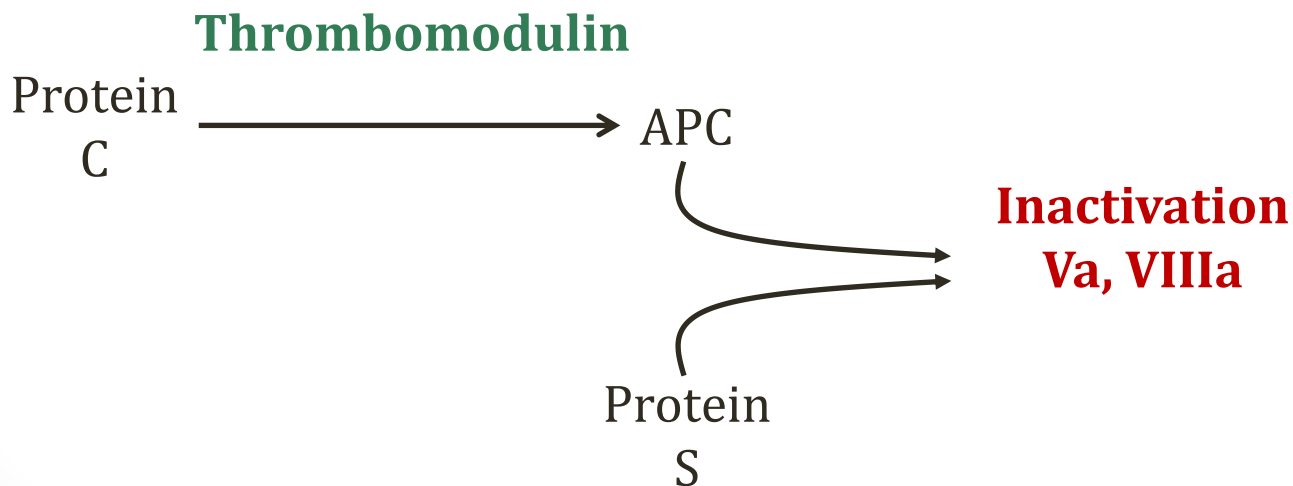


Antithrombin III Deficiency

- Inherited deficiencies due to gene mutations
- Acquired deficiencies:
 - Impaired production (liver disease)
 - **Protein losses (nephrotic syndrome)**
 - Consumption (DIC)
- Classically presents as **heparin resistance**
 - Escalating dose of heparin
 - No/little change in PTT

Protein C or S Deficiency

- Protein C: associated with **warfarin skin necrosis**
- Initial warfarin therapy → ↓ protein C (short half life)
- If protein C deficient → marked ↓ protein C
- Result: **thrombosis of skin tissue**
- Large dark, purple skin lesions



Antiphospholipid Syndrome

- Caused by antiphospholipid **antibodies**
- Occur in association with **lupus** or as primary disease

Antiphospholipid Syndrome

- Three important clinical consequences of antibodies
- “Antiphospholipid syndrome”
 - #1: Increased risk of **venous and arterial thrombosis**
 - Most commonly DVT
 - Also CNS: stroke
 - **Recurrent fetal loss**
 - #2: **Increased PTT**
 - #3: **False positive** syphilis (RPR/VDRL)

Antiphospholipid Syndrome

- **Anti-cardiolipin**
 - False positive RPR/VDRL
 - Syphilis also produces these antibodies
- **“Lupus anticoagulant”**
 - Interferes with PTT test (silica activation of XII)
 - False elevation
- **Anti- β 2 glycoprotein**

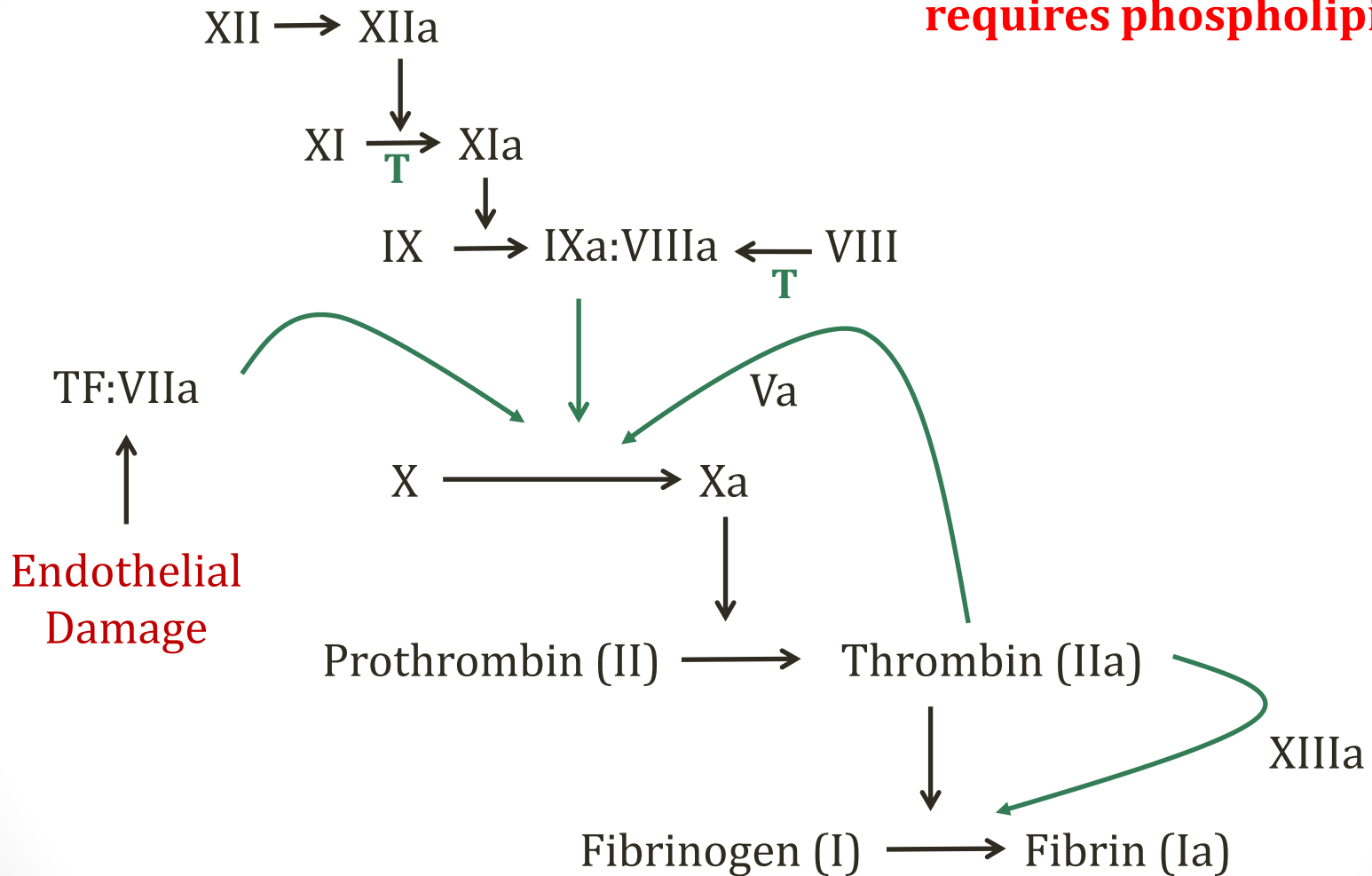
Antiphospholipid Syndrome

Antibody Detection

- **Anti-cardiolipin, Anti- β 2 glycoprotein**
 - Enzyme-linked immunosorbent assay (ELISA) testing
- **“Lupus anticoagulant”**
 - Detected indirectly through coagulation assays

Lupus Anticoagulant

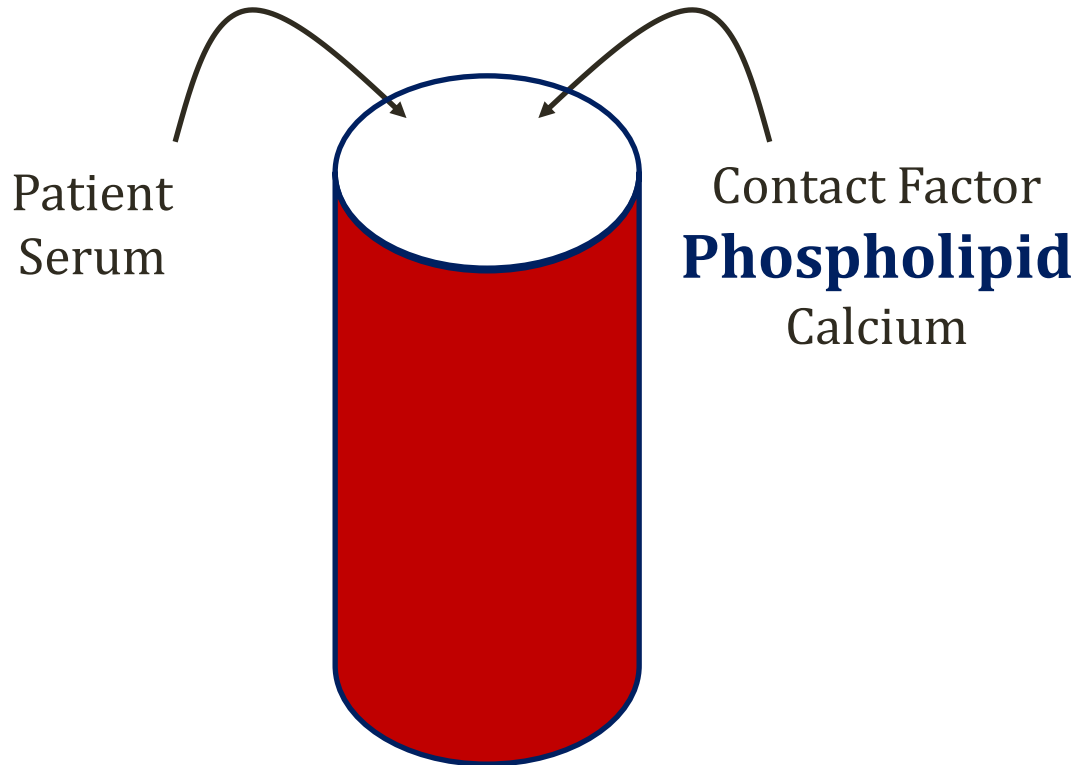
**Coagulation cascade
requires phospholipids**



Lupus Anticoagulant

PTT Testing

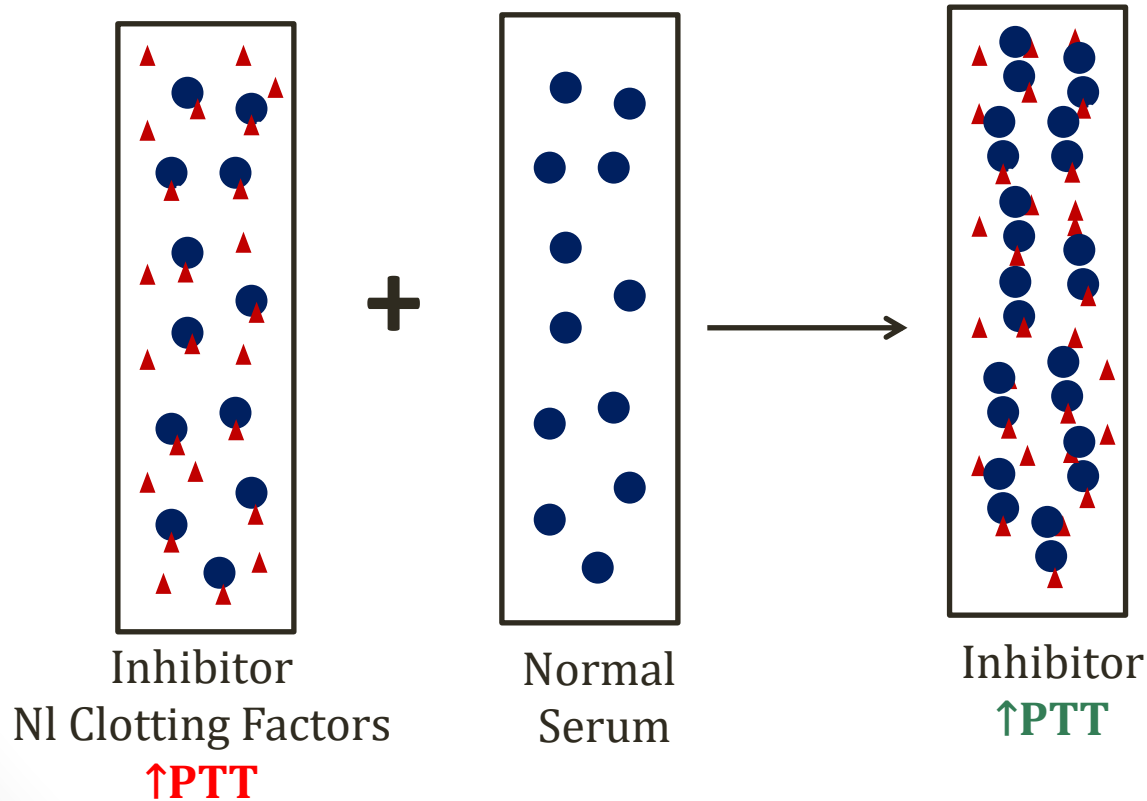
- Lupus anticoagulant binds phospholipid \rightarrow \uparrow PTT



Lupus Anticoagulant

Mixing Study

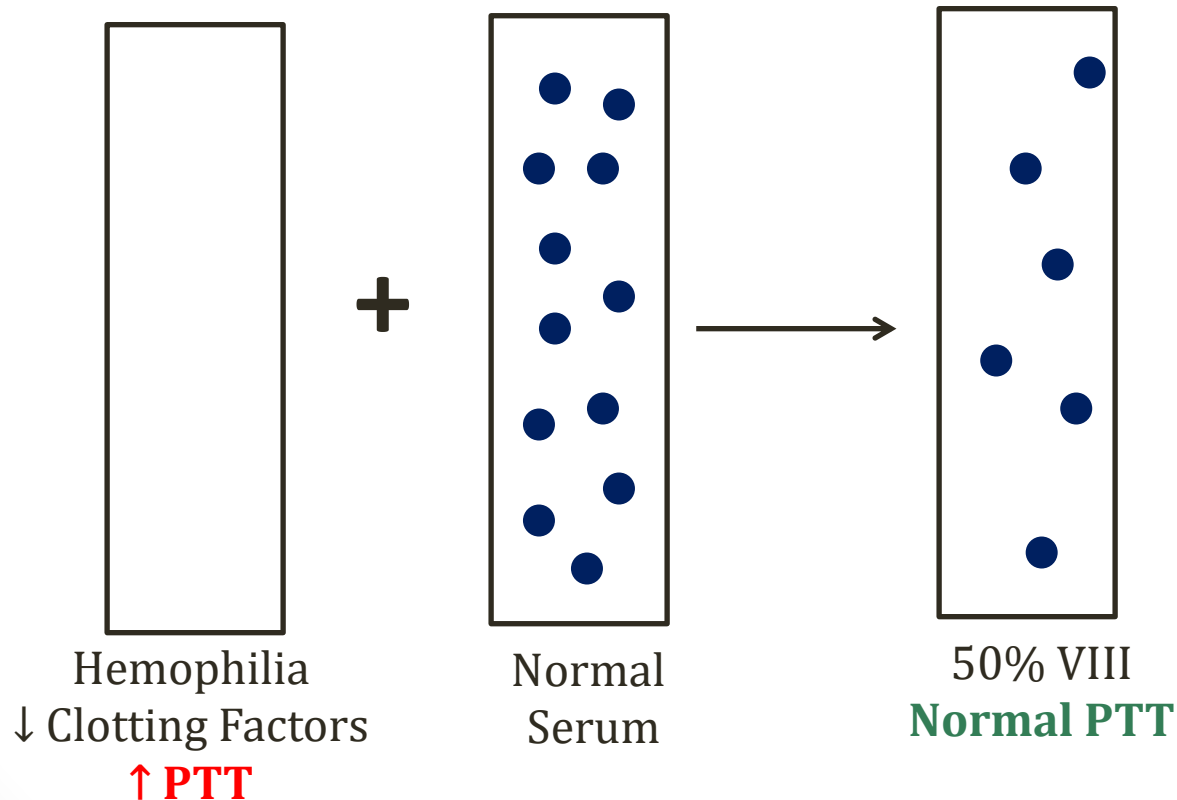
- Can show presence of lupus anticoagulant (inhibitor)



Lupus Anticoagulant

Mixing Study

- Clotting factor deficiency: PTT corrects to normal
- Clotting factors ~50% normal → normal PT/PTT



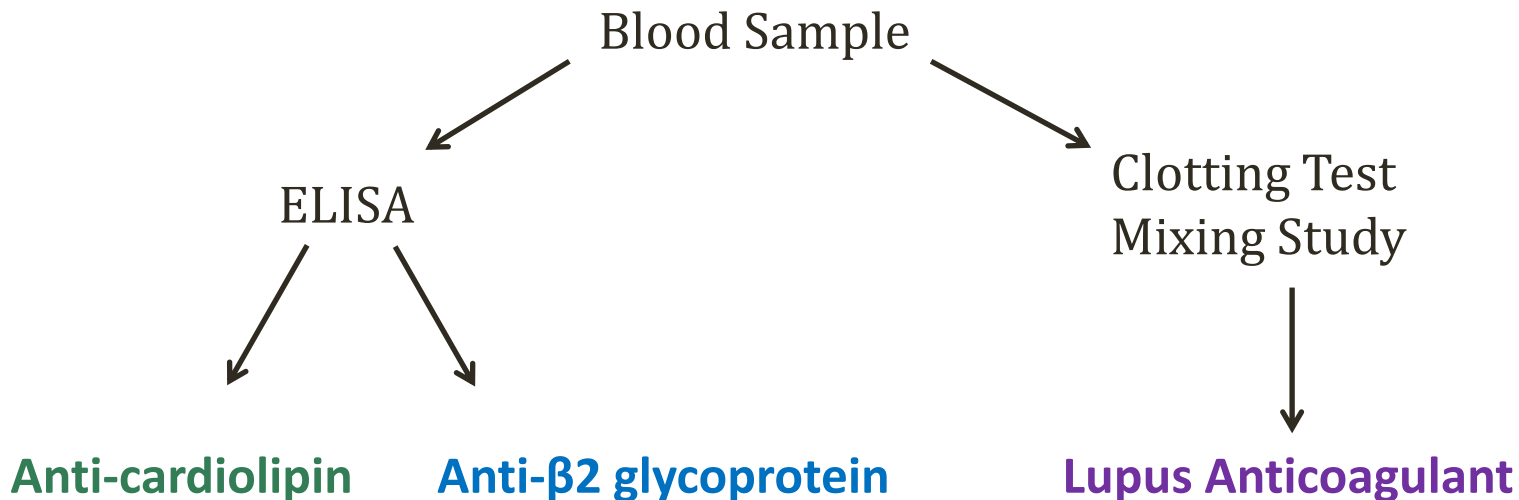
Lupus Anticoagulant

Other Tests

- Only ~50% patients with LA have ↑PTT
- Other coagulation tests sometimes used
 - Dilute Russell viper venom time
 - Kaolin clotting time
- Time to clot will be prolonged if LA present
- Time to clot will not correct with mixing study

Antiphospholipid Antibodies

Testing



Antiphospholipid Syndrome

- Syndrome = one laboratory plus one clinical criteria
- Lab criteria (2 positive results >12 weeks apart):
 - Lupus anticoagulant
 - Anti-cardiolipin
 - Anti- β 2-glycoprotein
- Clinical criteria:
 - Arterial or venous thrombosis
 - Fetal death after 10 weeks of normal fetus
 - ≥ 3 consecutive fetal losses before 10 weeks

Hypercoagulable Workup

- Panel of tests for hypercoagulable states
- Sometimes performed in:
 - Unprovoked DVT/PE
 - Stroke/MI at an early age
- Controversial
 - Expensive
 - Rarely changes management
 - Few data on management of identified states
 - Risk of bleeding with indefinite anticoagulation
- Some tests altered by thrombus or blood thinners

Hypercoagulable Workup

- Antithrombin level
- Protein C and S levels
- Factor V Leiden gene mutation
- Prothrombin gene mutation
- Antiphospholipid antibodies
- Cancer screening

Coagulopathies

Jason Ryan, MD, MPH

Bleeding Disorders

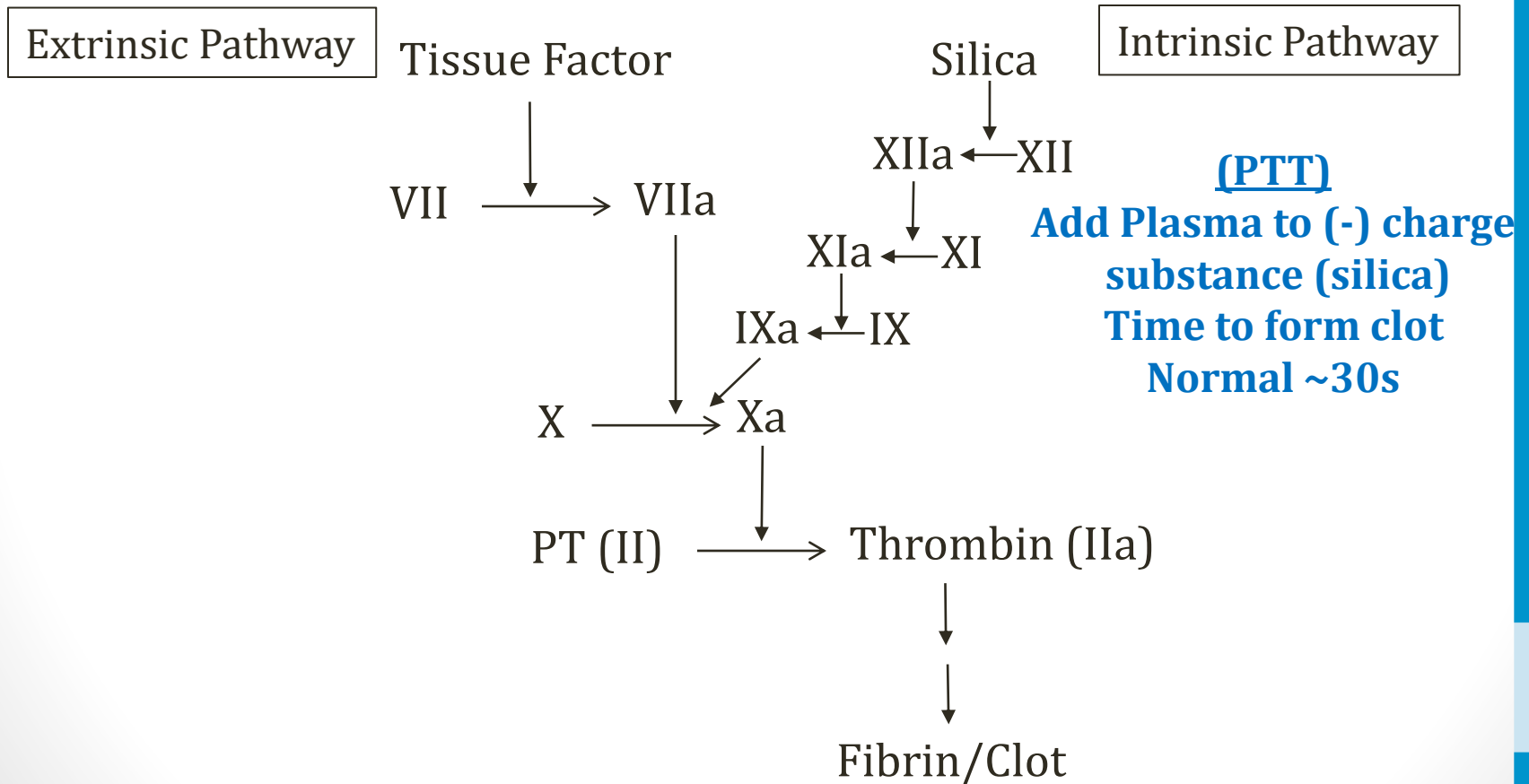
- Abnormal coagulation cascade
 - Hemophilia, Vitamin K deficiency
- Abnormal platelets
 - Bernard-Soulier, Glanzmann's Thrombasthenia
 - ITP, TTP
 - Uremia
- Mixed Disorders
 - Von Willebrand Disease, DIC, Liver disease

Bleeding Time

- Test of **platelet function**
- Small cut to patient's arm
- Filter paper applied/removed until bleeding stops
- Rarely done in modern era

PTT

Activated Partial Thromboplastin Time



PT

Prothrombin Time

Extrinsic Pathway

Tissue Factor

VII → VIIa

Silica

Intrinsic Pathway

XIIa ← XII

XIa ← XI

IXa ← IX

X → Xa

PT (II) → Thrombin (IIa)

Fibrin/Clot

Prothrombin Time (PT)

Add Plasma to TF

Time to form clot

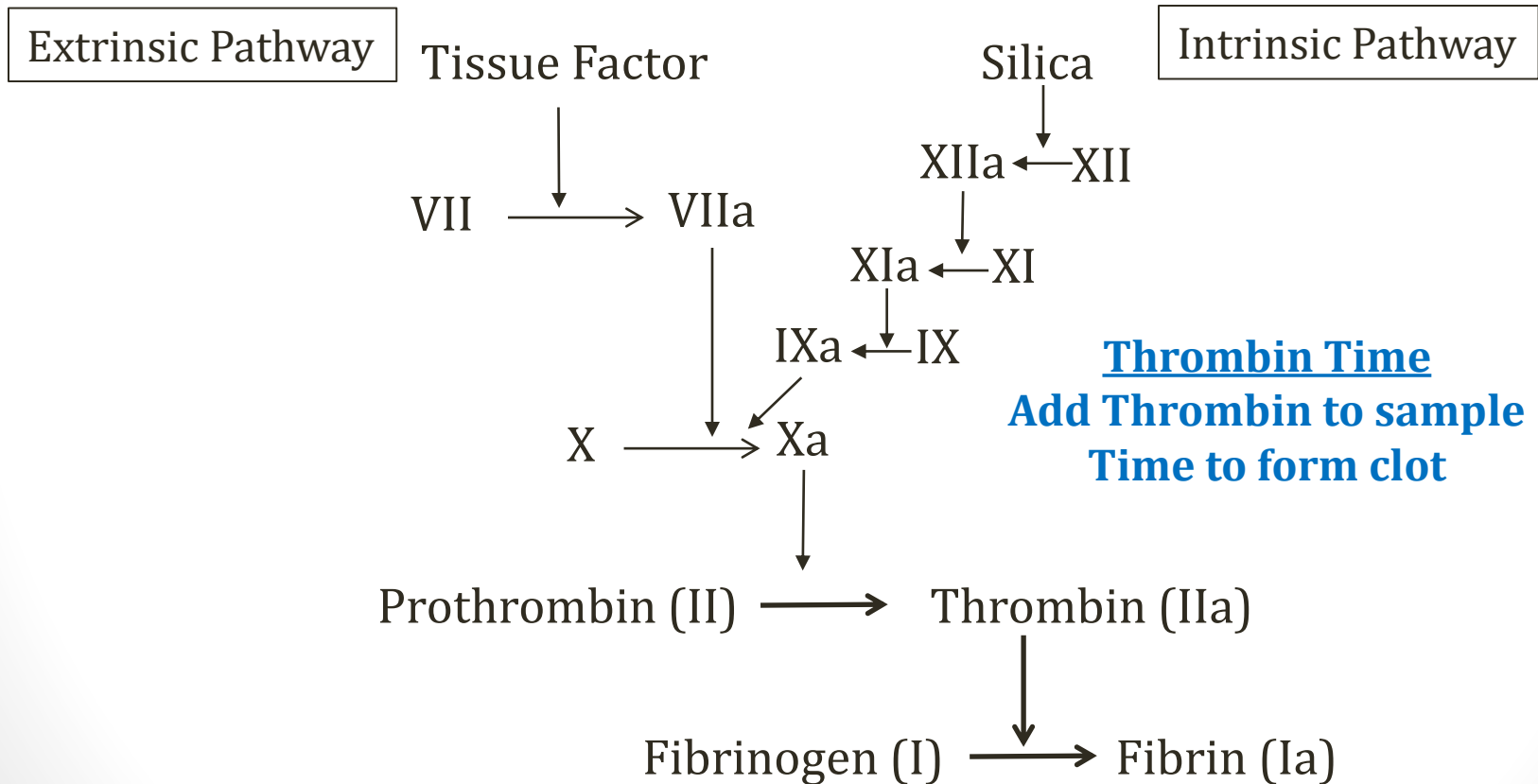
Normal ~10s

$INR = \frac{\text{Patient PT}}{\text{Control PT}}$

Normal = 1

Therapeutic = 2-3

Thrombin Time



Type of Bleeding

- Abnormal **platelets**
 - Mucosal bleeding, **skin** bleeding, petechiae
- Abnormal **coagulation factors**
 - **Joint** bleeding, deep tissue bleeding



Hemophilias

- X-linked recessive diseases
- Gene mutations: Run in families; also occur de novo
- **Hemophilia A:** Deficiency of factor VIII
- **Hemophilia B:** Deficiency of factor IX
 - Also called Christmas disease

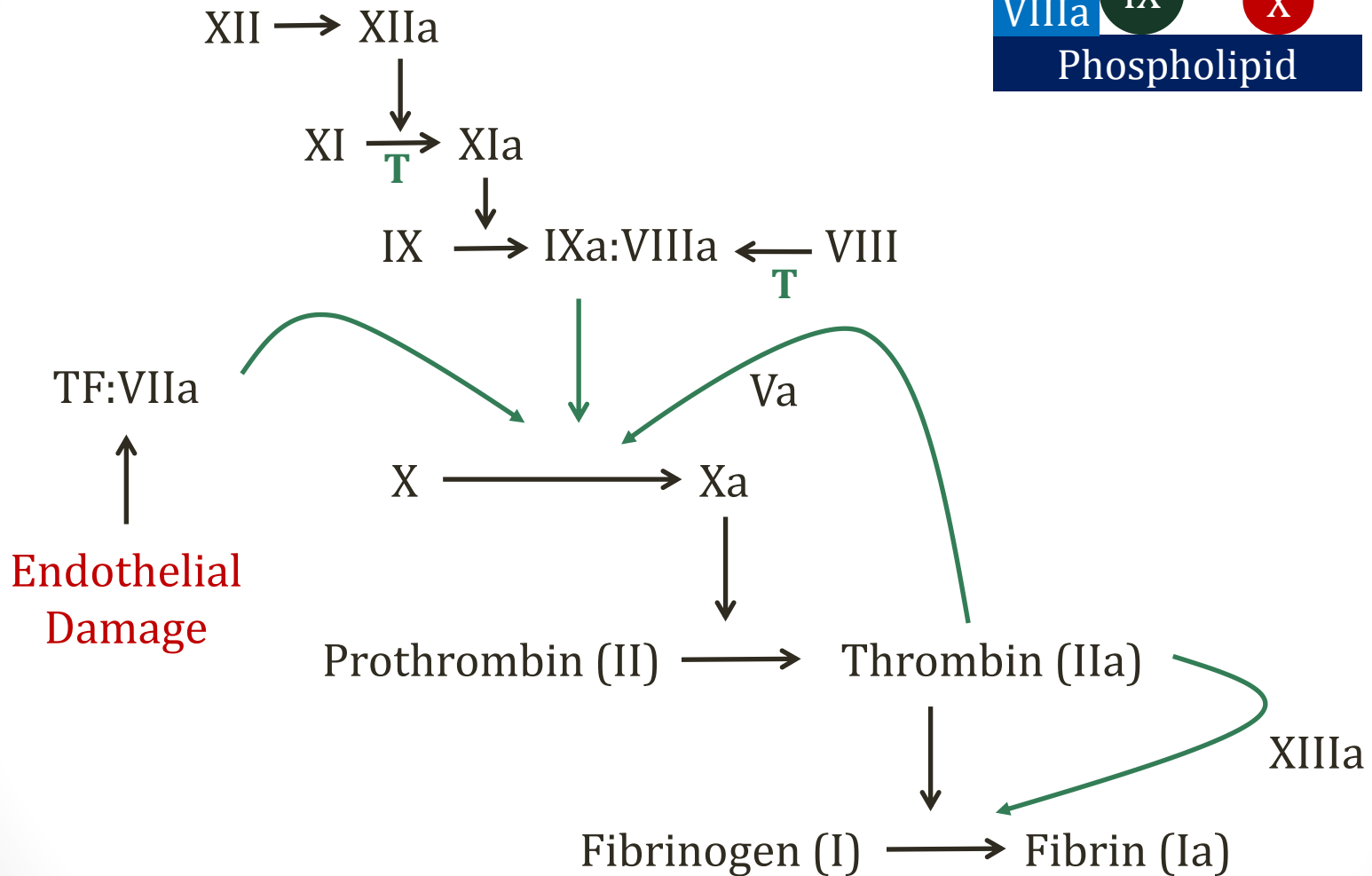
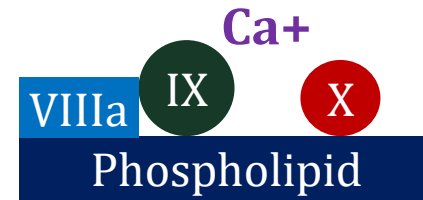
Alexei Nikolaevich



Hemophilias

- Present with spontaneous or easy bruising
- Recurrent **joint bleeds** is common presentation
- Screening: **PTT will be prolonged**
 - Factors VIII, IX both part of intrinsic pathway
- **PT, bleeding time, platelet count all normal**

Coagulation Cascade



Hemophilias

Treatment

- Replacement factor VIII and IX



Biggishben~commonswiki

Hemophilias

Treatment

- Desmopressin (dDAVP)
 - Used in mild **hemophilia A**
 - Analogue of vasopressin (ADH) with no pressor activity
 - **Increases vWF and factor VIII levels**
 - Releases VIII from **Weibel-Palade bodies** (endothelial cells)

Desmopressin

- Also has vasodilating properties
- Key side effects: **flushing, headache**
- Other uses:
 - von Willebrand disease
 - Central diabetes insipidus (mimics ADH)
 - Bedwetting (decreases urine volume)

Hemophilias

Treatment

- **Aminocaproic acid**
 - Antifibrinolytic drug
 - Inhibits plasminogen activation → plasmin
 - Less breakdown of formed clots

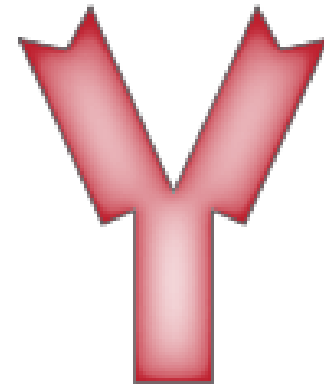
Cryoprecipitate

“Cryo”

- Obsolete therapy for hemophilia A
- Precipitate that forms when FFP is thawed
- Separated from plasma by centrifugation
- Contains **factor VIII, fibrinogen**
- Also factor XIII and von Willebrand factor (VWF)
- Often used as **source of fibrinogen**
 - DIC
 - Massive trauma with blood transfusions

Coagulation Factor Inhibitors

- Antibodies
- Inhibit activity or increase clearance of clotting factor
- Inhibitors of **factor VIII** most common
- Often occur in association with:
 - Malignancy
 - Post-partum
 - Autoimmune disorders
- Can be treated with **prednisone**

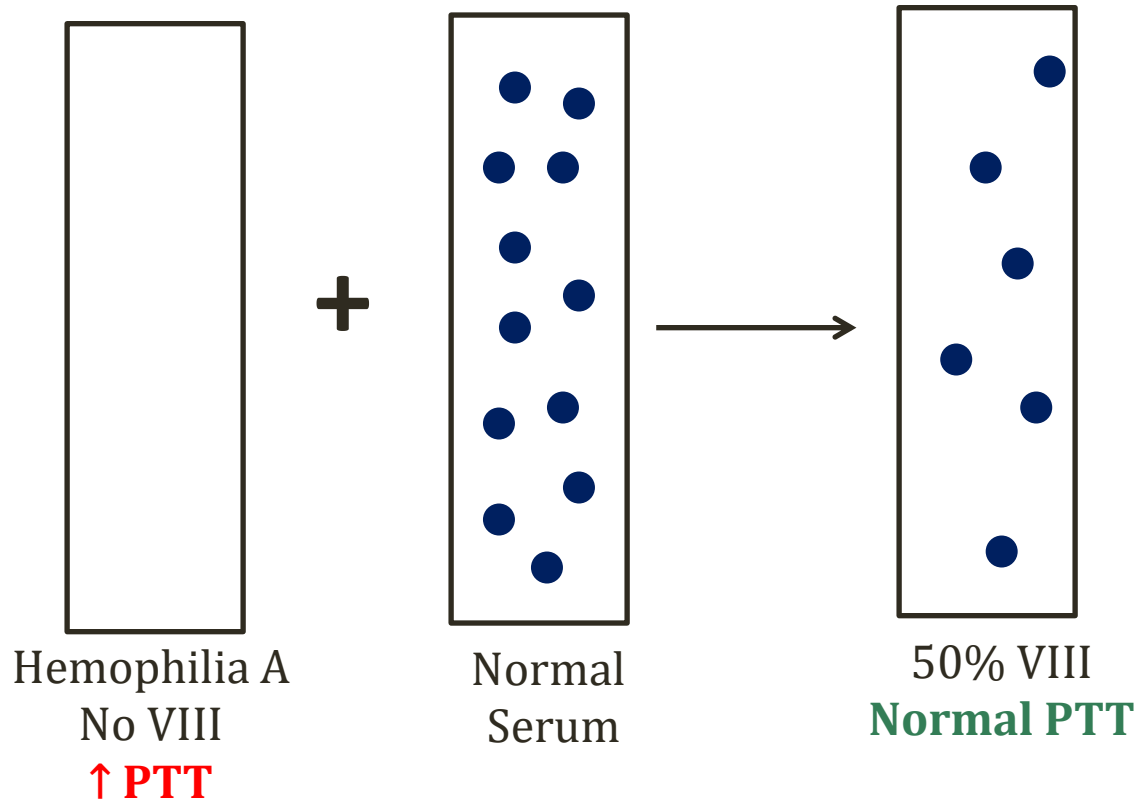


Coagulation Factor Inhibitors

- Can present similar to hemophilia
 - Deficient activity of VIII → bleeding
 - Prolonged PTT
- **Mixing study** will differentiate from hemophilia A

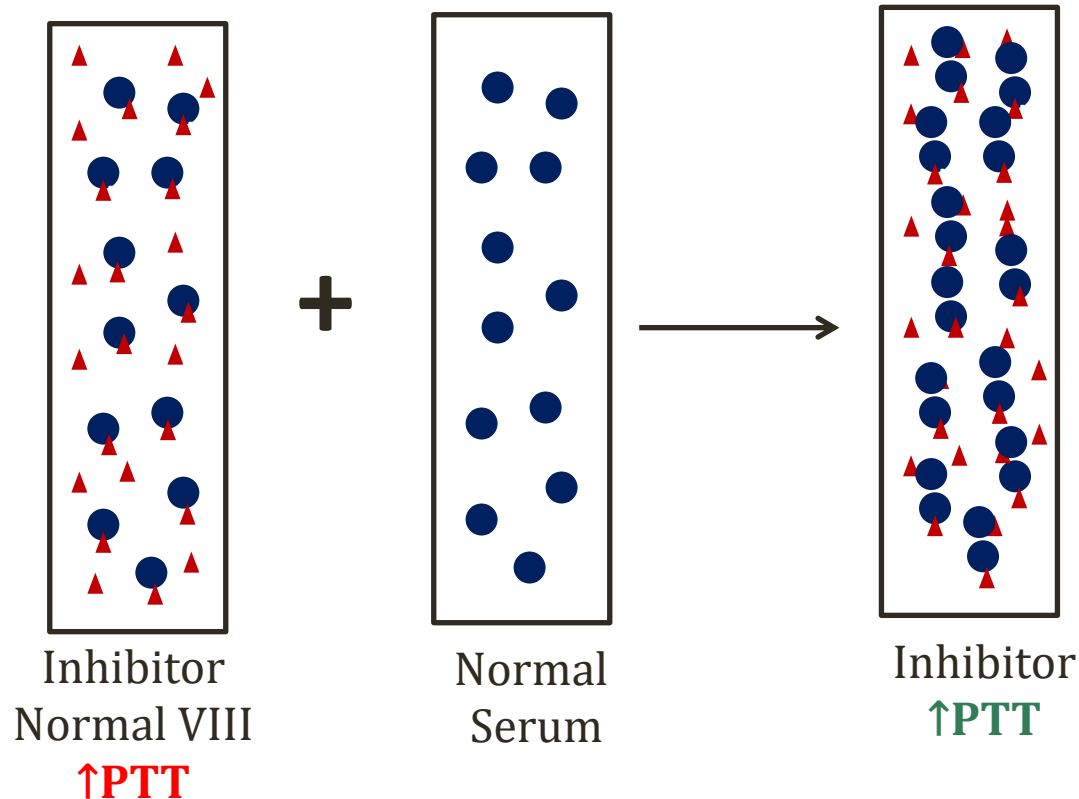
Mixing Study

- Clotting factors ~50% normal → normal PT/PTT



Mixing Study

- Clotting factors ~50% normal \rightarrow normal PT/PTT



Vitamin K Deficiency

- Results in **bleeding**
- Deficiency of vitamin K-dependent factors
 - II, VII, IX, X
- Key lab findings:
 - **Elevated PT/INR**
 - Can see elevated PTT (less sensitive)
 - Normal bleeding time

Vitamin K Deficiency

- Dietary deficiency rare
- GI bacteria produce sufficient quantities
- Common causes:
 - Warfarin
 - **Antibiotics** (deplete GI bacteria)
 - Newborns (sterile GI tract)
 - Malabsorption (Vitamin K is fat soluble)

Blood Transfusion

- Large volume transfusions → dilution clotting factors
- Packed RBCs: devoid of plasma/platelets
 - Removed after collection
- Saline or IVF: No clotting factors
- Treated with **fresh frozen plasma**



"Blausen gallery 2014".
Wikiversity Journal of Medicine.

Liver Disease

- Loss of **clotting factors**
 - Advanced liver disease → ↓ clotting factor synthesis
 - Most clotting factors produced in liver
 - Exception: Factor VIII produced in endothelial cells
 - **PT more sensitive** to liver disease (vitamin K)
- **Thrombocytopenia** also common
 - Decreased hepatic synthesis of **thrombopoietin**
 - Platelet sequestration in spleen from portal hypertension

Platelet Disorders

Jason Ryan, MD, MPH

Bleeding Disorders

- Abnormal coagulation
 - Hemophilia, Vitamin K deficiency
- Abnormal platelets
 - Bernard-Soulier
 - Glanzmann's Thrombasthenia
 - ITP, TTP
 - Uremia
- Mixed Disorders
 - Von Willebrand Disease, DIC

Type of Bleeding

- Abnormal **platelets**
 - Mucosal bleeding, **skin** bleeding, petechiae
- Abnormal **coagulation factors**
 - **Joint** bleeding, deep tissue bleeding



Bleeding Time

- Test of **platelet function**
- Small cut to patient's arm
- Filter paper applied/removed until bleeding stops
- Rarely done in modern era

Inherited Platelet Disorders

Inherited Platelet Disorders

Glanzman's
Thrombasthenia
Deficiency IIB/IIIA

Bernard-Soulier
Deficiency IB

Wiscott-Aldrich
Immunodeficiency

Glanzmann's Thrombasthenia

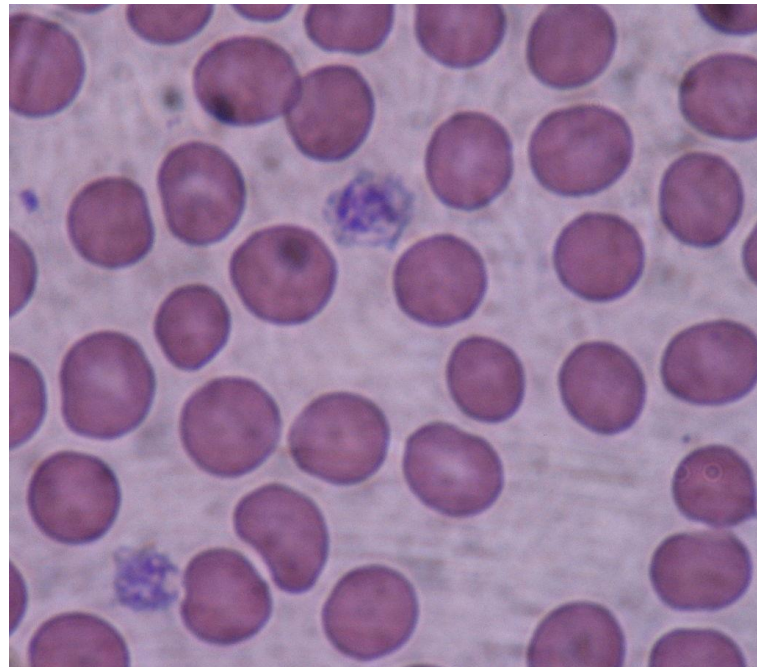
- Autosomal recessive disorder
- Functional **deficiency of GPIIb/IIIa receptors**
- Bleeding, often epistaxis
- Key diagnostic finding:
 - Prolonged bleeding time
 - Blood smear: **Isolated platelets** (no clumping)
 - **Absent platelet aggregation** in response to stimuli
 - Abnormal platelet aggregometry
 - Platelets mixed with ADP, arachidonic acid

Bernard-Soulier Syndrome

- Autosomal recessive disorder
- **Deficiency of GPIb platelet receptors**
- Platelets cannot bind vWF
- Also results in **large platelets**
- Bleeding, often epistaxis or menorrhagia
- Key lab findings:
 - Prolonged bleeding time
 - **Thrombocytopenia**
 - Large platelets on blood smear

Giant Platelets

- Can be seen in association with thrombocytopenia
- Caused by rare inherited disorders
 - Bernard-Soulier, others



Bobjgalindo/Wikipedia

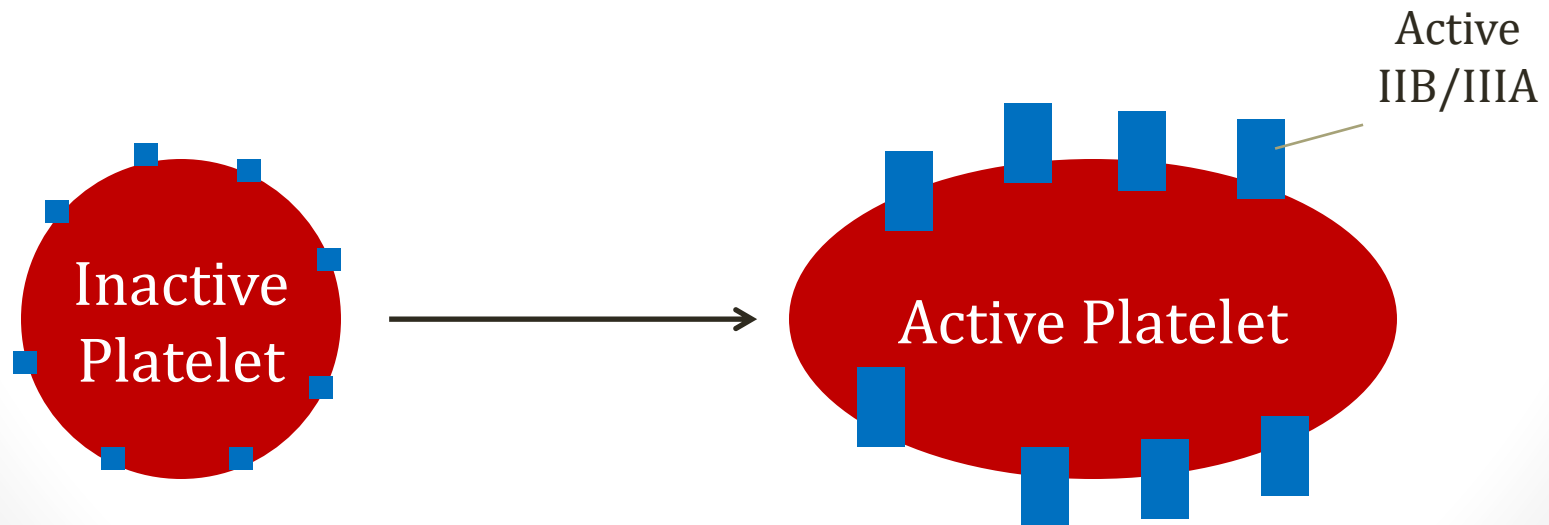
Wiskott-Aldrich Syndrome

- Immunodeficiency syndrome of infants
- X linked disorder of WAS gene (WAS protein)
 - Necessary for T-cell **cytoskeleton** maintenance
- Triad:
 - **Immune dysfunction**
 - **↓ platelets**
 - **Eczema**

ITP

Idiopathic thrombocytopenic purpura

- Disorder of decreased platelet survival
- Commonly caused by **anti-GPIIB/IIIA antibodies**
- Consumption **splenic macrophages**



ITP

Idiopathic thrombocytopenic purpura

- Diagnosis of exclusion
 - Rule out other causes of bone marrow suppression
- Treatment:
 - Steroids
 - IVIG (blocks Fc receptors in macrophages)
 - Splenectomy

TTP

Thrombotic thrombocytopenic purpura

- Disorder of **small vessel thrombus** formation
- Consumes platelets → thrombocytopenia
- ↓ activity of vWF cleaving protease **ADAMTS13**

Von Willebrand Factor

Multimers

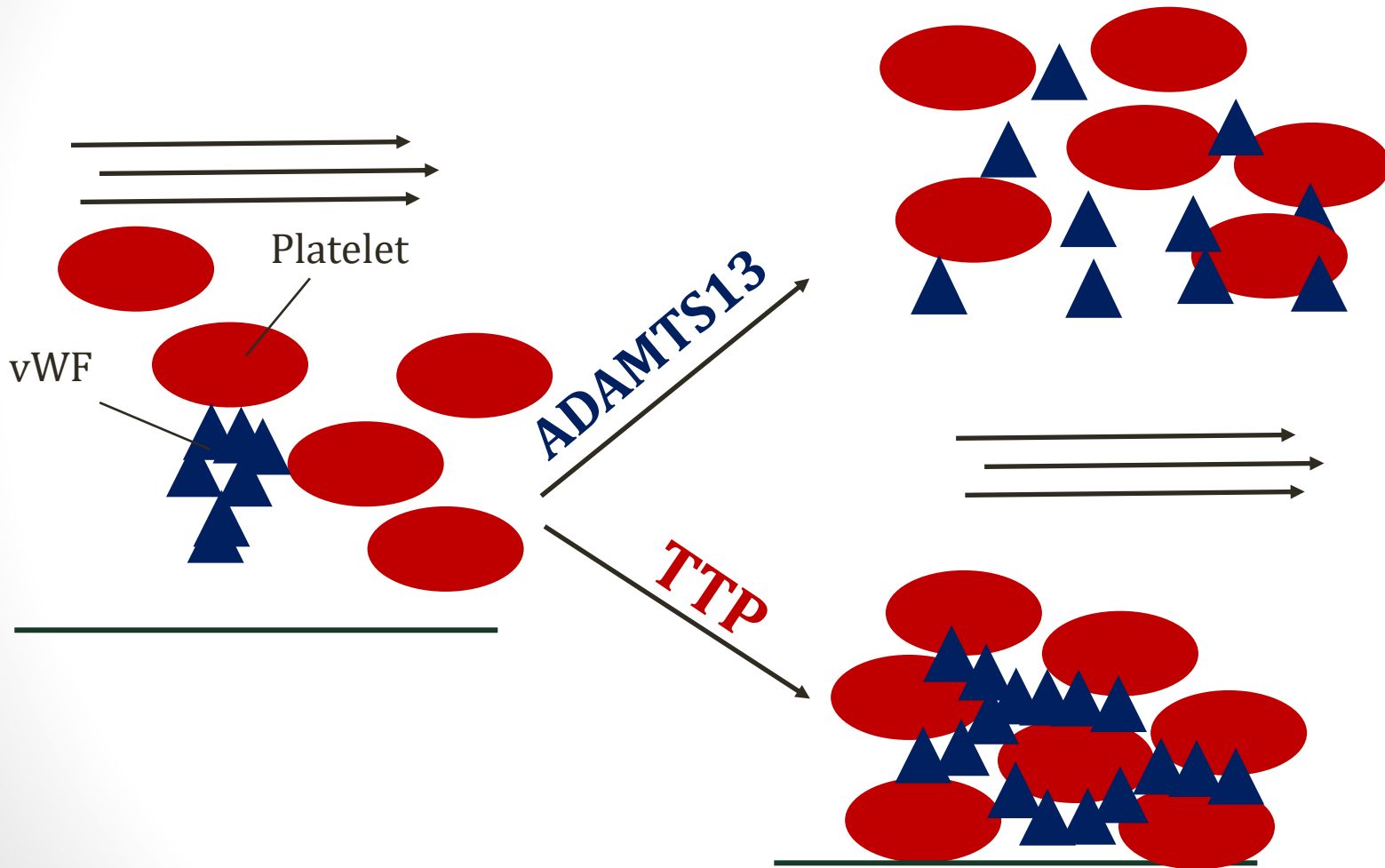
- vWF synthesized a protein **monomer**
 - Occurs in endothelial cells and megakaryocytes
- Monomers link in endoplasmic reticulum → dimers
- vWF dimers move to Golgi → multimers

Von Willebrand Factor

Multimers

- Large multimers stored:
 - Endothelial Weibel–Palade bodies
 - Platelet α -granules
- Large multimers can obstruct blood flow
- **ADAMTS13** prevents obstruction
 - Enzyme (metalloprotease)
 - **Breaks down multimers of vWF**
 - Prevents thrombotic occlusion

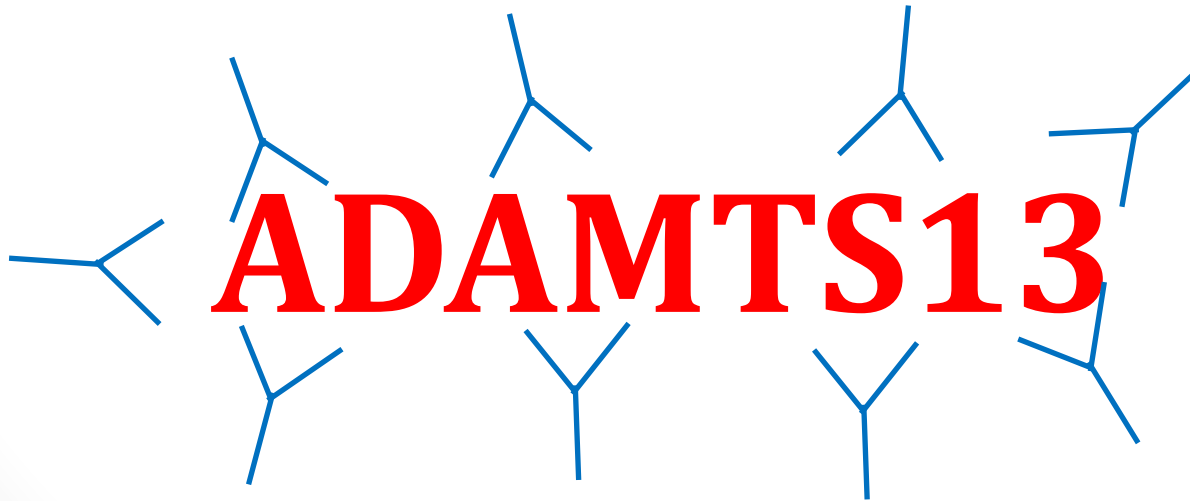
ADAMTS13



TTP

Cause

- Severe ADAMTS13 deficiency
 - Usually <10% normal activity
- Usual cause: **acquired autoantibody** to ADAMTS13
- Result: vWF multimers in areas of high shear stress
- Obstruction small vessels



MAHA

Microangiopathic hemolytic anemia

- Hemolytic anemia (\uparrow LDH, \downarrow haptoglobin)
- Caused by **shearing of RBCs** as they pass through thrombi in small vessels
- Blood smear: schistocytes
- Seen in:
 - TTP
 - HUS
 - DIC



Paulo Henrique Orlandi Mourao

TTP

Thrombotic thrombocytopenic purpura

- Fever
 - Inflammation from small vessel occlusion and tissue damage
- **Neurological symptoms**
 - Headache, confusion, seizures
- Renal failure
- **Petechiae** and bleeding

TTP

Thrombotic thrombocytopenic purpura

- Lab tests:
 - Hemolytic anemia
 - Thrombocytopenia
 - Schistocytes on blood smear
- PT/PTT should be normal
 - Contrast with DIC
- May see elevated d-dimer

TTP

Treatment

- **Plasma exchange**: removes antibodies
- **Platelet counts** monitored to determine efficacy



Mr Vacchi /Wikipedia

Hemolytic Uremic Syndrome

HUS

- Many similarities with TTP
- Also caused by platelet-rich thrombi in small vessels
- MAHA, thrombocytopenia, acute kidney injury
 - Usually no fever or CNS symptoms
 - Renal thrombi → kidney injury
- Commonly seen in **children**
- Commonly follow GI infection E. Coli O157:H7
 - **Shiga-like toxin** causes microthrombi

DIC

Disseminated Intravascular Coagulation

- Widespread activation of clotting cascade
- Diffuse thrombi (platelets/fibrin) → ischemia
- Consumption of clotting factors and platelets
- Destruction of red blood cells → anemia

DIC

Disseminated Intravascular Coagulation

- Occurs secondary to another process
- Obstetrical emergencies
 - Amniotic fluid contains **tissue factor**
 - DIC seen in conjunction with amniotic fluid embolism
- Sepsis
 - **Endotoxin** -> activates coagulation cascade
 - Cytokines

DIC

Disseminated Intravascular Coagulation

- Leukemia
 - Especially acute promyelocytic leukemia (APML)
 - Cancer: well-described hypercoagulable state
 - Excess coagulation: DIC
- Rattlesnake bites
 - **Thrombin-like glycoproteins** within venom
 - Diffuse activation of clotting



Andy king50/Wikipedia

DIC

Disseminated Intravascular Coagulation

- **Elevated PT/PTT/Thrombin time**
 - Consumption of factors
- **Low platelets**
 - Consumption of platelets
- **Low fibrinogen** (consumption)
- Microangiopathic hemolytic anemia
 - Low RBC (**anemia**)
 - Schistocytes on blood smear
- Elevated **D-dimer**

DIC

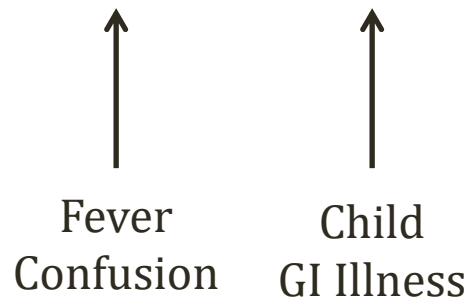
Disseminated Intravascular Coagulation

- Treatment: underlying disorder
- Fresh frozen plasma: replace clotting factors
- RBCs, platelets
- Cryoprecipitate (for **low fibrinogen**)

ITP, TTP, HUS, DIC



	ITP	TTP	HUS	DIC
↓ Platelets	+	+	+	+
Hemolytic Anemia	--	+	+	+
↑PT/PTT	--	--	--	+



Uremia

- Renal dysfunction → bleeding
- Poor aggregation and adhesion of platelets
- Caused by uremic toxins in plasma
 - Uremic platelets work normally in normal serum
- Prolonged **bleeding time**
- Normal platelet count
- Normal coagulation testing

Thrombocytopenia

- Decreased production of platelets
 - Chemotherapy, leukemia
 - Sepsis (bone marrow suppression)
- Platelet sequestration
 - Splenomegaly
 - Portal hypertension
- Platelet destruction
 - ITP, TTP

Thrombocytopenia

- Normal platelet count: 150,000/ml to 400,000/ml
- Bleeding occurs when **<10,000**
- Treatment: Platelet transfusions

Von Willebrand Disease

- Deficient function of von Willebrand Factor
 - Large glycoprotein
 - Synthesized by **endothelial cells** and **megakaryocytes**
 - Present in platelets
- Two key roles in hemostasis
 - **Carrier of factor VIII** (intrinsic coagulation pathway)
 - **Binds platelets** to endothelium and other platelets

Von Willebrand Disease

- **Most common inherited bleeding disorder**
 - Affects up to 1 percent of population
- Gene mutations → ↓ level or function of vWF
 - Most cases autosomal dominant (males=females)

Von Willebrand Disease

- Usually mild, non-life-threatening bleeding
- Easy bruising
- Skin bleeding
- Prolonged bleeding from mucosal surfaces
 - Severe nosebleeds
 - **Menorrhagia**

Von Willebrand Disease

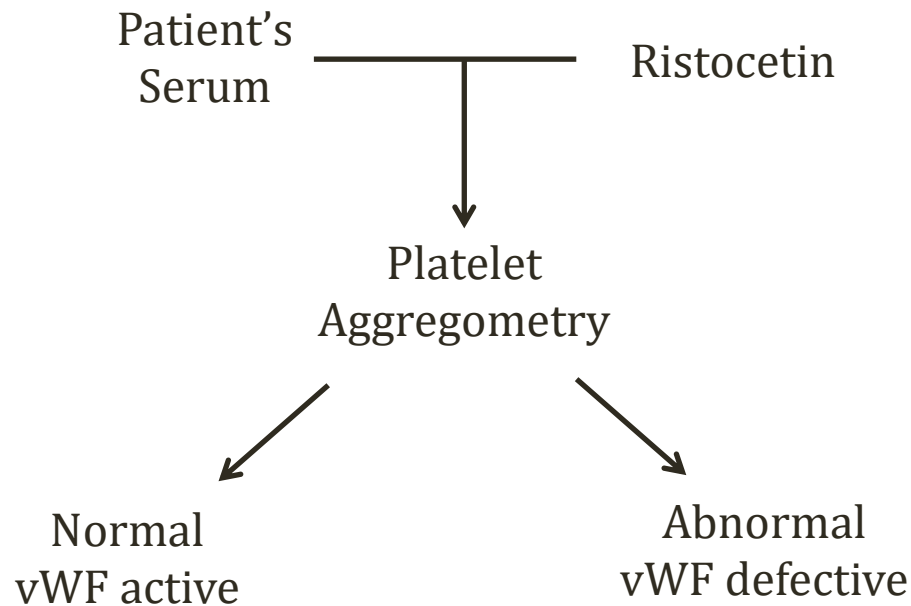
Diagnosis

- Normal platelet count
- Normal PT
- **Increased PTT** (depending on severity)
 - Usually no joint/deep tissue bleeding
- **Increased bleeding time**

Von Willebrand Disease

Diagnosis

- **Ristocetin cofactor activity assay**
- Ristocetin: antibiotic off market due to ↓platelets
- Binds vWF and platelet glycoprotein Ib
- Causes platelet aggregation if vWF present



Von Willebrand Disease

Treatment

- vWF concentrate
- **Desmopressin**
 - **Increases vWF and factor VIII levels**
 - Releases vWF from endothelial cells
- **Aminocaproic acid**
 - Antifibrinolytic drug
 - Inhibits plasminogen activation → plasmin
 - Less breakdown of formed clots

Heyde's Syndrome

- **GI bleeding** associated with **aortic stenosis**
- Angiodysplasia
 - Vascular malformations of GI tract
 - Prone to bleeding
 - Commonly occur in aortic stenosis patients
- Deficiency of **von Willebrand factor**
 - High shearing force caused by aortic stenosis
 - Uncoiling of vWF multimers
 - Exposes cleavage site for **ADAMTS13**
- Improves after aortic valve surgery

Antiplatelets

Jason Ryan, MD, MPH

Thrombus Formation

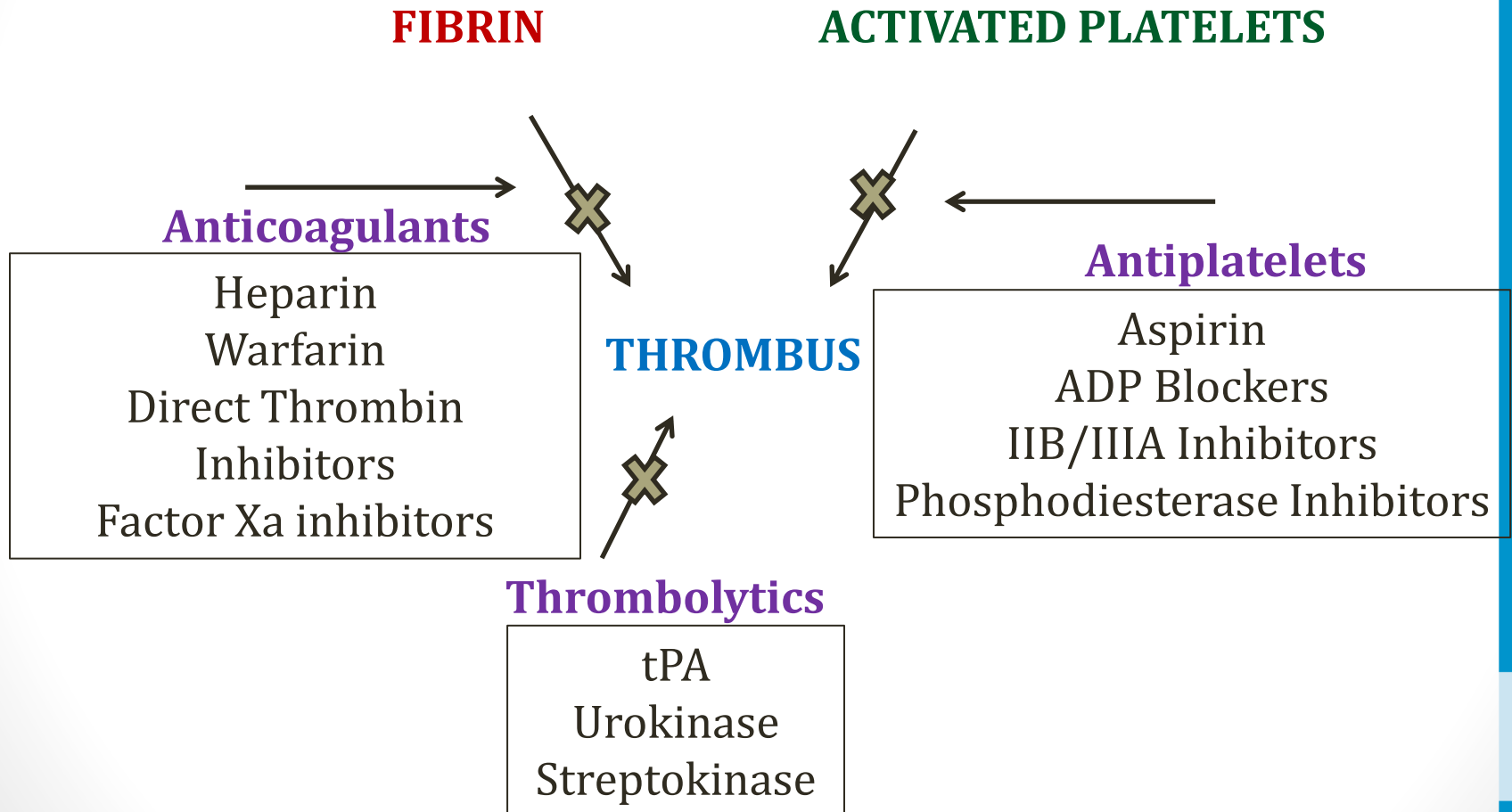
FIBRIN

ACTIVATED PLATELETS

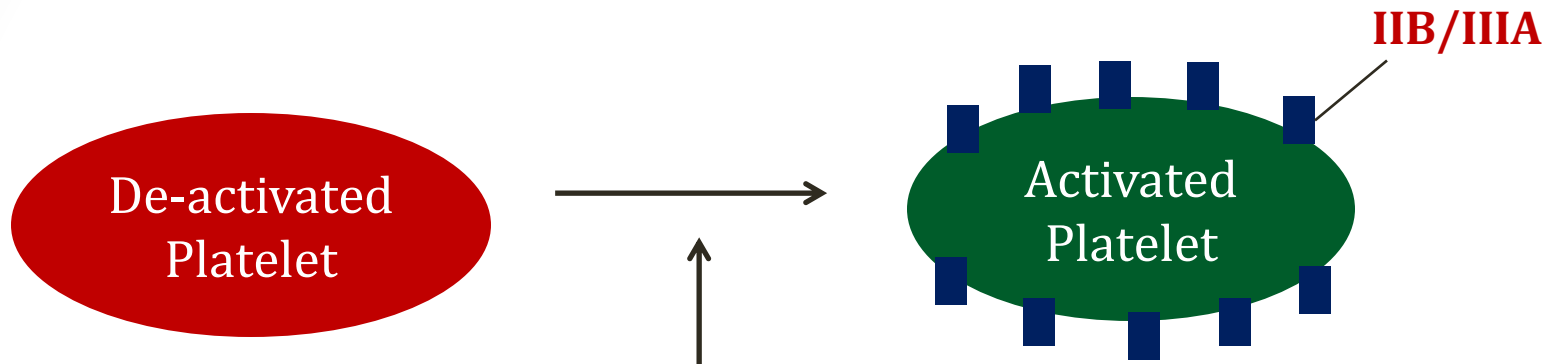


THROMBUS

Thrombus Formation

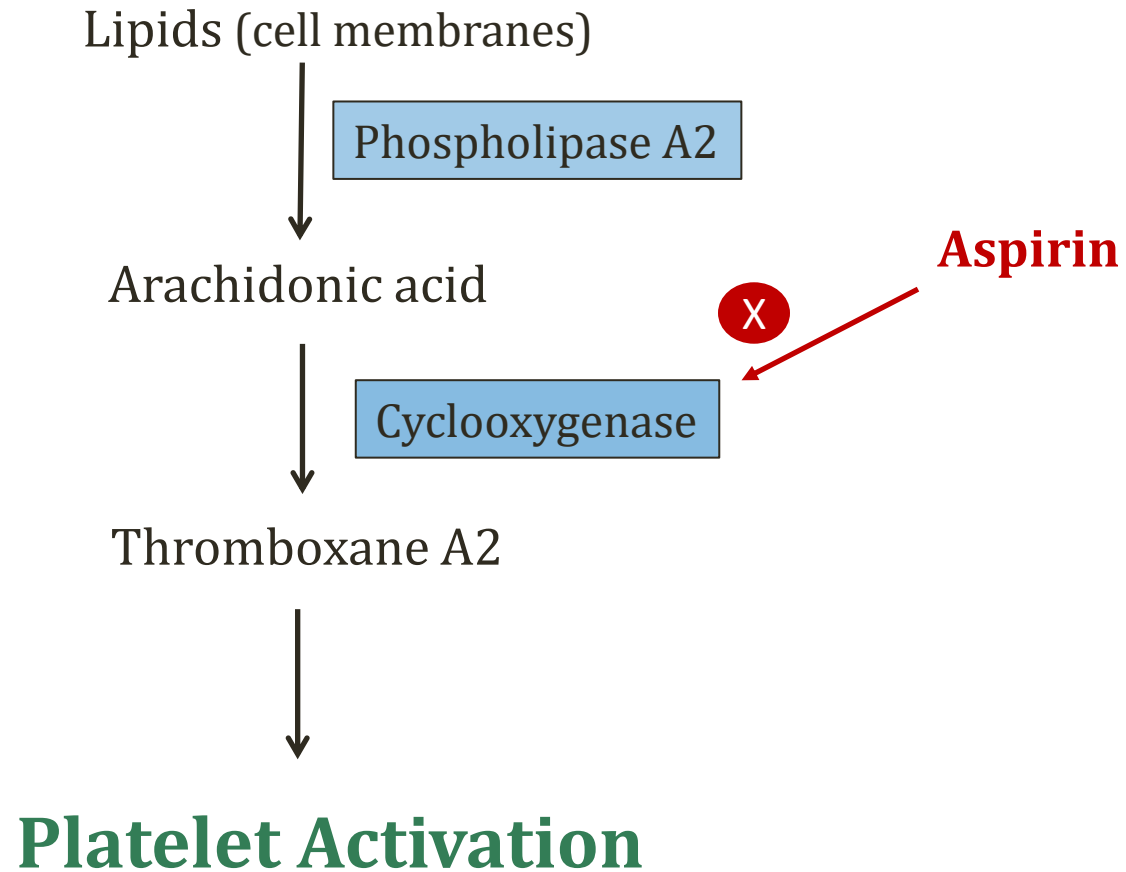


Antiplatelets



Thromboxane (from arachidonic acid)
Adenosine diphosphate (ADP)
↓cAMP (via ADP)

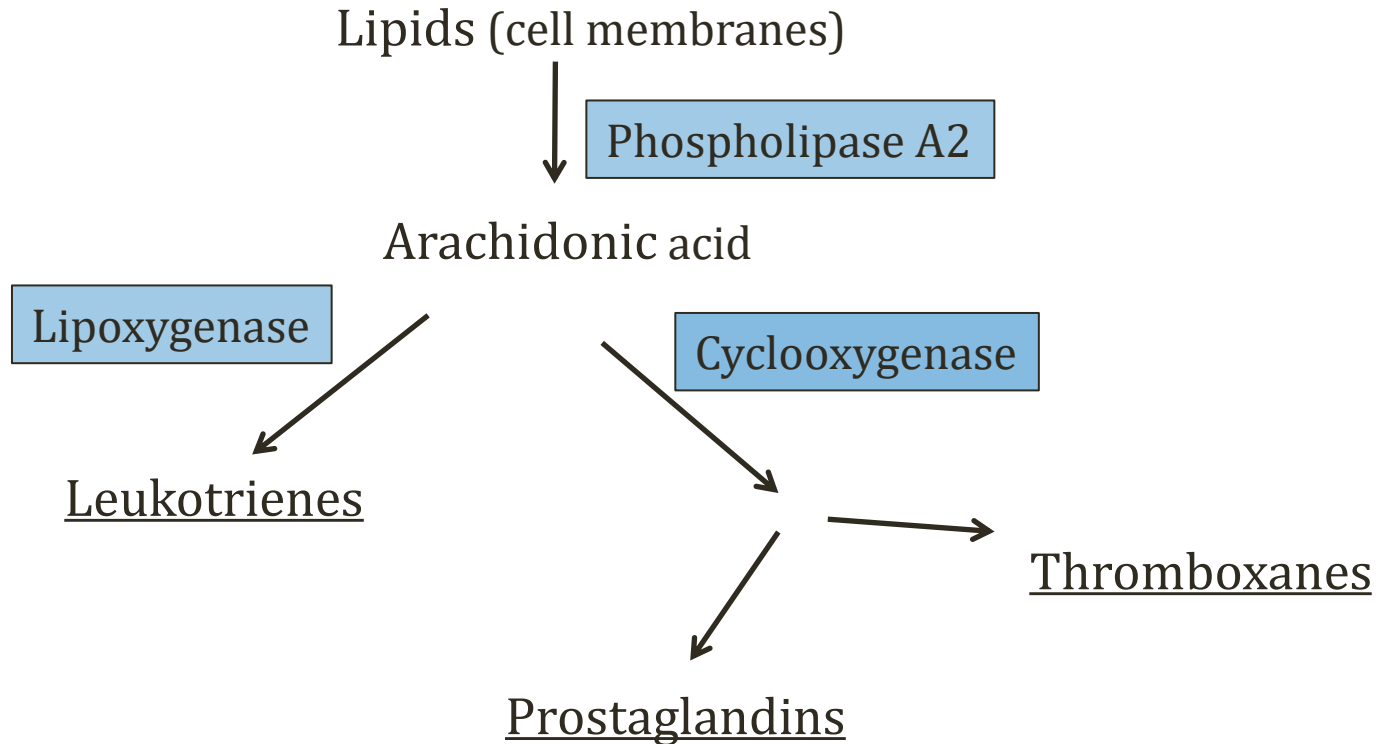
Aspirin



Aspirin

- Inhibits COX-1 and COX-2
 - Both found in platelets
- Blunts conversion of AA to TXA₂
- **↓ platelet activity**
- Also inhibits production of prostaglandins

Eicosanoids



Eicosanoids

Mediator	Effects
PGE ₂	Redness (vasodilation) Edema (permeability) Fever (hypothalamus) Pain (nerves) Renal vasodilation (afferent)
PGE ₂ /PGI ₂	Protect GI mucosa
TXA ₂	Platelet activation

Ricciotti E, FitzGerald G; **Prostaglandins and Inflammation**
Arterioscler Thromb Vasc Biol. 2011 May; 31(5): 986–1000.

NSAIDs

Ibuprofen, naproxen, indomethacin, ketorolac, diclofenac

- Aspirin is technically NSAID
- NSAIDs **reversibly** inhibit COX-1 and COX-2
- Aspirin **irreversibly** inhibits COX-1 and COX-2
- Decreases activity for lifetime of platelet (7-10days)
- All NSAIDs may cause bleeding
- All NSAIDs reduce pain, inflammation via ↓ PGs

Aspirin

Common antiplatelet uses

- **Coronary disease**
 - Acute myocardial infarction/unstable angina
 - Secondary prevention
- **Stroke**
 - Acute ischemic stroke
 - Secondary prevention

Aspirin

Adverse Effects

- **Bleeding**
- Gastritis/Ulcers
 - COX important for maintenance of **GI mucosa**
- **Tinnitus**
 - Caused by salicylate (aspirin metabolite: salicylic acid)
 - Alters cochlear nerve function
 - Rare: Usually occurs with very high doses
 - Resolves with discontinuation

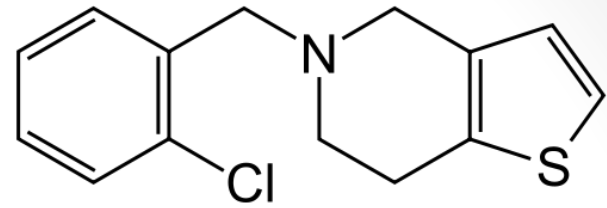
Aspirin

Adverse Effects

- Reye's syndrome
 - **Liver failure** and **encephalopathy**
 - Associated with aspirin use in children
 - Aspirin not generally used in kids (exception: Kawasaki)

Thienopyridines

Ticlopidine, clopidogrel, prasugrel



Ticlopidine

- **Irreversible** P2Y₁₂ receptor blockers
- Block effects of ADP on platelets
- Used in aspirin allergy
- Added to aspirin for prevention of MI, Stroke
- Major adverse effect is **bleeding**
- Rare, dangerous adverse effect: **TTP**

TTP

Thrombotic Thrombocytopenic Purpura

- Associated with thienopyridine drugs
- Severe thrombocytopenia
- Microangiopathic hemolytic anemia
- Neurologic abnormalities
- Deficient activity of ADAMTS13
- Antibodies to ADAMTS13

Ticagrelor

- Cyclo-pentyl-triazolo-pyrimidine (CPTP)
 - NOT a thienopyridine
- **Reversible** antagonist to P2Y₁₂ receptor
- Unique side effect: **Dyspnea**
 - Mechanism unclear

Phosphodiesterase Inhibitors

PDE Inhibitors

- Inhibit phosphodiesterase III in platelets
- PDE breaks down cAMP
- \uparrow cAMP \rightarrow \downarrow platelet activation
- Two drugs in class: **dipyridamole, cilostazol**

Dipyridamole

- PDEIII inhibitor
- Inhibits platelet activation
- Also blocks adenosine uptake by cells
 - Adenosine = vasodilator
 - **Raises adenosine levels → vasodilation**
- Used with aspirin for **stroke prevention** (antiplatelet)
- Used in chemical **cardiac stress testing** (vasodilator)

Cilostazol

- PDEIII inhibitor
- Inhibits platelet activation
- Also raises cAMP in vascular smooth muscle
- Vasodilator
- Rarely used for anti-platelet effects
- Used in **peripheral arterial disease**

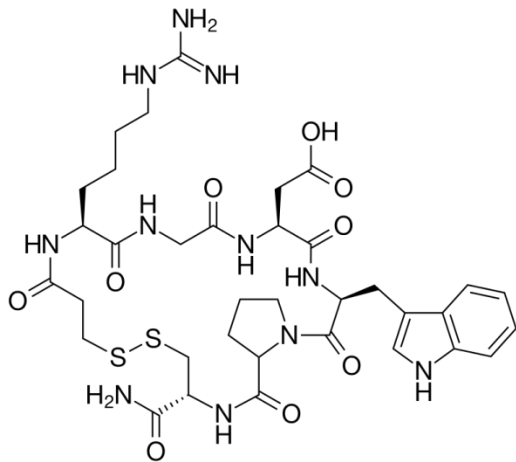
Phosphodiesterase Inhibitors

Dipyridamole, Cilostazol

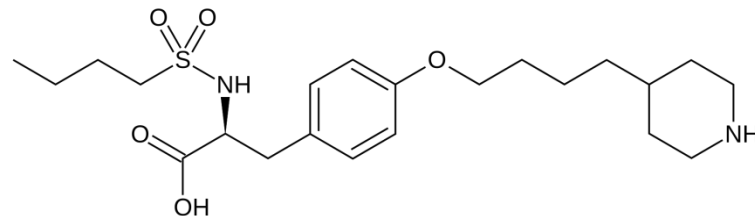
- Many side effects related to vasodilation
 - **Headache**
 - **Flushing**
 - **Hypotension**

IIB/IIIA Receptor Blockers

- **Abciximab, eptifibatide, tirofiban**
- Bind and block IIB/IIIA receptors
- Abciximab: Fab fragment of antibody to IIB/IIIA
- IV drugs used in acute coronary syndromes/stenting



Eptifibatide

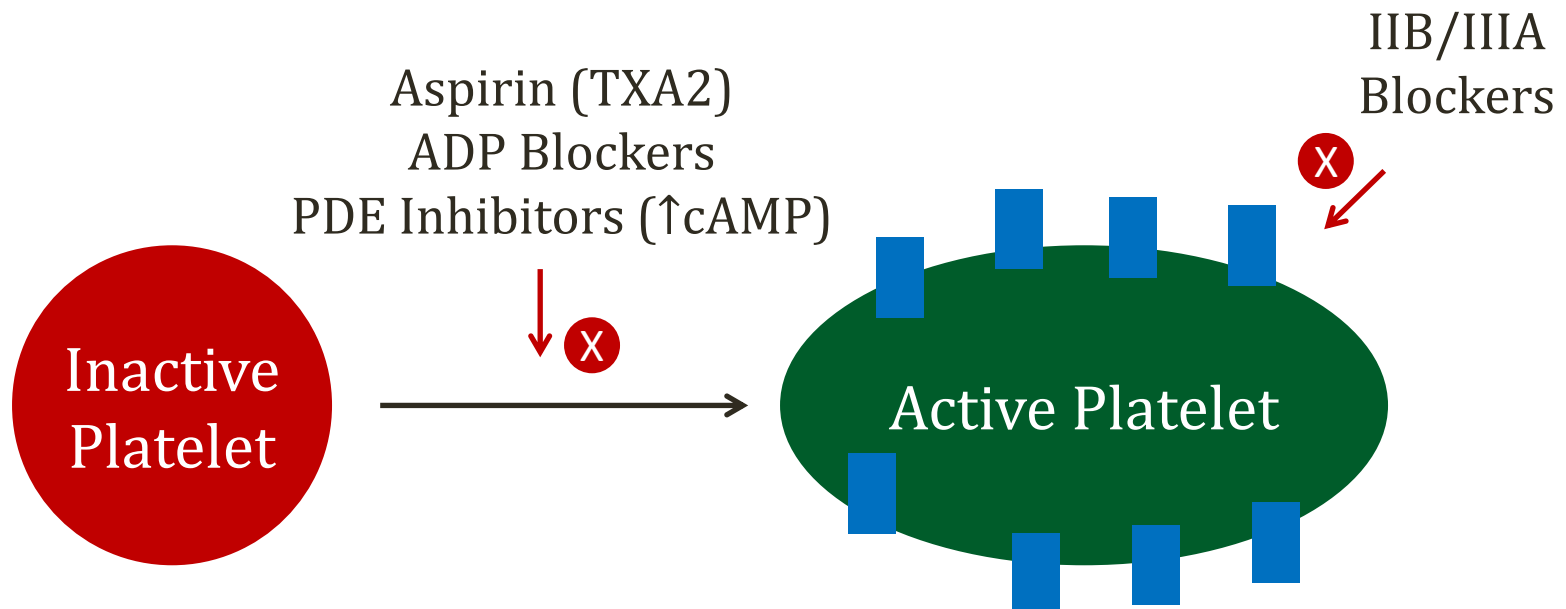


Tirofiban

IIB/IIIA Receptor Blockers

- Main adverse effect is bleeding
- Can cause **thrombocytopenia**
 - May occur within hours of administration
 - Mechanism poorly understood
 - **Must monitor platelet count** after administration

Antiplatelet Drugs



Anticoagulant Drugs

Jason Ryan, MD, MPH

Thrombus Disorders

<u>Disease</u>	→	<u>Thrombus Location</u>
• Atrial Fibrillation		• Left atrial appendage
• Myocardial Infarction		• Coronary artery
• DVT/PE		• Deep vein/pulm artery
• Stroke		• CNS circulation
• Critical Limb Ischemia		• Peripheral circulation

Antithrombotic Drugs

- Acute therapy: Help eliminate clot already formed
- Prevention: Lower risk of clot in high risk patients

Thrombus Formation

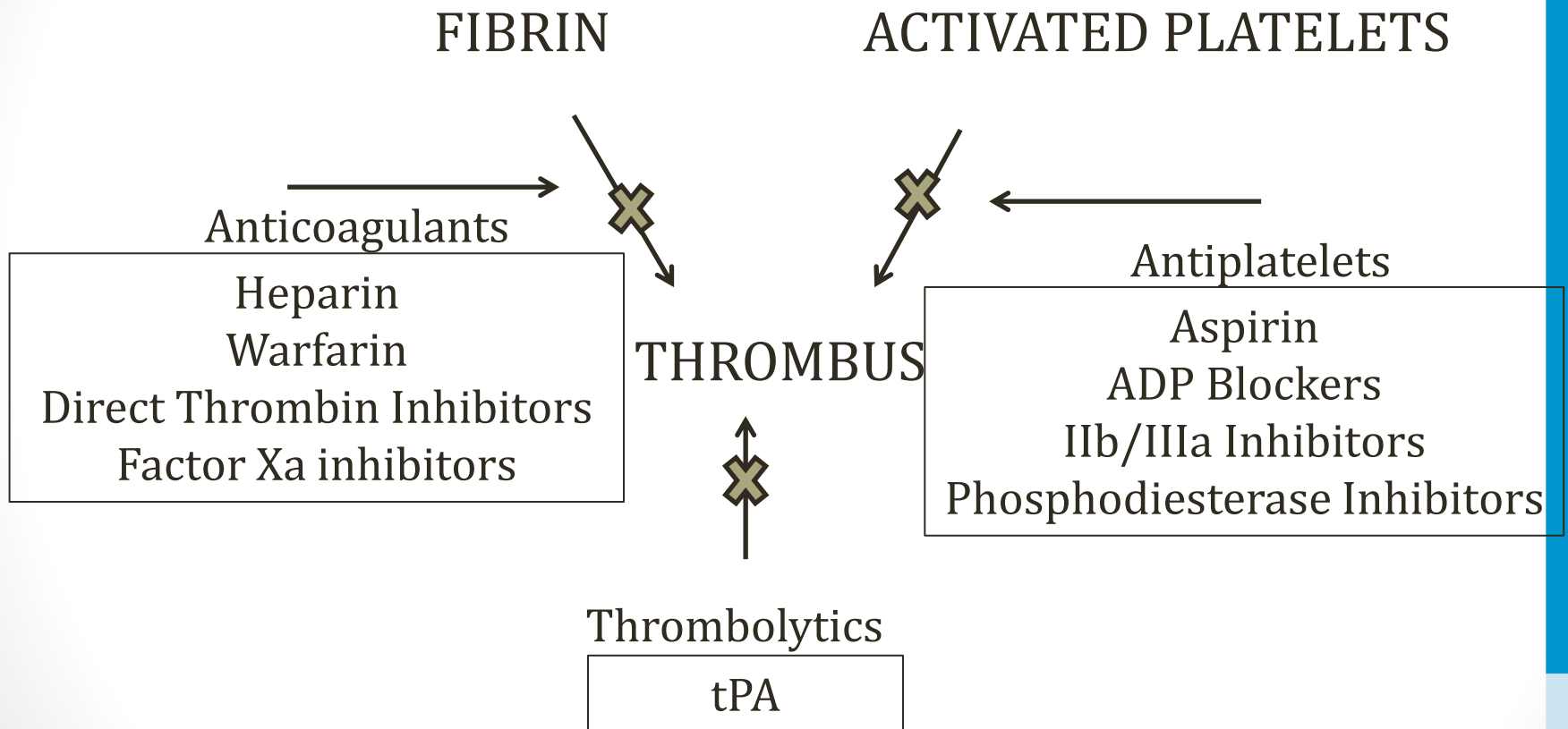
FIBRIN

ACTIVATED PLATELETS



THROMBUS

Blood thinners



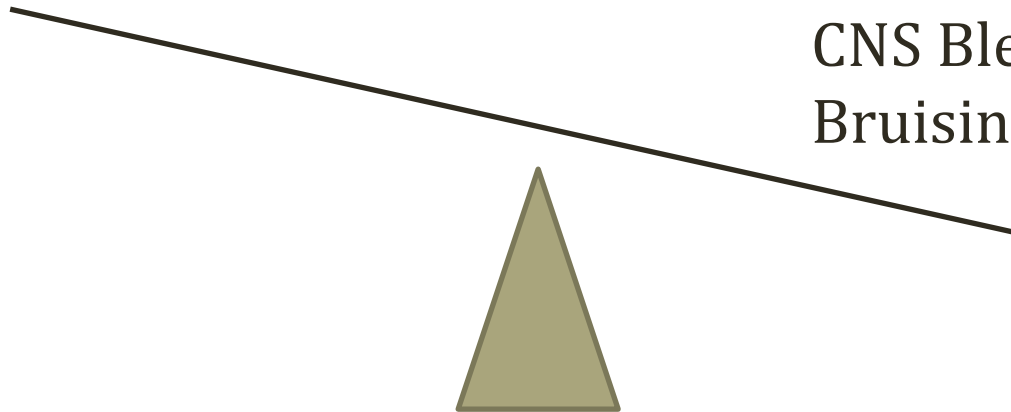
Bleeding

- Thrombus formation very beneficial
- Prevents/stops bleeding
- **BLEEDING:** common side effect
- Can occur with all antithrombotic/antiplatelet drugs

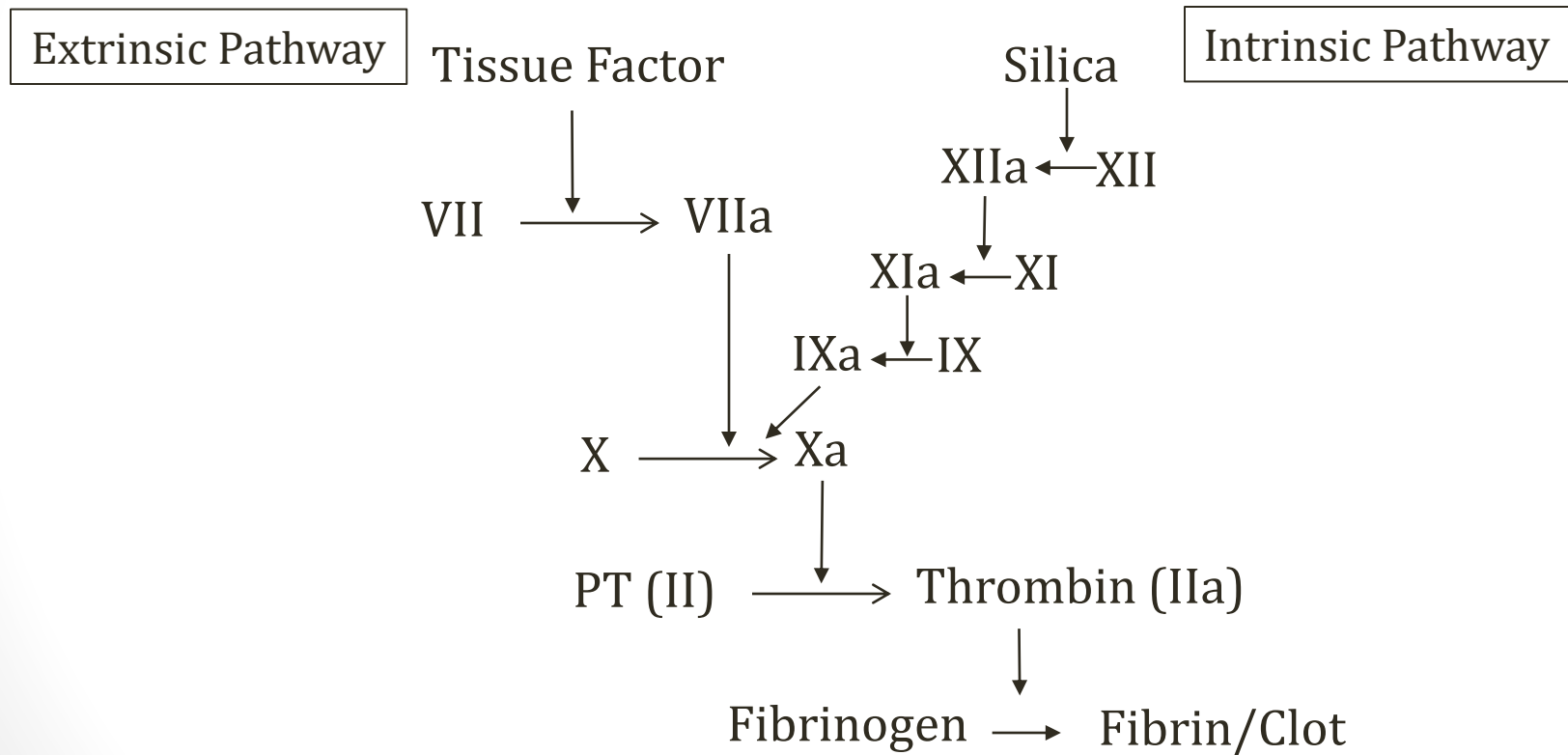
Clotting versus Bleeding

Myocardial Infarction
Pulmonary Embolism
Deep Vein Thrombosis
Stroke

GI Bleeding
CNS Bleeding
Bruising

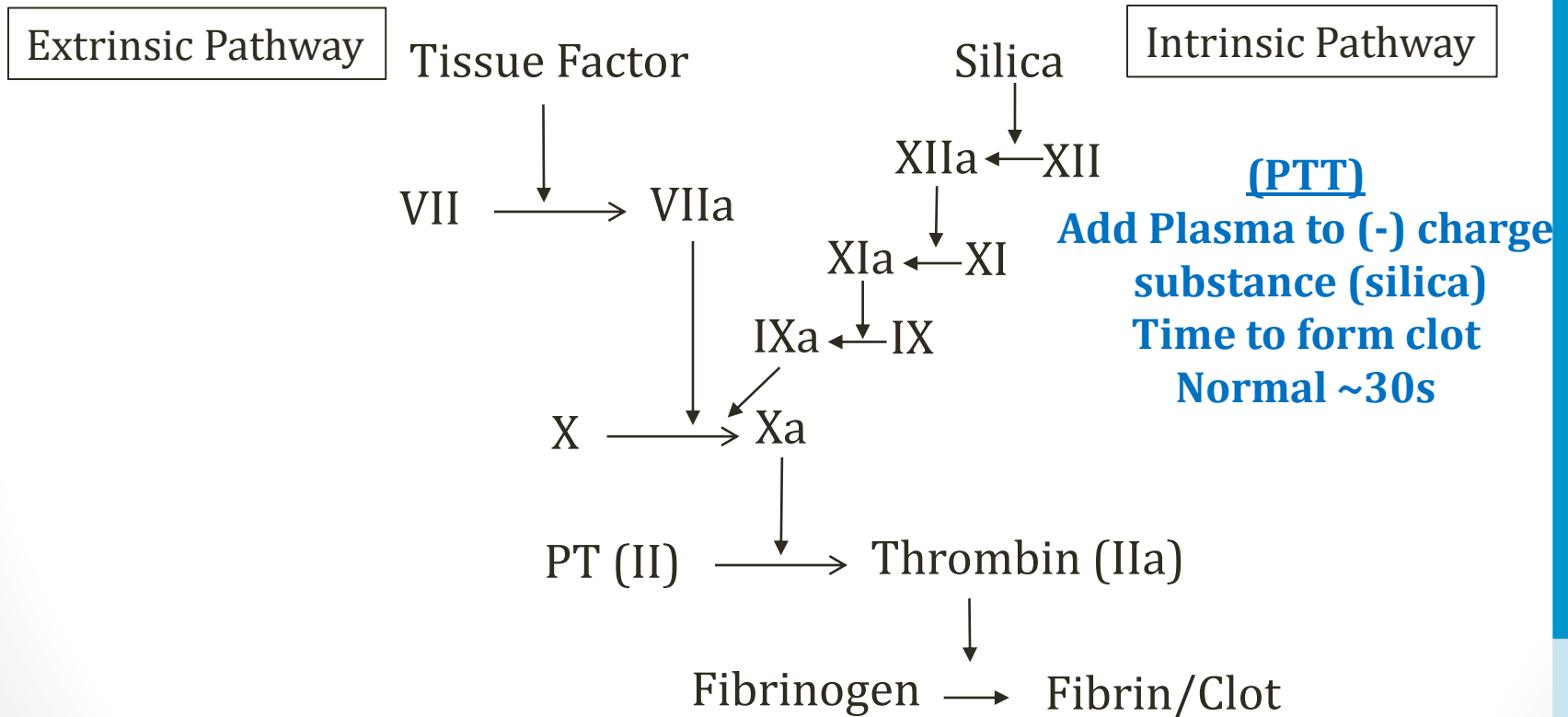


Coagulation Cascade



PTT

Activated Partial Thromboplastin Time



PT

Prothrombin Time

Extrinsic Pathway

Tissue Factor

VII → VIIa

Silica

Intrinsic Pathway

XIIa ← XII

XIa ← XI

IXa ← IX

X → Xa

PT (II) → Thrombin (IIa)

Fibrinogen → Fibrin/Clot

Prothrombin Time (PT)

Add Plasma to TF

Time to form clot

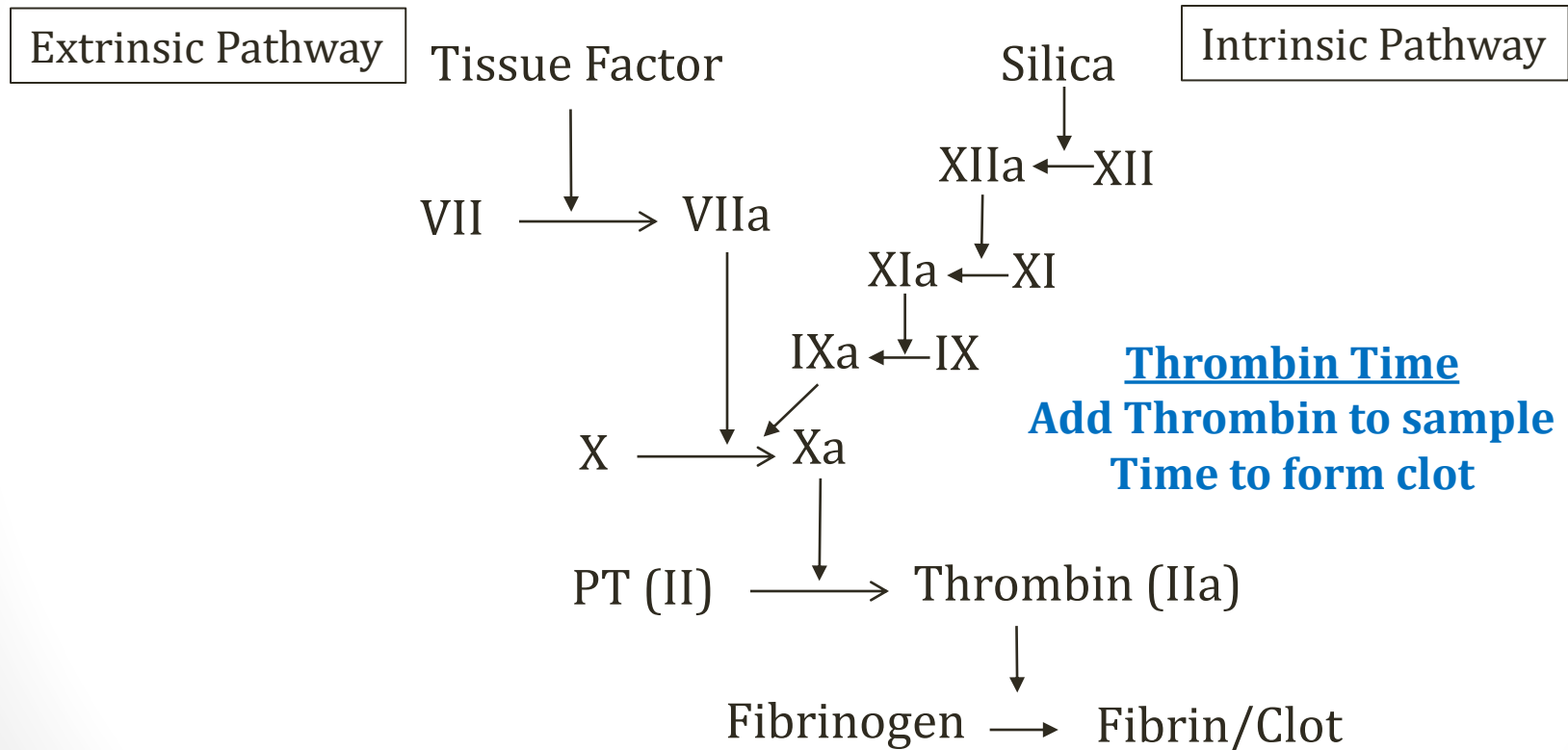
Normal ~10s

$INR = \frac{\text{Patient PT}}{\text{Control PT}}$

Normal = 1

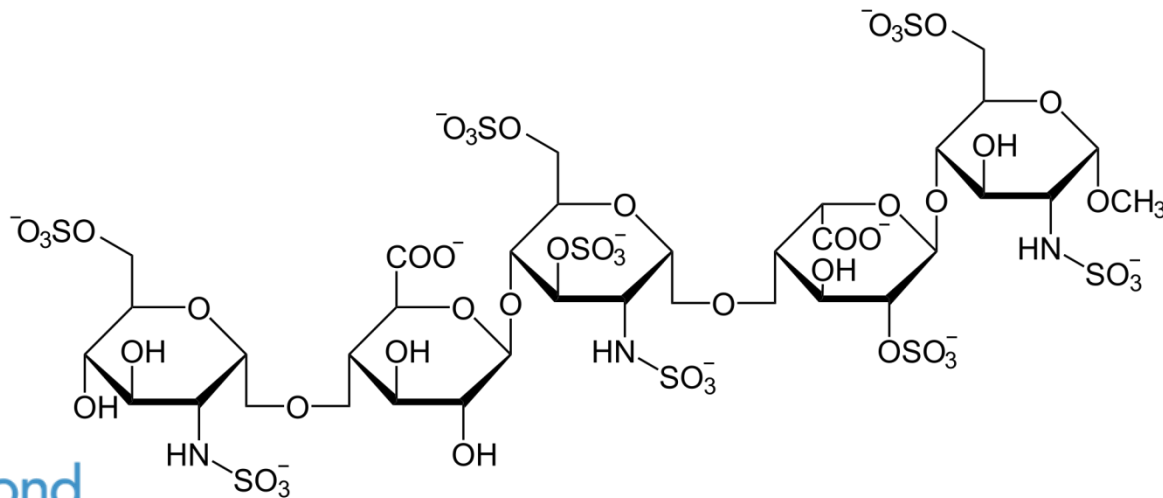
Therapeutic = 2-3

Thrombin Time

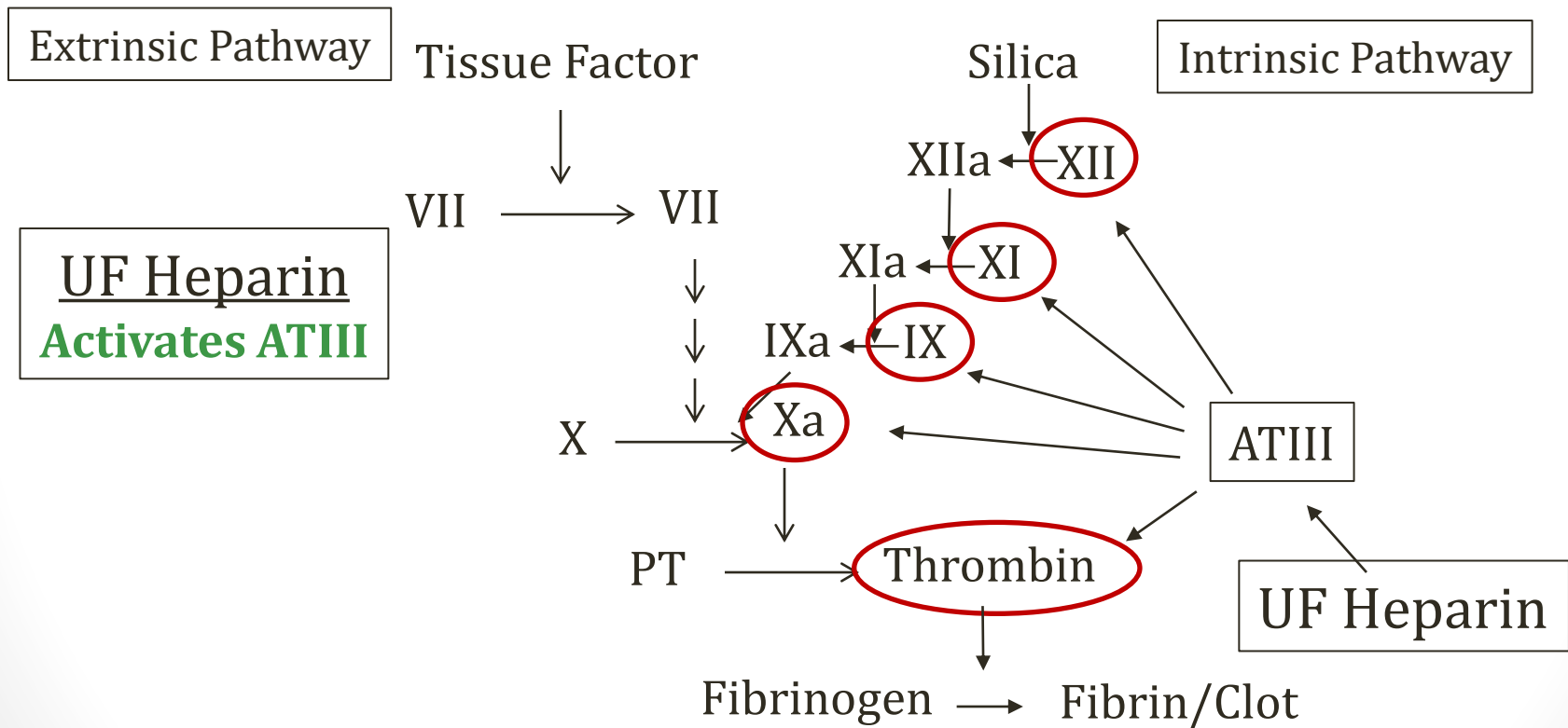


Heparin

- Polymer (glycosaminoglycan)
- Occurs naturally (found in **mast cells**)
- Molecules with varying chain lengths
- Used in two forms:
 - **Unfractionated**: widely varying polymer chain lengths
 - **Low molecular weight**: Smaller polymers only



Unfractionated Heparin



Unfractionated Heparin (UFH)

- Given IV or SQ → acute onset
- **Increases PTT**
 - Effects many components of intrinsic pathway
 - HeparIN = INtrinsic (PTT)
- Will also increase thrombin time
- Can increase PT at high dosages
- Lots of binding to plasma proteins, cells
 - Highly variable response from patient to patient
 - **Dose must be adjusted** to reach goal PTT

Protamine

- Reversal agent for unfractionated heparin
 - Less effective with LMWH
- Binds heparin → neutralizes drug
- Used in heparin overdose
- Used in cardiac surgery
 - High dose heparin administered for heart-lung bypass
 - Quick reversal at completion of case

Unfractionated Heparin (UFH)

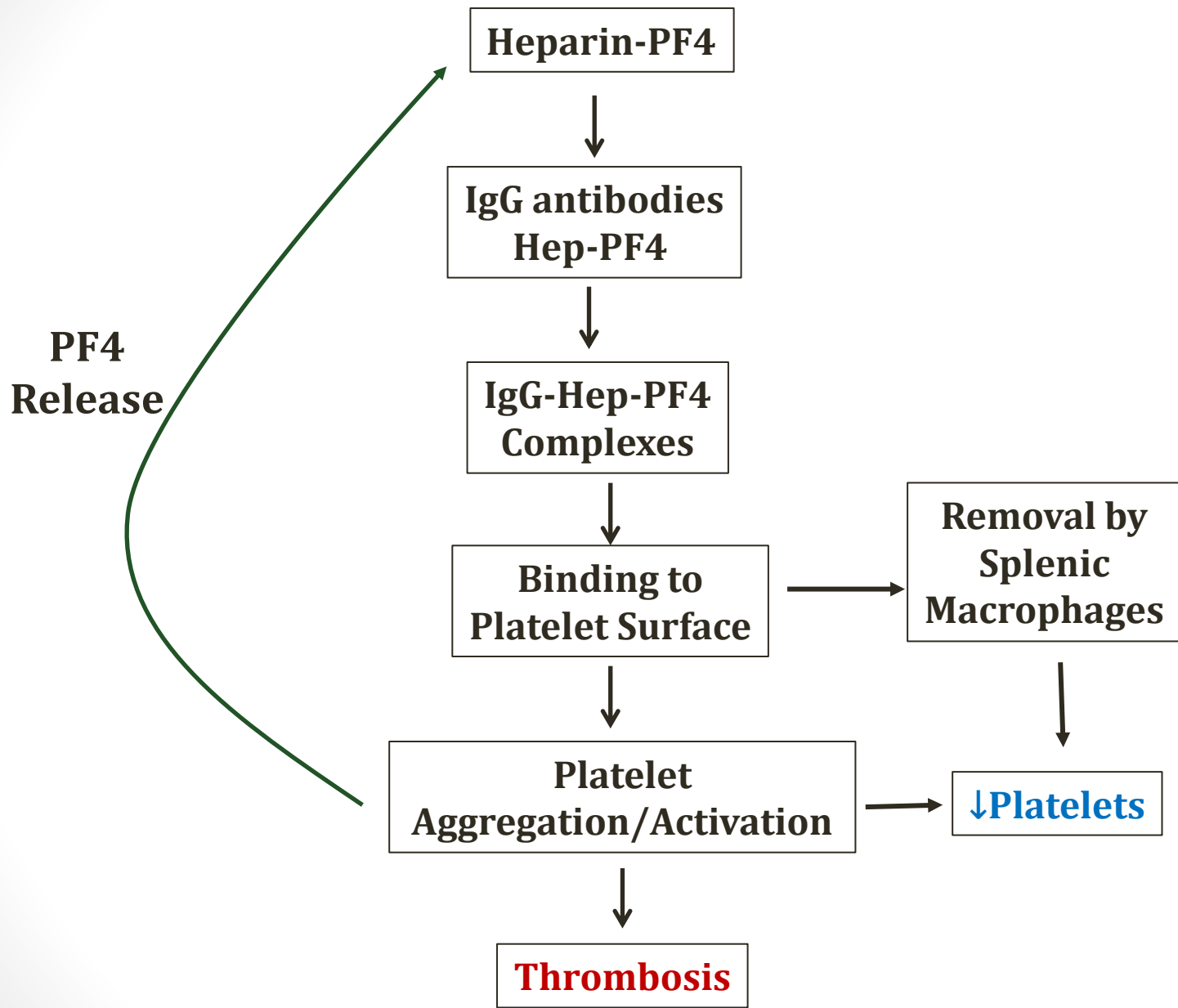
- Uses:
 - Acute management: DVT/PE, MI, Stroke
 - Prophylaxis for DVT in hospitalized patients (SQ)

Unfractionated Heparin (UFH)

- Side Effects
 - Mainly bleeding and thrombocytopenia
 - Osteoporosis (long term use)
 - Elevated AST/ALT (mild)

Heparin and Thrombocytopenia

- Many patients → mild (10-20%) ↓ platelets
 - “Non-immune” thrombocytopenia
 - Direct suppressive effect platelet production
- Heparin-induced thrombocytopenia (HIT)
 - Immune-mediated reaction
 - Immune complexes bind **platelet factor 4-heparin**
 - **Type II hypersensitivity reaction**



HIT

Heparin-induced thrombocytopenia

- 5-10 days after exposure to heparin
- Abrupt fall in platelets (>50%)
- **Arterial/vein thrombosis**
- Rare: 0.2 – 5% Heparin patients
- Patients with HIT must use alternative drugs
 - Lepirudin, Bivalirudin (direct thrombin inhibitors)

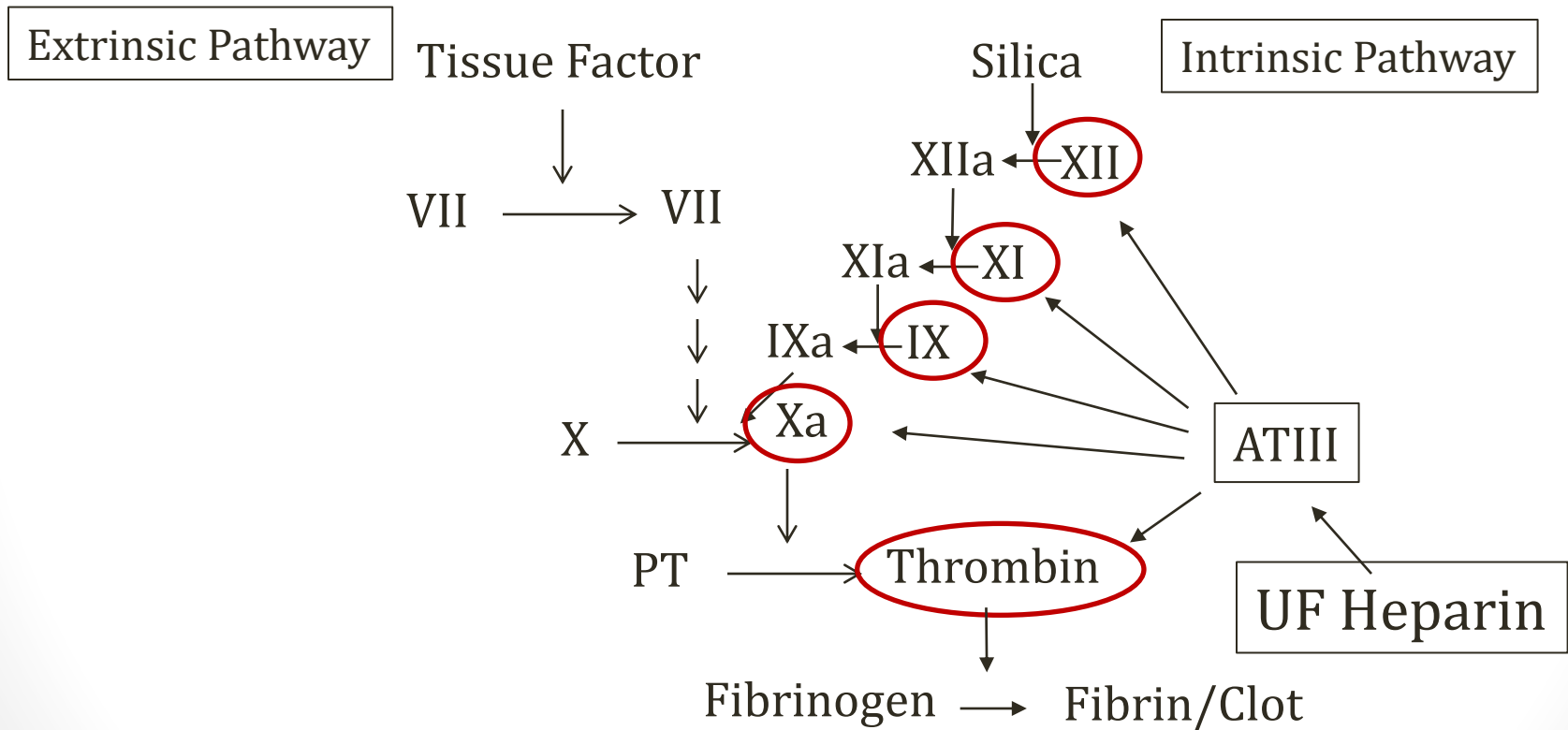
HIT

Heparin-induced thrombocytopenia

- Presumptive diagnosis:
 - Significant drop in platelet count
 - Thrombosis formation
- Definitive diagnosis: HIT antibody testing
 - Autoantibodies to platelet factor 4 complexed with heparin

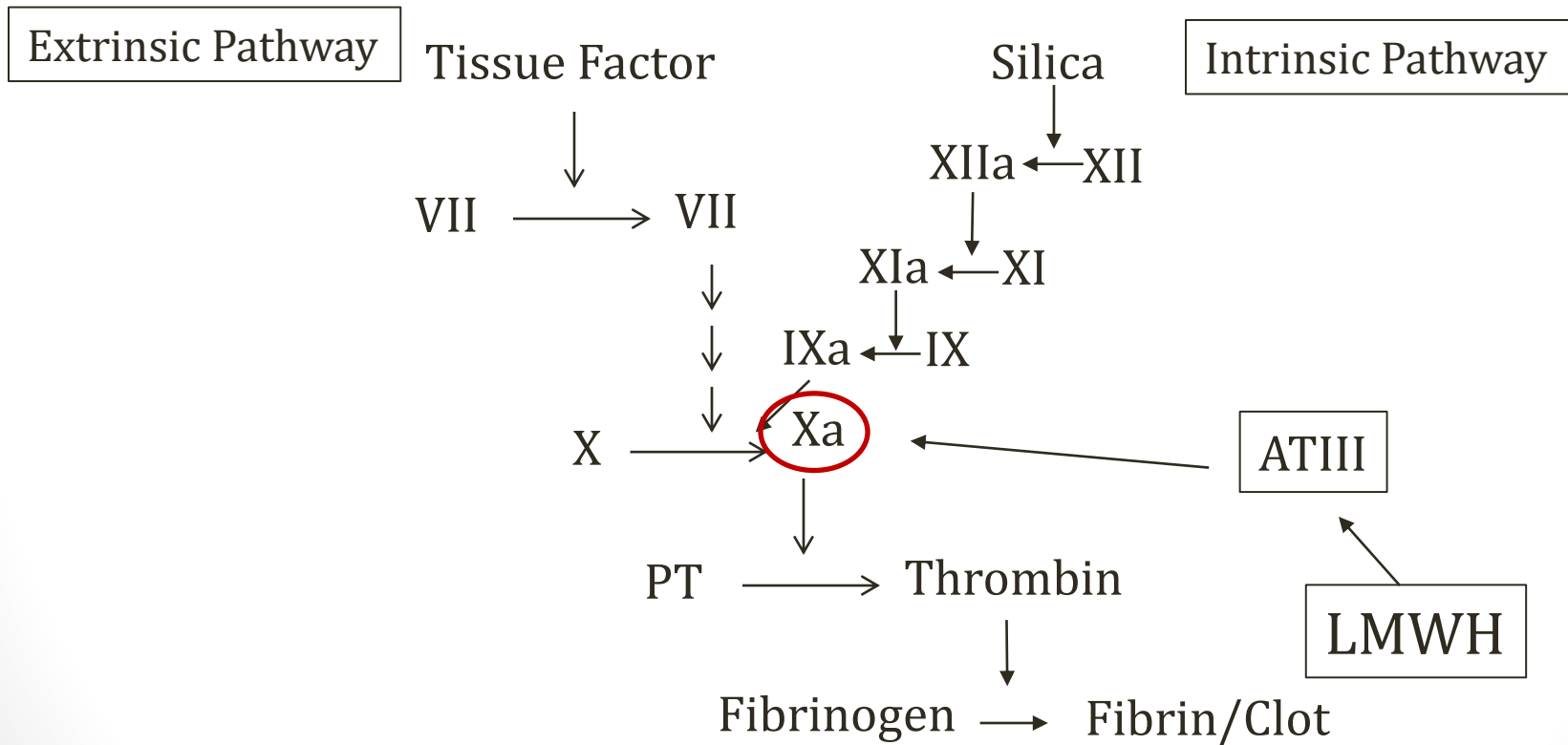
Low Molecular Weight Heparin

Enoxaparin



Low Molecular Weight Heparin

Enoxaparin



Low Molecular Weight Heparin

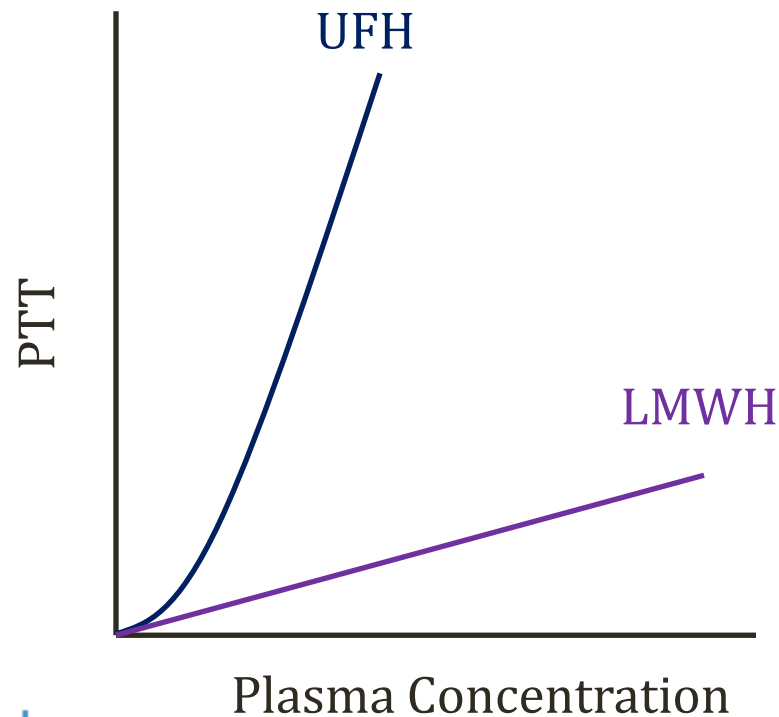
Enoxaparin

- Dose based on weight – no titrating
 - Reduced binding to plasma proteins and cells
- Given SQ
- Lower incidence of HIT (but may still cause!)

Low Molecular Weight Heparin

Enoxaparin

- Will not affect thrombin time (like UF heparin)
- PTT not sensitive to LMWH-induced changes
 - Unlike UF heparin, only factor X effected

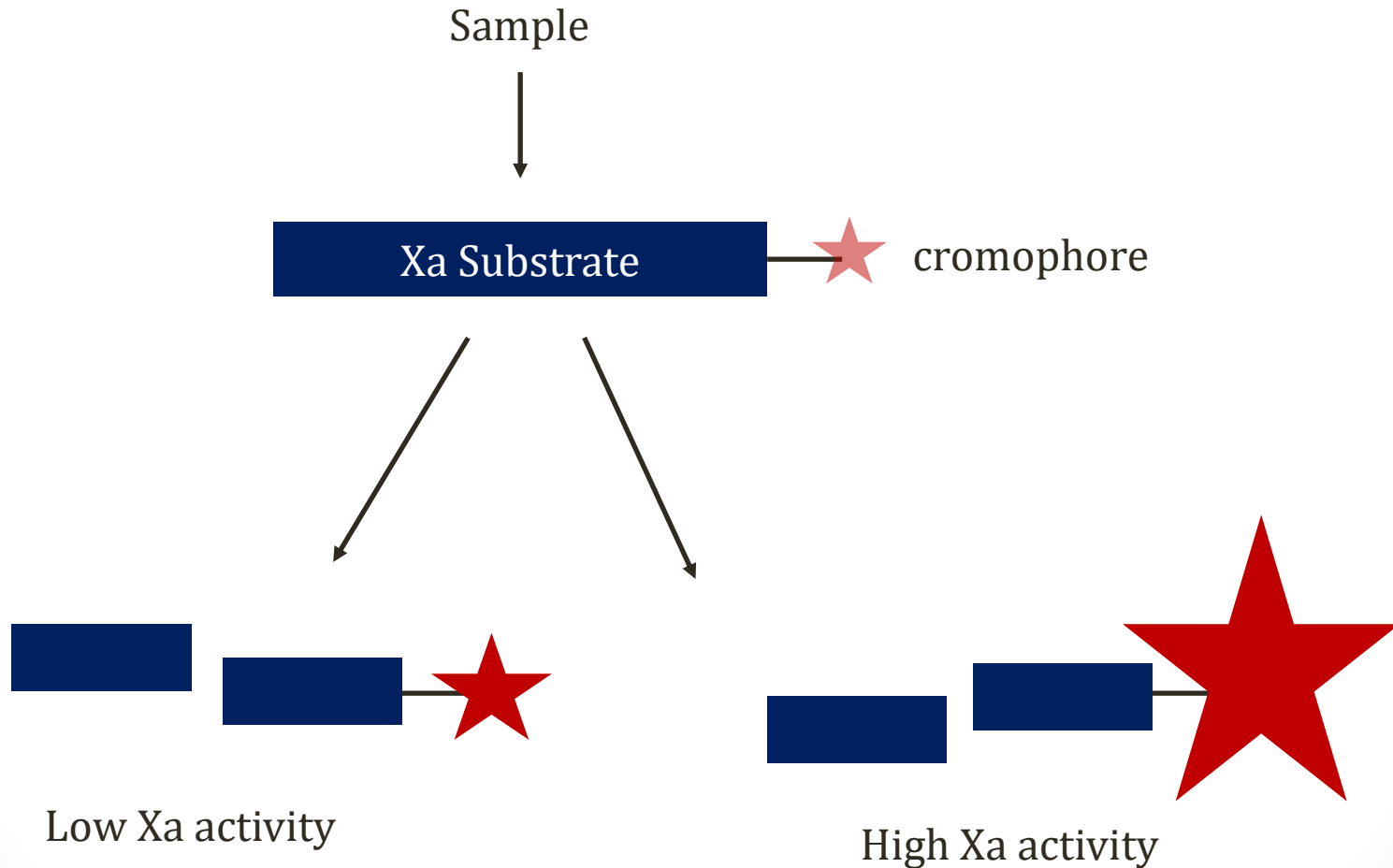


Low Molecular Weight Heparin

Enoxaparin

- If monitoring required, must check **anti Xa levels**
 - Limited/insensitive affect on PTT
 - Standard dose based on weight
 - Usually no monitoring used
 - Exceptions: Obesity and renal failure

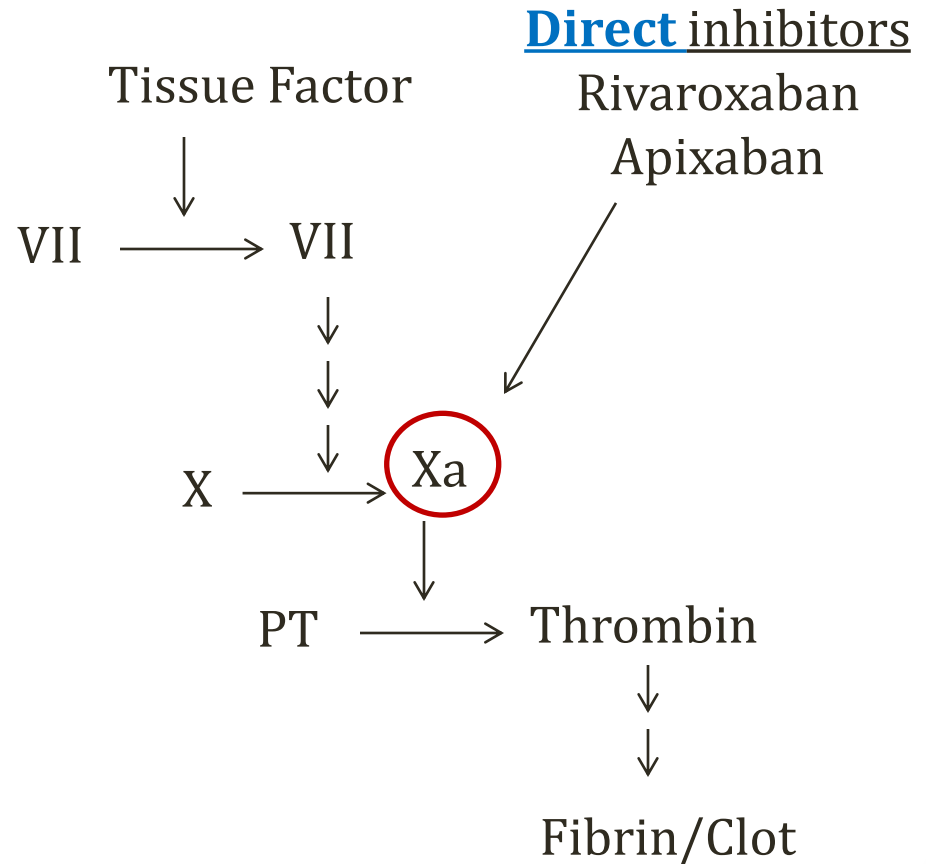
Anti-Xa Level



Factor Xa Inhibitors

Indirect inhibitors (ATIII)

Unfractionated Heparin
LMWH

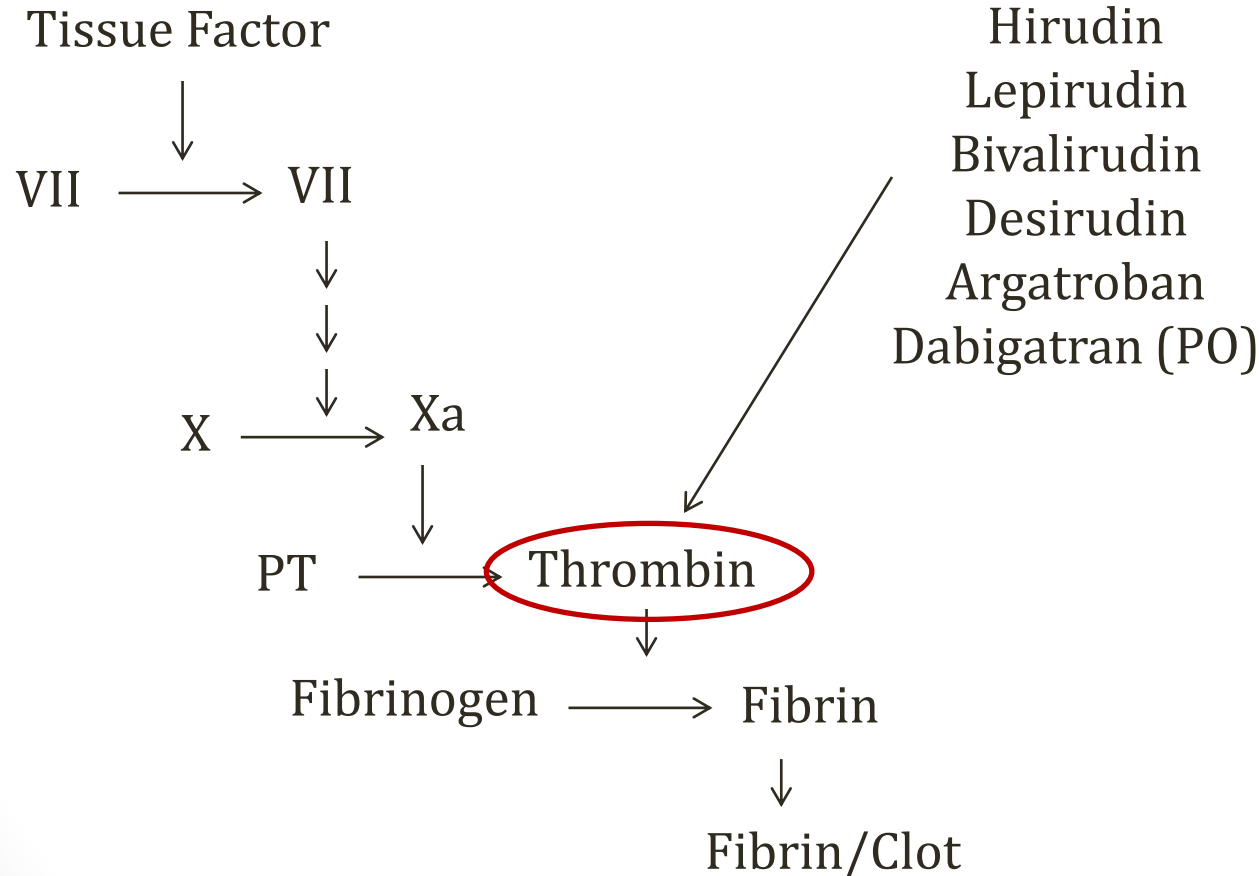


Factor Xa Inhibitors

- Rivaroxaban, Apixaban
- Used in atrial fibrillation as alternatives to warfarin
 - Do not require monitoring of PT/INR
 - Standard dosing
- Can increase PT and PTT (Xa in both pathways)
- Will not affect thrombin time

Direct Thrombin Inhibitors

Direct Thrombin Inhibitors (DTIs)



Direct Thrombin Inhibitors

Uses

- Can prolong **PT, PTT, and thrombin time**
- Thrombin activity common to all tests
- Only **UF heparin and DTIs** prolong thrombin time
 - Requires an inhibitor of thrombin function
 - UF Heparin: ATIII
 - DTIs: Direct drug effect

Direct Thrombin Inhibitors

Uses

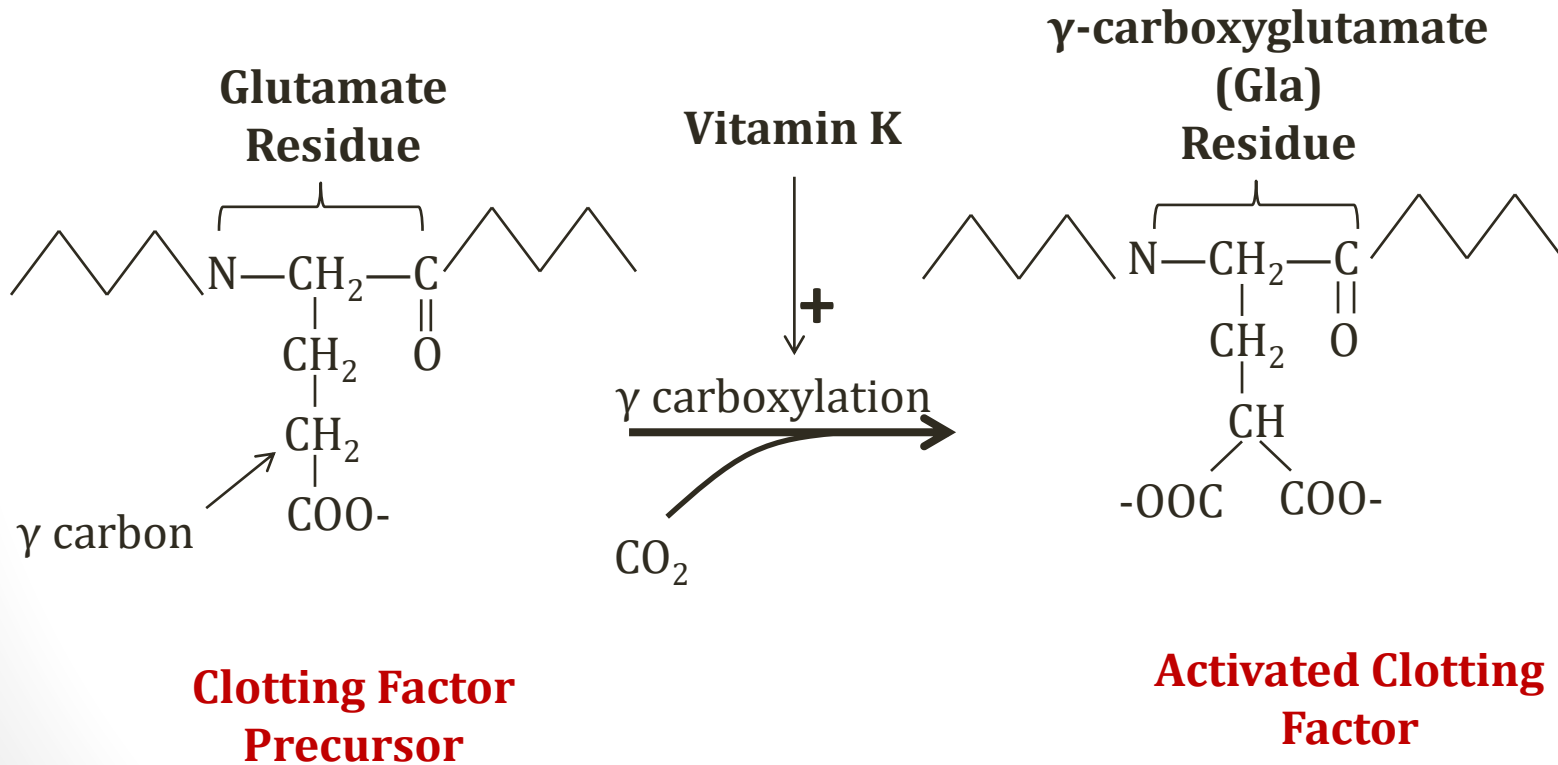
- Patients with HIT
 - Hirudin, lepirudin, bivalirudin, desirudin, argatroban
 - Stop heparin, start DTI
 - PTT often monitored
- Acute coronary syndromes, coronary interventions
 - Bivalirudin
- Atrial fibrillation
 - Dabigatran (oral)
 - Standard dosing: does not require PT/INR monitoring

Warfarin

- Vitamin K Factors: II, VII, IX, and X
- Warfarin: **Antagonist to vitamin K**
- ↓ levels of all vitamin K dependent factors

Vitamin K

- Forms **γ -carboxyglutamate (Gla) residues**

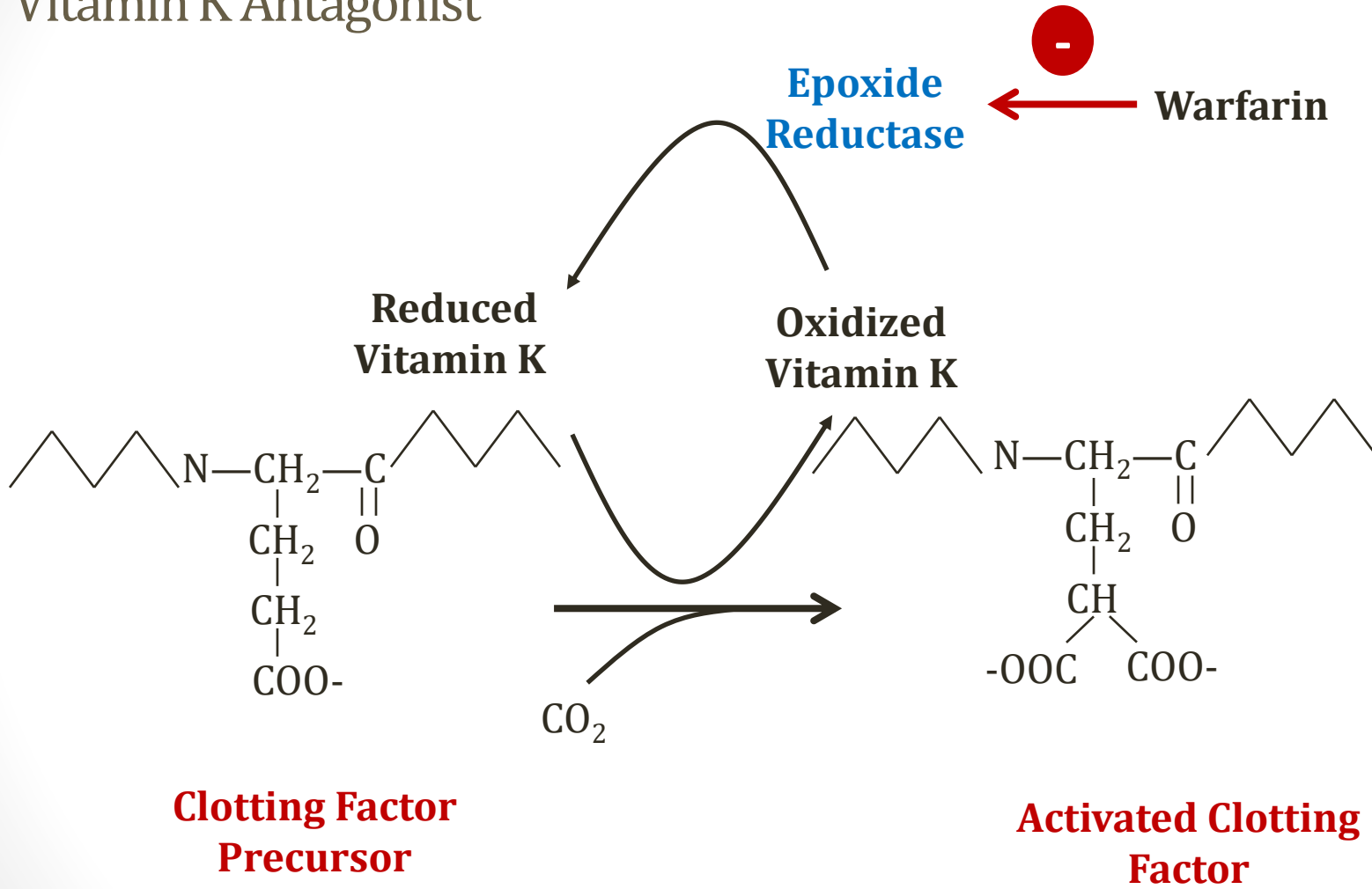


Vitamin K

- Found in **green, leafy vegetables** (K1 form)
 - Cabbage, kale, spinach
 - Also egg yolk, liver
- Also synthesized by **GI bacteria** (K2 form)

Warfarin

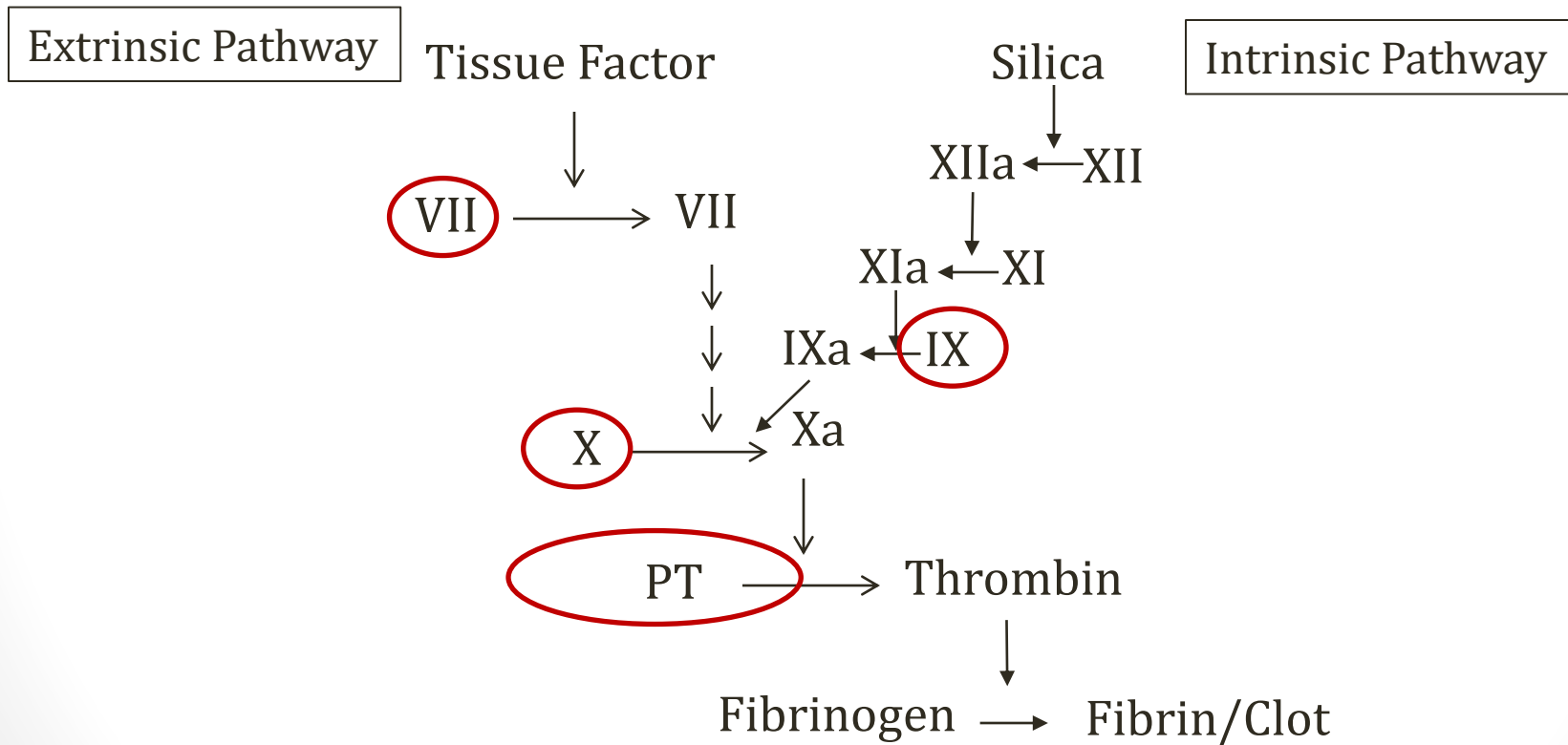
Vitamin K Antagonist



Warfarin

- Takes days to achieve its effects
 - Time required for clotting factor levels to fall
- Dose adjusted to reach target **PT/INR**
 - Drug's effect varies with diet (vitamin K)
 - Antibiotics may ↓ GI bacteria → ↓ vitamin K → ↑ INR
 - Some drugs interfere with metabolism

Vitamin K Dependent Factors



Vitamin K Dependent Factors

- **Factor VII has shortest half life**
 - First level to fall after Warfarin administration
- **Only PT captures factor VII activity**
- PTT less sensitive to Warfarin
- Thrombin time normal

Protein	Half-life (hours)
Factor VII	4-6
Protein C	8-10
Factor X	24-40
Protein S	40-60
Prothrombin (II)	60 - 72

Warfarin

Prothrombotic Effects

- Protein C: anti-clotting factor with **short half-life**
- Also **vitamin K dependent**
- Initial warfarin Rx → protein C deficient
 - This is pro-thrombotic
 - Brief...eventually other factors fall → antithrombotic

Protein	Half-life (hours)
Factor VII	4 - 6
Protein C	8 - 10
Factor X	24 - 40
Protein S	40 - 60
Prothrombin (II)	60 - 72

Warfarin

Prothrombotic Effects

- Should you start another drug (heparin) anytime you start warfarin?
 - Yes, but this is usually not an issue
 - For clot disorders (DVT/PE) heparin used for acute onset
 - Heparin → anticoagulation during initial warfarin therapy
- One exception: Atrial fibrillation
 - No active clot; just risk of clot
 - Often start warfarin without heparin
 - Brief increase in risk of clot is very low

Warfarin

Adverse Effects

- **Crosses placenta**
 - Avoided in pregnancy
 - Fetal warfarin syndrome: abnormal fetal development
 - Unfractionated heparin often used (does not cross)
- **Side Effects:**
 - Mainly bleeding
 - Skin necrosis

Warfarin Skin Necrosis

- Rare complication of therapy
- Occurs in patients with **protein C deficiency**
- Can also occur with very high dosages
- Initial exposure to warfarin → ↓ protein C
- Result: **thrombosis of skin tissue**
- Large dark, purple skin lesions

Warfarin

Uses

- Stroke prevention atrial fibrillation
- Mechanical heart valves
- DVT/PE

Chronic Oral Anticoagulation

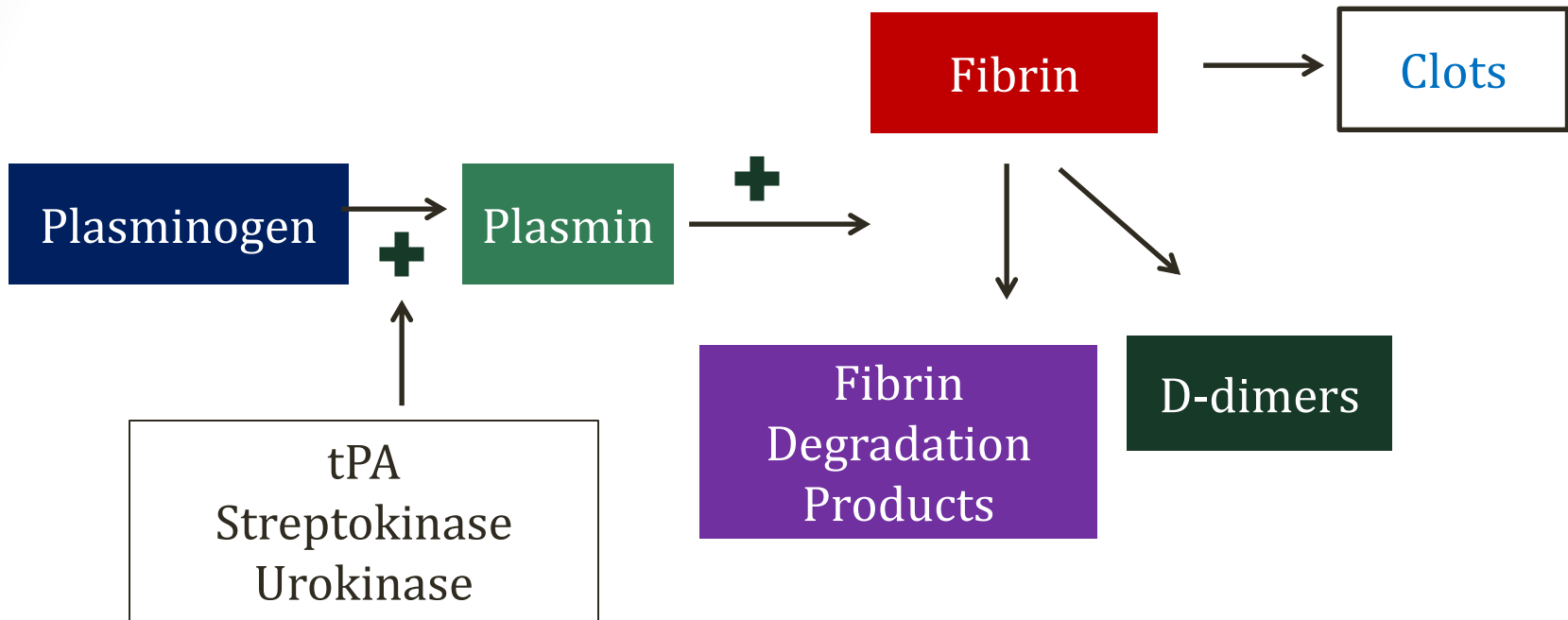
- Several Indications
 - Atrial Fibrillation
 - Mechanical heart valve
 - Prior DVT or PE
- Prior Standard: Warfarin
 - Oral drug, Low Cost
 - Downside: Requires INR checks (monthly blood draw)

Novel Oral Anticoagulants (NOACs)

Alternatives to Warfarin

- Factor Xa inhibitors
 - #1: Rivaroxaban
 - #2: Apixaban
- Direct Thrombin inhibitors
 - #3: Dabigatran
- Upside: No INR checks...consistent dose
- Downsides
 - Cost \$\$
 - Reversal agents - Idarucizumab

Thrombolysis



Powerful, “clot busters”
Used in acute MI, stroke
MAJOR bleeding risk

Reversal of drugs

- **Fresh Frozen Plasma (FFP)**
 - Plasma after removal of RBC, WBC, and Plt
 - Frozen for storage
 - Once thawed, must be used within 24hrs
 - Clotting factors degrade
 - Corrects deficiencies of any clotting factor
 - PT/PTT will normalize after infusion

Reversal of drugs

- Vitamin K
 - Reverses warfarin
 - Used with \uparrow INR in absence of serious bleeding
 - Given PO or IV
 - IV can cause anaphylaxis
- INR 3-5: Hold warfarin
- INR 5-9: Hold warfarin, Oral vitamin K
- INR >9 : Consider IV vitamin K, FFP

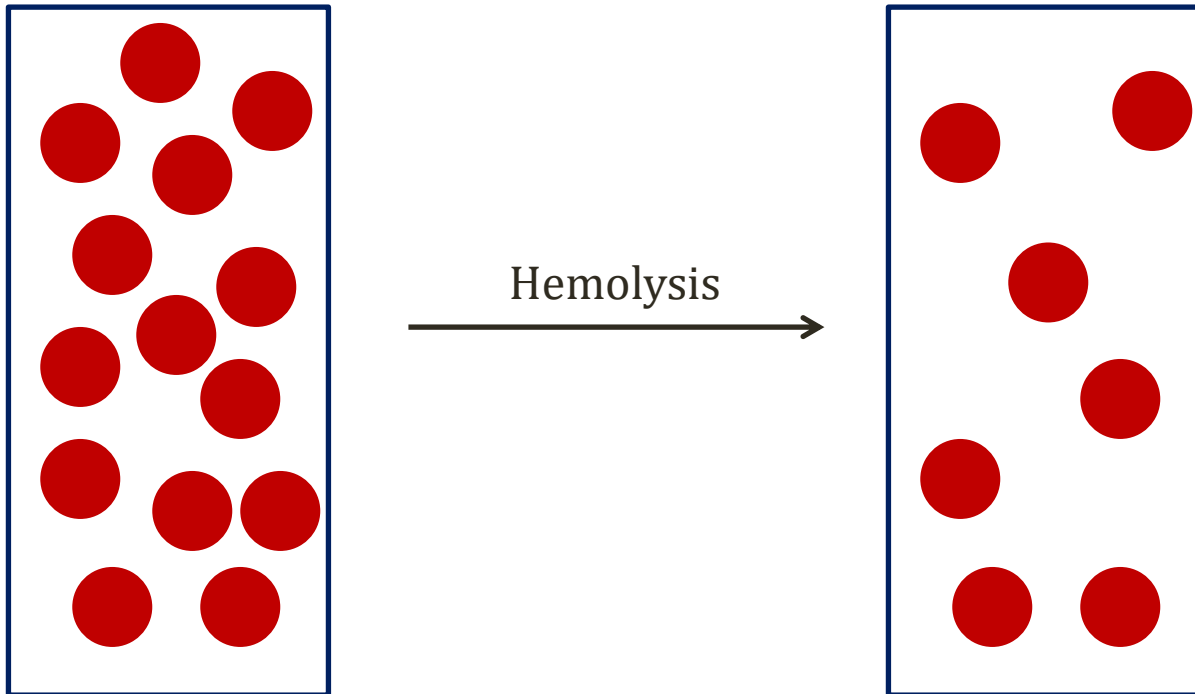
Severe bleeding + \uparrow INR = administer FFP

Hemolysis Basics

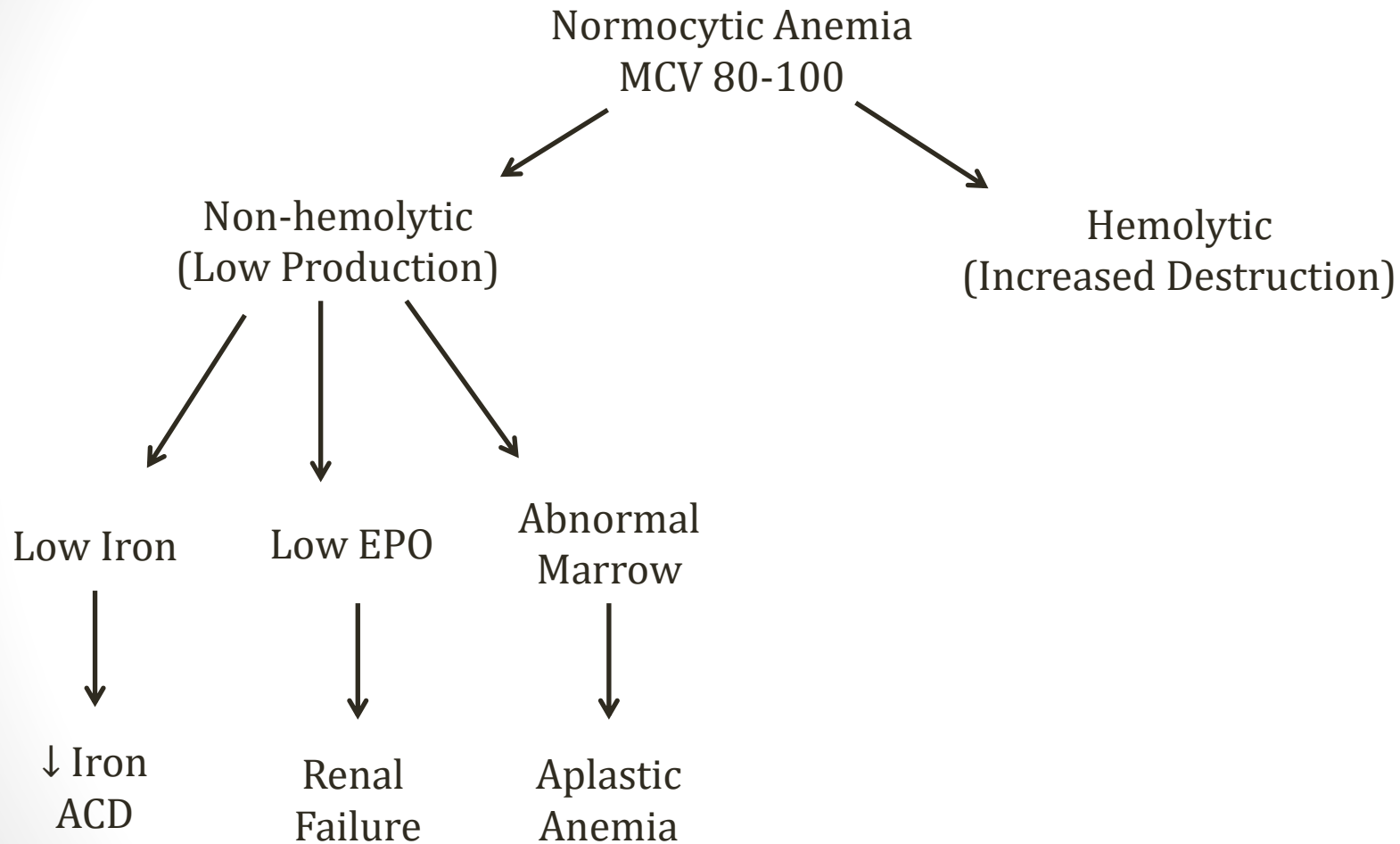
Jason Ryan, MD, MPH

Hemolysis

- Destruction of red blood cells
- Causes a **normocytic anemia**



Normocytic Anemias

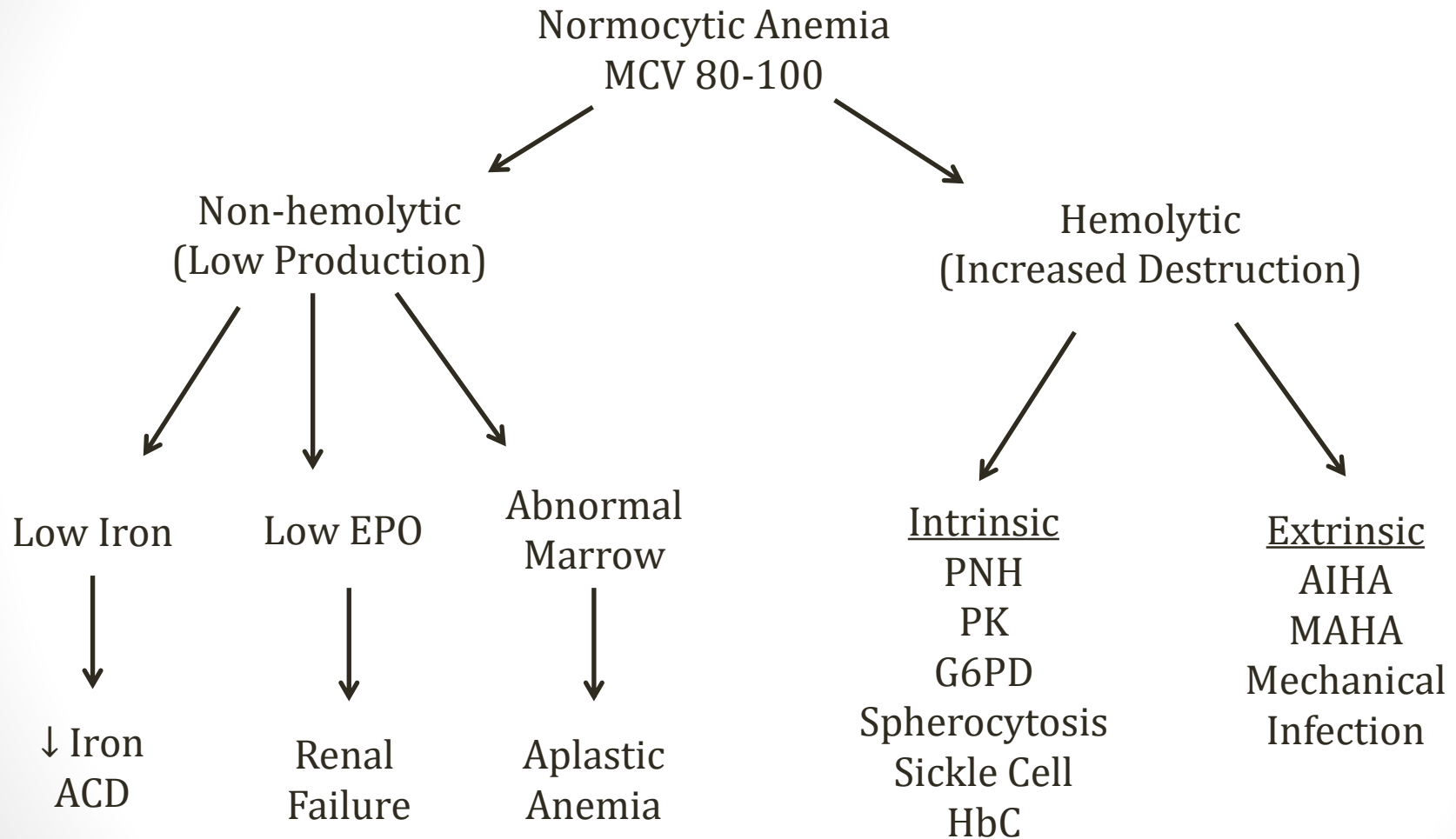


Hemolysis

Extrinsic versus Intrinsic

- Extrinsic cause
 - Cause is extrinsic to the red cell
 - **Antibodies**
 - Mechanical trauma (narrow vessels)
 - RBC infection
- Intrinsic cause
 - Cause is intrinsic to red blood cells
 - Failure of membrane, hemoglobin, or enzymes
 - Membrane: Hereditary spherocytosis
 - Enzyme: G6PD deficiency
 - Hemoglobin: Sickle cell anemia (Abnormal Hgb)

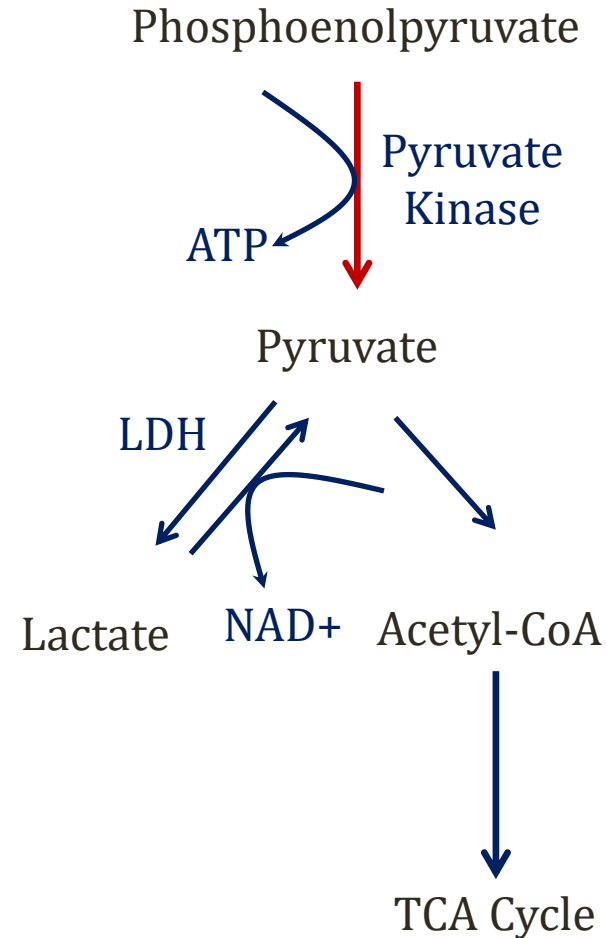
Normocytic Anemias



Hemolysis

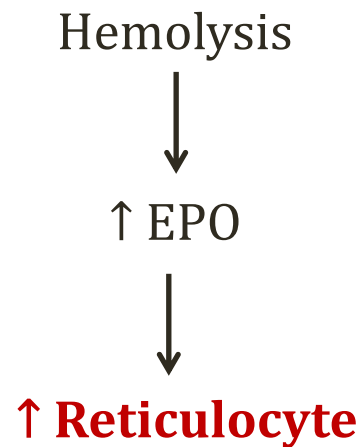
Consequences

- Normocytic anemia
- **Elevated plasma LDH**
 - Lactate dehydrogenase
 - Glycolysis enzyme
 - Converts pyruvate → lactate
 - Spills out of RBCs



Reticulocytes

- Immature red blood cells
- Usually about 1-2% of RBCs in peripheral blood
- Increased reticulocyte count: Hallmark of hemolysis



Reticulocyte Count

- ↑ reticulocytes: normal marrow response to anemia
- Key blood test in **normocytic anemias**
- Normocytic anemia: ↓ production or ↑ destruction
- Reticulocyte count **differentiates between causes**
 - Low retic count: Underproduction
 - High retic count: Increased destruction (hemolysis)

Reticulocyte Count

- Normal: 1 to 2 %
- Anemia: 4-5%
- Must be **corrected** for degree of anemia
- If <2% → **inadequate bone marrow response**

Hct 45 (normal) Hct 11
Retic 1% (normal) Retic 8%

—————→

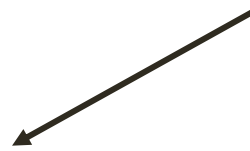
$$\text{Corrected RC} = 8\% * (11/45) = 2\%$$

Reticulocyte Production Index

- Normal reticulocytes circulate ~1day
- In anemia → premature release of reticulocytes
- **Can live longer** → circulate longer
- RPI corrects for longer life of reticulocytes in anemia
- RPI < 2% seen with bone marrow failure

$$\text{RPI} = \frac{\text{Corrected Retic \%}}{\text{Maturation Time}}$$

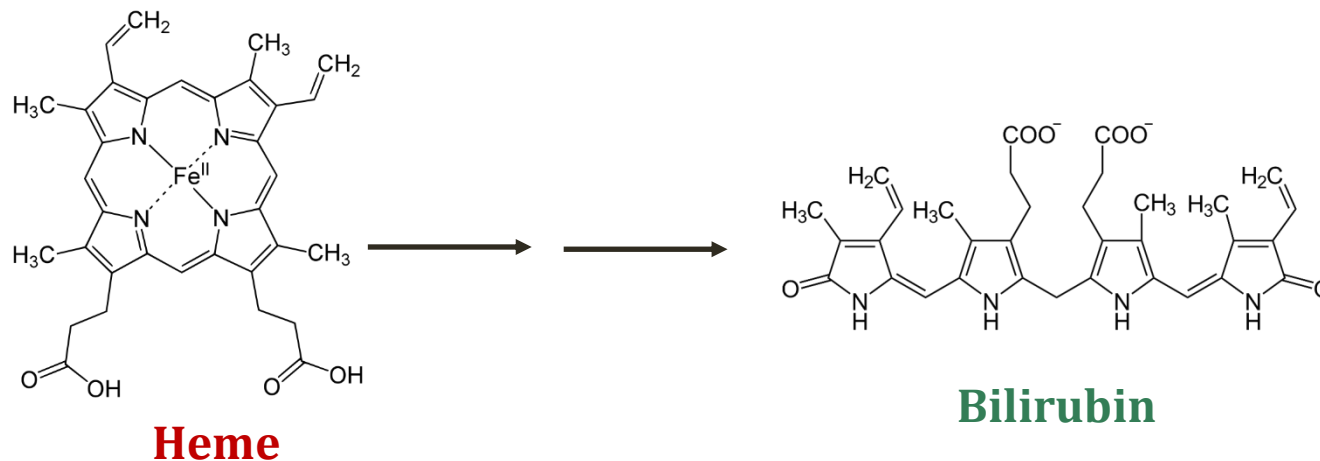
Hgb	MT
15	1
12	1.5
8	2
5	2.5



Hemolysis

Consequences

- **Elevated unconjugated (indirect) bilirubin**
- Not water soluble
- Bound to albumin in plasma



Jaundice



Gallstones

- ↑ risk in hemolysis
- Pigment stones
 - Contain bilirubin
 - Less common type of gallstone (more common: cholesterol)



Emmanuelm/Wikipedia

Hemolysis

Intravascular versus Extravascular

- **Intravascular** hemolysis
 - Occurs inside blood vessels
- **Extravascular** hemolysis
 - Occurs in liver and spleen
- Both cause normocytic anemia and \uparrow retic count

Extravascular Hemolysis

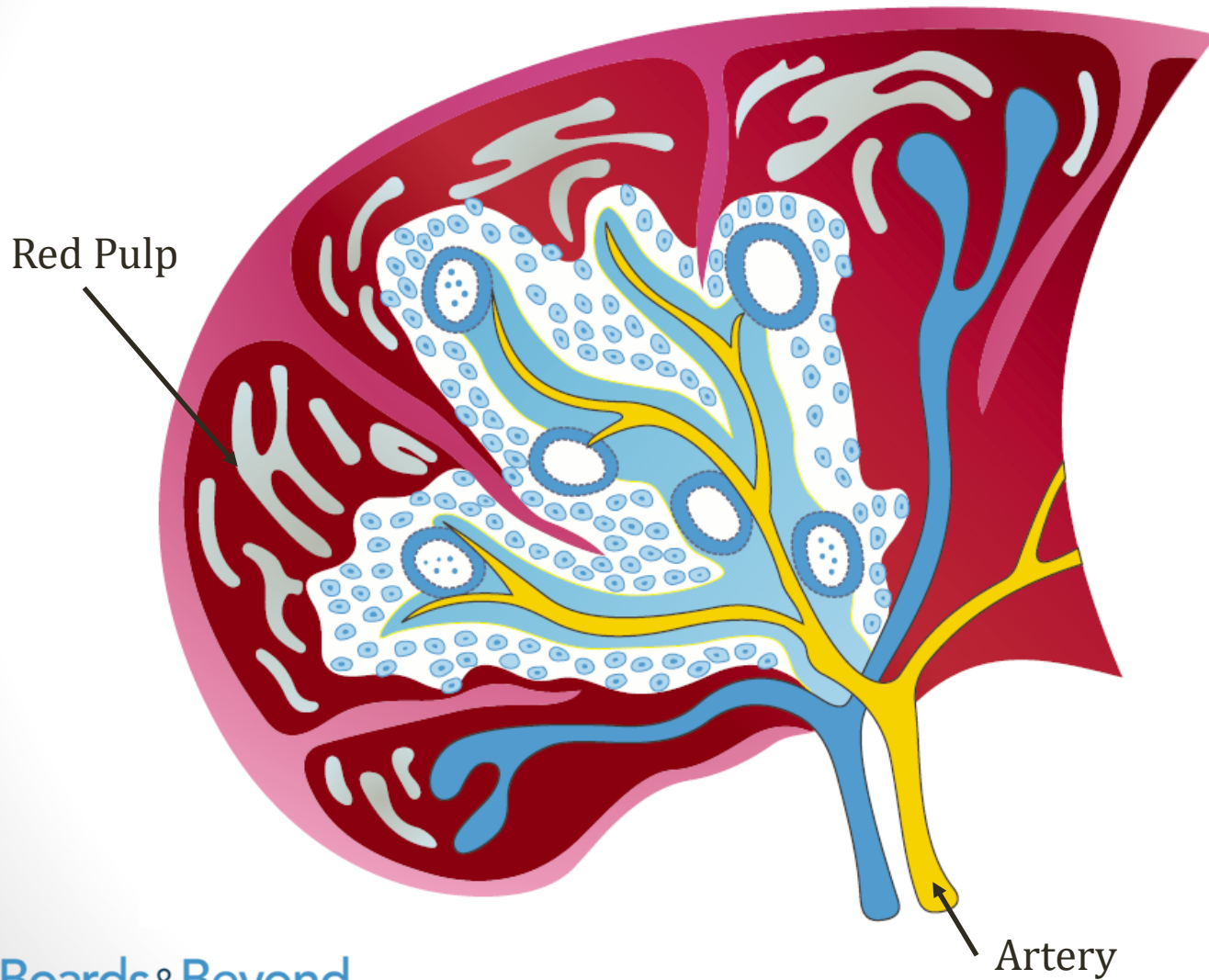
- **Liver**

- Receives large portion cardiac output
- Can remove severely damaged RBCs

- **Spleen**

- Destroys poorly deformable RBCs
- **Cords of Billroth**: Vascular channels that end blindly
- Found in **red pulp** of spleen
- RBCs must deform to pass through slits in walls of cords
- Old (“senescent”) or damaged RBCs remain in the cords
- Phagocytosed by the macrophages
- Hemolysis disorders → ↑ splenic removal of RBCs

Spleen



Intravascular Hemolysis

- Destruction of RBCs inside blood vessels
 - Outside of spleen
- Mechanical trauma
 - Narrowed vessels
 - Small vessels: thrombus (“microangiopathic”)
 - Large vessels: mechanical heart valves

Haptoglobin

- Plasma protein
- **Binds free hemoglobin**
- Haptoglobin-hemoglobin complex removed by **liver**
- **↓ serum haptoglobin with hemolysis**

Haptoglobin

- Intravascular: Hgb released directly into plasma
 - Haptoglobin **very low or undetectable**
- Extravascular: Some Hgb released from spleen
 - Haptoglobin can be **low or normal**
- Classically taught as low in intravascular only
- Studies show can be low in both types

Kormoczi G. **Influence of clinical factors on the haemolysis marker haptoglobin.**
Eur J Clin Invest 2006 Mar;36(3)

Haptoglobin

- Produced by the liver
- **Acute phase reactant**
- Increased levels with inflammation
- Decreased levels in cirrhosis

Hemolysis

Urine findings

- No bilirubin
 - Unconjugated bilirubin not water soluble
 - Cannot pass into urine

Hemolysis

Urine findings

- Intravascular hemolysis
 - Haptoglobin saturation → **free excess hemoglobin**
 - “Hemoglobinemia”
 - Filtered in kidneys → **hemoglobinuria**
 - Some reabsorbed in proximal tubules
 - Iron converted into ferritin → **hemosiderin** in tubular cells
 - Tubular cells slough into urine
 - Prussian blue stain on sediment shows hemosiderinuria

Hemolysis

Urine findings

- Hemoglobin part of urine dipstick
- Hgb may turn urine red/brown
 - Also occurs in rhabdomyolysis
 - Myoglobin from muscle damage
- **No red cells plus + Hgb**



James Heilman, MD -

Urine test strip

Leukocytes

Nitrite

Urobilinogen

Protein

pH

Haemoglobin

Specific gravity

Ketone

Bilirubin

Glucose



Hemolysis

Classic Findings

- Normocytic anemia
- ↑ LDH
- ↑ Indirect bilirubin
- ↑ Reticulocyte count
- ↓ Haptoglobin (lower in intravascular)
- Urine Hgb and hemosiderin (intravascular)

Parvovirus B19

- DNA virus
- Replicates in **RBC progenitor cells**
- ↓erythropoiesis

Parvovirus B19

- Healthy patients:
 - RBC production returns 10 to 14 days; mild/no anemia
- Hemolysis patients
 - Increased RBC turnover
 - Lack of erythropoiesis leads to severe anemia
 - Pallor, weakness, and lethargy

Parvovirus B19

- “Aplastic Crisis” in patients with chronic hemolysis
 - Sickle cell anemia
 - Hereditary spherocytosis
 - Beta thalassemia major
- Classic scenario:
 - Worsening anemia with **LOW reticulocyte count**

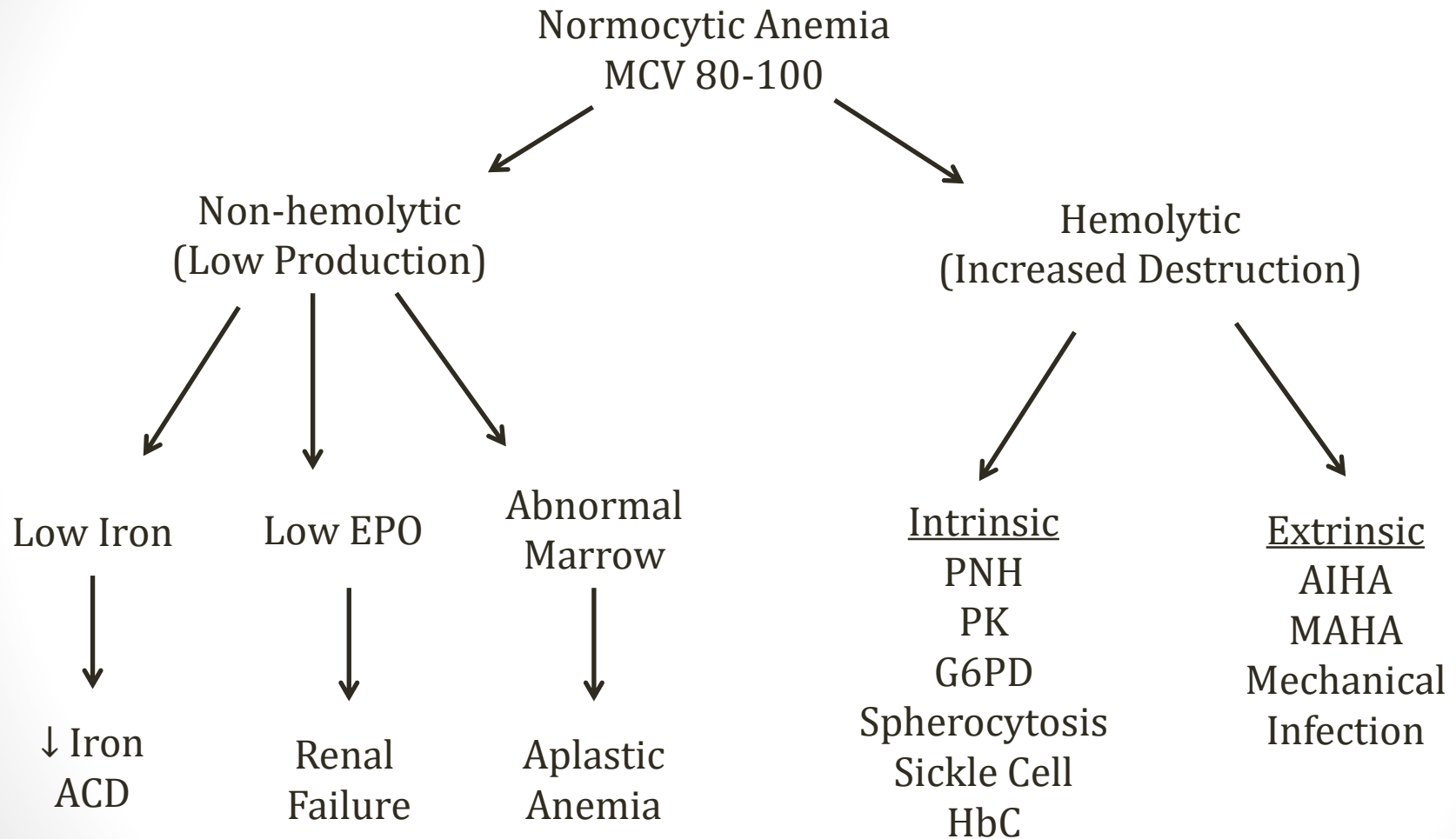
Back and Abdominal Pain

- Seen in some hemolytic syndromes
- Abdominal pain can be caused by splenomegaly
- May be due to smooth muscle spasm
- **Nitric oxide**: scavenged by free hemoglobin
- Common in some hemolytic disorders
 - Paroxysmal nocturnal hemoglobinuria
 - G6PD deficiency

Extrinsic Hemolysis

Jason Ryan, MD, MPH

Normocytic Anemias



Extrinsic Hemolysis

- Antibodies
- Trauma/shearing
- Red cell infections

AIHA

Autoimmune Hemolytic Anemia

- Red cell destruction from **autoantibodies**
- Results in extravascular hemolysis
- Red cell membrane removed in pieces by spleen
- Can be “warm” or “cold”

Warm AIHA

- Most common type of AIHA
- Antibodies bind at **body temp 37°C** (“warm”)
- **IgG antibodies** against RBC surface antigens

Warm AIHA

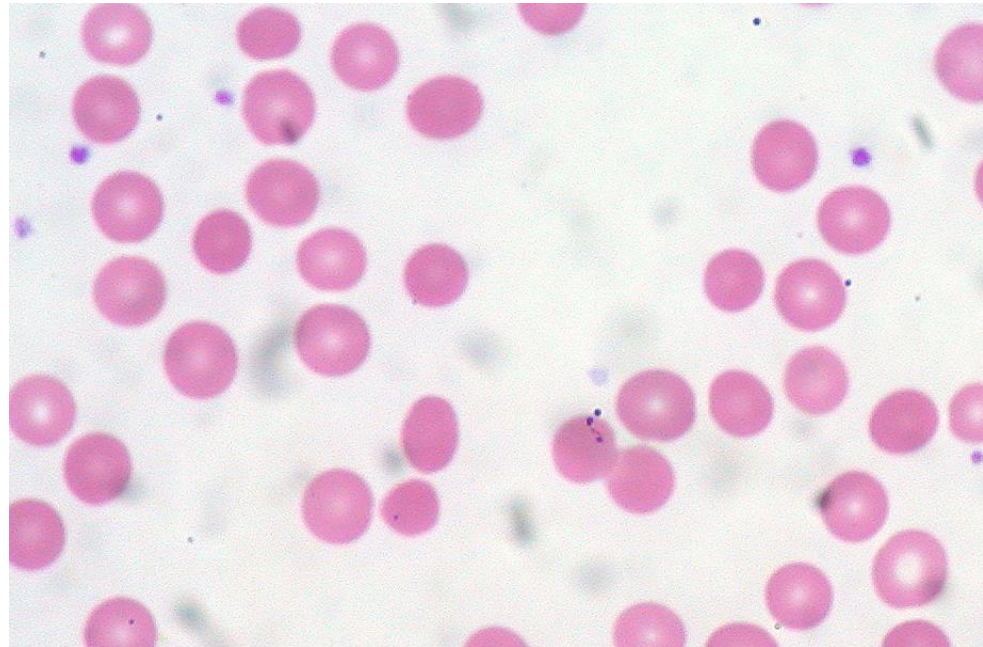
Signs and symptoms

- **Anemia**
 - Fatigue
 - Pallor (pale skin)
 - Dyspnea
 - Tachycardia
- **Extravascular hemolysis**
 - Jaundice
 - Splenomegaly

Warm AIHA

Diagnostic Findings

- **Spherocytes**
 - Smaller than normal RBCs
 - Spherical



Ed Uthman

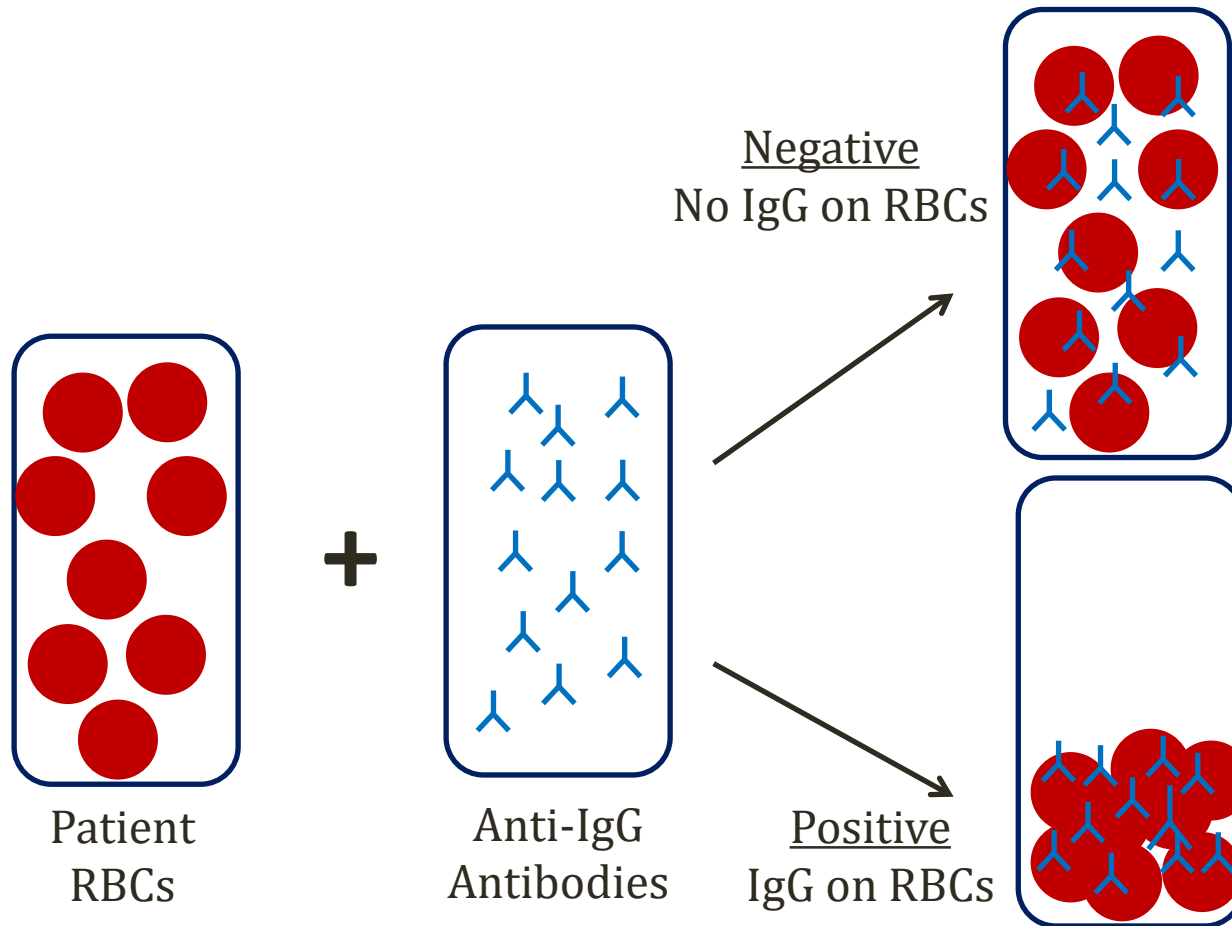
Direct Antiglobulin Test

DAT or Coombs Test

- Test for red blood cell antibodies
- Patient RBCs plus **anti IgG** antiserum
- Positive if agglutination occurs
- Indicates patient's **RBCs covered with IgG**

Direct Antiglobulin Test

DAT or Coombs Test



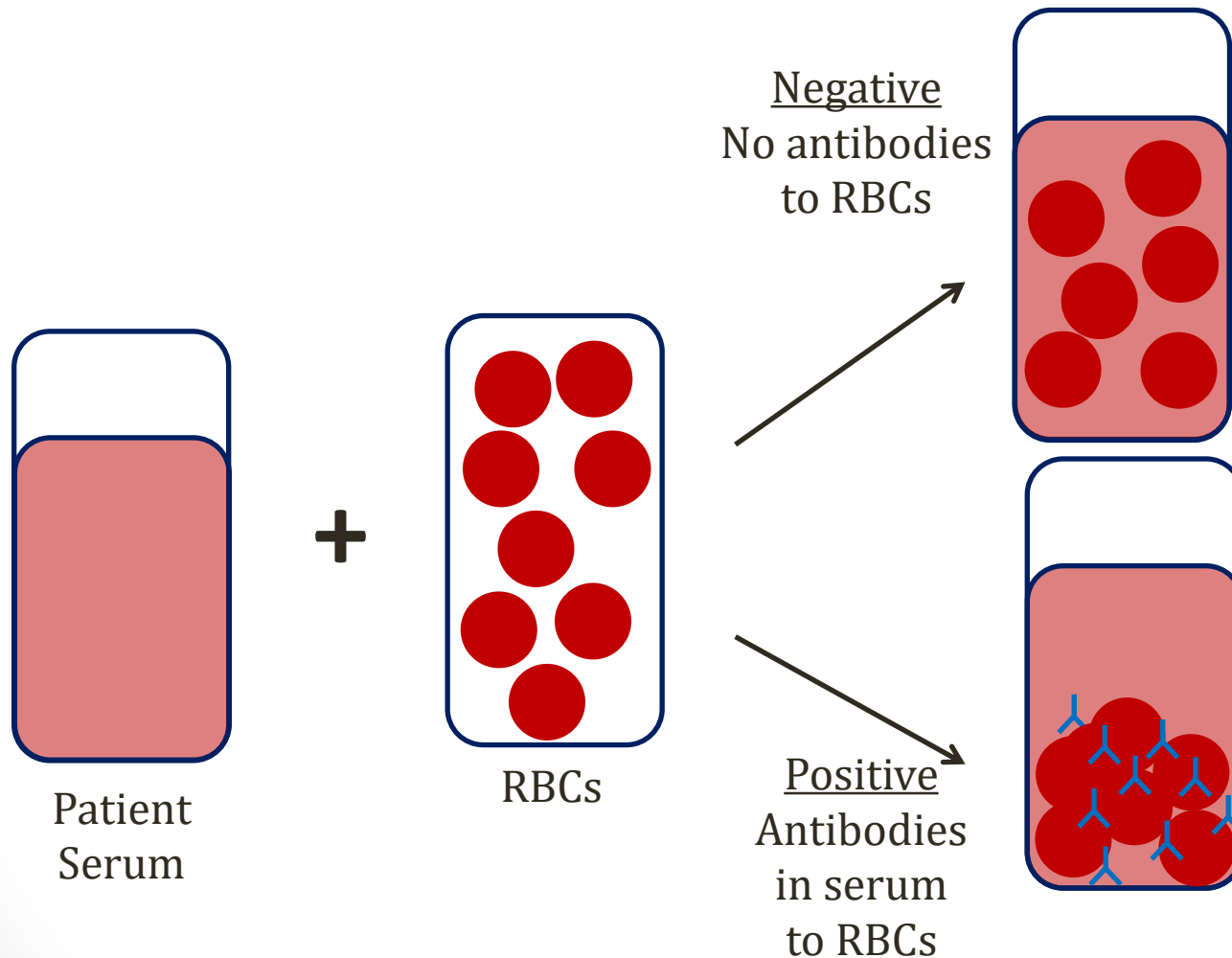
Indirect Antiglobulin Test

Indirect Coombs

- Also a test for red blood cell antibodies
- Not generally used in warm/cold AIHA
- Tests for **antibodies in the serum**
- Patient's serum (not RBCs) tested
- Added to RBCs
- Indicates **antibodies to RBC components**

Indirect Antiglobulin Test

Indirect Coombs



Antiglobulin Tests

- Direct antiglobulin test
 - Test for **antibodies bound to RBCs**
 - Commonly used in **hemolytic anemias**
- Indirect antiglobulin test
 - Test for **antibodies in serum**
 - Will serum react with RBCs?

Warm AIHA

Associated Conditions

- Most cases idiopathic
- Associated with:
 - Lupus
 - Non-Hodgkin lymphoma
 - Chronic lymphocytic leukemia (CLL)

Methyldopa

α methyldopa

- **Antihypertensive drug of choice in pregnancy**
 - Agonists to CNS α_2 receptors
 - Synapses believe too much sympathetic outflow
 - Decrease sympathetic tone in body
- **Associated with warm AIHA**



Øyvind Holmstad/Wikipedia

Methyldopa

α methyldopa

- Triggers production of RBC antibodies
 - Unclear mechanism
 - Drug may alter Rh antigens on red cells
 - **Red cells bind antibody in absence of drug**
- **Direct Coombs test: positive**

Penicillin

- High doses can lead to hemolytic anemia
- PCN binds to surface RBCs (“haptens”)
 - Elicits immune response *only when bound*
- **Antibodies against PCN bound to RBCs**
- **Direct Coombs test: positive**

Warm AIHA

Treatment

- **Glucocorticoids**
- Immunosuppressants
- **Splenectomy**

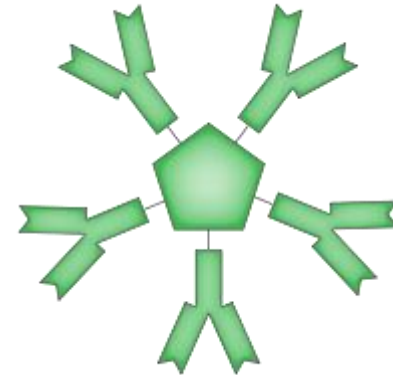
Cold AIHA

- Less common type of AIHA
- Antibodies bind at $<30^{\circ}\text{C}$ (“cold”)
 - Occurs in **limbs**
 - Also **fingertips, toes, nose, ears**
- May present with painful fingers/toes
 - **Purple discoloration**
- Symptoms associated with cold exposure

Cold AIHA

Cold Agglutinin Disease

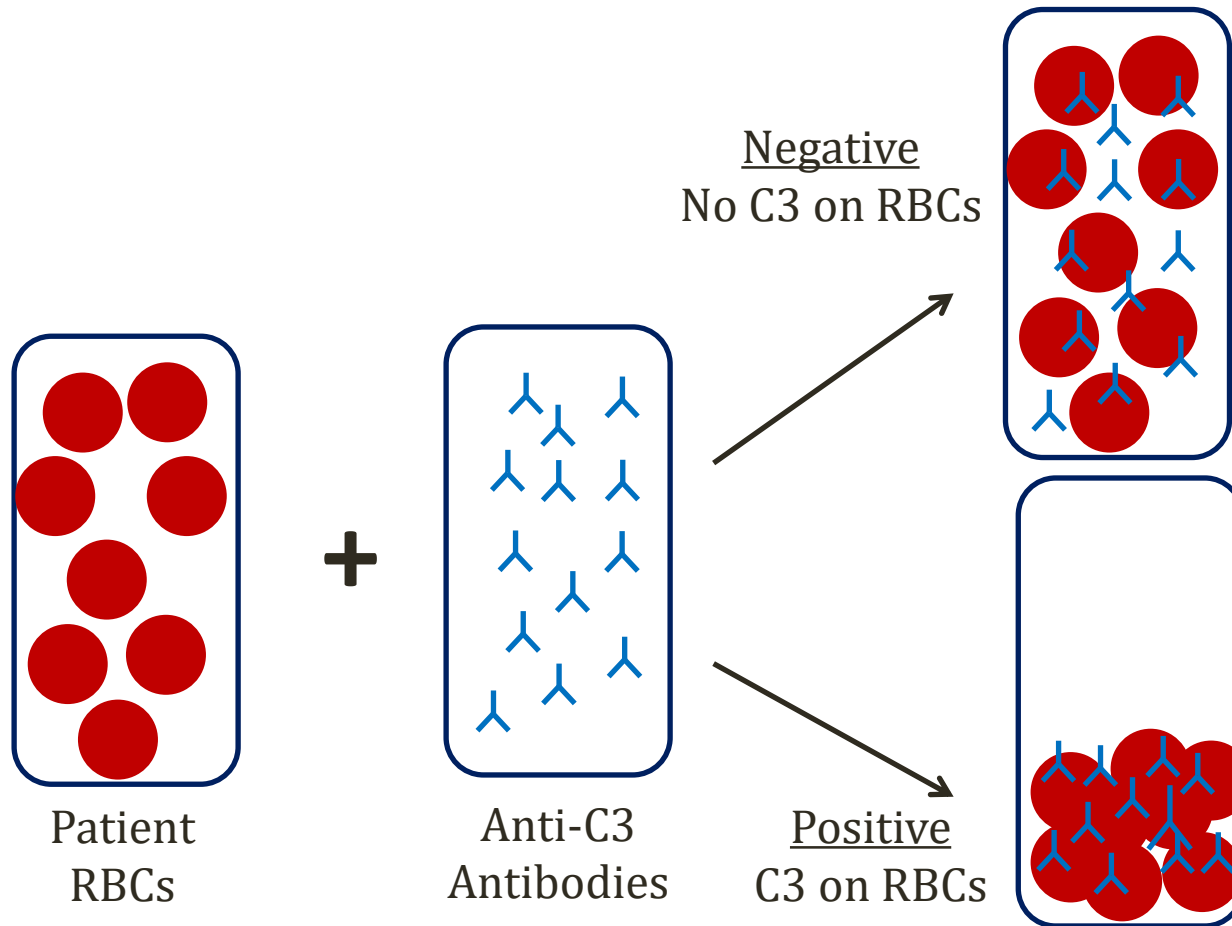
- Caused by IgM antibodies that agglutinate RBCs
- RBCs warmed in central organs → IgM lost
- Leaves bound C3 on RBCs
- **DAT positive only for C3**



Martin Brändli /Wikipedia

Direct Antiglobulin Test

DAT or Coombs Test



Cold AIHA

Cold Agglutinin Disease

- Usually causes **extravascular hemolysis**
 - C3 coated RBCs removed by spleen
 - Often engulfed whole
 - Spherocytosis less common than in warm AIHA
- Intravascular hemolysis rarely occurs
 - Complement usually does not activate
 - RBCs: complement inhibitory molecules (CD55/CD59)
 - Complement must be significantly activated to lyse cells

Cold AIHA

Associated conditions

- Can be seen in chronic lymphocytic leukemia (CLL)
- Often occurs secondary to infection
 - **Mycoplasma pneumonia**
 - Epstein–Barr virus (**Infectious mononucleosis**)

Cold AIHA

Treatment

- Avoid cold (stay warm!)
- Immunosuppressants

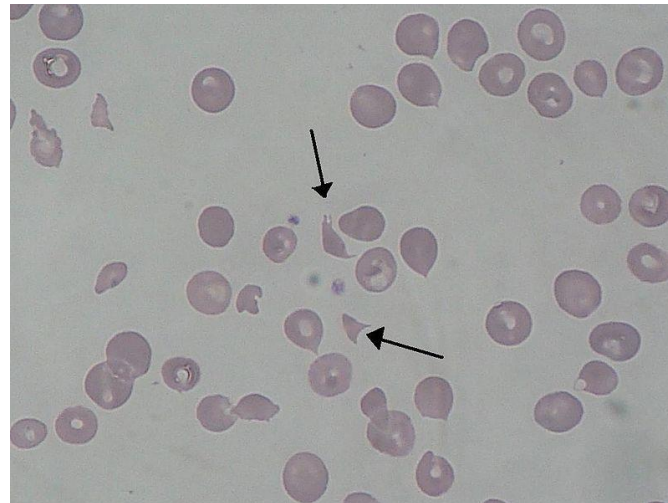


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MAHA

Microangiopathic hemolytic anemia

- **Shearing of RBCs in small blood vessels**
- Thrombi in microvasculature → narrowing
- Blood smear: schistocytes
- Seen in:
 - TTP
 - HUS
 - DIC



Paulo Henrique Orlandi Mourao

Malignant Hypertension

- Associated with MAHA
- Endothelial injury → thrombus formation
- Improved with BP control



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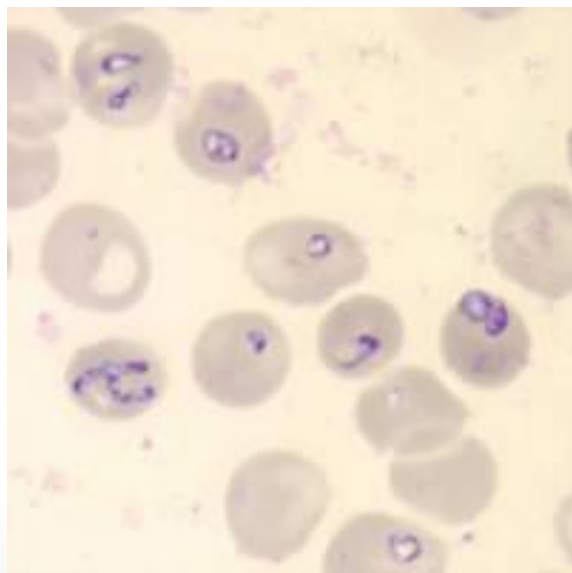
Mechanical Hemolysis

- **Shear forces** destroy RBCs in **large** blood vessels
- Seen in:
 - **Aortic stenosis**
 - **Mechanical heart valves**
 - Left ventricular assist devices
- Hemolytic anemia may occur
- Schistocytes can be seen on blood smear

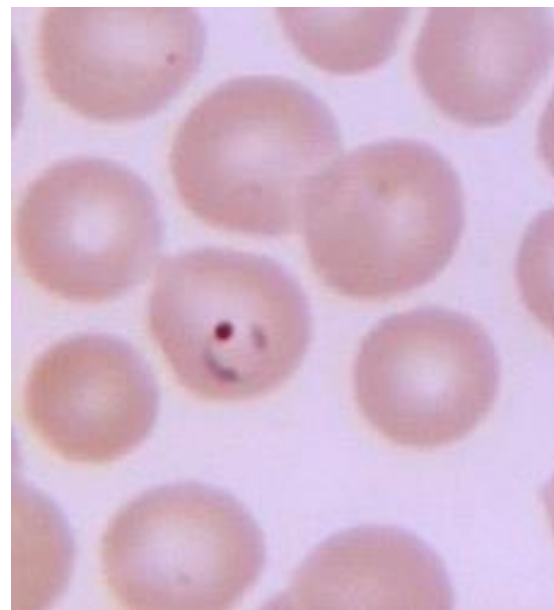
Red Blood Cell Infections

- May cause hemolytic anemia
- Classic infectious agents: **Malaria**, **Babesia**

Babesia
Ring Forms



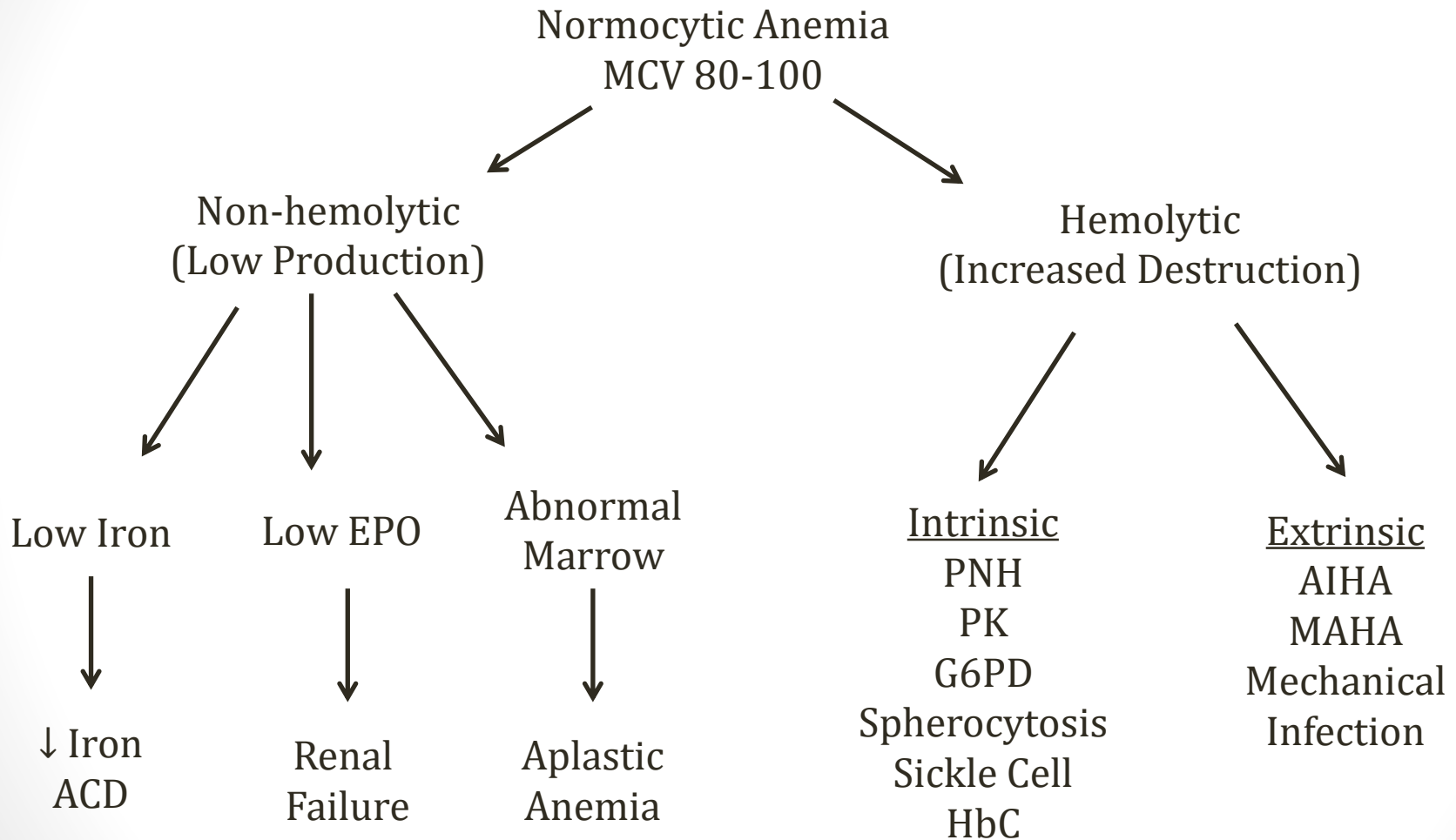
Malaria
Trophozoite Ring



Intrinsic Hemolysis

Jason Ryan, MD, MPH

Normocytic Anemias



PNH

Paroxysmal Nocturnal Hemoglobinuria

- RBC destruction via **complement system**
- Loss of protective proteins in RBC membrane
 - Decay Accelerating Factor (DAF/CD55)
 - MAC inhibitory protein (CD59)
- Predominantly **intravascular hemolysis**
- Some extravascular hemolysis
 - Macrophage destruction of RBCs opsonized with C3 fragments

PNH

Paroxysmal Nocturnal Hemoglobinuria

- **Acquired** genetic mutation in stem cell
 - Loss of **glycosylphosphatidylinositol (GPI) anchor**
 - Attaches proteins to cell surface
 - Lead to loss of DAF/CD59 on RBC cell membranes
- Platelets/WBCs may also have lysis

PNH

Paroxysmal Nocturnal Hemoglobinuria

- Classically causes sudden **hemolysis at night**
 - Slowing of respiratory rate with sleep
 - Also shallow breathing
 - Mild $\uparrow\text{CO}_2 \rightarrow$ mild resp. acidosis $\rightarrow \uparrow$ complement activity
- Fatigue, dyspnea
 - Anemia from hemolysis
 - May also lose iron in urine
 - **Iron-deficiency is common**

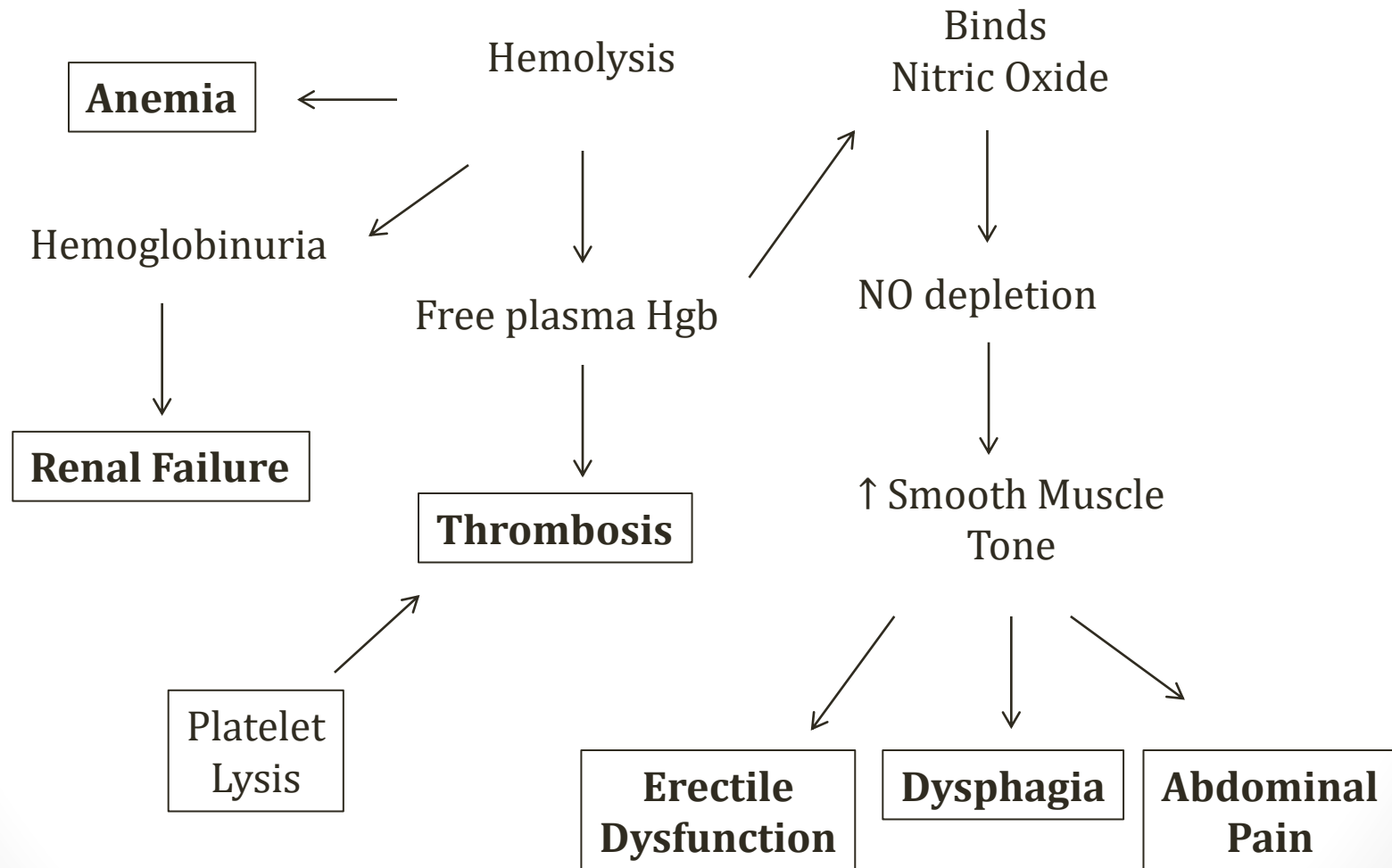
PNH

Paroxysmal Nocturnal Hemoglobinuria

- Abdominal pain (smooth muscle tension)
- Thrombosis
 - **Leading cause of death**
 - Usually venous clots
 - Unusual locations: portal, mesenteric, cerebral veins
- Some patients develop acute myeloid leukemia (AML)
 - Stem cell mutation progresses to acute leukemia
 - Lifetime risk: 5 percent or less

PNH

Paroxysmal Nocturnal Hemoglobinuria



PNH

Diagnosis

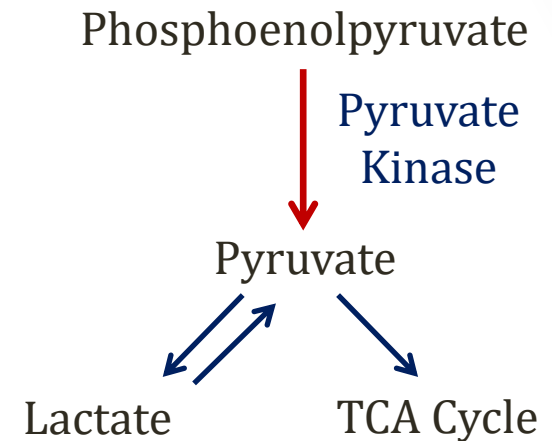
- Suspected with hemolysis, unexplained thrombosis
- Labs may show evidence of hemolysis
 - LDH, Low haptoglobin
 - Urine hemoglobin or hemosiderin
- Direct antibody testing (Coombs) will be negative
- **Flow cytometry** confirms diagnosis
 - Monoclonal antibodies to GPI-anchored proteins
 - Cells will be deficient in GPI-anchored proteins

Eculizumab

- **Anti-complement therapy**
- **Antibody** that binds to complement component C5
 - Prevents cleavage to C5a and C5b
- Blocks formation of membrane attack complex (MAC)
- Protects against intravascular hemolysis
- Does not protect against extravascular hemolysis
 - C3 fragments still bind RBCs → spleen
 - Treated patients may still have mild anemia
- Results in stable Hgb levels, **fewer transfusions**

Pyruvate Kinase Deficiency

- Deficiency of pyruvate kinase
- Key enzyme in **glycolysis**
- **RBCs** most effected
 - No mitochondria
 - Require PK for anaerobic metabolism
- **Membrane failure** → phagocytosis in spleen



Pyruvate Kinase Deficiency

- Autosomal recessive disorder
- Usually presents as **newborn**
- **Extravascular hemolysis**
- Splenomegaly
- Disease severity ranges based on enzyme activity

G6PD Deficiency

Glucose-6-Phosphate Dehydrogenase

- Key enzyme in **HMP shunt**
- HMP shunt necessary for generation of **NADPH**
- NADPH protects RBCs from **oxidative damage**

G6PD Deficiency

Glucose-6-Phosphate Dehydrogenase

- H_2O_2 toxic to RBCs
- H_2O_2 generation triggered by:
 - Infections
 - Drugs
 - Fava beans
- Need NADPH to degrade H_2O_2
- Absence of required NADPH → **hemolysis**

G6PD Deficiency

Glucose-6-Phosphate Dehydrogenase

- **X-linked** recessive disorder (males)
- Most common human enzyme disorder
- **Recurrent hemolysis** after exposure to trigger
 - Red cells become rigid
 - Consumed by splenic macrophages (**extravascular**)
 - Some lysis in blood vessels (intravascular)

**Exposure
to
Trigger**



Hemolysis

G6PD Deficiency

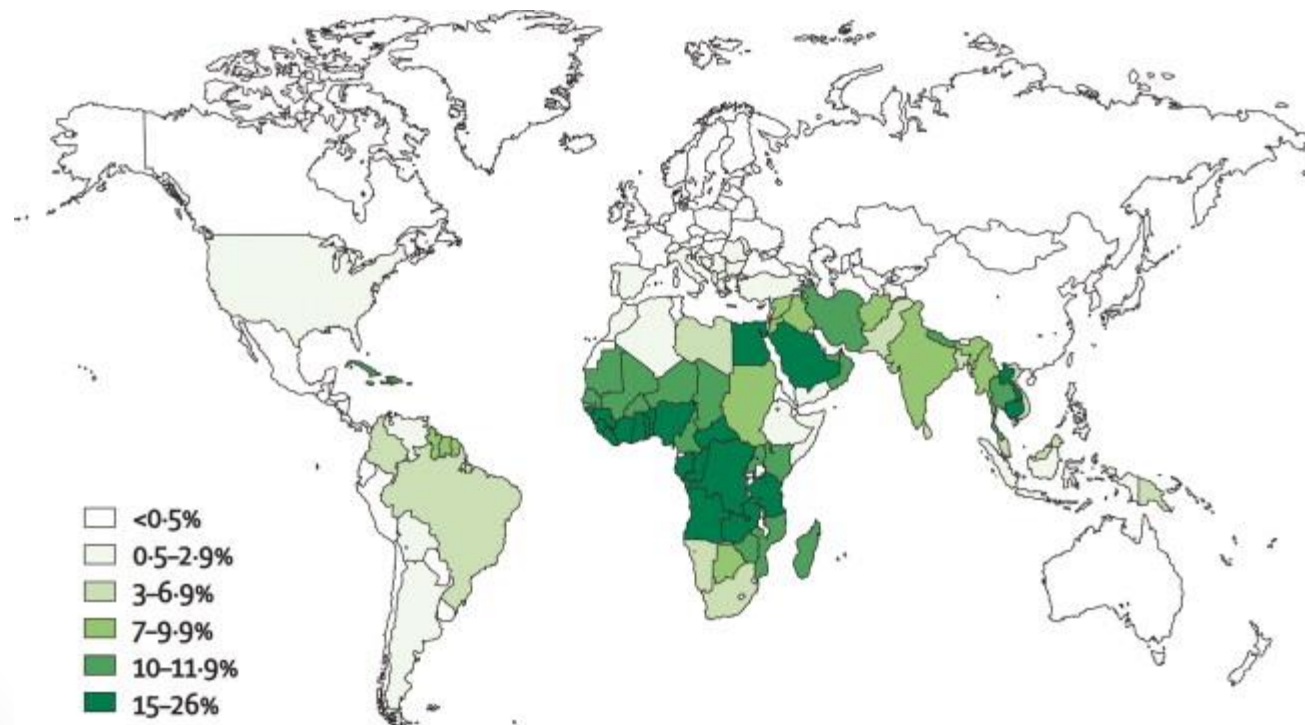
Triggers

- Infection: Macrophages generate free radicals
- Fava beans: Contain oxidants
- Drugs:
 - Antibiotics (**sulfa drugs**, dapsons, nitrofurantoin, INH)
 - **Anti-malarials** (primaquine, quinidine)
 - Aspirin, acetaminophen (rare)

G6PD Deficiency

Glucose-6-Phosphate Dehydrogenase

- High prevalence in **Africa**, Asia, the Mediterranean
 - May protect against malaria



Cappellini, Fiorelli, Lancet 2008; 371: 64-74

G6PD Deficiency

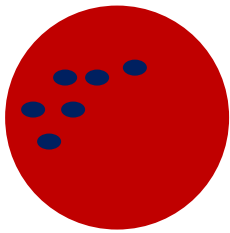
Classic presentation

- Patient from Africa
- Jaundice, dark urine, anemia
- May have back pain (free Hgb)
- Onset after exposure to trigger

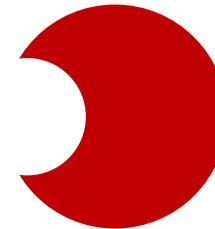
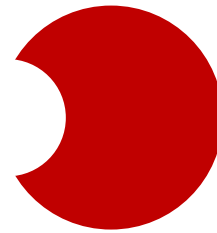
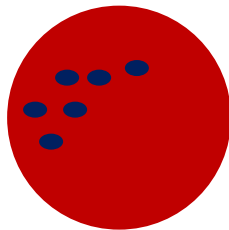
G6PD Deficiency

Glucose-6-Phosphate Dehydrogenase

- Classic findings: **Heinz bodies** and **bite cells**
- Heinz bodies: oxidized Hgb precipitated in RBCs
 - Seen with Heinz body stain (“Heinz body preparation”)
- Bite cells: phagocytic removal by splenic macrophages



Heinz bodies



Bite cells

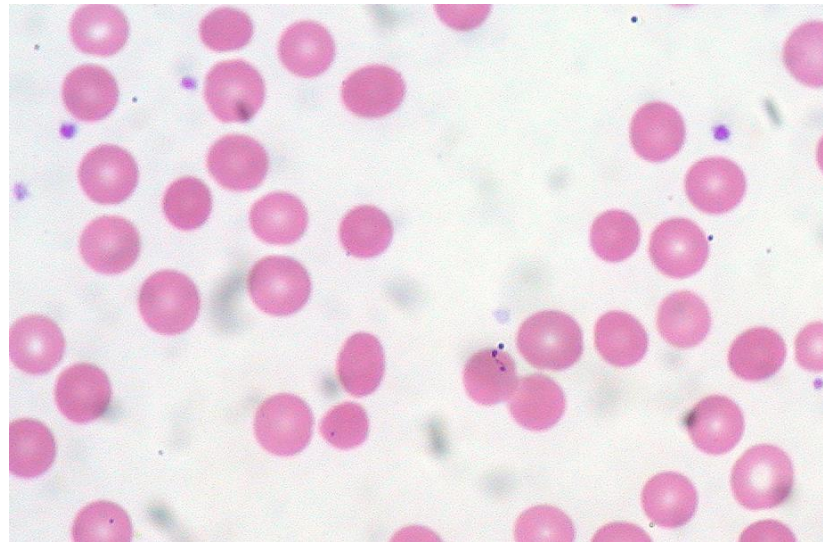
G6PD Deficiency

Diagnosis and Treatment

- **Diagnosis:**
 - Fluorescent spot test
 - Detects generation of NADPH from NADP
 - Add glucose-6-phosphate and NADP to red cells
 - Positive test if blood spot **fails to fluoresce** under UV light
- **Must test outside of acute attack**
 - Triggers → destruction of enzyme-poor cells
 - Remaining cells may have normal enzyme levels
- **Treatment:**
 - Avoidance of triggers

Hereditary Spherocytosis

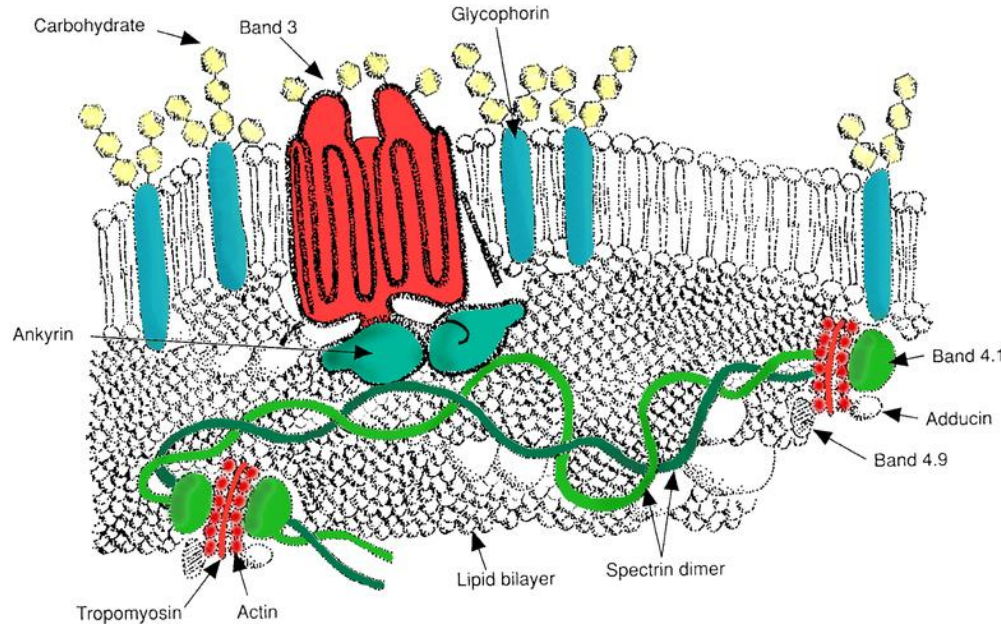
- Hereditary disorder
 - Can be autosomal dominant or recessive
- Results in **spherocytes**
 - Slightly smaller than normal RBCs
 - Spherical shape
 - Lacks central pallor



Ed Uthman

Hereditary Spherocytosis

- Cytoskeleton abnormality
 - Abnormal **proteins that tie cytoskeleton to RBC membrane**
 - Common involves **spectrin**
 - Other proteins: ankyrin, band 3, band 4.2



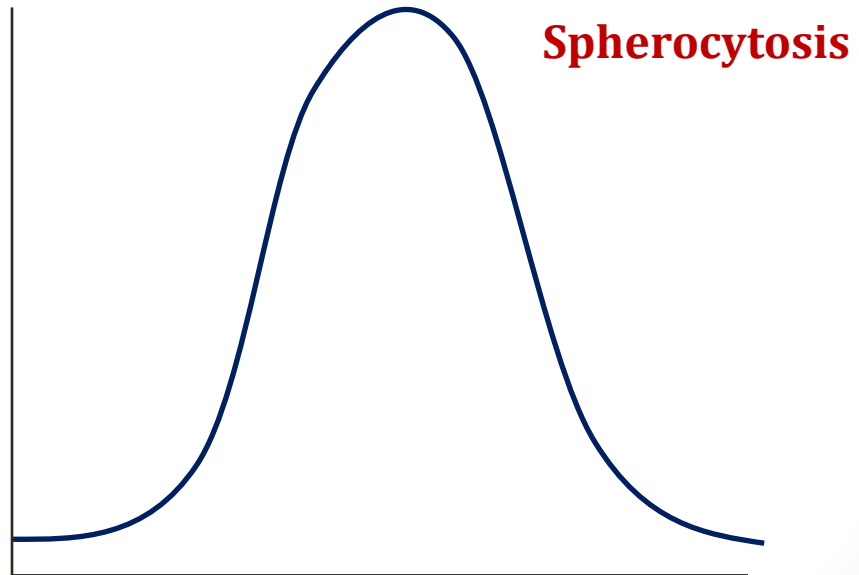
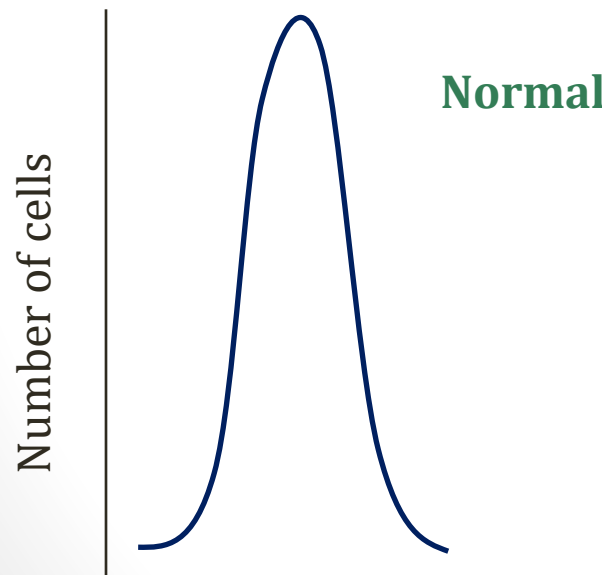
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Hereditary Spherocytosis

- O₂ carrying function of spherocytes normal
- Disease from **chronic destruction in spleen**
 - Splenomegaly (growth of splenic macrophages)
 - Increased bilirubin
 - Jaundice
 - Bilirubin gallstones

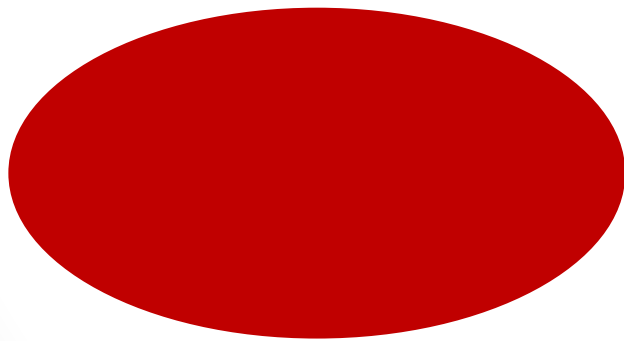
Hereditary Spherocytosis

- **Progressive** loss of cell membrane
- Over time, more and more membrane lost
- Results in a **high RDW**

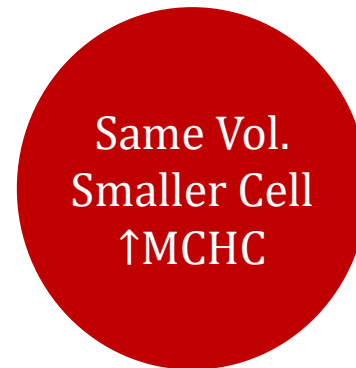


Hereditary Spherocytosis

- Volume does not change over time
- Results in a **high MCHC**
- MCV usually normal or low
 - Spherocytes: low MCV
 - Reticulocytes: high MCV



Normal



Spherocyte

Hereditary Spherocytosis

- Loss of membrane flexibility → more rigid cells
- High **resistance to blood flow** in small vessels

$$R = \frac{\Delta P}{Q} = \frac{8 \eta \text{ (viscosity)} L \text{ (length)}}{\pi r \text{ (radius)}^4}$$

Poiseuille's Law

Hereditary Spherocytosis

- Risk of aplastic crisis with **parvovirus B19 infection**
 - Patients dependent on marrow to replace hemolyzed cells
- Initial presentation may be a child with infection
 - Hemolysis compensated until B19 exposure
 - Spherocytosis seen on blood smear
 - Don't confuse with G6PD

Hereditary Spherocytosis

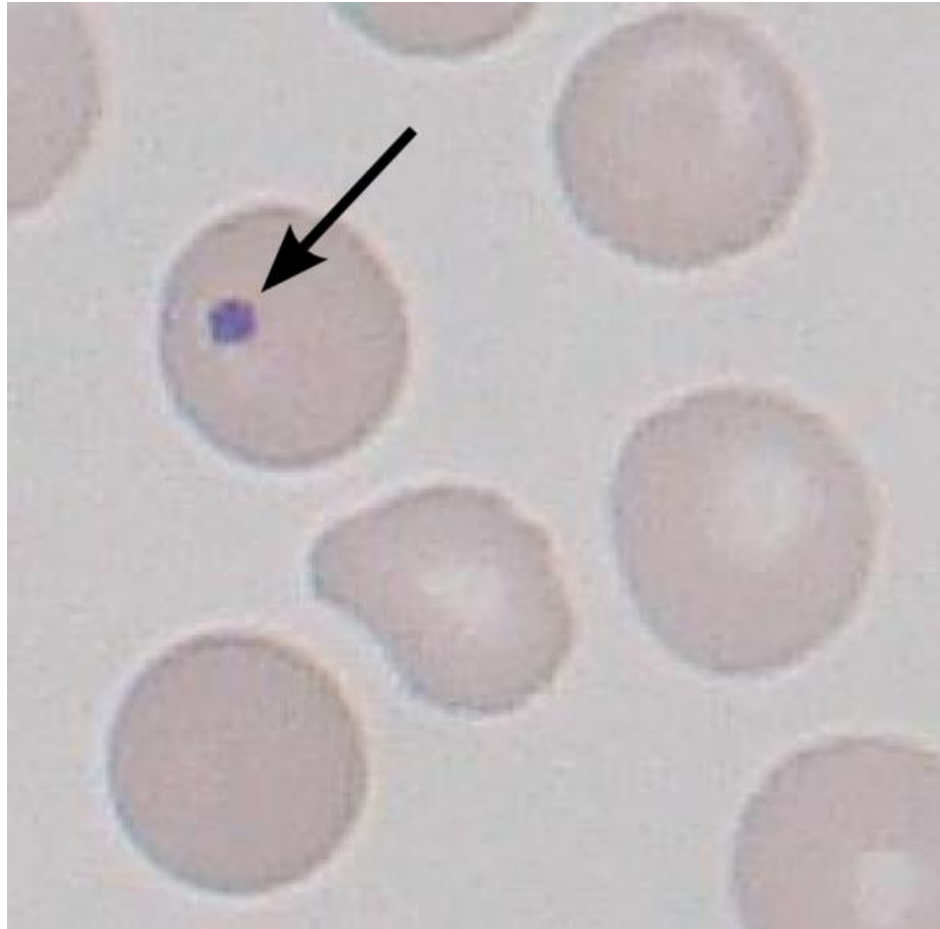
Diagnosis

- **Osmotic fragility test**
- Spherocytes: susceptible to osmotic lysis
 - Poor ability to swell like normal RBCs
 - Will lyse in hypotonic solution
- Measure Hgb release in hypotonic solution
- Osmotic fragility will be \uparrow if spherocytosis present

Hereditary Spherocytosis

- Treatment: **Splenectomy**
- Spherocytes will persist but hemolysis resolves
- Howell–Jolly bodies appear
 - Some RBCs leave marrow with nuclear remnants
 - Normally cleared by spleen
 - Presence in peripheral blood indicates splenic dysfunction
- Classic finding: spherocytes and Howell-Jolly bodies
 - Indicates patient post-splenectomy for spherocytosis

Howell-Jolly Bodies



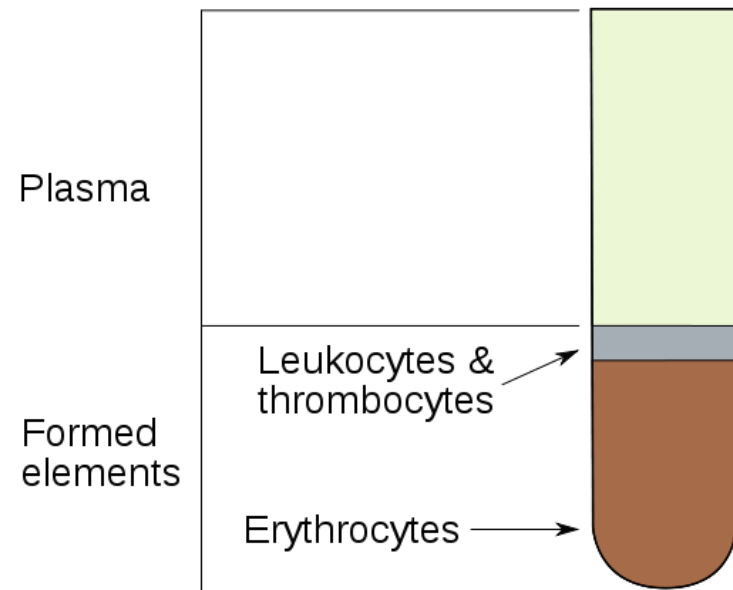
Paulo Henrique Orlandi Mourao /Mikael Häggström

Microcytic Anemias

Jason Ryan, MD, MPH

Red Blood Cell Measurements

- RBC count
 - Part of CBC with white cell count and platelets
- Hemoglobin
 - Concentration in g/dl
- Hematocrit
 - Volume % of red cells



[MesserWoland/Wikipedia](#)

Rule of 3

- $\text{Hgb} = 3 \times \text{Red Blood Cell Count}$
- $\text{Hct} = 3 \times \text{Hgb}$

Normal Values

RBC = 5 million cells/ul

Hgb = 15g/dl

Hct = 45%

RBC Indices

- Measured by automated blood counters
- Measures of **mean** characteristics of RBCs
- Used in evaluation of anemias

RBC Indices

- **Mean corpuscular volume (MCV)**
 - Normal range: 80 to 100 femtoliters
- **Mean corpuscular hemoglobin (MCH)**
 - Amount (mass) of hemoglobin per red cell
 - Usually reported in picograms (per cell)
- **Mean corpuscular Hgb concentration (MCHC)**
 - Concentration of Hgb in red cells
 - Usually reported g/dL

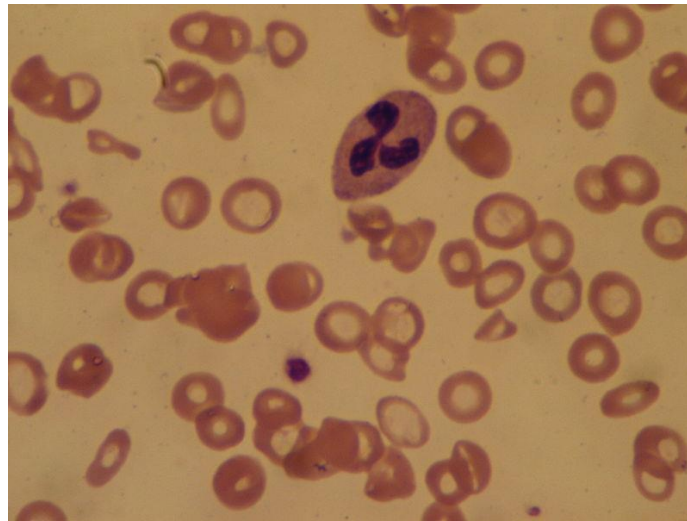
Anemia Classification

- MCV commonly used to classify anemias

Microcytic MCV<80	Normocytic MCV 80-100	Macrocytic MCV>100
Iron deficiency Anemia Chronic Disease Thalassemia Lead poisoning Sideroblastic Anemia	Iron deficiency Anemia Chronic Disease Hemolysis Aplastic anemia Kidney disease	Folate/B12 deficiency Orotic Aciduria Liver disease Alcoholism Reticulocytosis

Microcytic Anemias

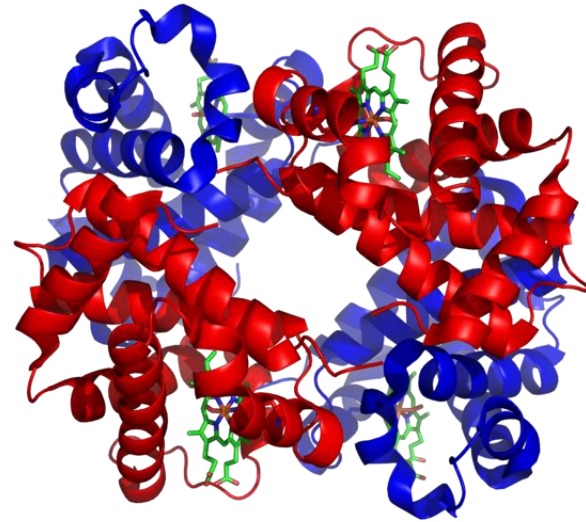
- Usually due to ↓ **hemoglobin** in red cells
- Usually associated with ↓ MCH and MCHC
- Low hemoglobin → hypochromic RBCs on smear



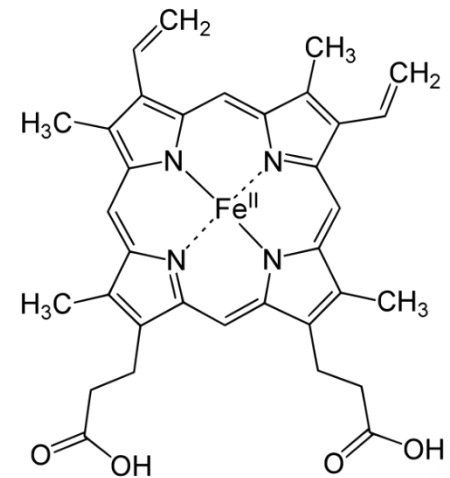
Roberto J. Galindo

Hemoglobin

- **Globin chains**
 - Proteins
 - 4 chains in 2 pairs
- **Protoporphyrin**
- **Iron**
- Microcytic anemia
 - Loss of iron
 - Loss of globins (thalassemia)
 - Loss of heme (lead, sideroblastic)



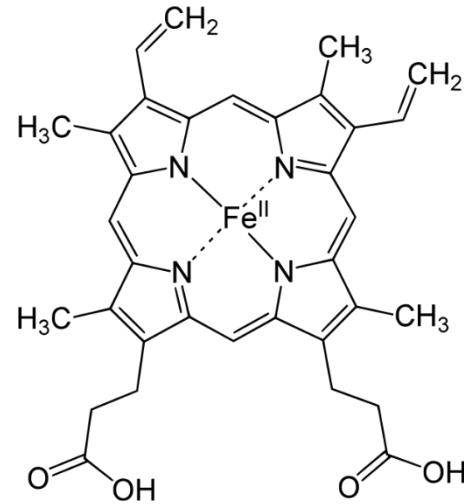
Richard Wheeler and Zephyris



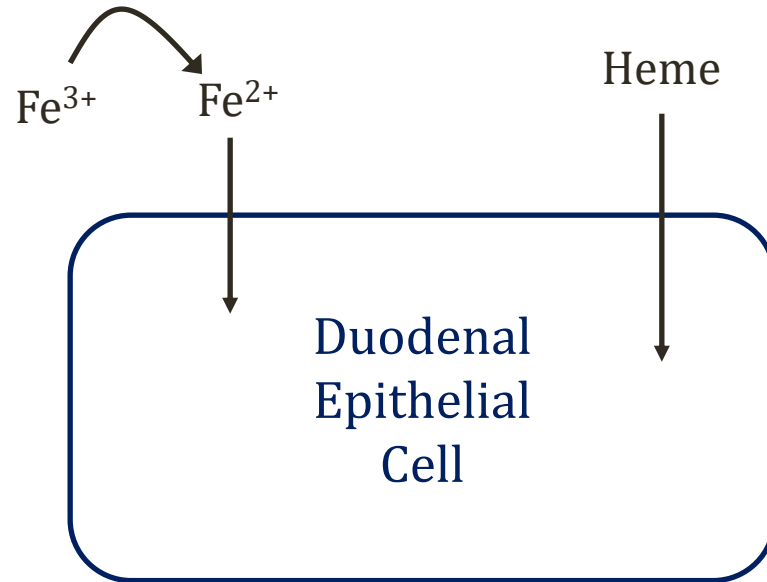
Heme

Iron Absorption

- Heme iron
 - Found in meats
 - Easily absorbed
- Non-heme iron
 - Absorbed in Fe^{2+} state
 - Aided by vitamin C

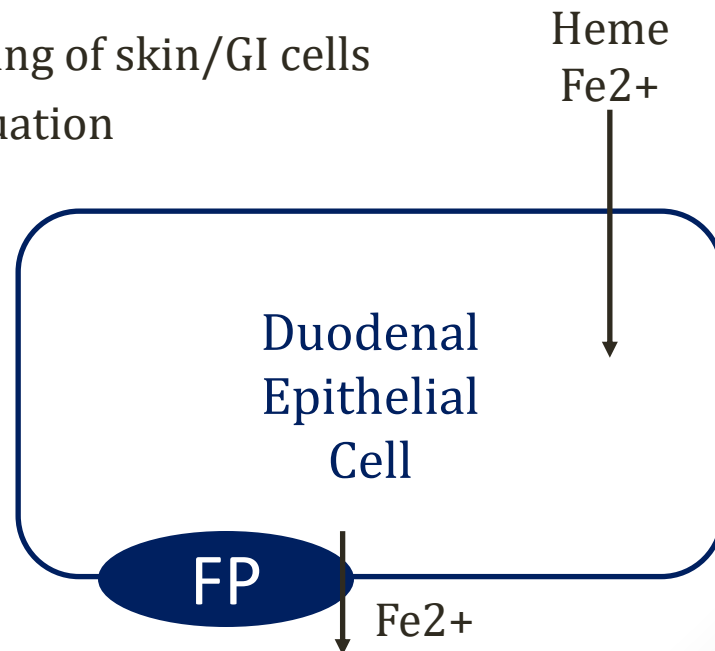


Vitamin C



Iron Metabolism

- Iron consumed in diet
- Uptake to plasma **regulated by enterocytes**
 - Iron transporter: **ferroportin**
 - Transports iron out of enterocytes and other cells
- Few mechanisms to excrete excess iron
 - Small amount in sweat, sloughing of skin/GI cells
 - Women lose iron from menstruation



Iron Metabolism

- Iron always bound to a protein
- Transport protein: **transferrin**
 - Transported in blood via transferrin
 - ↑ transferrin when iron stores are low
- Storage protein: **ferritin**
 - Stored intracellularly as ferritin
 - Stored in **macrophages** of **liver and bone**

Clinical Iron Measurements

Test	Interpretation
Serum iron	Iron level
Total Iron Binding Capacity	Amount of transferrin in serum
Serum ferritin	Amount of storage iron
% saturation	Amount of transferrin bound to Fe

Iron Deficiency

- Lack of iron from gut
- Loss of iron (usually as blood)

Iron Deficiency

Inadequate GI uptake

- **Babies**
 - Iron stores depleted ~ 6months
 - Recommendation: add iron-containing foods
 - Exclusive breast feeding → iron deficiency



Achoubey/Wikipedia

Iron Deficiency

Inadequate GI uptake

- **Malabsorption**
 - Any disease affecting duodenum or acid production
 - Loss of acid → **more Fe³⁺**
 - Status post gastrectomy
 - Proton pump inhibitors
- Rarely malnutrition

Iron Deficiency

Loss of iron

- **Bleeding**
 - Menorrhagia
 - Peptic ulcers
 - Colon cancer
- **Adult or post-menopausal female with iron deficiency must have work-up for colon cancer**

Iron Deficiency

Other causes

- **Pregnancy**
 - “Negative iron balance” in pregnancy
 - Expansion in mothers Hgb mass
 - ↑ demand of fetal growth
 - Prenatal vitamins often contain **iron and folate**

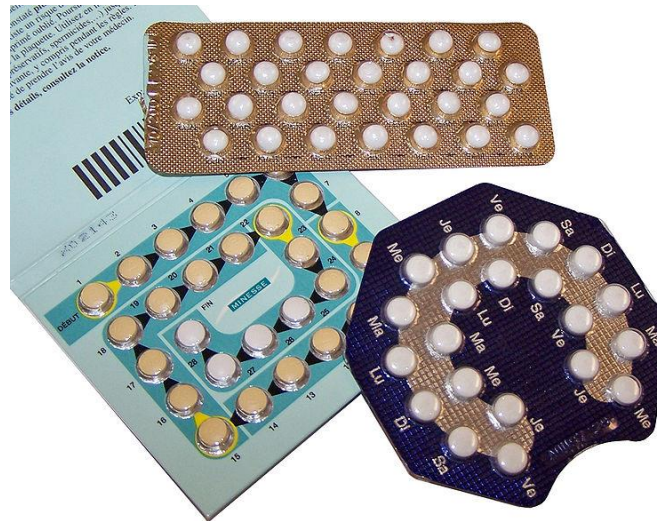


Øyvind Holmstad/Wikipedia

Pregnancy/OCPs

$$\% \text{ Sat} = \frac{\text{Iron}}{\text{TIBC}}$$

- **Increase plasma transferrin**
- Percent saturation may be low
- **Low ferritin** often used to diagnose iron deficiency

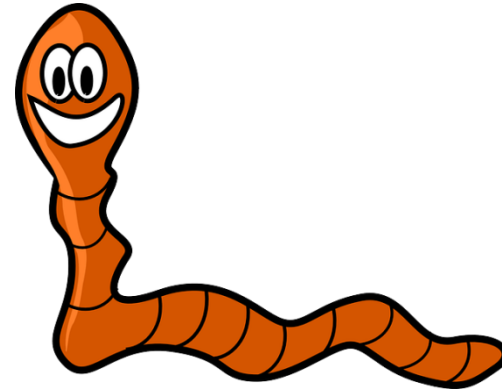


Ceridwen/Wikipedia

Iron Deficiency

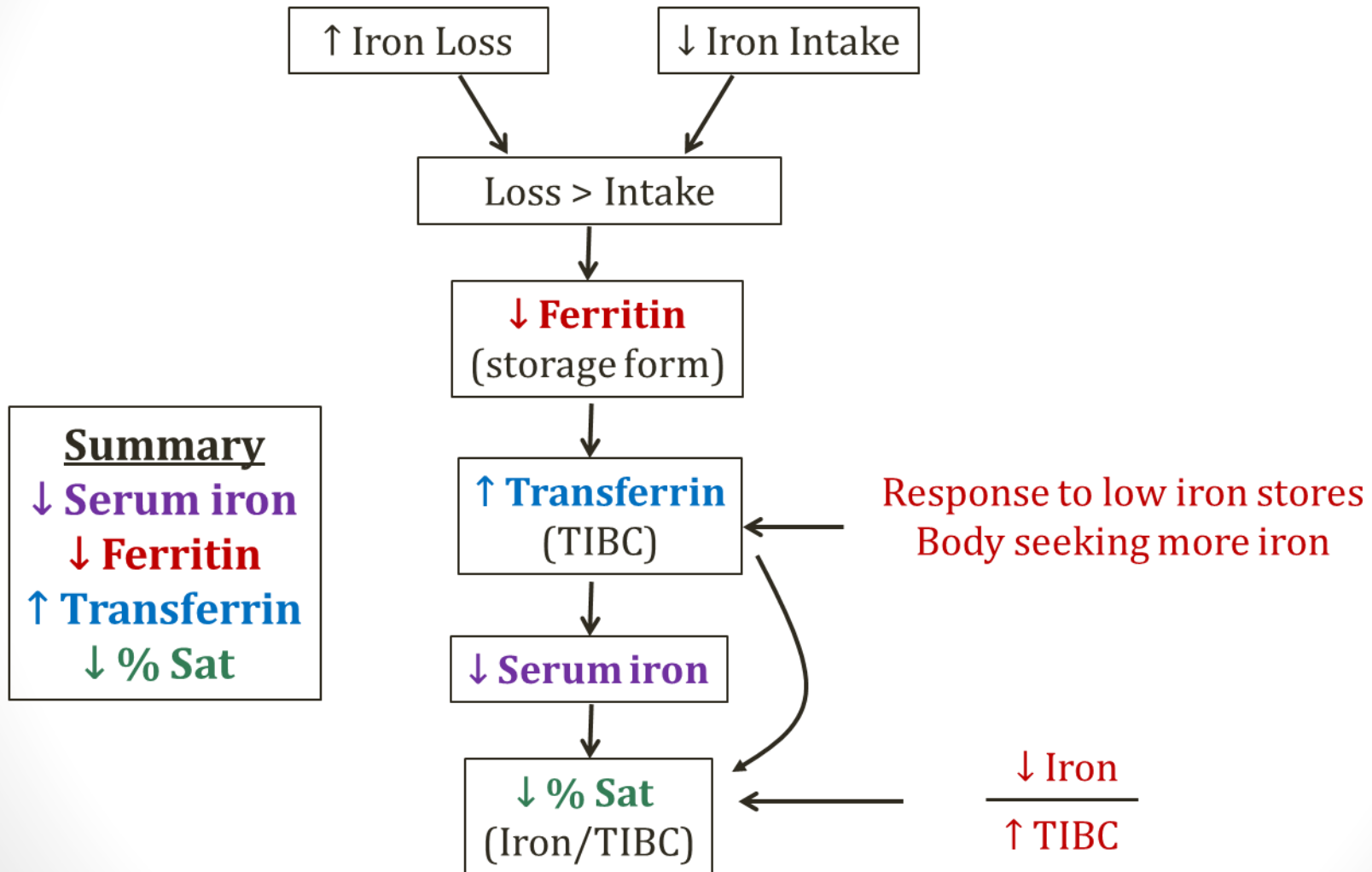
Rare causes

- **Hookworms**
 - Consume blood in intestines
 - *Ancylostoma duodenale*
 - *Necator americanus*
- **Plummer–Vinson syndrome**
 - Rare condition; poorly understood cause
 - Iron deficiency anemia, beefy red tongue, **esophageal webs**



Pixabay/Public Domain

Iron Deficiency



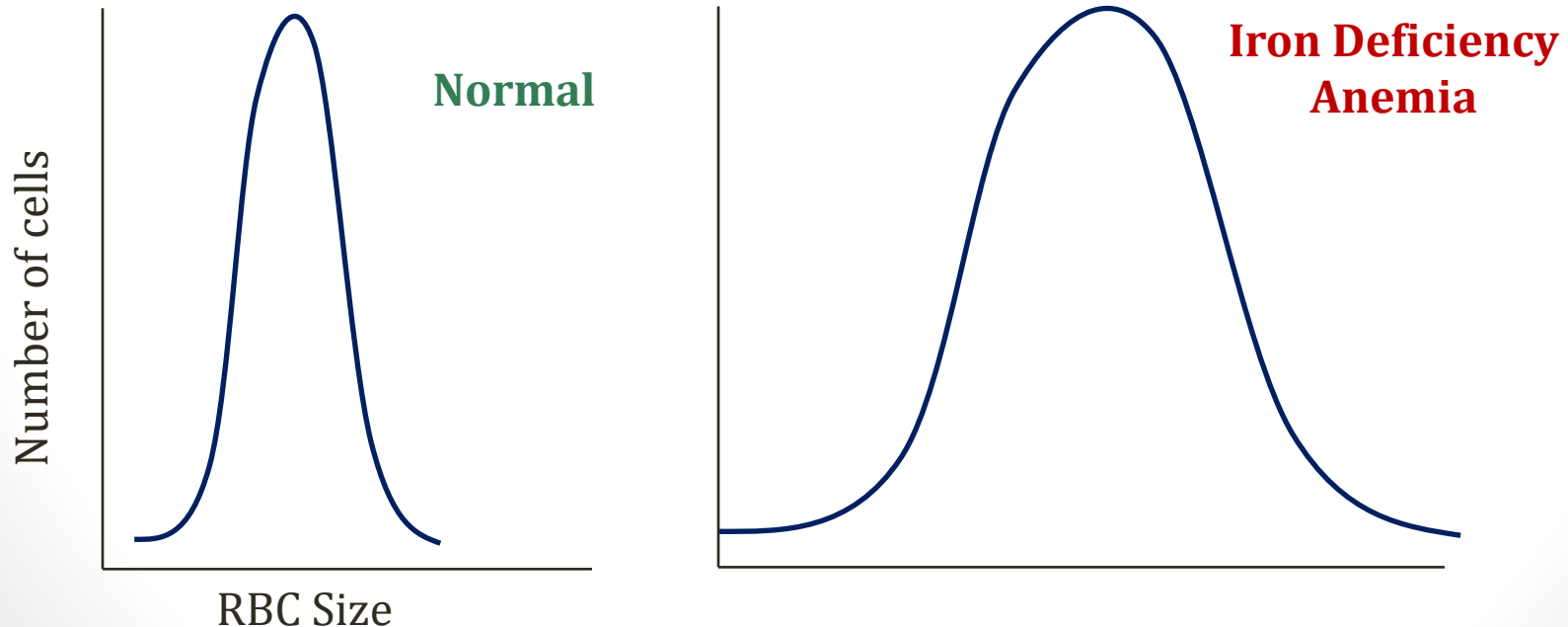
Iron Deficiency Anemia

- **Microcytic, hypochromic** anemia
 - ↓ RBCs (anemia)
 - Small cells
 - Hypochromic (low hemoglobin)
 - ↓ MCV, MCH, MCHC
- **Initially may be normocytic**
 - Marrow makes fewer RBCs; maintains Hgb

Red Cell Distribution Width

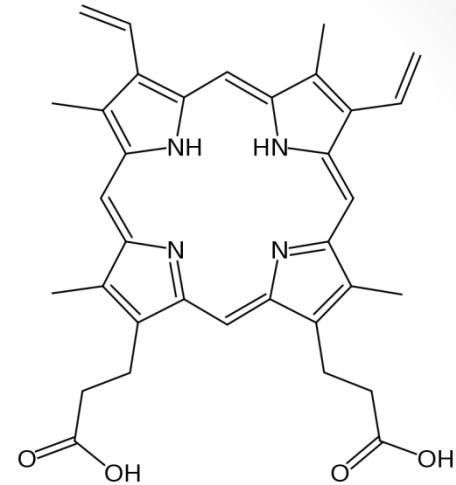
RDW

- Spectrum of RBC size
- Often wider in **iron, B12/Folate deficiency**
 - Normal RDW makes iron deficiency unlikely
- Can be normal in mild thalassemia



Protoporphyrin

- Heme = Iron + protoporphyrin
- **Erythrocyte protoporphyrin level**
 - Rarely used blood test
 - Will be **elevated in iron deficiency**
 - No Fe for protoporphyrin to bind with
- Also elevated in **lead poisoning**
 - Inhibits addition of iron to protoporphyrin
- Major uses: screening
 - Iron deficiency or lead poisoning



Iron Deficiency Anemia

Treatment

- **Iron supplementation**
- Usually oral therapy
- Rarely IV iron can be used

Anemia of Chronic Disease

- Anemia in association with **inflammation**
 - Common in rheumatoid arthritis, lymphoma
 - Many other chronic conditions
- Usually a **mild** anemia (Hgb > 10g/dL)
- Symptoms from anemia are rare

Anemia of Chronic Disease

Mechanisms

- Triggered by **cytokines**
- Mild decrease in RBC survival
- Inadequate EPO level/response
 - Lower EPO than expected for degree of anemia
 - Less increase in RBC production by EPO
- Lack of **availability** of iron
 - Trapped in storage form
 - Key mediator: **hepcidin**

Hepcidin

- Acute phase protein
 - Produced in liver
 - Has anti-bacterial properties
- Affects iron metabolism
 - Inhibits iron transport
 - Binds to **ferroportin** in enterocytes, macrophages
- **Iron trapped in cells as ferritin**
- Contributes to anemia of chronic disease
- Key finding ACD: **↑ ferritin**

Anemia of Chronic Disease

- Usually normocytic/normochromic
- Microcytic/hypochromic in about 25% cases
 - Low iron availability may lead to small red cells
 - MCV usually mildly decreased (70-80)
- Important to distinguish from iron deficiency
 - Does not respond to iron
- First line therapy: **treat underlying disease**

Anemia of Chronic Disease

Diagnosis

- **Serum iron is low**
 - Thought to be protective
 - Bacteria may use iron for growth/metabolism
- **Ferritin is usually increased**
 - Iron trapped in storage form
 - Ferritin is acute phase reactant
 - Increase may not represent increased storage iron
- Transferrin (TIBC) is usually decreased
 - Transferrin rises when total body iron low
- % saturation usually normal

Iron Studies

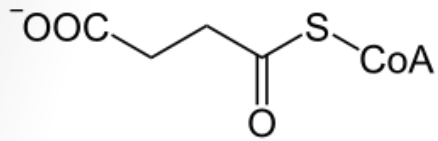
	Iron	Ferritin	TIBC	% Sat
Iron Deficiency	↓	↓	↑	↓↓
Anemia Chronic Disease	↓	↑	↓	--
Hemochromatosis	↑	↑	↓	↑↑

↑
Elevated
when body
storage iron
is low

Lead Poisoning

- Exposure to lead:
 - Adults: **Inhalation** from industrial work (battery factory)
 - Children: **Eating lead paint** (old house)
- **Inhibits heme synthesis** via two enzymes in RBCs
 - Delta-aminolevulinic acid (δ -ALA) dehydratase
 - Ferrochelatase
- \downarrow heme synthesis \rightarrow microcytic, hypochromic anemia
- Iron studies: **normal or low**

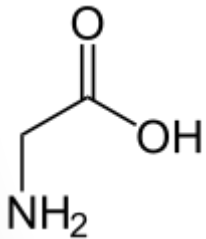
Heme Synthesis



Succinyl-CoA

δ-ALA Synthase

Glycine



δ-ALA

δ-ALA dehydratase

Porphobilinogen

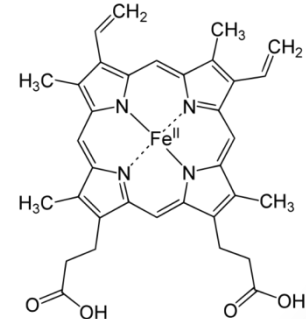
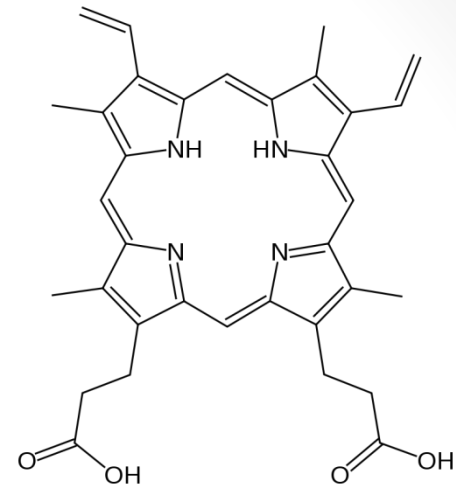
Protoporphyrin

Ferrochelatase

Fe²⁺

Heme

ALA Synthase: Rate-limiting Step
 ↓ Heme → ↑ ALA Synthase
 Build up of: δ-ALA, Protoporphyrin



Lead Poisoning

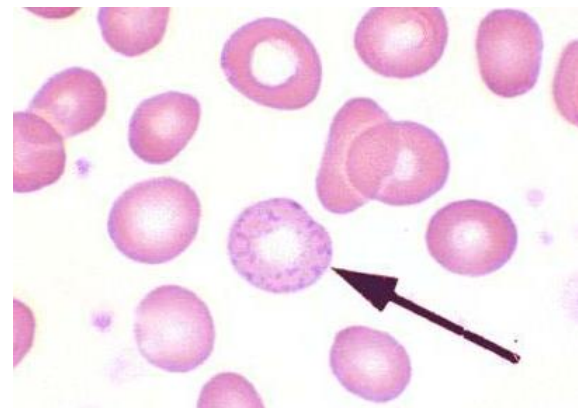
Diagnosis

- **Plasma lead level**
- ↑ delta-aminolevulinic acid (δ -ALA)
- ↑ erythrocyte protoporphyrin

Lead Poisoning

Diagnosis

- Blood smear: **basophilic stippling**
 - Blue granules in cytoplasm of red cells
 - Lead inhibits pyrimidine 5' nucleotidase
 - Normally digests pyrimidines in ribosomes/RNA
 - Leads to accumulation of pyrimidines/RNA in RBCs
- Also seen in thalassemia, other anemias



Lead Poisoning

Symptoms

- **Abdominal pain** (“lead colic”)
- Constipation
- Headache
- **“Lead lines”**
 - Blue pigment at gum-tooth line
 - Caused by reaction of lead with dental plaque
- Nephropathy
 - Injury to proximal tubules (Fanconi-type syndrome)
 - Glucose, amino acids, and phosphate wasting
- Neuropathy
 - Common symptom: Drop wrist and foot

Lead Poisoning

Symptoms

- Children may have prominent neurologic effects
 - **Behavioral issues**
 - Developmental delay
 - Failure to reach milestones (i.e. language)
- Many states screen children with lead level testing
 - Usually at 1-2 years of age

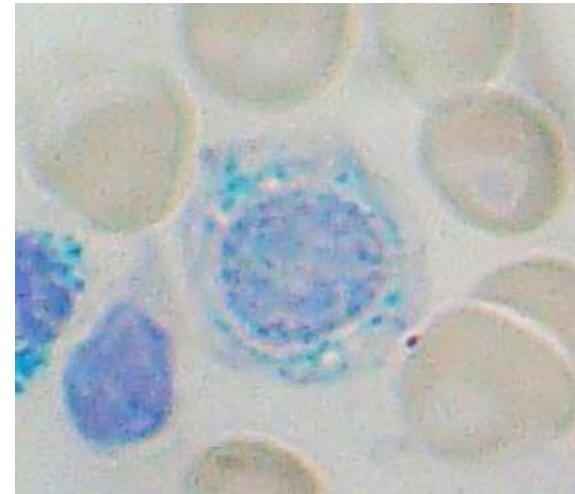
Lead Poisoning

Treatment

- Removal of exposure to lead
- Chelation therapy
 - **Dimercaprol** (2,3-dimercapto-1-propanol)
 - **Calcium disodium EDTA** (ethylenediaminetetraacetate)
 - **DMSA** (2,3-dimercaptosuccinic acid; succimer)

Sideroblastic Anemia

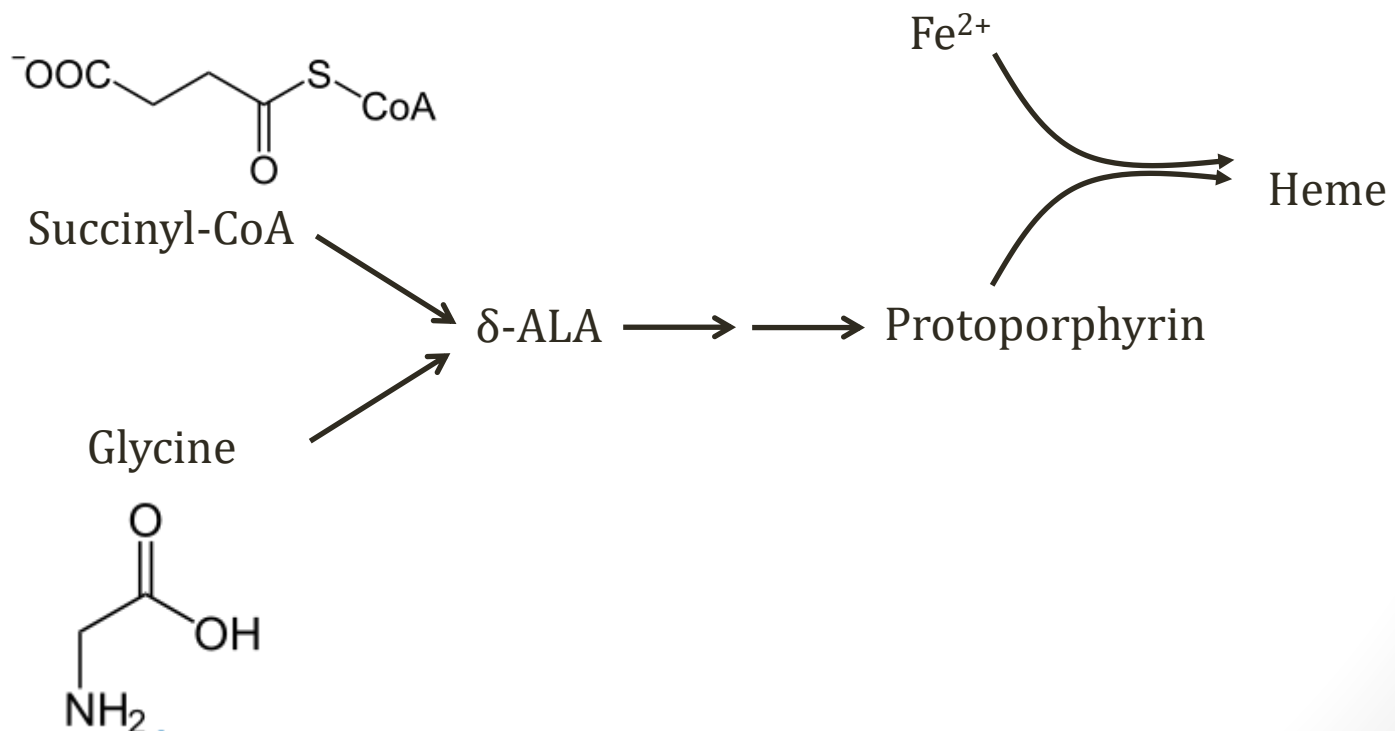
- Ring Sideroblasts:
 - Nucleated red cell precursors
 - **Iron-loaded mitochondria** seen with Prussian blue stain
 - Perinuclear ring of blue granules
- Sideroblastic anemia
 - Usually microcytic anemia
 - Ring sideroblasts in marrow



Paulo Henrique Orlandi Mourao

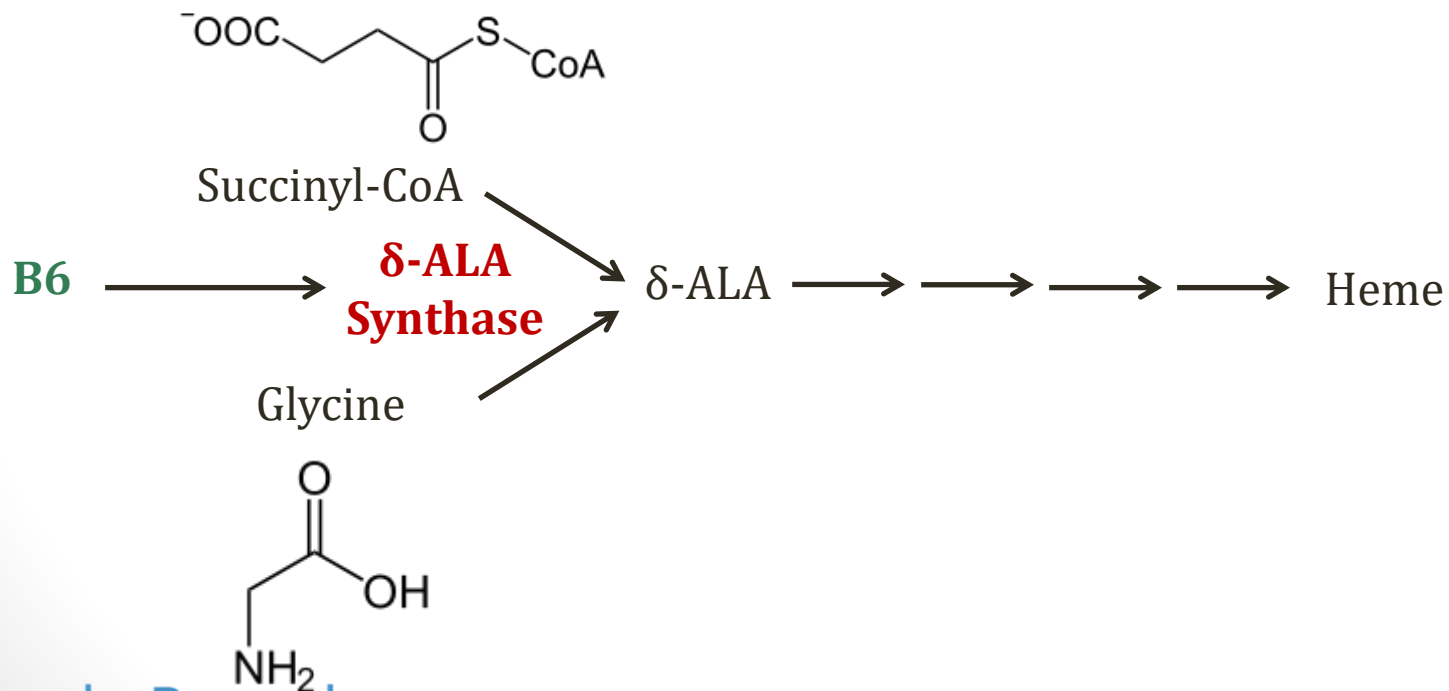
Sideroblastic Anemia

- **Failure to make protoporphyrin**
- Iron cannot bind → heme
- Iron accumulation in **mitochondria**



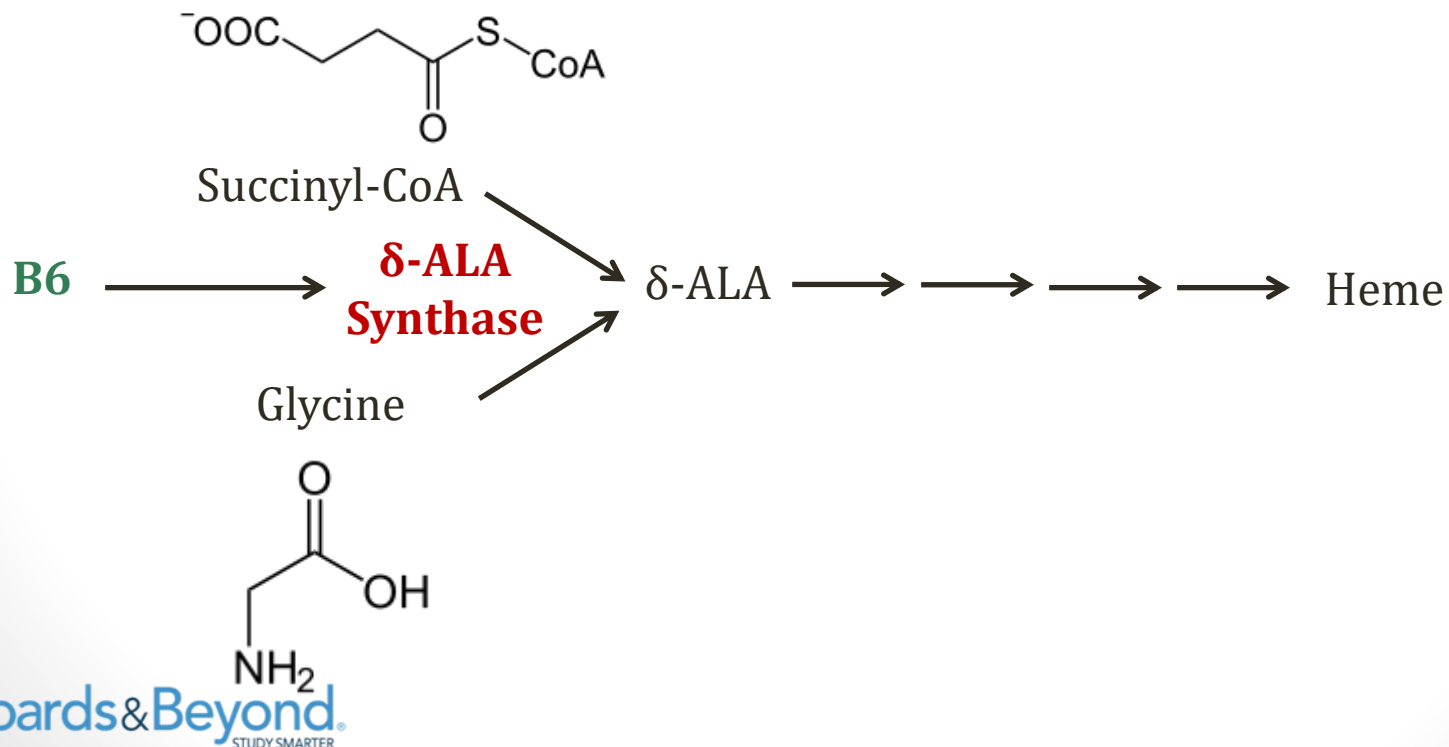
Sideroblastic Anemia

- Usually **secondary** to a toxin
 - Alcohol (mitochondrial poison)
 - Vitamin B6 deficiency (Isoniazid)
 - Lead poisoning (controversial)



Sideroblastic Anemia

- **X-linked sideroblastic anemia**
 - Rare, inherited deficiency of ALA synthase
 - Most common hereditary sideroblastic anemia
- Often responds to treatment with **vitamin B6**



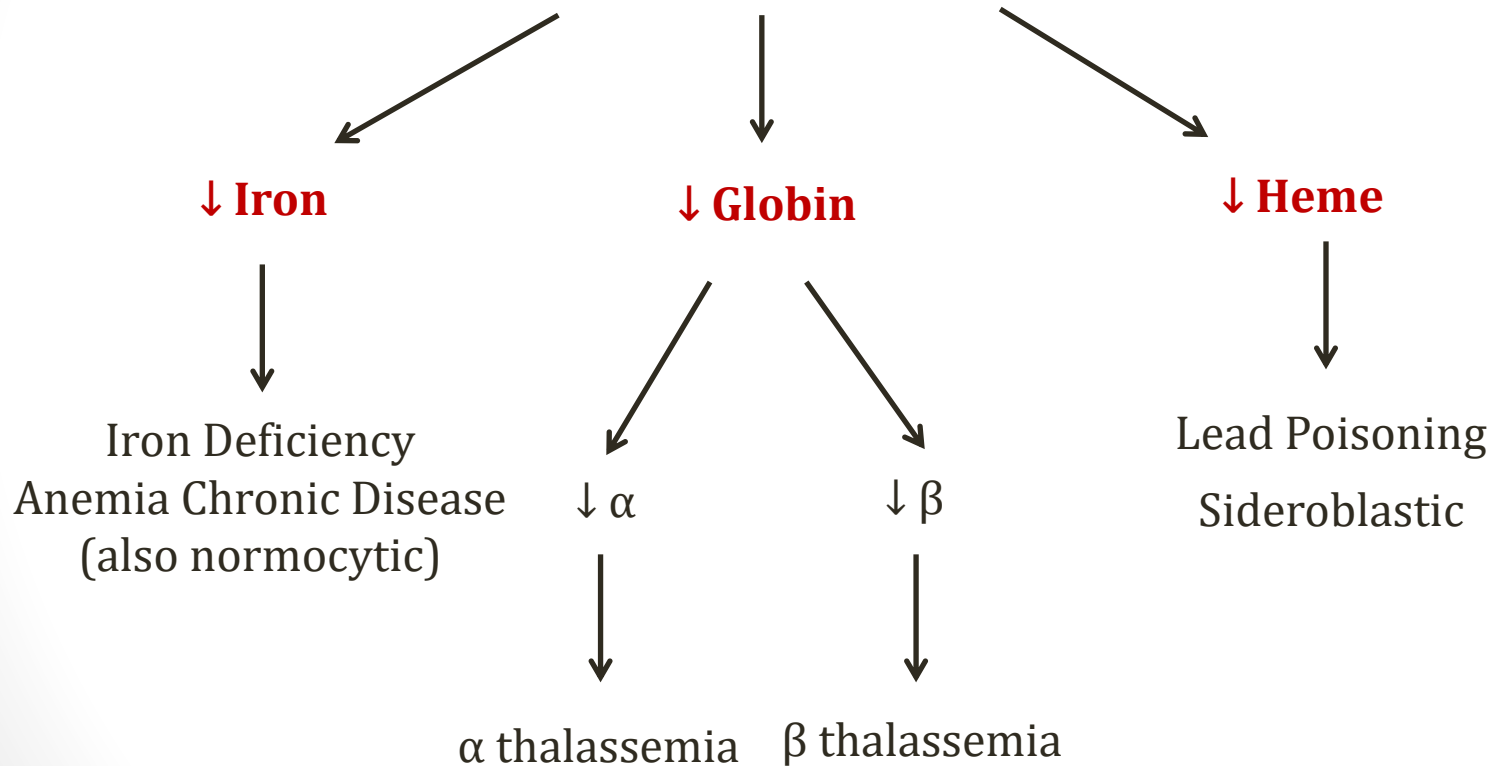
Sideroblastic Anemia

Lab Findings

- Microcytic, hypochromic anemia
- Iron studies show **iron overload**
 - ↑ serum iron
 - ↑ ferritin
 - ↓ TIBC (transferrin)
- **Low erythrocyte protoporphyrin levels**

Microcytic Anemias

Microcytic Anemia



Thalassemias

Jason Ryan, MD, MPH

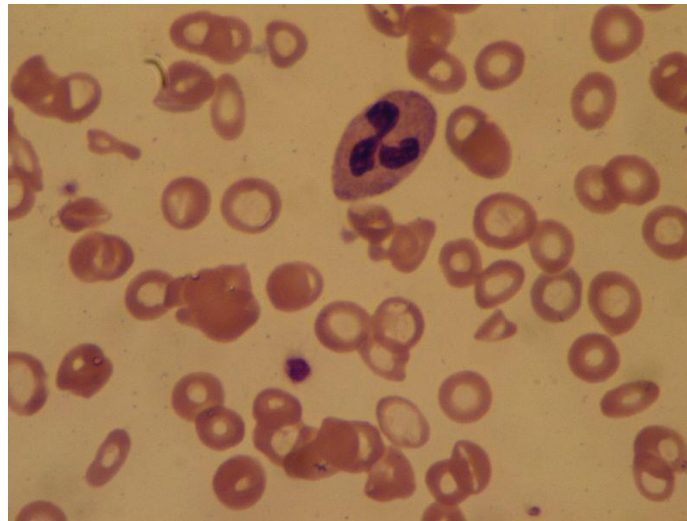
Anemia Classification

- MCV commonly used to classify anemias

Microcytic MCV<80	Normocytic MCV 80-100	Macrocytic MCV>100
Iron deficiency Anemia Chronic Disease Thalassemia Lead poisoning Sideroblastic Anemia	Iron deficiency Anemia Chronic Disease Hemolysis Aplastic anemia Kidney disease	Folate/B12 deficiency Orotic Aciduria Liver disease Alcoholism Reticulocytosis

Microcytic Anemias

- Usually due to ↓ **hemoglobin** in red cells
- Usually associated with ↓ MCH and MCHC
- Low hemoglobin → hypochromic RBCs on smear

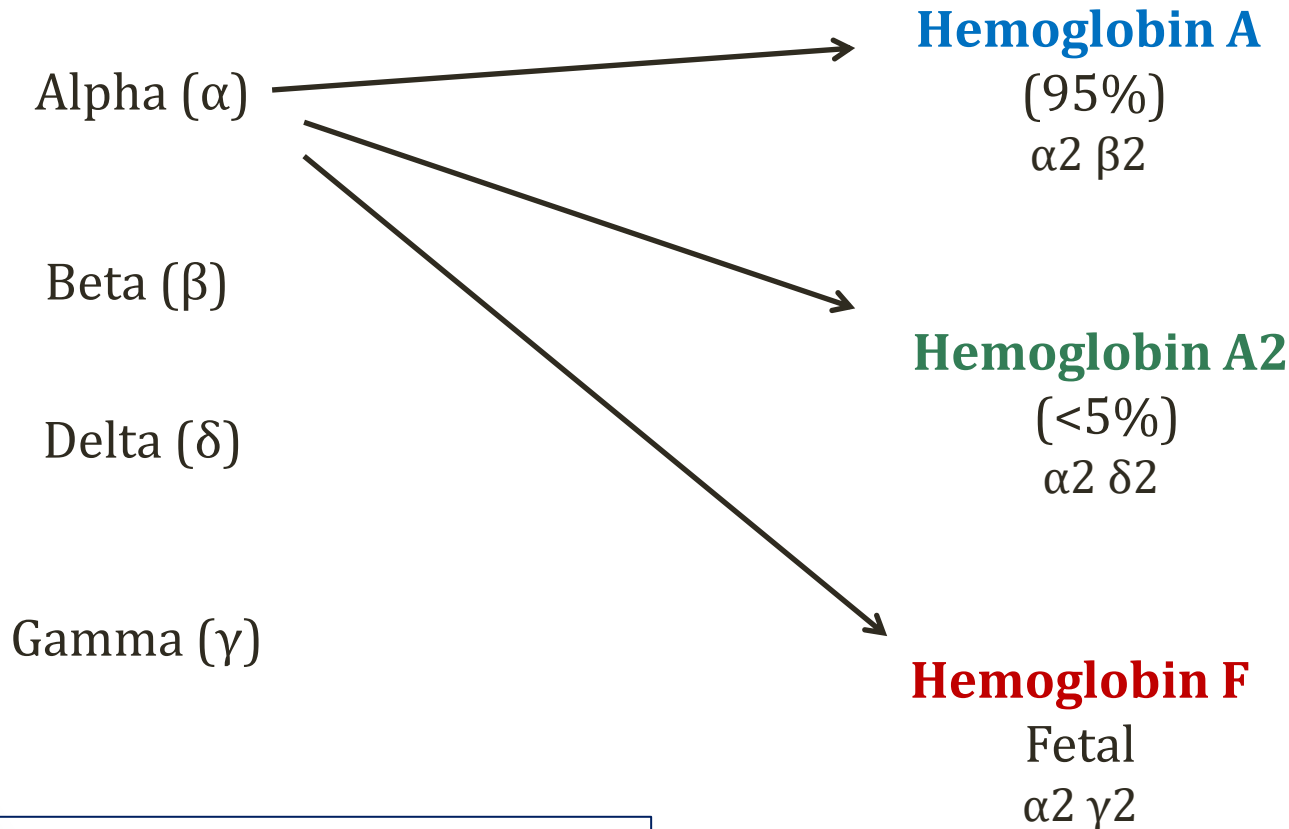


Roberto J. Galindo

Thalassemia

- Decreased or absent **production of globin chains**
 - Alpha thalassemia: alpha globin
 - Beta thalassemia: beta globin

Globins and Hemoglobin



**All Hgb has two alpha globins
Other chain determines type**

Globins and Hemoglobin

Alpha (α)

Beta (β)

Delta (δ)

Gamma (γ)

Hemoglobin A

(95%)

$\alpha_2 \beta_2$

Hemoglobin A2

(<5%)

$\alpha_2 \delta_2$

Hemoglobin F

Fetal

$\alpha_2 \gamma_2$

**All Hgb has two alpha globins
Other pair determines type**

Globins and Hemoglobin

Alpha (α)

Beta (β)

Delta (δ)

Gamma (γ)

Hemoglobin A

(95%)

$\alpha_2 \beta_2$

Hemoglobin A2

(<5%)

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Hemoglobin F

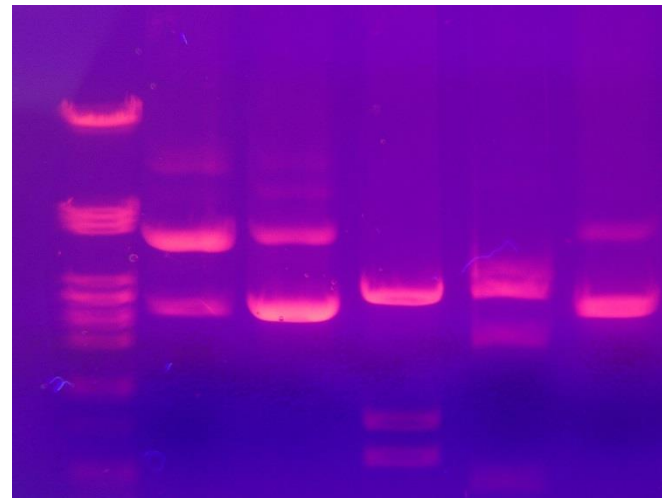
Fetal

$\alpha_2 \gamma_2$

**All Hgb has two alpha globins
Other pair determines type**

Hgb Electrophoresis

- Electrical charge applied to sample on gel
- Different hemoglobin → different distances moved
- Determines HgbA, HgbA2, HgbF, HgbS
- Used to diagnose hemoglobinopathies
 - Thalassemia
 - Sickle cell disease

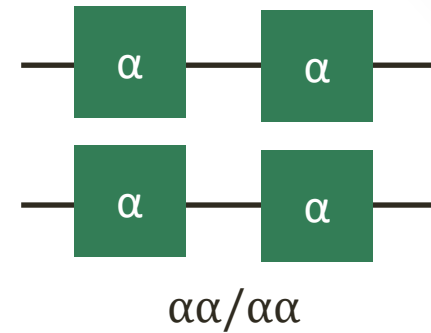


Mnolf/Wikipedia

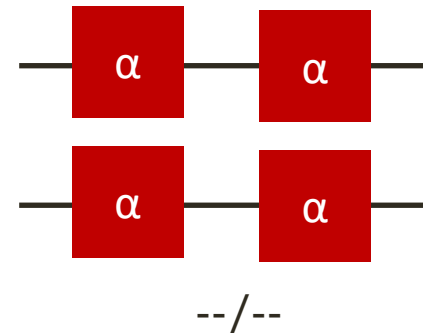
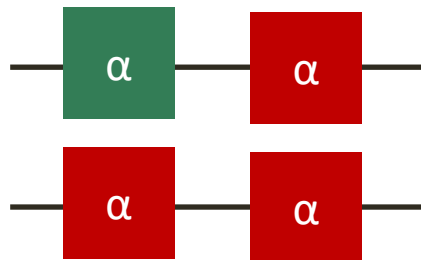
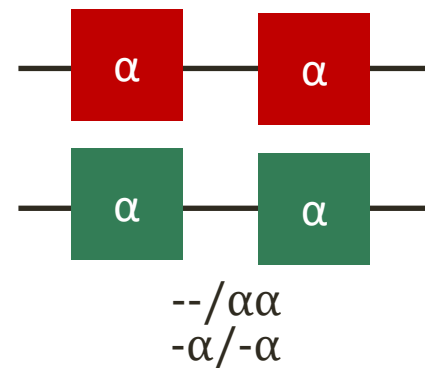
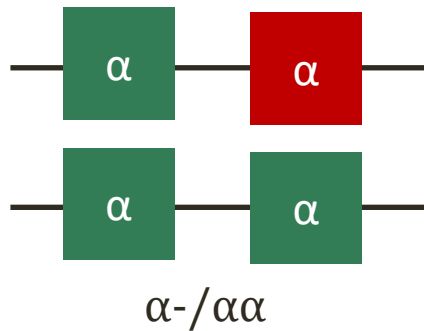
Thalassemia

- Spectrum of severity
- Thalassemia **minor**
 - Often asymptomatic
 - Identified on routine blood testing or blood smear
- Thalassemia **major**
 - Severe loss of globin production
 - Lifelong transfusions or death

Alpha Thalassemia

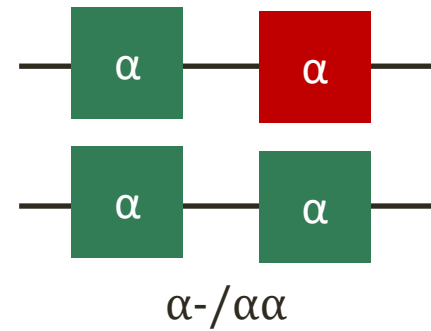


- **Four genes** code for alpha chains
 - Two on each copy of chromosome 16
- **Gene deletions** → ↓α chains → alpha thalassemia



Alpha Thalassemia Minima

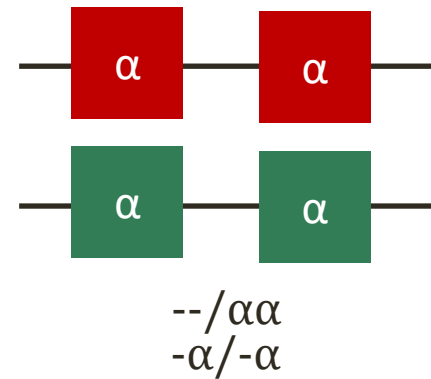
- Normal red cells
- No symptoms
- Carrier state



Alpha Thalassemia Minor

Alpha Thalassemia Trait

- No symptoms
- Can have normal red cells
- Sometimes mild anemia
 - \downarrow MCV/MCH/MCHC



Alpha Thalassemia Minor

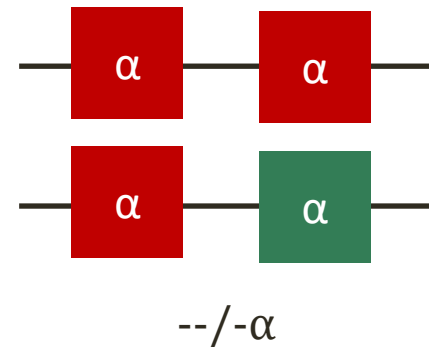
Alpha Thalassemia Trait

- Common among Asians and Africans
- Alpha minor can be cis ($\alpha\alpha/--$) or trans ($\alpha-/ \alpha-$)
- Asians more commonly have cis type
- Africans: trans



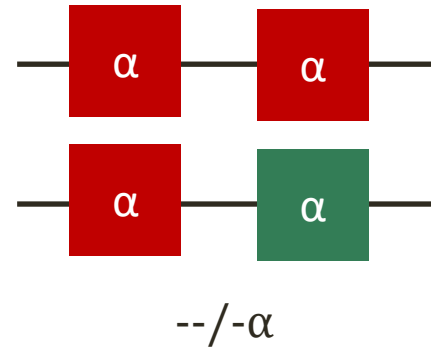
HbH Disease

- Very little alpha globin production
- Excess beta globin
- HbH forms: **4 beta chains**
 - Easily damaged
 - Affinity for oxygen 10x HbA
 - Useless for oxygen delivery
- HbH forms after birth
 - No β chains in HbF
 - More β produced \rightarrow HbH



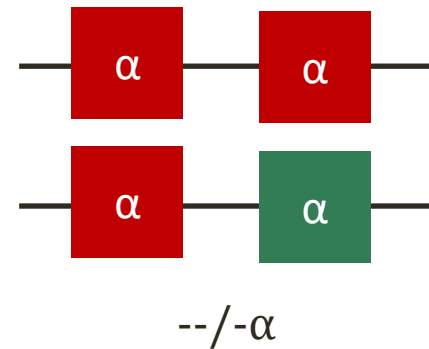
HbH Disease

- Hypochromic, microcytic anemia
- Low MCV, MCH, MCHC



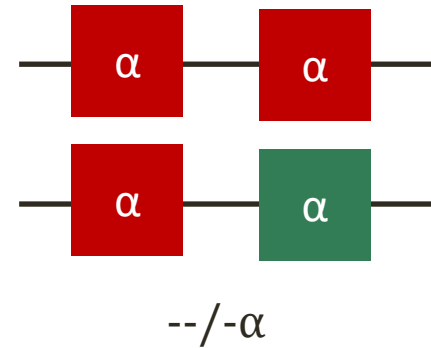
HbH Disease

- Abnormal RBC **deformability**
- **Extravascular hemolysis**
 - Splenomegaly
 - Indirect hyperbilirubinemia
 - Elevated LDH
- HBH easily oxidized
 - Risk for intravascular hemolysis
 - Occurs with oxidant stressors (infection, drugs)
 - Similar glucose-6-phosphate dehydrogenase deficiency



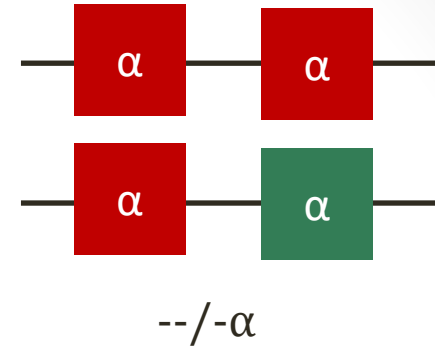
HbH Disease

- Diagnosis: **DNA testing**
- Electrophoresis insensitive
 - Some production A and A2
 - **May see HbH depending on amount**



HbH Disease

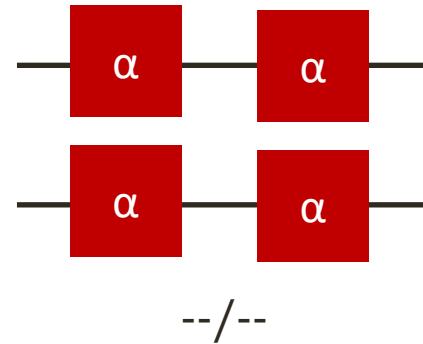
- Treatment:
 - Splenectomy
 - Transfusions
- Long term risk: **iron overload**



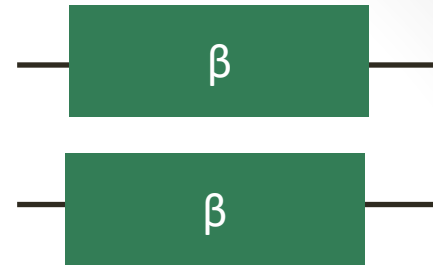
Public Domain

Hgb Barts

- **No α globin**
- Cannot form HbF
- Hgb Barts forms in utero
 - **Four gamma globin chains**
- Cannot release oxygen to tissues
 - Affinity for oxygen 10x HbA
- Hydrops fetalis
 - Massive total body edema
 - High output heart failure
- Fetal death usually occurs or death hours after birth

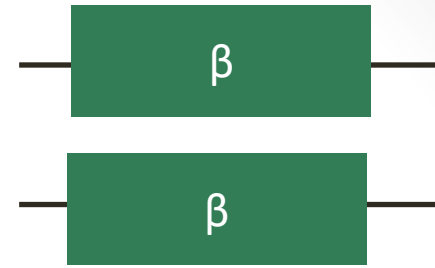


Beta Thalassemia

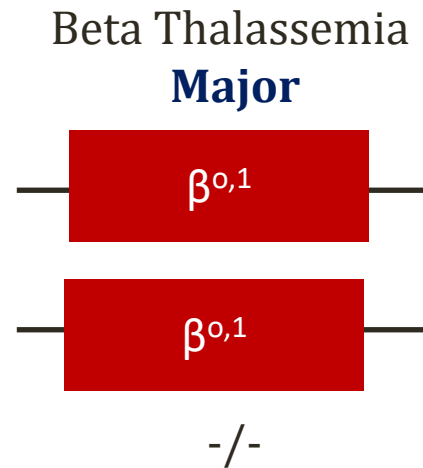
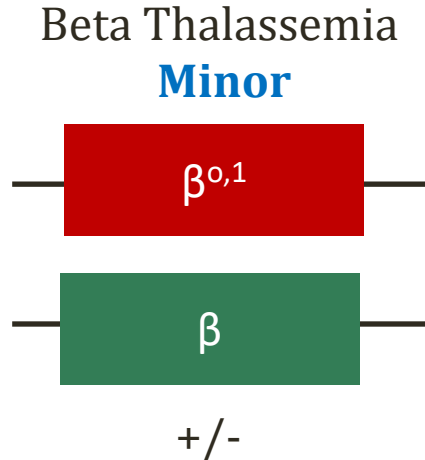


- \downarrow β globin chain synthesis
- **Two genes** code for beta chains
- One on each copy of chromosome 11

Beta Thalassemia



- Often caused by **mutations** (NOT deletions)
- Wide spectrum of disease depending on mutation
 - β^0 = no function; β^1 = some function



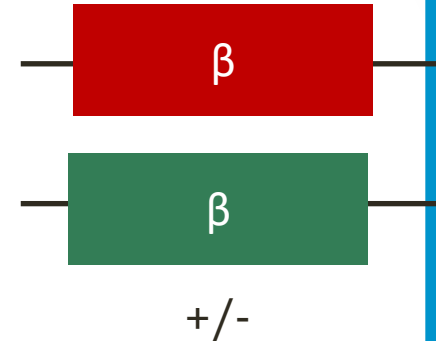
Beta Thalassemia



Beta Thalassemia Minor

- Also called beta thalassemia trait
- Heterozygotes: single abnormal gene
- Reduced β globin production
- **Asymptomatic**
- May see mild anemia on routine blood work
- Diagnosis by **electrophoresis**
 - \uparrow HgbA2 ($\alpha_2\delta_2$ – no beta chains)
 - Normal $<5\%$

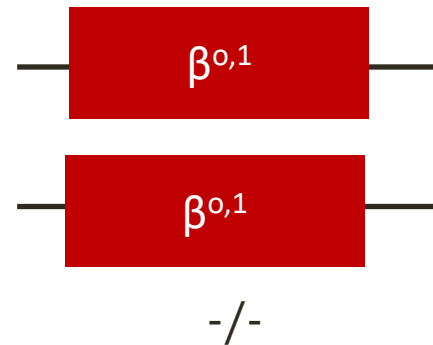
Beta Thalassemia
Minor



Beta Thalassemia Major

Cooley's Anemia

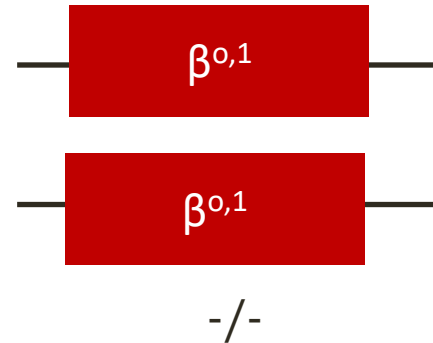
- No or severely limited β globin production
- Anemia beginning **1st year of life**
 - HgbF ($\alpha_2\gamma_2$) production wanes
- Ineffective erythropoiesis
 - Alpha chains form tetramers
 - Precipitate \rightarrow RBC damage
 - Failure to produce RBCs
- Splenomegaly
 - Spleen clears any abnormal RBCs in plasma



Beta Thalassemia Major

Cooley's Anemia

- Hypochromic, microcytic anemia
- Abnormal red blood cells shapes
- Erythroid hyperplasia
- Extramedullary hematopoiesis

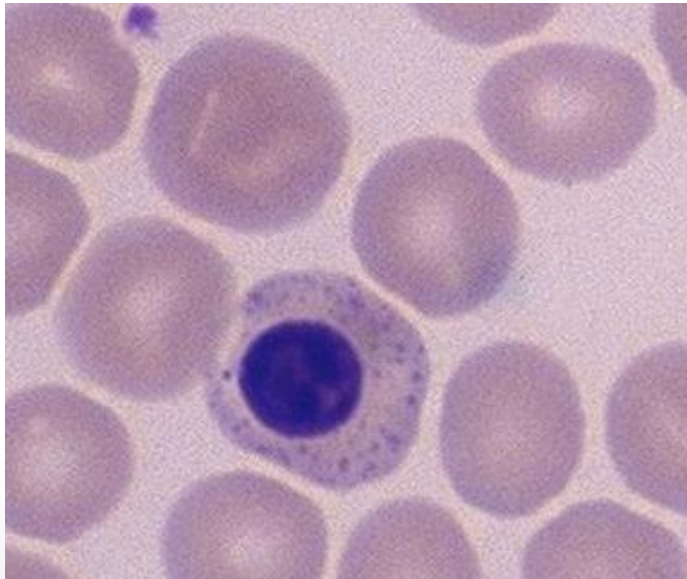


RBC Abnormalities

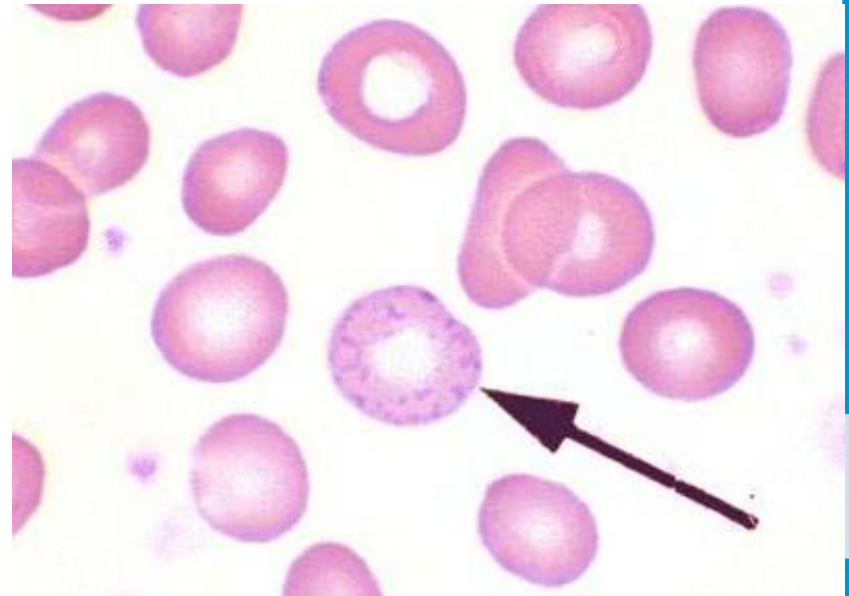
- Microcytosis (small RBCs)
- Hypochromia (loss of Hgb)
- Anisocytosis
 - Wide variation in sizes of RBCs
 - Increased red cell distribution width (RDW)
- Poikilocytosis (abnormal shapes)
- Basophilic stippling
- Nucleated RBCs
- Target cells

Basophilic Stippling

- Residual RNA in red cells
- Often seen with nucleated RBC
- Seen in thalassemia
- Also lead poisoning



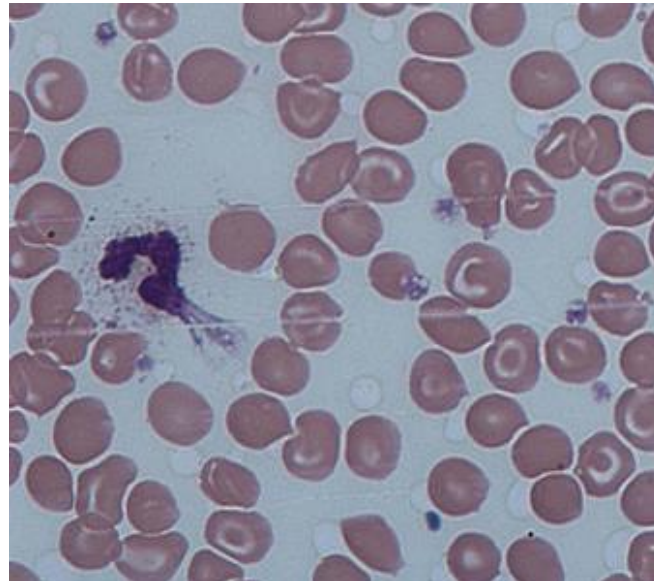
Prasad CSBR



isis325/Flickr

Target Cells

- Target formed in center of RBC
 - Small dark area in center of cell
- Due to **↑ surface area-to-volume ratio**
- Extra cell membrane → target appearance



Dr Graham Beards

Target Cells

- Decreased cell volume
 - **Thalassemia**
 - Can be seen in iron deficiency
- Increased cell membrane
 - Liver disease (↑ cholesterol in membrane)
 - Splenic dysfunction (↓ removal excess membranes)

Erythroid Hyperplasia

- Massive expansion of bone marrow
- ↑↑ EPO without normal response
- Consequence of severe anemia in beta major disease
- Abnormalities of skull and facial bones
 - “**Chipmunk facies**”
 - **Crew cut appearance** of skull on x-ray
- Delayed skeletal maturation
- Widening of marrow spaces → osteoporosis

Extramedullary hematopoiesis

- Hematopoiesis **outside of bone marrow**
- Consequence of severe anemia in beta major disease
- Liver and spleen produce RBCs
- **Hepatosplenomegaly**
- Often produces **nucleated RBCs**

Parvovirus B19

- Infection may cause aplastic crisis
- Beta major patients highly dependent bone marrow

B19

Beta Thalassemia Major

Cooley's Anemia

- Diagnosis: **Electrophoresis**
- Increased Hgb forms that do not require beta chains
- ↓ or absent HbA ($\alpha_2\beta_2$)
- ↑ HbA2 ($\alpha_2\delta_2$)
- ↑ HbF ($\alpha_2\gamma_2$)

Beta Thalassemia Major

Cooley's Anemia

- Treatment: **Blood transfusions**
- Long term risk: **iron overload**



Public Domain

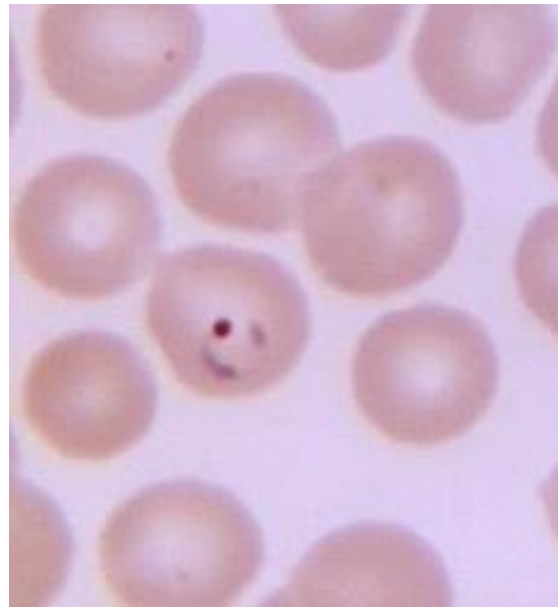
Beta Thalassemia Intermedia

- **Symptomatic** beta thalassemia
- Does not require transfusions
- Chronic hemolytic anemia
- Bone marrow expansion
- Hepatosplenomegaly

Malaria

- Alpha and beta thalassemia protective vs. malaria
- ↓ growth in RBCs of **plasmodium falciparum**

Trophozoite Ring

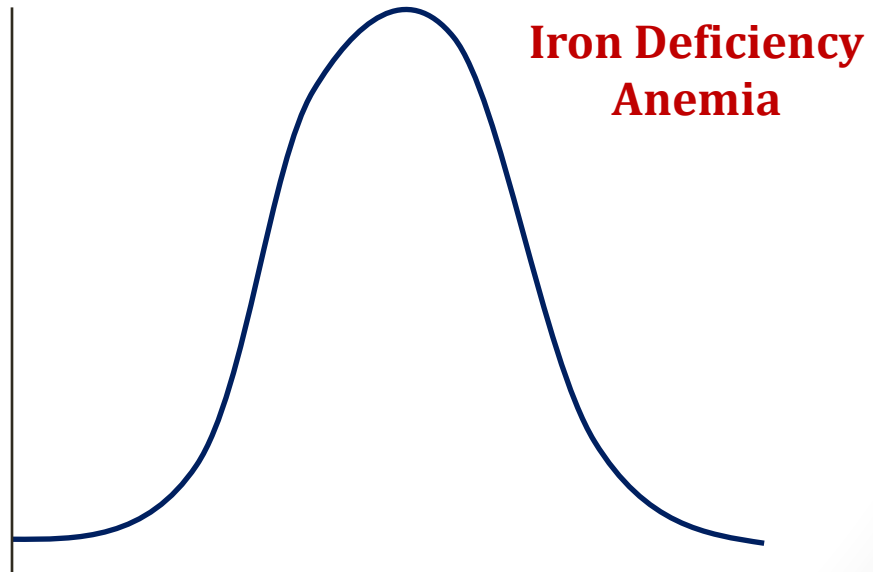
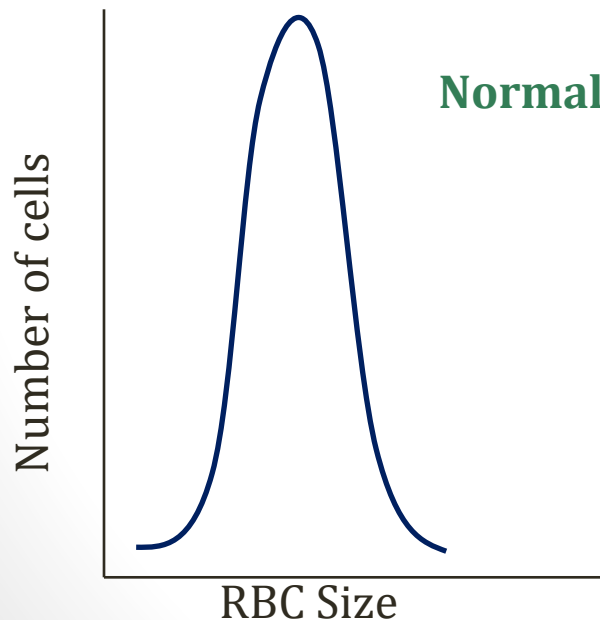


CDC/Public Domain

Red Cell Distribution Width

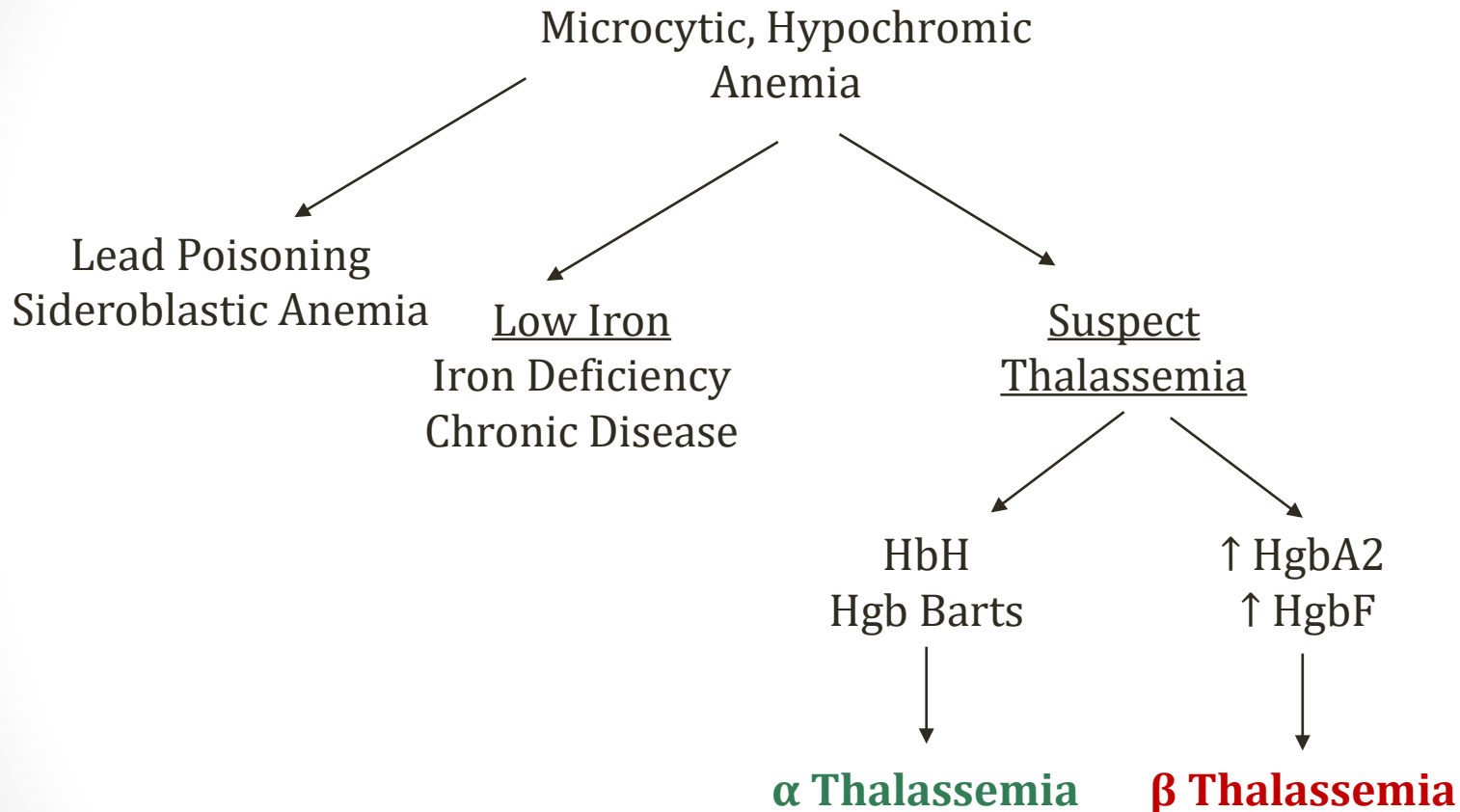
RDW

- Spectrum of RBC size
- Wider in **iron deficiency**
- Can be normal in mild thalassemia
- Normal RDW makes iron deficiency unlikely



Thalassemia

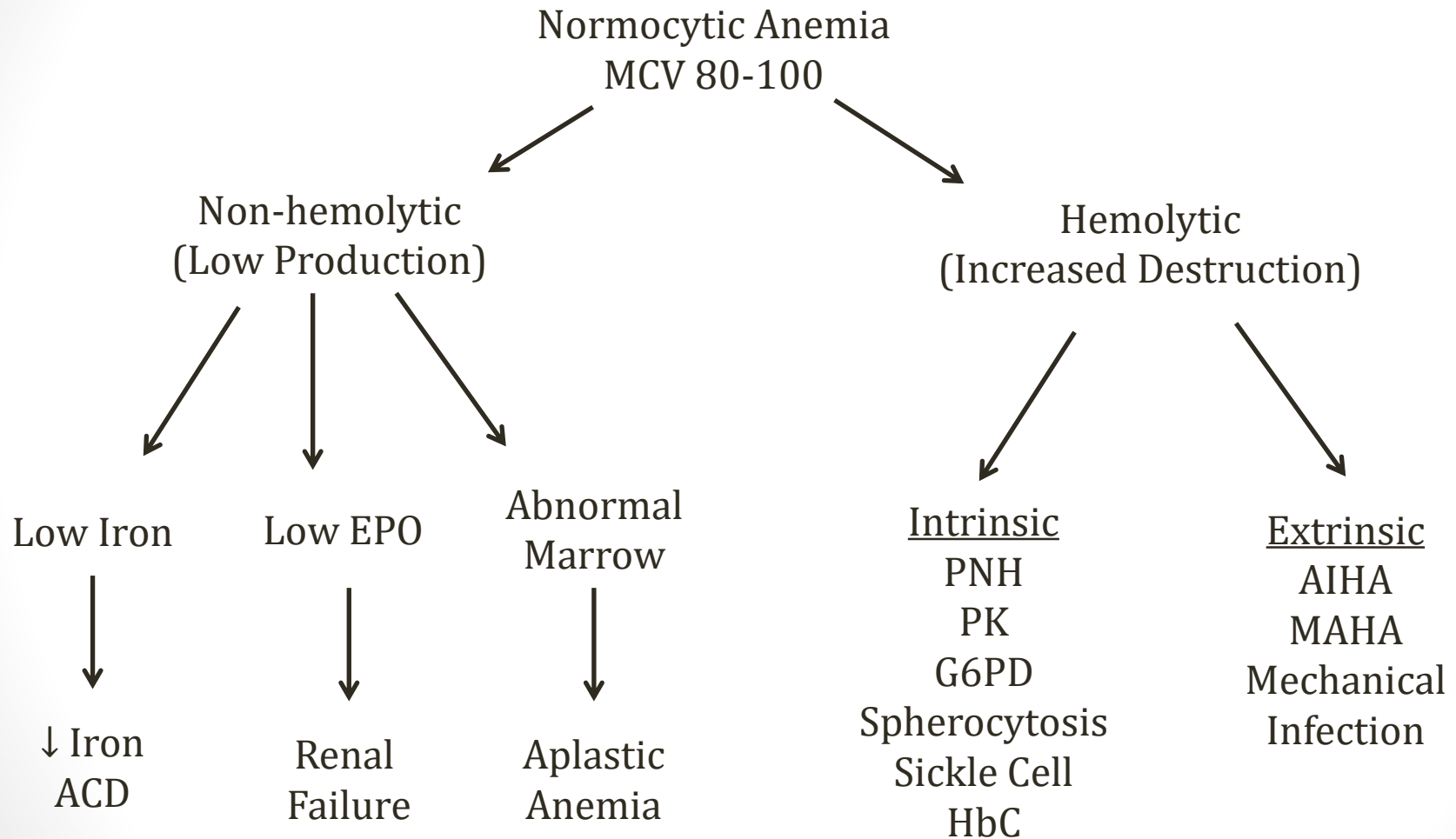
Key Points



Sickle Cell Anemia

Jason Ryan, MD, MPH

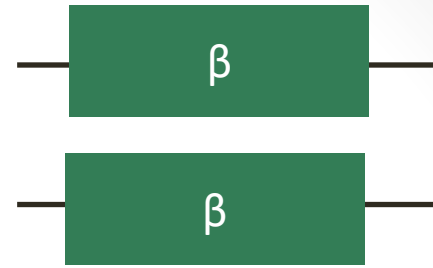
Normocytic Anemias



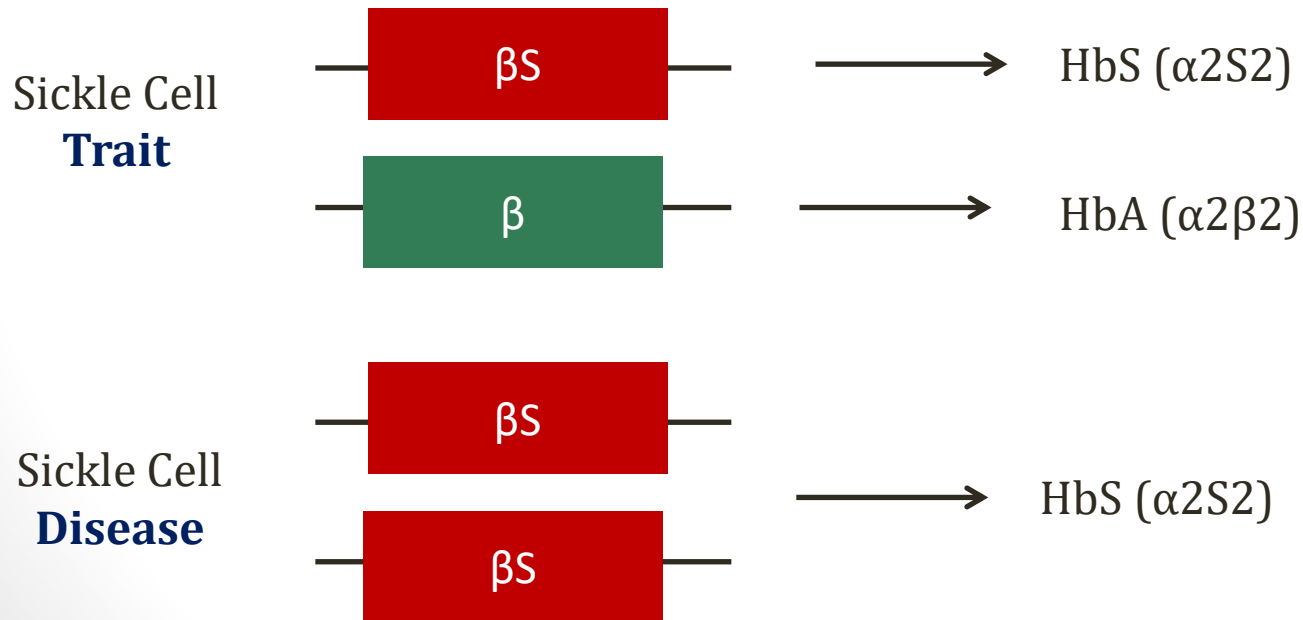
Sickle Cell Anemia

- Autosomal recessive disorder
- **Abnormal β hemoglobin chains**
 - Beta chains found in hemoglobin A ($\alpha_2 \beta_2$)
 - Makes up 95% of Hgb

Sickle Cell Anemia

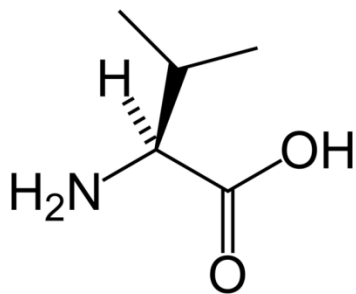


- Root cause is abnormal beta globin gene
- **Single base substitution** 6th codon of β gene
- Adenine changed to thymine
- Abnormal genes produce HbS

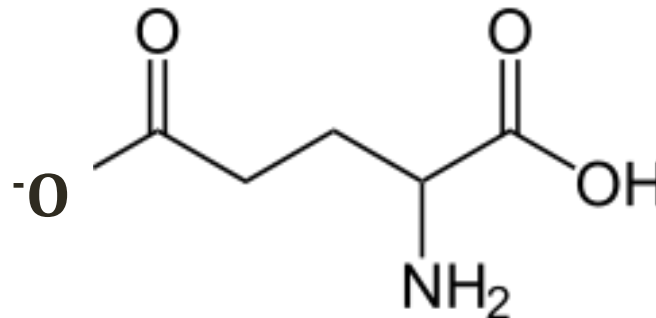


Sickle Cell Anemia

- Substitution of **valine** for **glutamate** in beta chains
 - Glutamate: polar (hydrophilic)
 - Valine: non-polar (hydrophobic)
- Alters shape of beta chains



Valine
(HbS)



Glutamate
(Normal Hgb)

Globins and Hemoglobin

Alpha (α)

Beta (β)

Delta (δ)

Gamma (γ)

Hemoglobin A

(95%)

$\alpha_2 \beta_2$

Hemoglobin A2

(<5%)

$\alpha_2 \delta_2$

Hemoglobin F

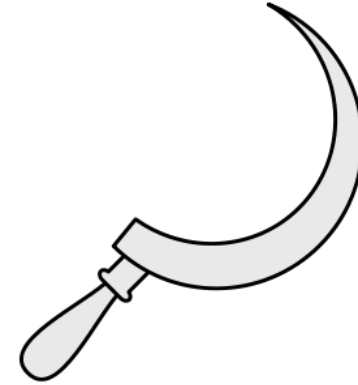
Fetal

$\alpha_2 \gamma_2$

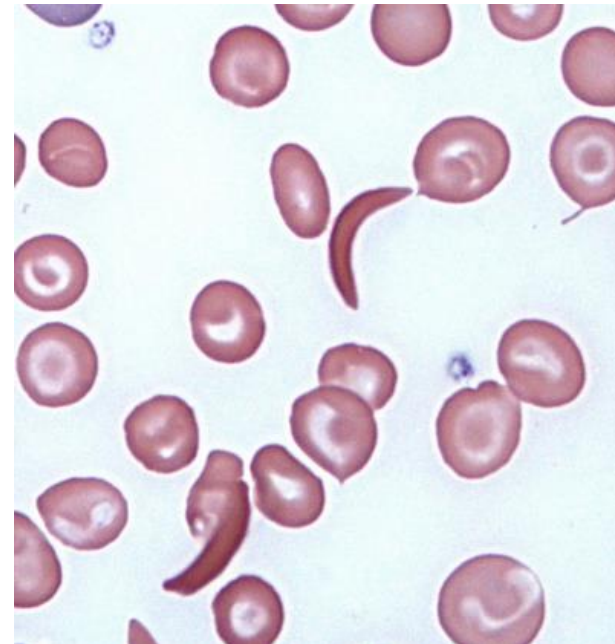
In utero and at birth: \uparrow HbF \downarrow HbA

Sickle Cell Anemia

- Deoxygenated HbS is poorly soluble
- **Polymerization** when O₂ low
 - Also in dehydration, acidosis
- Red blood cells form crescents
- Appearance of a sickle
- Causes a ↓ ESR



Madboy74



Scooterdmu/Flickr

Sickle Cell Anemia

- Two major problems result from sickle cells
- #1: **Hemolytic anemia**
- #2: **Vaso-occlusion of small blood vessels**

Sickle Cell Anemia

Hemolysis

- Sickling is reversible
- Cells continuously sickle/de-sickle in circulation
- Over time this leads to **RBC membrane damage**
- Results in **extravascular hemolysis**
 - Anemia
 - Jaundice
 - Elevated unconjugated bilirubin
 - Pigment gallstones
- Some intravascular hemolysis may also occur

Sickle Cell Anemia

Erythroid Hyperplasia

- ↑↑ EPO
- Massive expansion of bone marrow
- Consequence of severe anemia:
 - Also seen in beta thalassemia major
- Abnormalities of skull and facial bones
 - **“Chipmunk facies”**
 - **Crew cut appearance** of skull on x-ray
- Delayed skeletal maturation
- Widening of marrow spaces → osteoporosis

Sickle Cell Anemia

Parvovirus B19

- Infection may cause aplastic crisis
- Crisis also seen in spherocytosis, thalassemia

B19

Sickle Cell Anemia

Vaso-occlusion

- Sickle cells may **occlude microvasculature**
- May affect any organ
- Classic clinical manifestation:
 - Swollen hands (“dactylitis”)
 - Acute pain crises
 - Spleen failure → infections
 - Acute chest syndrome
 - Renal dysfunction

Sickle Cell Anemia

Dactylitis

- Pain/swelling in hands or feet
- Fingers may look like “sausage” digits
- Common initial symptom among children

Sickle Cell Anemia

Avascular Necrosis

- Bone collapse
- Most commonly **femoral head**
- Also associated with long term steroid use



Jmarchn/Wikipedia

Sickle Cell Anemia

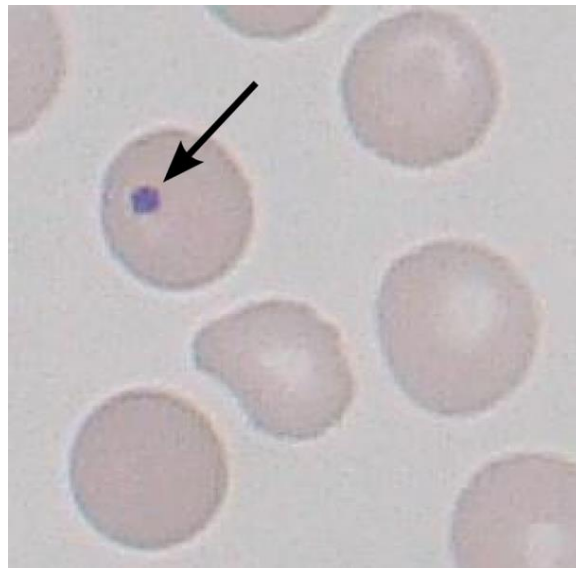
Pain Crises

- Episodes of acute pain (“sickle cell crisis”)
 - Sudden onset of pain
- Most common type of vaso-occlusive event
- May affect any part of body
 - Abdomen, bones, joints, soft tissue, fingers, toes
 - Swollen hands and/or feet especially in children
- Treatment: Hydration and pain medications

Sickle Cell Anemia

Splenic Failure

- Repeated splenic infarctions → functional asplenia
 - Early in disease: splenomegaly (macrophage hyperplasia)
 - Late in disease: Fibrosis and atrophy
- Howell-Jolly bodies will appear in peripheral blood



Paulo Henrique Orlandi Mourao /Mikael Häggström

Sickle Cell Anemia

Splenic Failure

- Increased risk of infections by encapsulated bacteria
- Strep pneumoniae and H influenza
 - Bacteremia/sepsis from S. Pneumoniae
 - Patients must be vaccinated
- Osteomyelitis from Salmonella species
 - Infection of infarcted bones
 - Most common cause SCA is Salmonella (usually S. Aureus)

Sickle Cell Anemia

Splenic Sequestration Crisis

- Vaso-occlusion in spleen → **pooling of red cells**
- Marked fall in hemoglobin level
- Rapidly enlarging spleen
- Risk of hypovolemic shock **especially in children**
 - Occurs in spleens yet to develop fibrosis
 - May occur before sickle cell disease is diagnosed

Sickle Cell Anemia

Chest Syndrome

- Leading cause of death in adults with SCD
- Vaso-occlusion of pulmonary microvasculature
- Often triggered by infection (pneumonia)
 - Increased sickling in lungs
 - Once begun → inflammation/acidosis → more sickling

Sickle Cell Anemia

Chest Syndrome

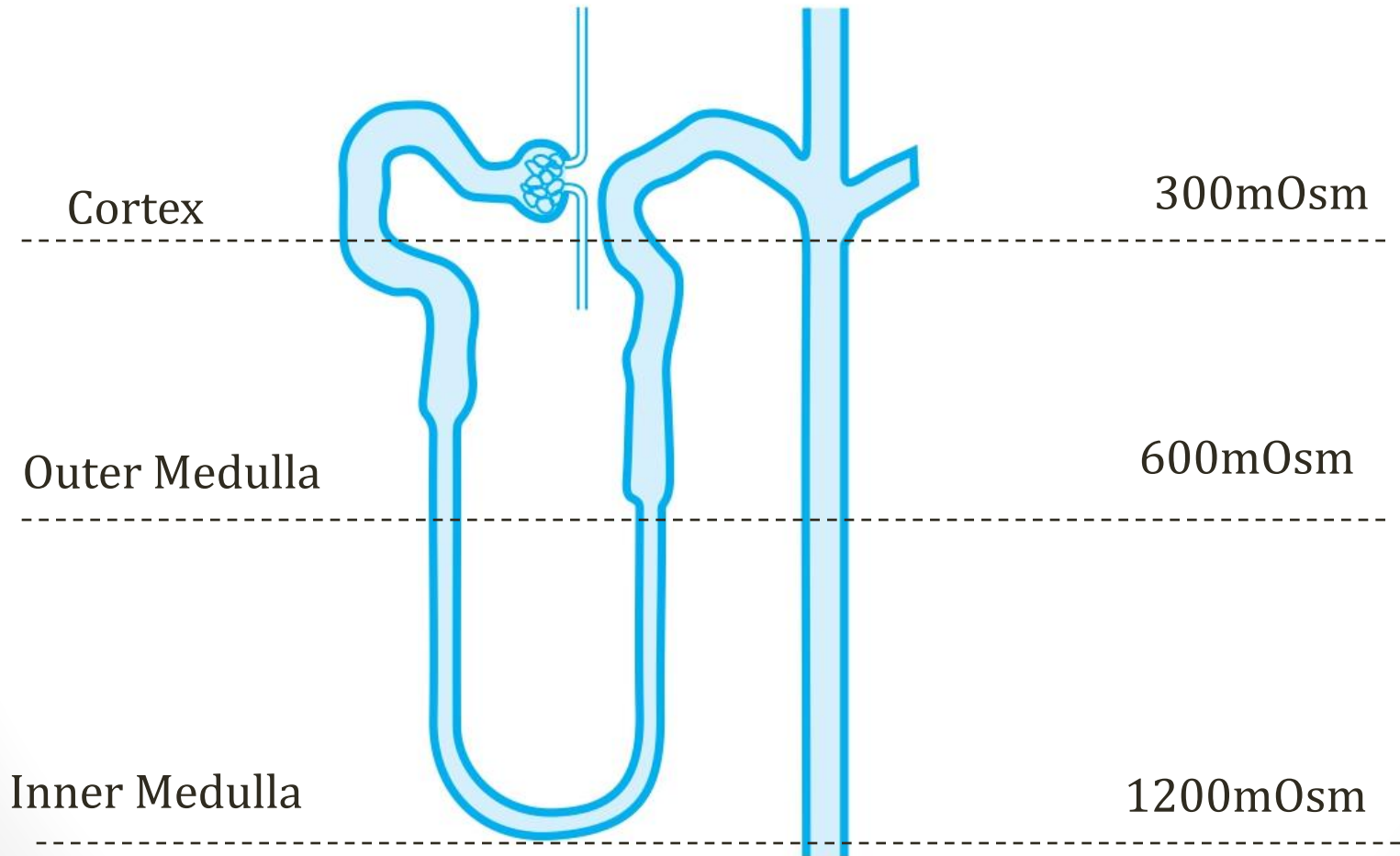
- Chest pain and shortness of breath
- Infiltrate on chest x-ray
- Looks like pneumonia
- Treatment:
 - Fluids and pain medication (similar to pain crisis)
 - Antibiotics, oxygen, bronchodilators
 - Transfusions as needed

Sickle Cell Anemia

Renal Dysfunction

- Occlusion of **vasa recta in renal medulla**
 - Medulla has low oxygen and high osmolality
 - Promotes sickling
- May impair concentrating ability
 - Cannot raise urine osmolality even with H₂O deprivation
 - Causes nocturia and polyuria

The Nephron



Sickle Cell Anemia

Renal Dysfunction

- Papillary necrosis
 - Sloughing of renal papilla due to renal vaso-occlusion
 - Usually painless
 - Gross hematuria and proteinuria

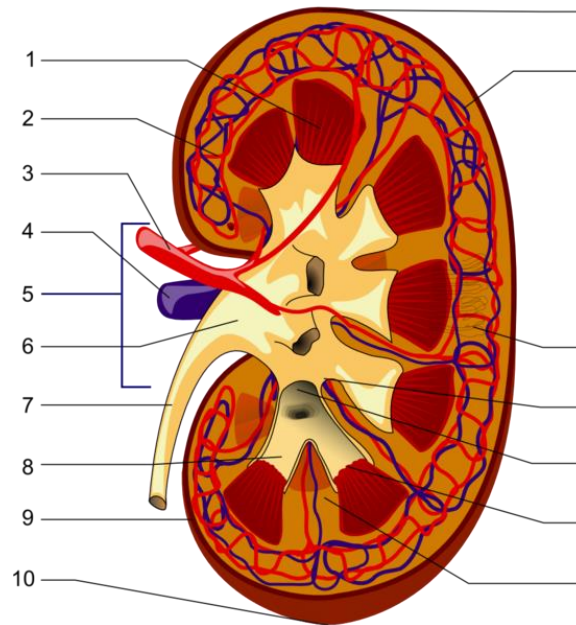


Image courtesy of Piotr Michał Jaworski

Sickle Cell Anemia

Treatment

- Immunizations
- Hydroxyurea
 - Raises amount of HbF
 - Mechanism unclear
- Transfusions
 - Iron overload may develop
- Bone marrow transplant is curative
- Median survival 42-48 years

Sickle Cell Trait

- One mutated beta globin gene
- Usually no sickling
 - Normal beta gene more effective → >50% beta globins
 - Need >50% HbS for sickling
- One exception: **Renal medulla**
 - May see loss of concentrating ability
 - ↑ risk of **renal medullary carcinoma** (> than sickle disease)



Sickle Cell Diagnosis

Disease or Trait

- Electrophoresis
 - Will show presence of HbS
 - Different amounts disease versus trait
- Sickling Test
 - Sodium metabisulphite reduces the oxygen tension
 - HbS becomes insoluble
 - Forms a turbid suspension → easily visualized
 - Other hemoglobin types remain in solution
 - Positive if any amount HbS present (disease or trait)

Electrophoresis

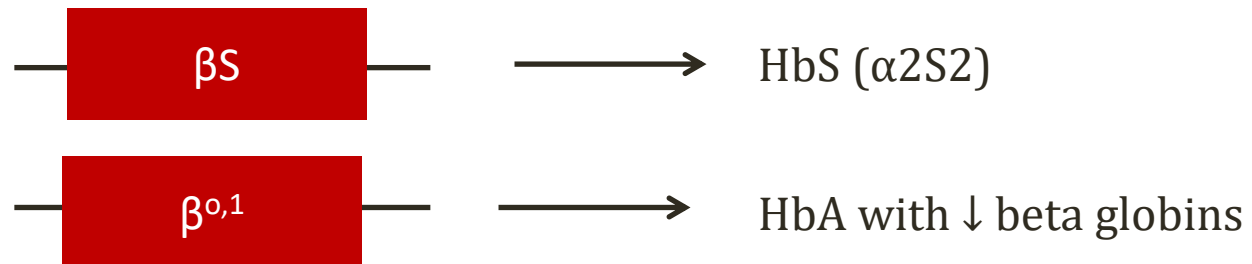
	Normal	Sickle Cell Disease	Sickle Cell Trait
HbA	97%	--	55%
HbA2	2%	2%	2%
HbF	1%	2-15%	1%
HbS	--	~90%	40%

Malaria

- Sickle trait **protective against p. falciparum**
 - Cells sickle when infected → ↑ clearance
 - **Does not protect against infection**
 - When infection does occur it is milder
 - Patients still need malaria prophylaxis
- African Americans: 8 to 10% have sickle cell trait
- Sub-Saharan Africa: ~30%

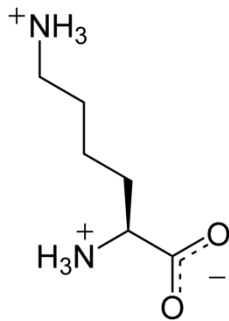
Sickle Cell/Beta Thalassemia

- One beta gene: sickle cell
- One beta gene: beta thalassemia
- Clinical manifestations similar to sickle cell
 - Vary depending on beta thalassemia gene function
 - β^0 : Similar to sickle cell disease
 - β^1 : Less severe

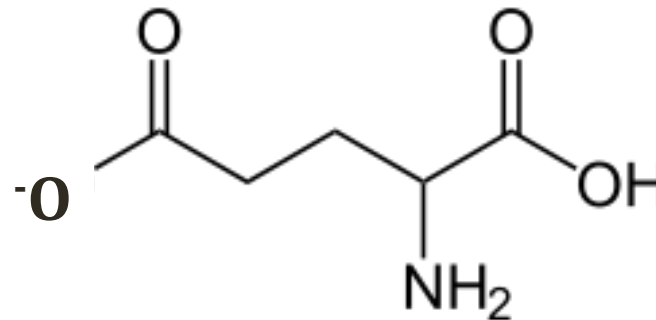


Hemoglobin C

- Rare mutation of beta gene (different from SCA)
- Glutamic acid replaced by lysine (not valine)
- Heterozygotes: Mild anemia (extravascular hemolysis)



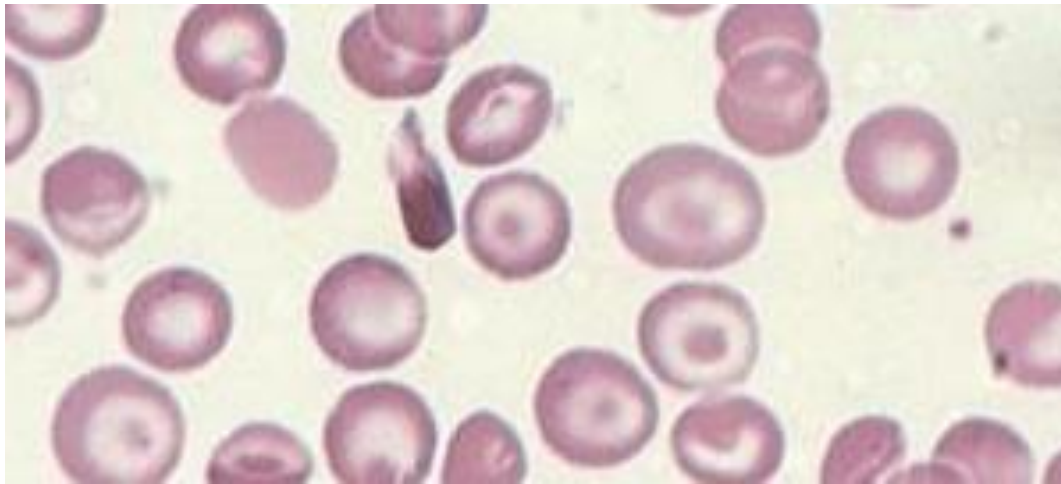
Lysine



Glutamate

Hemoglobin C

- Presence of **HbC crystals** on smear
- Induces red cell dehydration: **↑ MCHC**



Isis325/Flickr

Hemoglobin SC

- One HbS gene plus one HbC gene
- More common than homozygous HbC disease
- At risk for same complications as sickle cell disease
- Lower frequency of complications

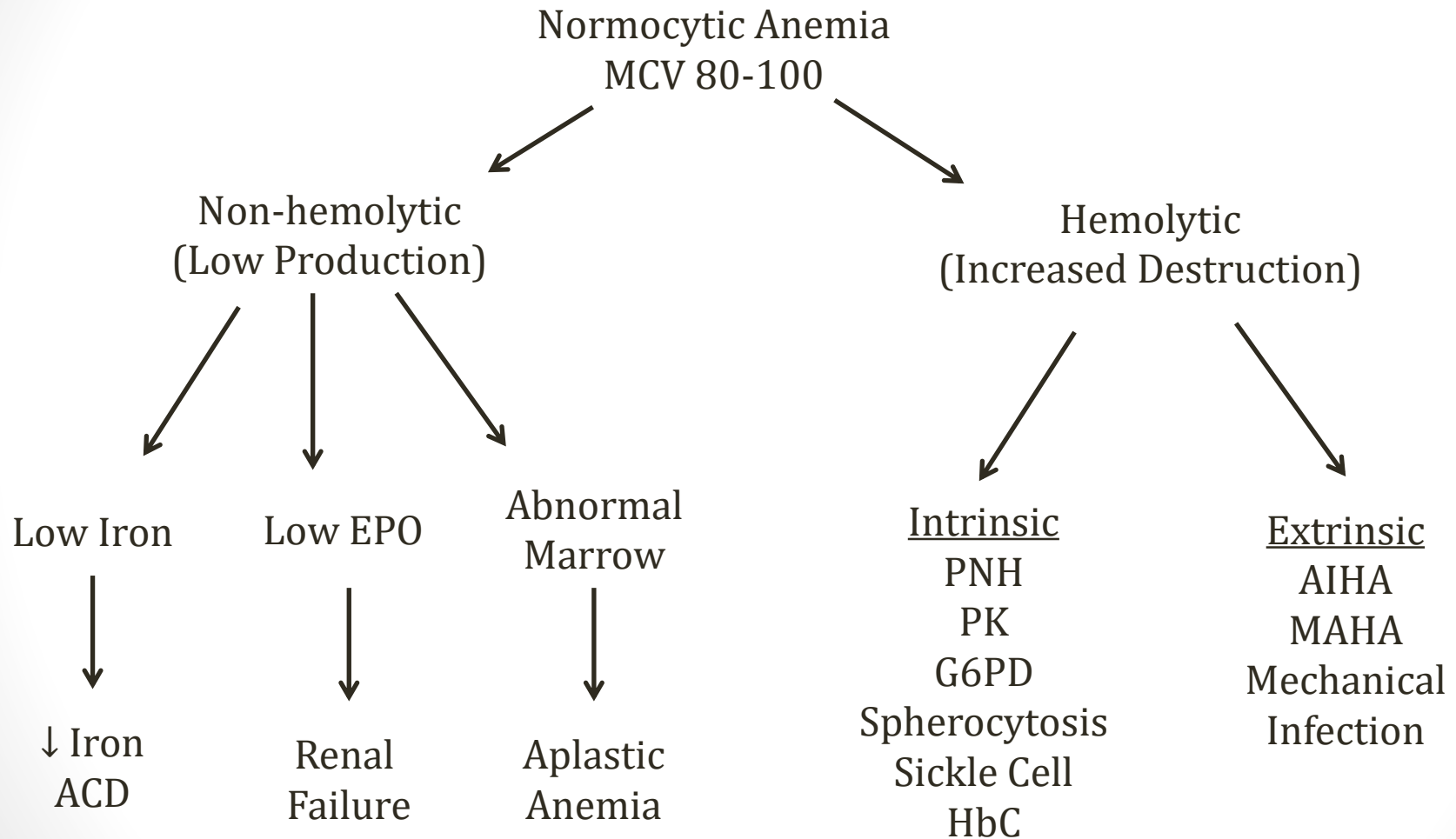
Other Anemias

Jason Ryan, MD, MPH

Anemias

- Microcytic
- Normocytic, hemolytic
- Normocytic, non-hemolytic
- Macrocytic

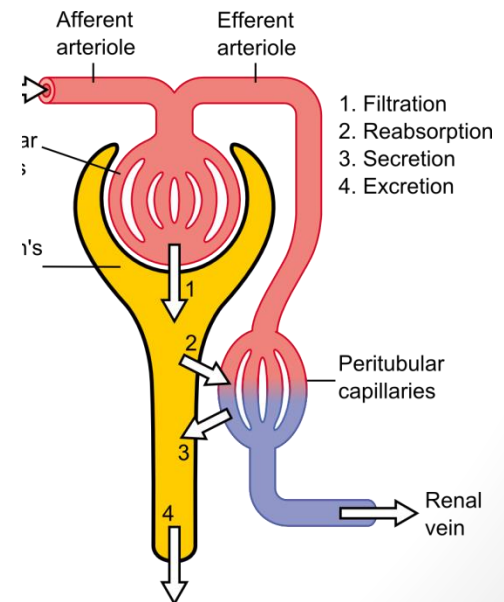
Normocytic Anemias



EPO

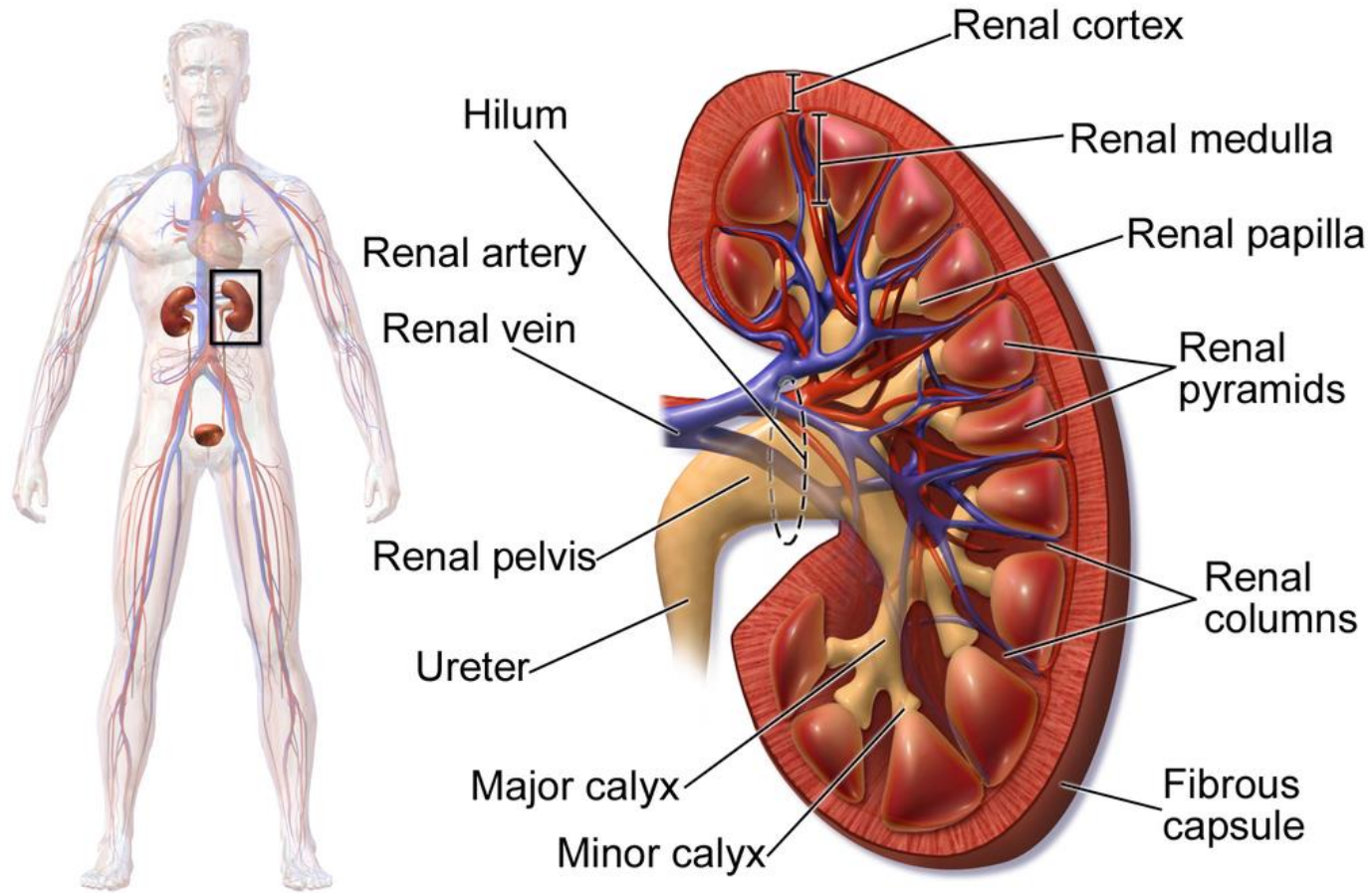
Erythropoietin

- Synthesized in the kidney
 - Interstitial cells peritubular capillary
 - Found in cortex of the kidney
- Released in response to hypoxia
- Decreased production in renal failure
- Results in a **normocytic anemia**



Madhero88/Wikipedia

Kidney Anatomy



Kidney Anatomy

EPO Injections

- Darbepoetin alfa (Aranesp)
- Epoetin alfa (Epogen)
- Used to treat anemia of chronic kidney disease
- FDA Black Box warning
- Generally reserved for patients with severe anemia

Aplastic Anemia

- **Loss of hematopoietic precursors** in bone marrow
- Results in pancytopenia
 - ↓ WBC, ↓ Platelets, ↓ RBC

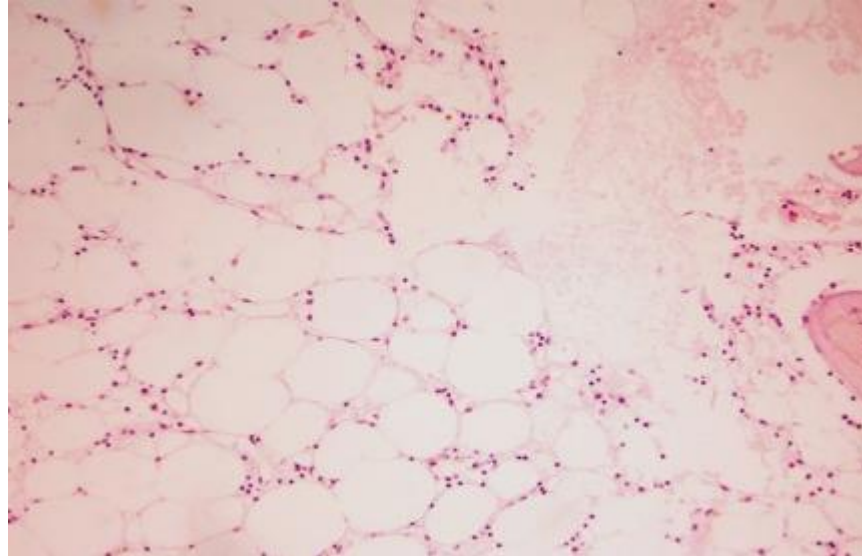
Vocabulary

- “Aplasia”: Defective or absent development
- Bone marrow failure
 - Bone marrow cannot produce cells
 - Results in pancytopenia
 - Many causes: fibrosis, tumors
 - “Myelophthisis:” displacement of bone-marrow tissue
- Aplastic anemia:
 - Specific type of bone marrow failure
 - Defective stem cells → acellular/hypocellular bone marrow

Aplastic Anemia

Hallmarks

- Pancytopenia
- Acellular or hypocellular bone marrow
 - Bone marrow biopsy for diagnosis
 - Absence of cells/replacement with fat



Chalin Drosera

Aplastic Anemia

Symptoms

- **Pancytopenia** (normal cells but not enough)
- Anemia
 - Fatigue, pallor
- Thrombocytopenia
 - Bleeding
- Leukopenia
 - Infections

Aplastic Anemia

Causes

- Most commonly **idiopathic**
- Radiation
- Drugs
- Viruses
- Inherited (Fanconi anemia)

Aplastic Anemia

Idiopathic

- Unknown trigger
- Strong evidence for **immune mediated mechanism**
- **T-cell mediated** destruction of stem cells

Aplastic Anemia

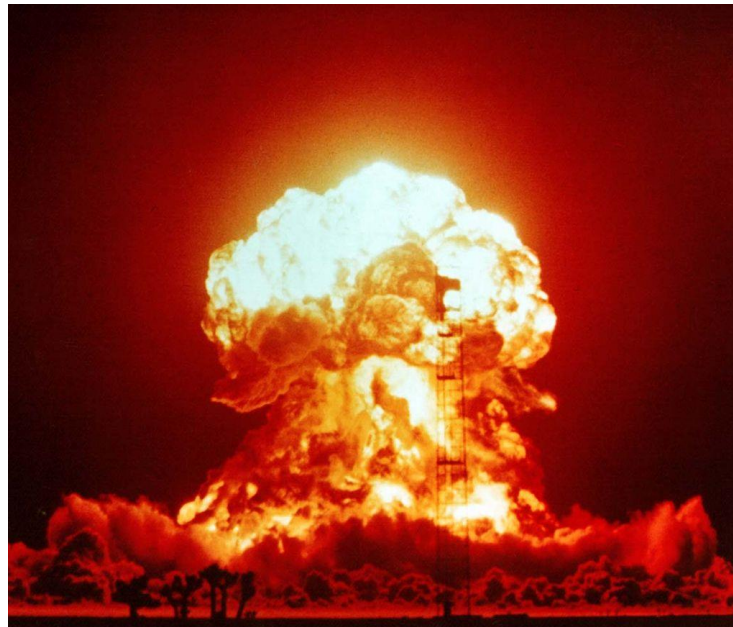
Idiopathic

- Can be treated with immunosuppression
- **Antithymocyte globulin**
 - Animal-derived antibodies against human T cells
 - Usually from rabbits or horses
 - Also can be used in kidney transplant patients
- **Cyclosporine**

Aplastic Anemia

Radiation

- Well-described cause of aplastic anemia
- Radiation → damage to stem cells → aplastic anemia

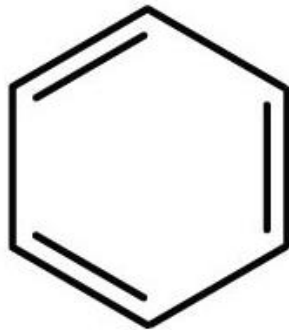


Public Domain

Aplastic Anemia

Chemicals

- **Benzene**: well-described cause of aplastic anemia
- Rubber factories, shoe repair shops
- Often with poor ventilation



Aplastic Anemia

Drugs

- Most cancer therapies
 - Anticipated effect
- **Chloramphenicol**
 - Rarely used antibiotic (bacterial protein synthesis inhibitor)
- Phenylbutazone
 - Old NSAID
 - Pulled from market due to cases of aplastic anemia
- **Methimazole, Propylthiouracil (PTU)**
 - Aplastic anemia cases reported (monitor WBCs)

Aplastic Anemia

Viruses

- **Parvovirus B19**
 - Infects proerythroblasts
 - Usually causes ↓ RBCs (“red cell aplasia”)
 - Pancytopenia can occur
 - ↑ risk: immunocompromised

Aplastic Anemia

Viruses

- **Acute Viral Hepatitis**
 - Can cause aplastic anemia
 - Reported after infection with HAV, HBV, HCV, HDV, and HEV
 - Often affects boys and young adult males
 - Aplasia develops weeks to months after acute hepatitis
- Others: HIV, EBV, CMV
- All probably NOT caused directly by virus
- Evidence suggests T-cell activation

Fanconi Anemia

- Inherited aplastic anemia
- Autosomal recessive or X-linked
- Usually presents in children <16 years old
- More than half of patients have **physical deformities**
 - Short stature
 - Cafe-au-lait spots
 - **Malformed thumbs**
 - Heart, renal, eye abnormalities described

Fanconi Anemia

- More than 13 genetic abnormalities identified
- Many involve **DNA repair enzymes**
 - Hypersensitivity to DNA damage
 - Especially vulnerable to abnormal DNA strand cross-links
- Increased risk of **malignancies**
 - Myelodysplastic syndrome (MDS)
 - Acute myeloid leukemia (AML)
 - Squamous cell carcinoma of head, neck or vulva

Aplastic Anemia

Treatment

- Stop offending agent
- Transfusions (red cells, platelets)
- Bone marrow stimulation
 - EPO, GM-CSF, G-CSF
- Immunosuppression
 - **Antithymocyte globulin**
 - **Cyclosporine**
- Bone marrow transplant

Pure Red Cell Aplasia

- Absence of erythroid precursors in bone marrow
- Marked reduction in reticulocytes
- Normal granulocytes, platelets
- Usually idiopathic
- Associated with some drugs, viral infections
- **Key association: Thymoma**
 - Present in 5 percent of PRCA cases

Macrocytic Anemias

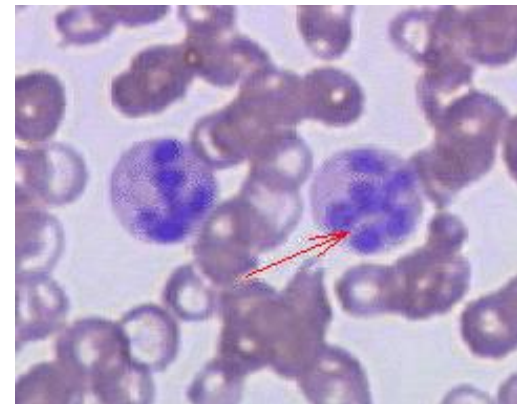
- MCV > 100
- Abnormal DNA synthesis
 - “Megaloblastic anemias”
- Other
 - Liver disease, alcohol, reticulocytosis

Megaloblastic anemias

- Red blood cell precursors grow but cannot divide
 - Contrast with microcytic anemias: divide too much
- Results from **abnormal DNA synthesis**
 - Cells cannot efficiently make DNA for cellular division

Megaloblastic Anemias

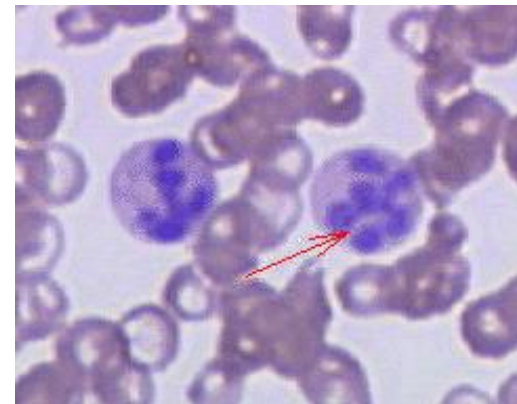
- Anemia (\downarrow Hct)
- Large RBCs (\uparrow MCV)
- **Hypersegmented neutrophils**
 - WBCs also cannot divide effectively due to \downarrow DNA synthesis
 - Result: hypersegmentation of nucleus (>5 lobes)



Wikipedia/Public Domain

Megaloblastic Anemias

- Causes of defective DNA production
 - **Folate deficiency**
 - **B12**
 - Orotic aciduria
 - Drugs (MTX, 5-FU, hydroxyurea)
 - Zidovudine (HIV NRTIs)

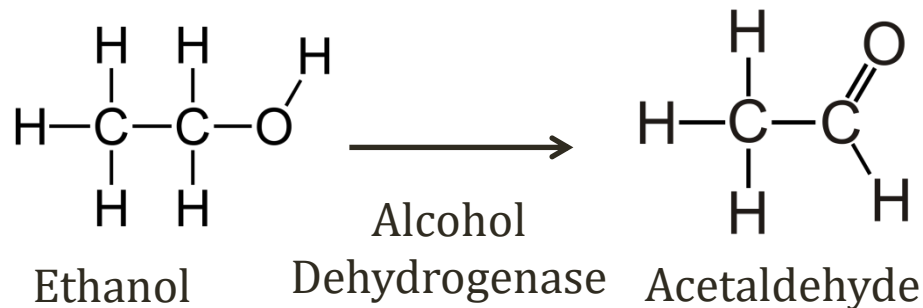


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Macrocytic Anemias

Non-megaloblastic

- Macrocytosis without impaired DNA synthesis
- **Liver disease**
 - Exact mechanism not known
 - Increased lipids seen in red cell membranes
- **Alcoholism**
 - Common cause of macrocytosis
 - Acetaldehyde may induce membrane changes in RBCs



Reticulocytosis

- Reticulocytes have MCV of 103 to 126fl
- Normal RBCs: 80 to 96 fL
- In theory **may cause macrocytosis**
 - But only about 20% bigger than normal cells
 - Need LOTS of reticulocytes to raise average MCV >100
 - Usually raise average MCV but should not reach >100

Blood Groups

Jason Ryan, MD, MPH

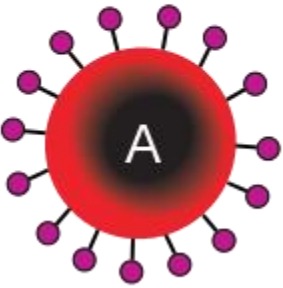
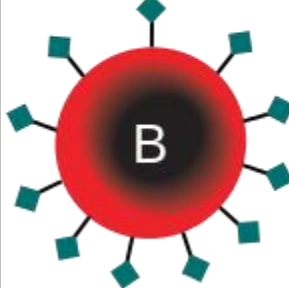
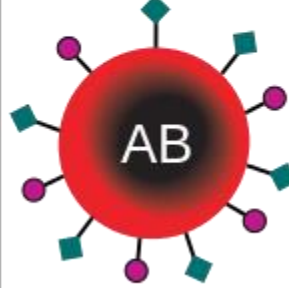
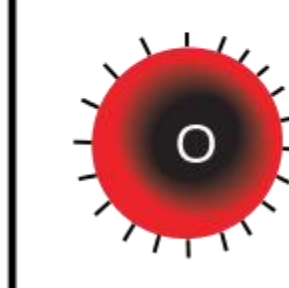
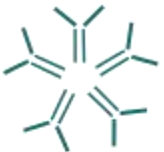

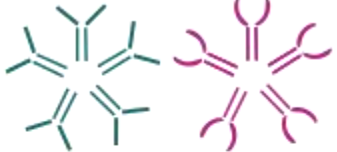



Blood Groups

- Antibodies form to RBC antigens
- “Blood group” defined by RBC antigens
- Important for safely administering blood transfusions
- Must match transfusion to “blood type”
- Two major blood groupings:
 - **ABO system**
 - **Rh system**

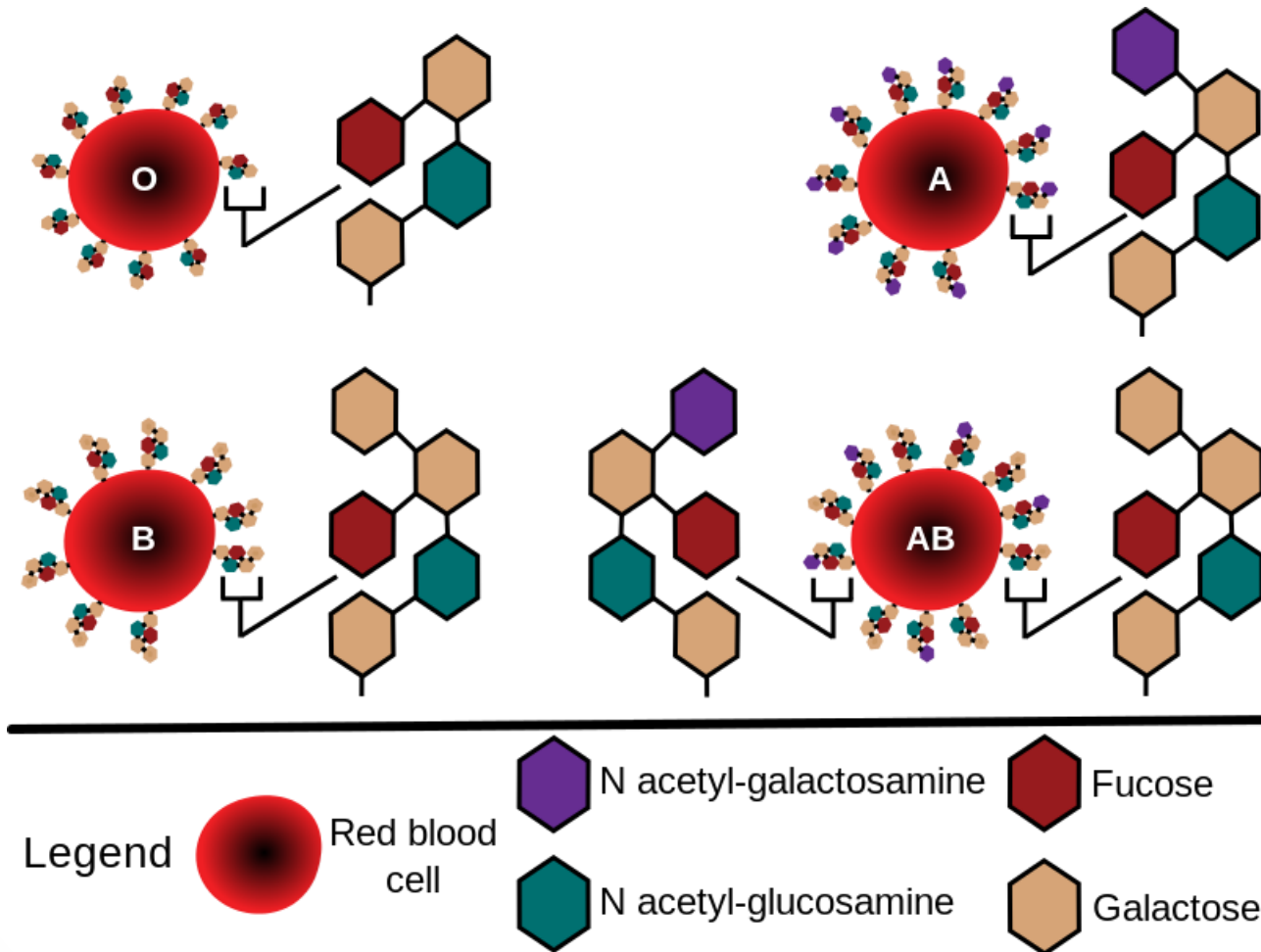
ABO System

- A and B antigens can be found on RBCs
- Patients who lack A or B generate antibodies
 - Appear in blood by 4-6 months
 - Exposure to **bacterial antigens** with similar structure
 - Occurs as the gut becomes colonized
- Antibodies: **IgM**
- Do not cross placenta
- Key point: A and B antibodies are **naturally occurring**

ABO System

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	 A antigen	 B antigen	 A and B antigens	None

ABO System



InvictaHOG/Wikipedia/Public Domain

Rh System

- Most important blood group system after ABO
- More than 50 antigens are part of Rh system
 - Names for letters following AB: C, D, E
- All are **transmembrane proteins**

Rh System

- **Presence/absence of D antigen is critical**
 - D antigen **highly immunogenic**
 - “Rh positive:” has the D antigen (of the Rh system)
 - “Rh negative:” lacks the D antigen (of the Rh system)
- Other Rh antigens not routinely tested: C, c, E, e

Rh System

- **Rh positivity is common**
 - Caucasians: 83%
 - Some Asian populations: 98%
- Rh negative may develop anti-D antibodies
- Only happens if **exposed to D⁺ RBC**
 - Transfusion
 - Pregnancy (Mom D⁻ with baby D⁺)
- Anti-D antibodies: **IgG**
- May cross placenta

Newborn Hemolytic Disease

- Classically caused by **anti-D (anti-Rh) antibodies**
- Can only occur in **D⁻ mother with D⁺ baby**
- D⁻ mother capable of developing anti-D antibodies
- If father is D⁺: baby may also be D⁺

Newborn Hemolytic Disease

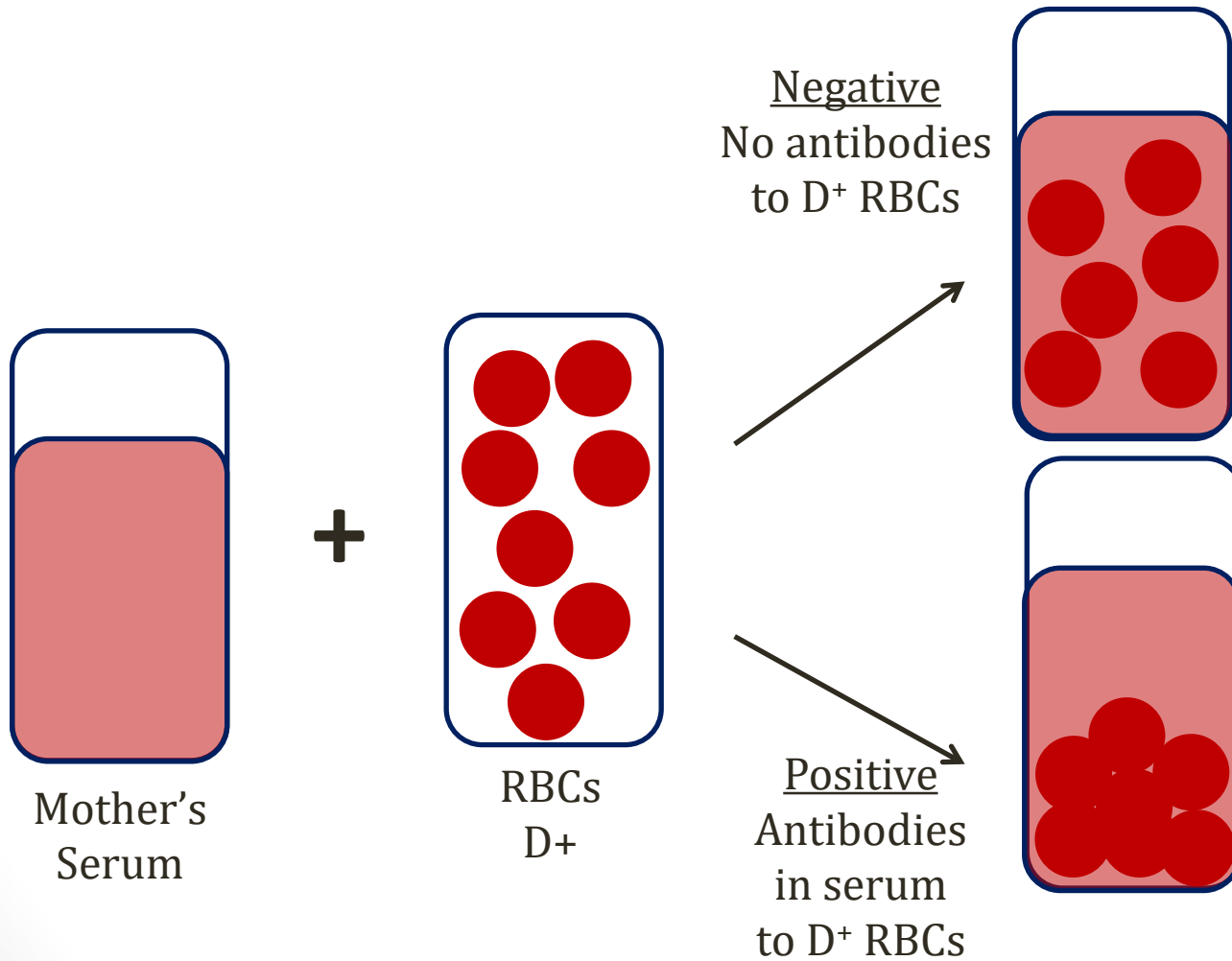
- First pregnancy: Mother exposed D⁺ RBCs **at delivery**
- 2nd pregnancy: Anti-D IgG in mother → fetus
- If 2nd baby also D⁺ hemolysis will occur in utero

Newborn Hemolytic Disease

- Mild cases present as hemolytic anemia
- Severe cases: **Hydrops fetalis**
- Massive **edema**: Pleural/pericardial effusion, ascites
- Mechanisms:
 - High-output congestive heart failure
 - ↑ RBC production by spleen/liver → obstruction
 - Results in portal hypertension
- Seen in other severe anemias of newborns
 - Hgb Barts (lack of alpha globins)

Maternal Antibody Screening

Indirect Coombs Test

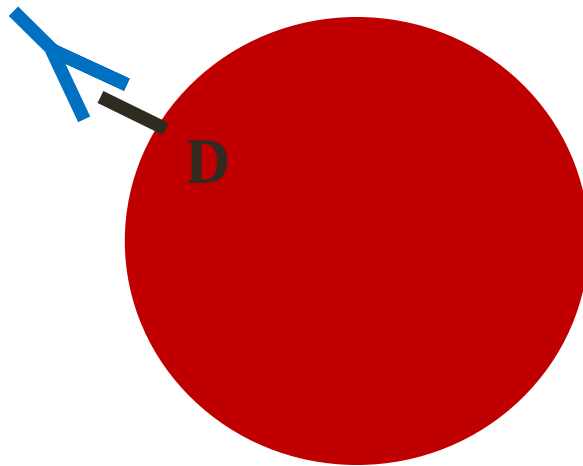


"Isoimmunization"

Newborn Hemolytic Disease

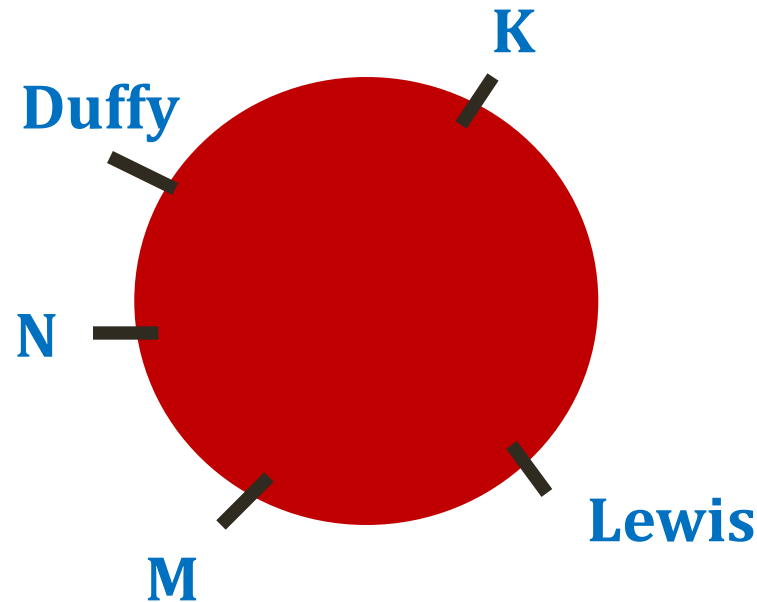
Prevention

- Anti-D immune globulin (“RhoGAM”)
- IgG **antibodies to D antigen**
- Rapid macrophage clearance of D⁺ RBCs
- Given in 3rd trimester to D⁻ women
- Blocks/prevents isoimmunization



Other Antigens

- Only ABO and Rh routinely tested
- Many other antigens on RBCs
- Only tested when patient has abnormal screening test
- Antibodies from **pregnancy or transfusion**



Transfusion Medicine

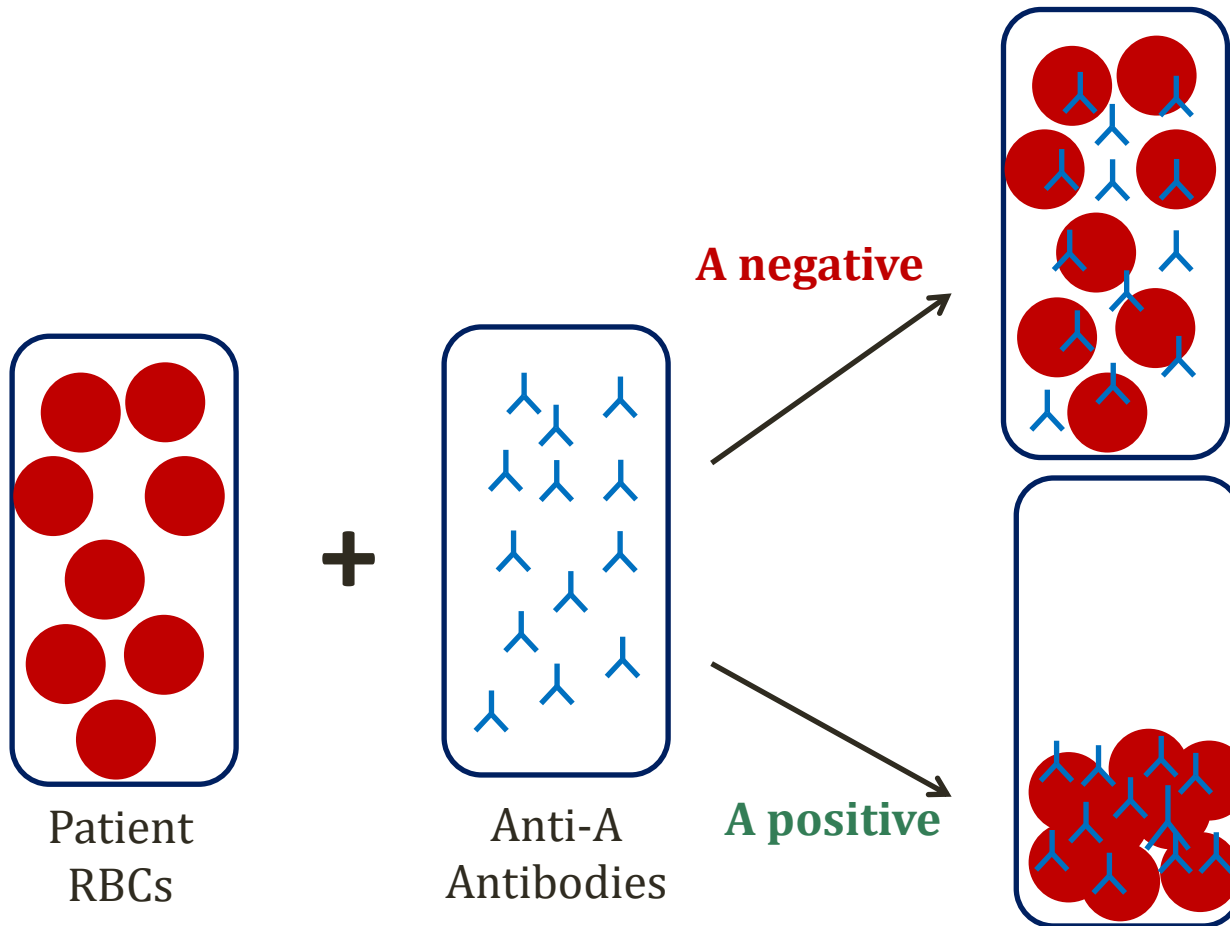
Common Tests

- Blood type (usually done with another test)
- Type and screen
 - Antibody screening test
 - Further testing if positive
- Type and crossmatch (“type and cross”)
 - Matching of donor blood to patient

Blood Type Testing

- **Patient RBCs** plus antibodies
 - Anti-A; Anti-B; Anti-D
- **Agglutination** indicates presence of antigen

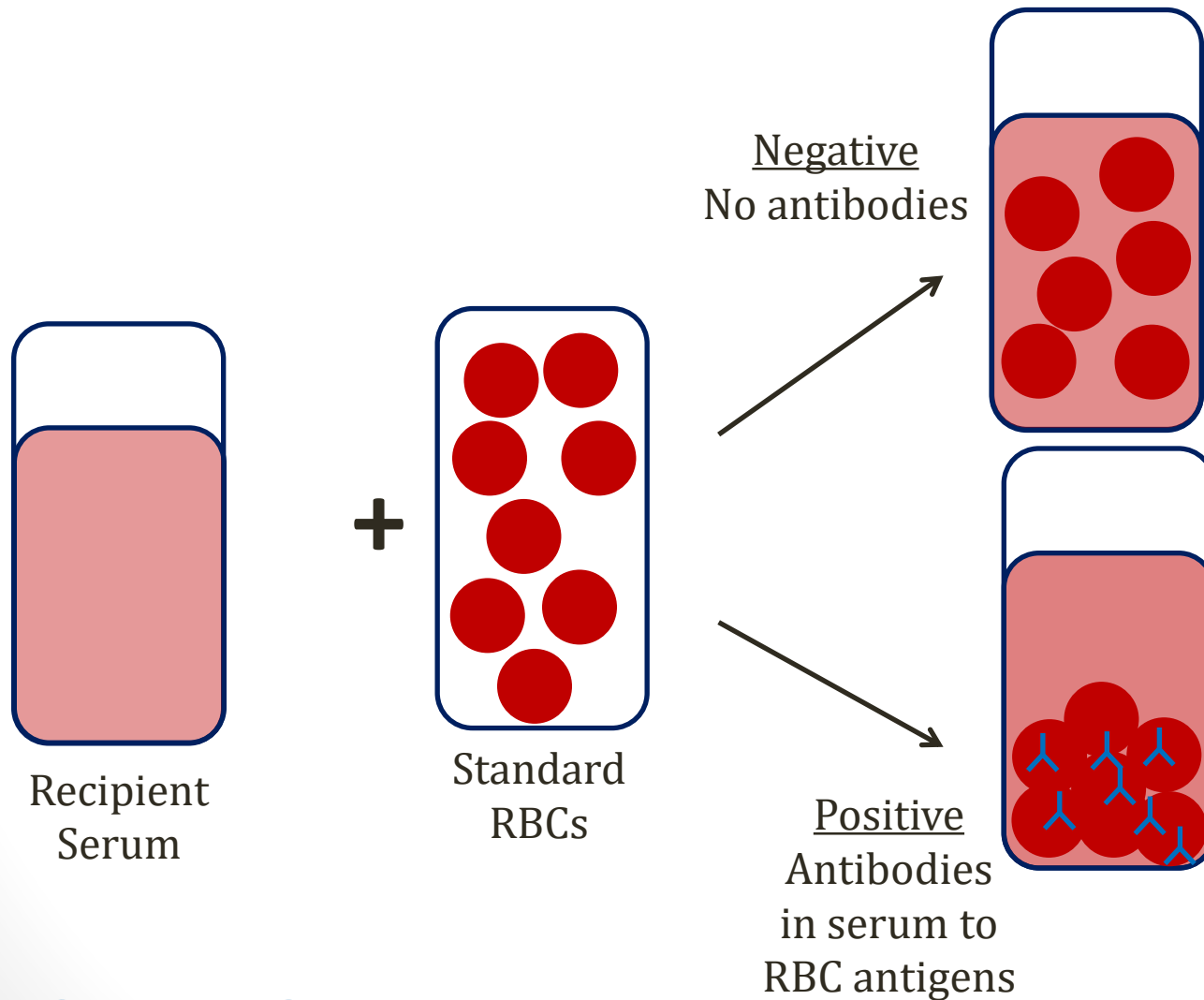
Blood Type Testing



Type and Screen

- **Recipient serum** plus standard RBCs
- Screen for patient antibodies to rare antigens
- Will only have antibodies if prior exposure
- **Reagent RBCs** contain many RBC antigens
 - No agglutination: Patient lacks antibodies
 - Agglutination: Antibodies to less common antigens present

Type and Screen

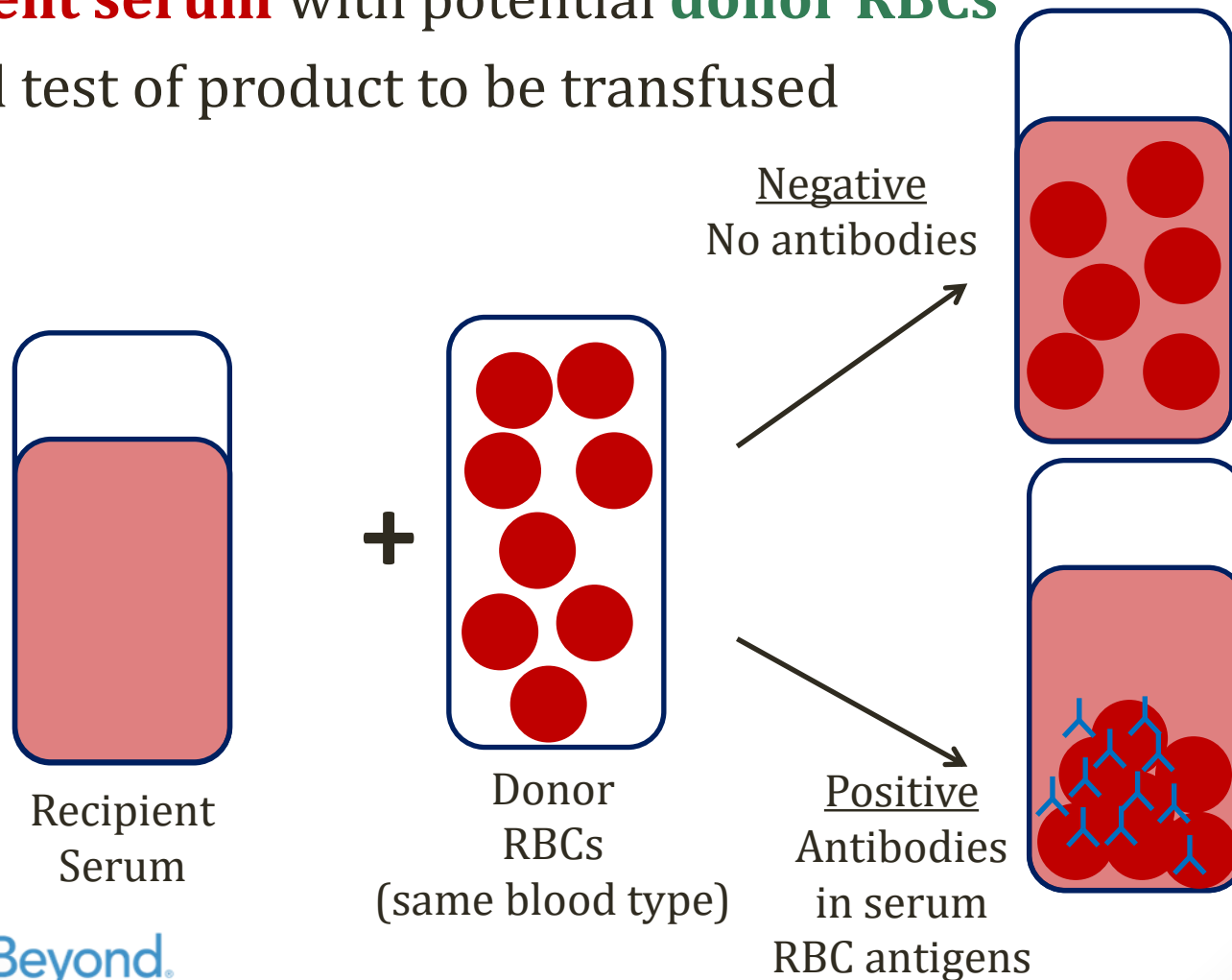


Abnormal Screen

- Determine **which antibody is present**
- Test patient's serum against large panel of antigens
- Subsequent transfusions: Test donor blood for antigen
- Challenging in patients with long transfusion history
 - Sickle cell anemia
 - Beta thalassemia major
- Key point: **Don't transfuse unless necessary**

Type and Cross

- **Patient serum** with potential **donor RBCs**
- Final test of product to be transfused



Blood Products

- **Packed RBCs**
 - RBCs with plasma removed
 - Usually administered instead of “whole blood”
 - Minimizes volume given to patient
- **Platelets**
 - Express ABO and HLA class I antigens
 - Do not express Rh or HLA class II
 - Reactions from mismatch less common than with RBCs

Blood Products

- **Fresh Frozen Plasma (FFP)**
 - Plasma after removal of RBC, WBC, and platelets
 - Frozen for storage
 - Once thawed, must be used within 24hrs
 - Clotting factors degrade
 - Corrects deficiencies of any clotting factor
 - PT/PTT will normalize after infusion
- **Cryoprecipitate**
 - Precipitate that forms when FFP is thawed
 - Contains lots of **fibrinogen**
 - Massive bleeding or rare ↓ fibrinogen disorders

Transfusion Reactions

- Acute hemolytic reaction
- Anaphylaxis
- Febrile reaction
- TRALI
- Many, many other potential reactions
 - Heart failure
 - Sepsis

AHTR

Acute hemolytic transfusion reaction

- Feared complication of blood transfusion
- Pre-formed antibodies → donor RBCs
 - **Type II hypersensitivity reaction**
 - Usually from transfusion of **incorrect blood product**

AHTR

Acute hemolytic transfusion reaction

- Life-threatening reaction
- **Acute hemolysis** of transfused RBCs
 - Intravascular (complement; anti-AB are IgM)
 - Extravascular (spleen)
- Can lead to DIC
- Fever, chills, flank pain, oozing from intravenous sites
- Jaundice, elevated bilirubin → dark urine
- Direct antiglobulin test (Coombs) will be positive

AHTR

Acute hemolytic transfusion reaction

- Usual cause: **system or clerical error**
- Transfusion of wrong blood product
- Numerous safety measures used to prevent:
 - Blood type, antibody screen, cross match
 - Careful patient identification

Anaphylaxis

- Allergic reaction (type I hypersensitivity)
- Hives, angioedema, wheezing, hypotension
- May occur in **IgA-deficient individuals**
 - Produce anti-IgA antibodies
 - React with IgA in transfused product
- Also occurs to plasma proteins in transfused product
- Treatment:
 - Stop transfusion
 - Epinephrine, anti-histamines

FNHTR

Febrile non-hemolytic transfusion reaction

- Fever, chills
- No other systemic symptoms
- Caused by **cytokines** in blood products
 - Especially IL-1
 - Generated by WBCs during storage
 - Accumulate in stored blood components
- Some blood products undergo “**leukoreduction**”

TRALI

Transfusion-related acute lung injury

- Sudden onset hypoxemia during transfusion
- Inflammatory reaction: Fever, chills are common
- Infiltrates on chest x-ray
- Results from **neutrophil activation** by blood products
 - Some patients predisposed with PMNs in lungs
 - PMNs release cytokines, reactive oxygen species, enzymes
 - Damage the pulmonary capillary endothelium
 - Exudative fluid loss → pulmonary edema

Transfusion Reaction

- Any suspected reaction: **STOP TRANSFUSION**



Acute Leukemias

Jason Ryan, MD, MPH

Leukemia

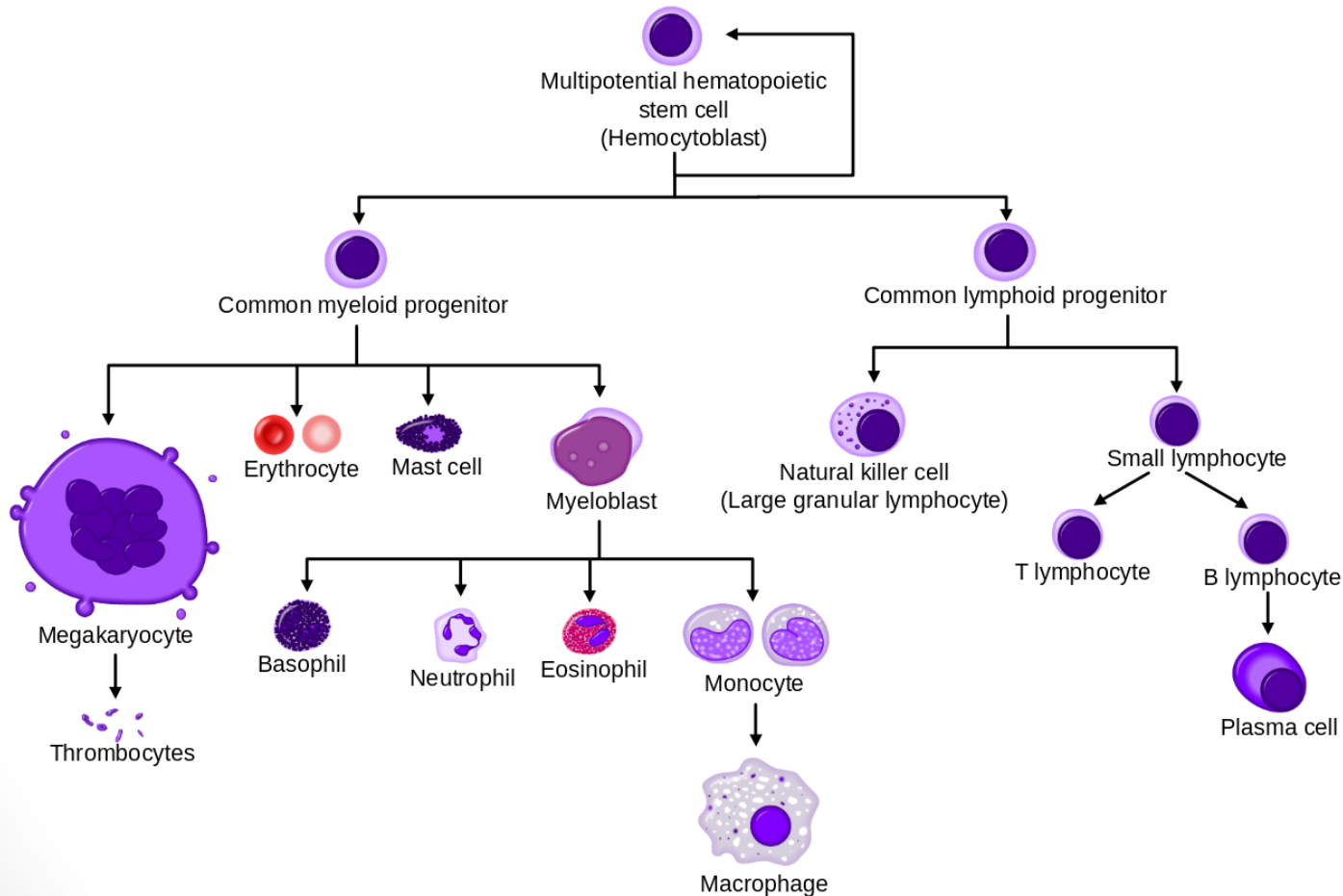
- Malignant proliferation of white blood cells
- Cells appear in blood (contrast with lymphoma)
- **Increased WBC**

Leukemias

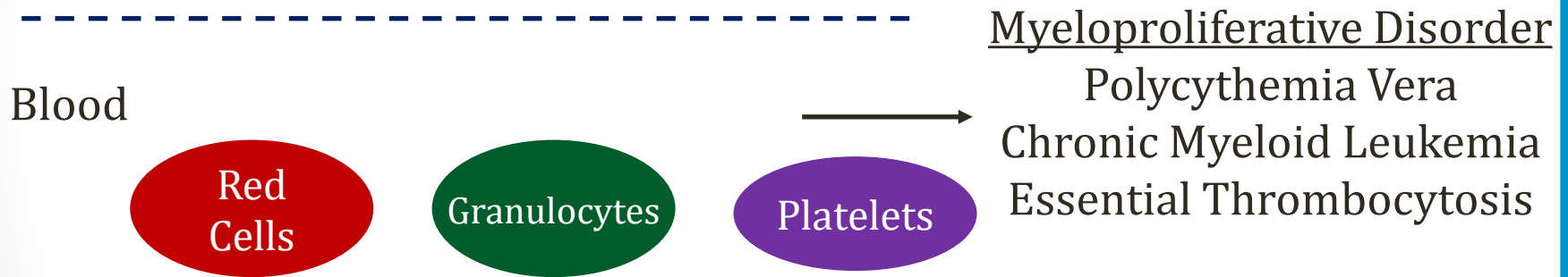
Classification

- Myeloid versus lymphoid
- Acute versus chronic
- Acute
 - Rapid onset of symptoms
 - Involves **blasts** in bone marrow
- Chronic
 - Slower onset of symptoms (or no symptoms)
 - Malignant cells are not blasts (more mature)

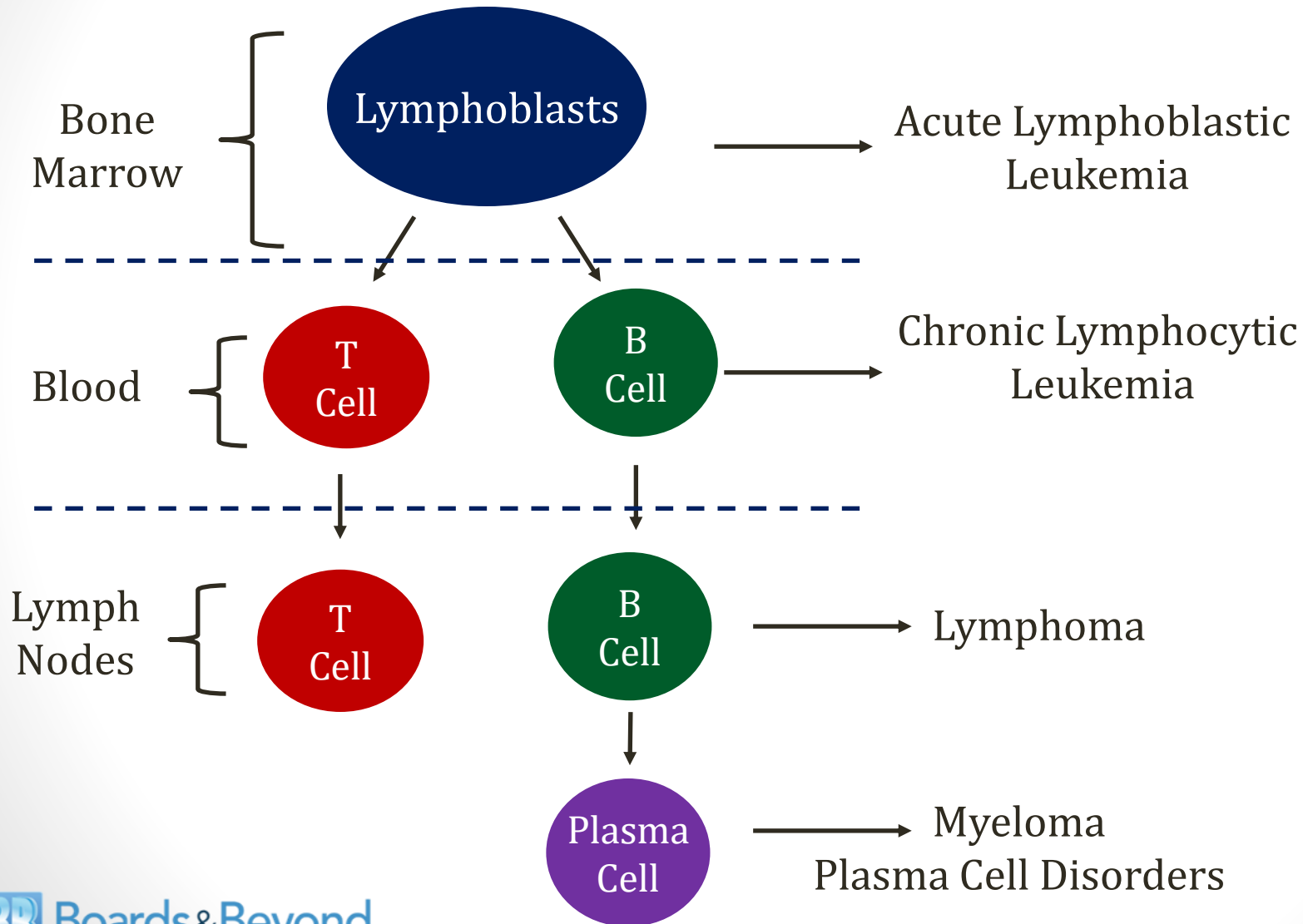
Hematopoiesis



Myeloid Disorders



Lymphoid Disorders



ALL

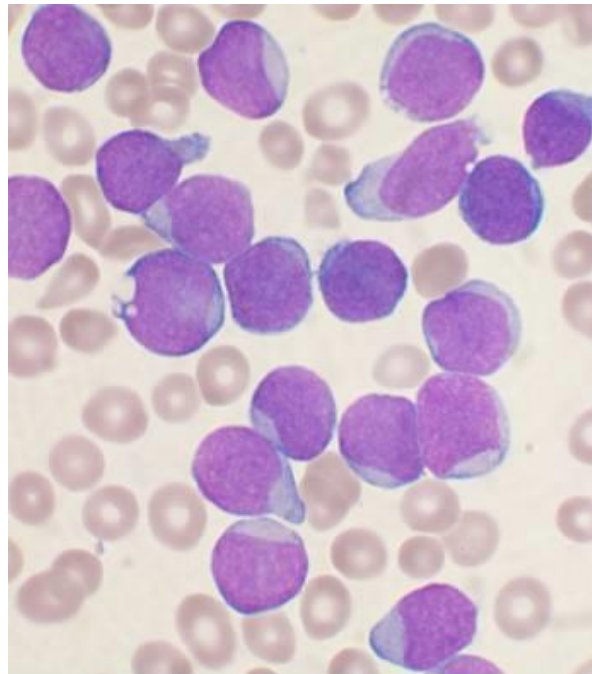
Acute Lymphoblastic Leukemia

- Disease of children
 - Peak incidence ~ 4 years old
- Fever
- **Bone pain** (marrow expansion)
- Lymphadenopathy, splenomegaly, hepatomegaly
 - Infiltration by malignant cells
- **Headache, vomiting**
 - Meningeal spread
- May cause bone marrow depression
 - Anemia, thrombocytopenia, neutropenia

ALL

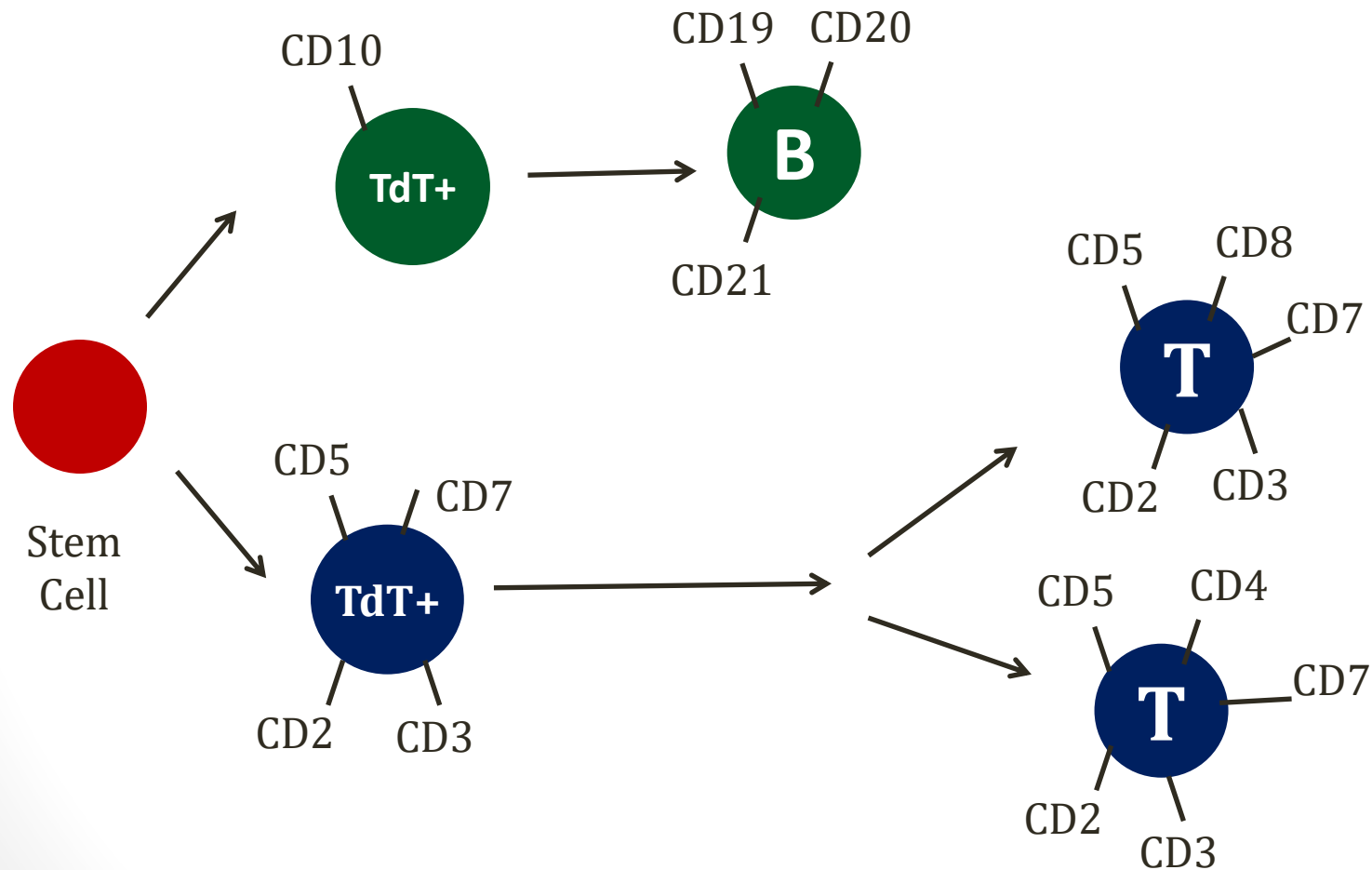
Acute Lymphoblastic Leukemia

- Peripheral blood: lymphoblasts
 - Can appear similar to myeloblasts
 - Special testing distinguish from myeloblasts



VashiDonsk /Wikipedia

Lymphocyte Development



Lymphocyte Antigens

Primarily T-Cell Associated	Primarily B-cell Associated
CD1	CD10
CD2	CD19
CD3	CD20
CD4	CD21
CD5	CD22
CD7	CD23
CD8	

ALL

Acute Lymphoblastic Leukemia

- Usually pre-B cell malignancy (~70 to 80% cases)
 - **CD10+**
 - “Common acute lymphoblastic leukemia antigen” or “CALLA”
 - Also CD19+, sometimes CD20+
- Terminal deoxynucleotidyl transferase (TdT)
 - DNA polymerase (found in nucleus)
 - Found only in pre-B and pre-T blasts
 - NOT seen in myeloblasts

ALL

Acute Lymphoblastic Leukemia

- Treated with chemotherapy
 - Cure rates >80% in many studies
- “Sanctuary sites”
 - Poor penetration by chemotherapy drugs
 - Relapse may occur in these locations
- **Testes**
- **Central nervous system**
- Special treatments (radiation/chemo) used
- Sterility may occur in boys

ALL

Acute Lymphoblastic Leukemia

- Many different translocations reported in B-ALL
- Philadelphia chromosome t(9;22)
 - 20 to 30% ALL in **adults**
 - 2 to 3 % ALL in children
 - Associated with a **poor prognosis**
- t(12;21)
 - Fusion product of two genes: TEL-AML1
 - TEL-AML1 impairs differentiation of blasts
 - **Good prognosis**
 - Most common rearrangement in children

ALL

Acute Lymphoblastic Leukemia

- **Down Syndrome**
 - Risk of ALL ↑↑ 10-20x
 - 1-3% ALL cases have Down



Vanellus Foto/Wikipedia

T-Cell ALL

T-cell acute Lymphoblastic Leukemia

- Less common form of ALL
- Common in adolescent males (teens to 20s)
- Presents as a **mass**
 - Lymphadenopathy
 - **Mediastinal mass**
 - Anterior with pleural effusions
- Tumor compression may occur
 - Superior vena cava syndrome
 - Tracheal obstruction



G. Ferretti

T-Cell ALL

T-cell acute Lymphoblastic Leukemia

- Pathology: Blasts
- Different markers from B-cell ALL
 - Usually CD7+
 - Can see CD2, CD3, CD5, CD4, CD8
 - Not CD10+

AML

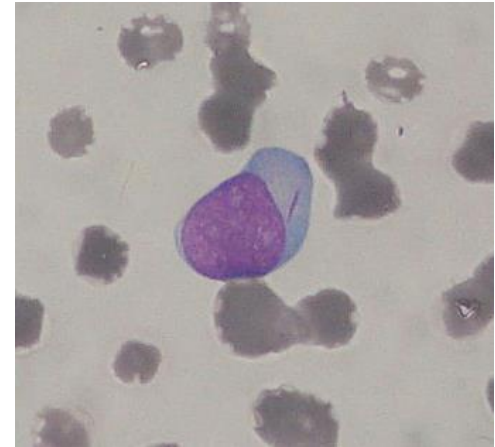
Acute Myelogenous Leukemia

- Malignancy of myeloblasts
- Common in adult males
 - Median age at diagnosis: 65
 - Male:female ratio: 5:3
- Symptoms from **bone marrow suppression**
 - Myeloblasts accumulate in marrow, suppress cell growth
 - Anemia: Fatigue, weakness, pallor
 - Thrombocytopenia: Bleeding (especially gums)
 - Neutropenia: Infections
- Enlarged nodes, spleen, liver less common than ALL

AML

Acute Myelogenous Leukemia

- Peripheral blood smear
 - Anemia, thrombocytopenia, blasts
- Myeloblasts
 - Myeloperoxidase (MPO) positive
 - Auer rods



Paulo Henrique Orlandi Mourao/Wikipedia

Auer Rods

- Pathognomonic AML
- Accumulation of MPO
- Can cause DIC



Paulo Henrique Orlandi Mourao/Wikipedia

AML

Acute Myelogenous Leukemia

- Classified into numerous subtypes (WHO system)
- Classified by morphology, surface markers, genetics
- Key subtype: **APML**

APML

Acute Promyelocytic Leukemia

- Defined by translocation $t(15;17)$
 - Creates a fusion gene: PML-RARA
 - Promyelocytic leukemia gene (chromosome 15)
 - **Retinoic acid receptor** alpha (chromosome 17)

APML

Acute Promyelocytic Leukemia

- #1: **Abnormal retinoic acid receptor (RAR)**
 - Prevents normal maturation of promyelocytes
 - Treatment: all trans retinoic acid (form of vitamin A)
 - Abnormal cells will mature

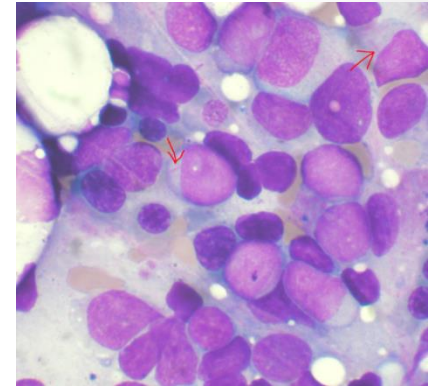
APML

Acute Promyelocytic Leukemia

- #2: **Disseminated intravascular coagulation**
 - Promyelocytes contains lots of MPO (Auer rods common)
 - Release → DIC (common initial presentation)

Myelodysplasia

Myelodysplastic Syndromes (MDS)



VashiDonsk /Wikipedia

- Abnormal **myeloid** progenitor cells
- Leads to ineffective hematopoiesis
 - Anemia, thrombocytopenia, neutropenia
- Diagnosis: Bone marrow biopsy
 - Dysplasia (abnormal) cells
 - Blasts <20% cells
- Can progress to AML (>20% blasts)

MDS
<20% cells
blasts



AML
>20% cells
blasts

Myelodysplasia

Myelodysplastic Syndrome (MDS)

- Associated with **environmental factors**
 - Prior radiation
 - Chemotherapy
 - Usually years after exposure



Dina Wakulchik/Wikipedia



Jenny Mealing/Wikipedia

Chronic Leukemias

Jason Ryan, MD, MPH

Leukemia

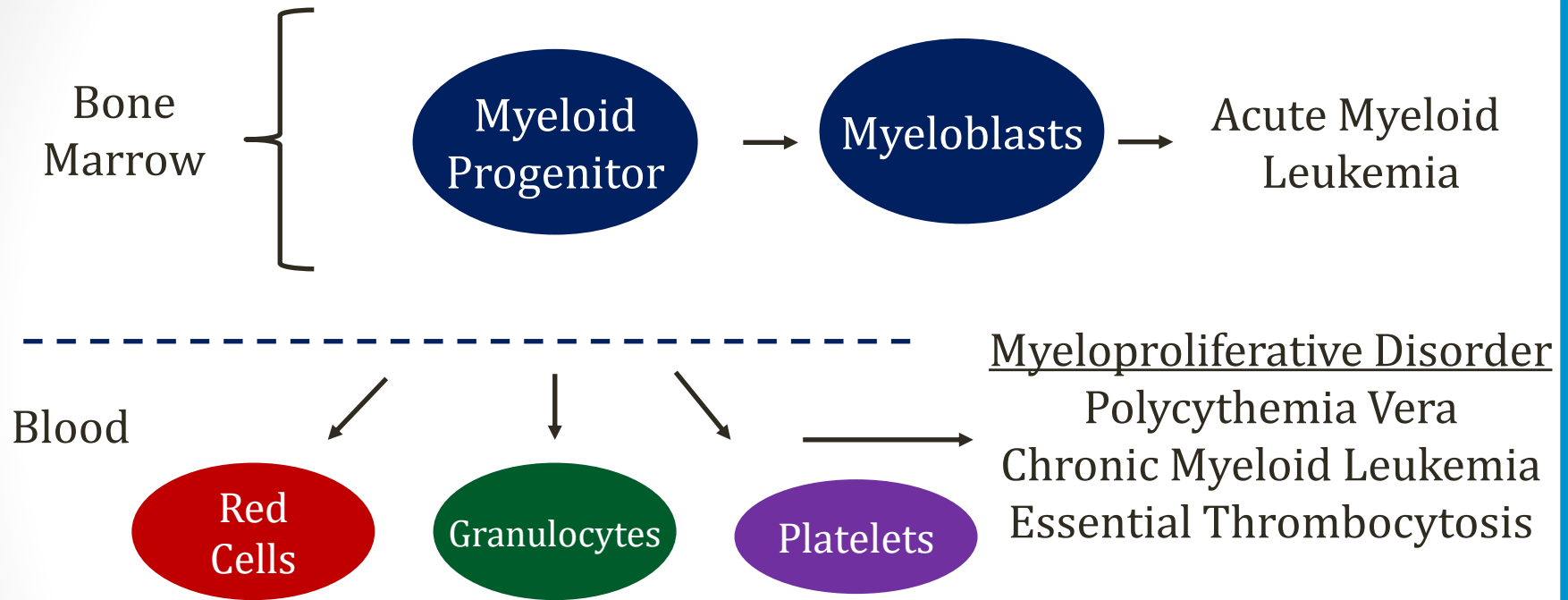
- Malignant proliferation of white blood cells
- Cells appear in blood (contrast with lymphoma)
- **Increased WBC**

Leukemias

Classification

- Myeloid versus lymphoid
- Acute versus chronic
- Acute
 - Rapid onset of symptoms
 - Involves **blasts** in bone marrow
- Chronic
 - Slower onset of symptoms (or no symptoms)
 - Malignant cells are not blasts (more mature)

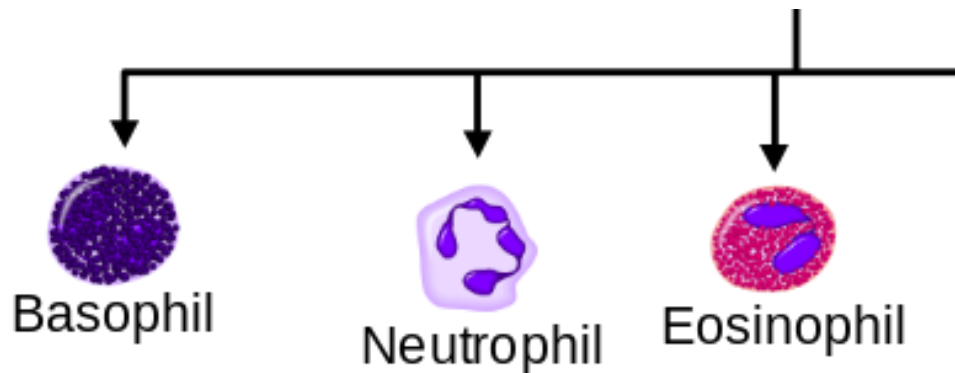
Myeloid Disorders



CML

Chronic Myelogenous Leukemia

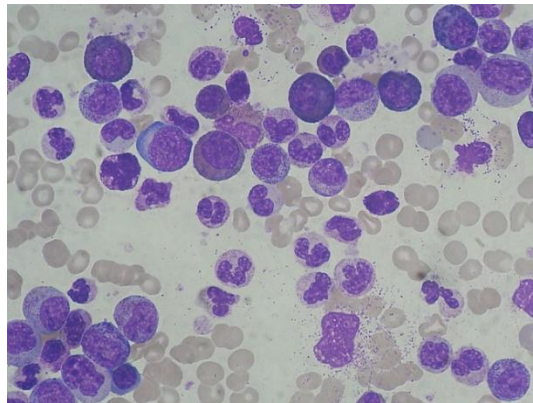
- Malignant disorder of myeloid progenitor cells
- Dysregulated production of **granulocytes**
 - Neutrophils, basophils, eosinophils
- Classified as a myeloproliferative disorder



CML

Chronic Myelogenous Leukemia

- Peripheral blood (chronic phase):
 - Leukocytosis (median WBC 100,000/microL)
 - ↑ neutrophils
 - ↑ myeloblasts, promyelocytes, myelocytes, bands
 - ↑ **basophils** (rare finding!)
 - ↑ eosinophils
- Mild anemia; normal or increased platelets



Paulo Henrique Orlandi Mourao/Wikipedia

CML

Chronic Myelogenous Leukemia

- Chronic phase (usually years)
 - Can be asymptomatic (↑WBC on blood testing)
 - Fatigue, malaise, weight loss, splenomegaly
 - Few blasts (usually <2%)
- Accelerated phase (usually months)
 - Treatment failure (rising WBC)
- Blast crisis
 - Acute leukemia (>20% blasts in periphery or marrow)
 - Usually myeloblasts (AML)
 - Less commonly lymphoblasts (ALL)

Left Shift

Leukemoid Reaction

- Normal response to infection
- More bands and neutrophils
- Must be distinguished from CML

	Normal	Infection
WBC	10,000/ μ L	17,000 / μ L
Neutrophils	55%	80%
Bands	5%	12%

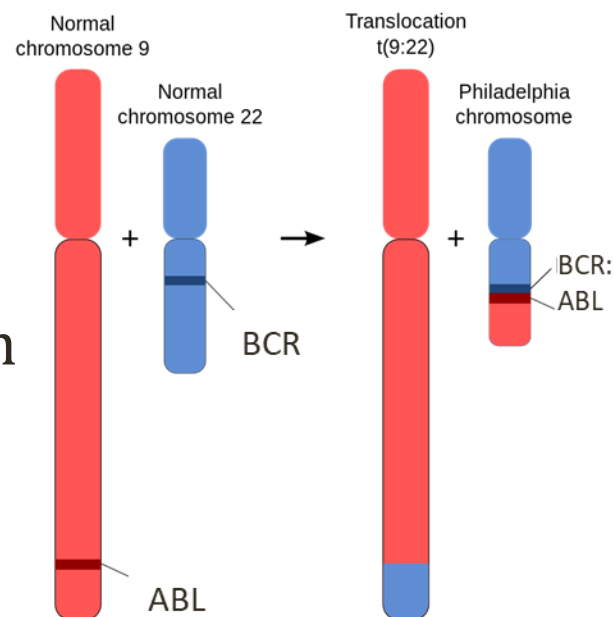
LAP

Leukocyte Alkaline Phosphatase

- Enzyme found in normal neutrophils
- Absent in neutrophils of CML
- Enzyme level assessed with **LAP score**
 - Low = CML
 - High = Leukemoid reaction
- Largely replaced by testing for Ph chromosome

Philadelphia Chromosome

- Genetic hallmark of CML
- 9;22 translocation
- BCR-ABL fusion gene
- Synthesis **tyrosine kinase** protein
- Long cell life → accumulation



Aryn89/Wikipedia

Tyrosine Kinase Inhibitors

Imatinib, Dasatinib, Nilotinib

- Used for treatment in CML (chronic phase)
- Long term control of disease (not curative)
- Bone marrow transplant often used after failure



[ProjectManhattan/Wikipedia](#)

CLL

Chronic Lymphocytic Leukemia

- Disorder of naïve lymphocytes
 - Not blasts
 - Newly produced by bone marrow
- Characteristic immunophenotype
 - **CD5+ B cells**
 - “Co-express CD20 and CD5”

SLL

Small lymphocytic lymphoma

- Same malignant cells as CLL
- Differentiated by degree of lymphocytosis (\uparrow WBC)
- CLL: Increased WBC
- SLL: normal or mild lymphocytosis
- SLL definition: lymphocyte count of <5000
- CLL definition: lymphocyte count of >5000

CLL

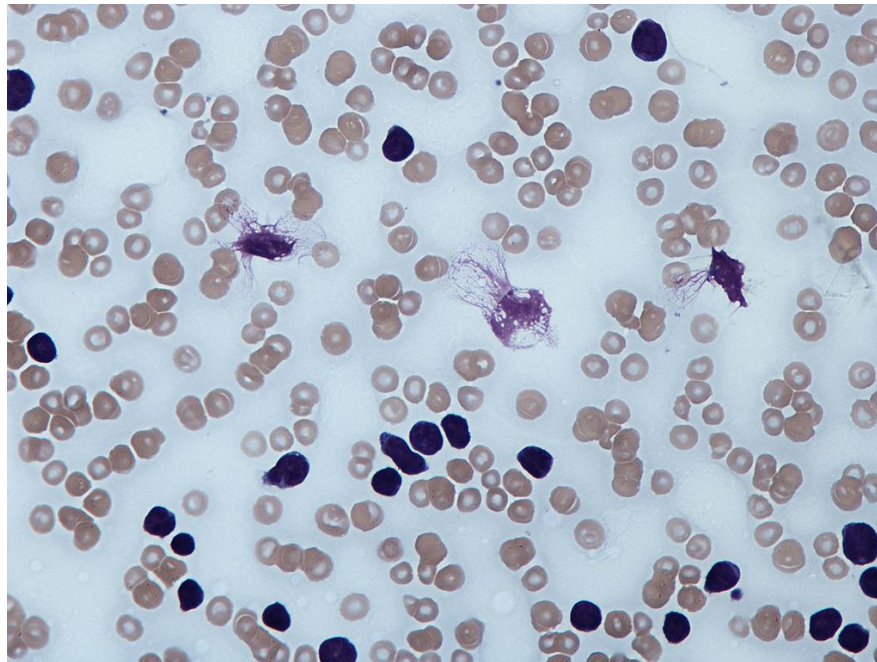
Chronic Lymphocytic Leukemia

- Median age 60
- Patients often **asymptomatic**
 - Routine CBC: Increased lymphocytes
 - 5-10% of patients have B symptoms (fevers, sweats)
- Signs
 - Lymphadenopathy, splenomegaly, hepatomegaly
- Many patients observed without treatment

CLL

Chronic Lymphocytic Leukemia

- Smudge cells
 - Peripheral lymphocytes are fragile
 - Disrupted during preparation of blood smear



Paulo Henrique Orlandi Mourao/Wikipedia

CLL

Chronic Lymphocytic Leukemia

- B-cell disruption
- Hypogammaglobulinemia
 - Usually ↓ IgG, IgA, IgM
 - **Increased susceptibility to bacterial infections**
- Autoantibodies
 - **Not produced by malignant cells**
 - Produced by non-neoplastic cells (self-reactive)
 - Autoimmune hemolytic anemia

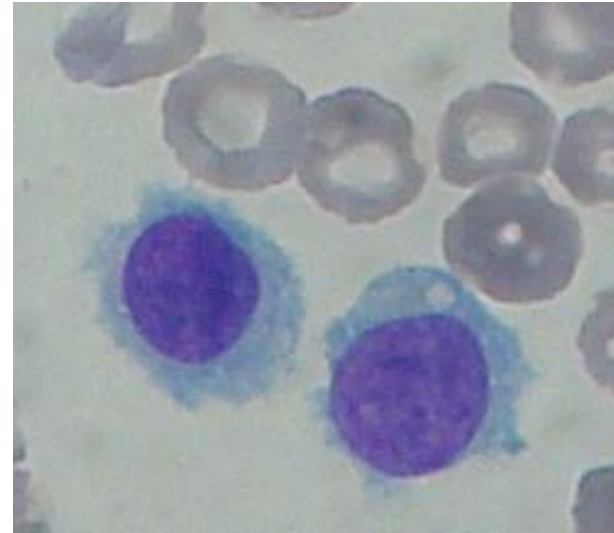
CLL

Chronic Lymphocytic Leukemia

- May transform into **diffuse large B cell lymphoma**
- Classic presentation
 - Patient with known CLL
 - Rapid growth of single lymph node
 - Biopsy: diffuse large B cell lymphoma

Hairy Cell Leukemia

- Rare chronic B-cell malignancy
 - Express CD19, CD20, CD22
 - CD103: sensitive marker
- Peripheral smear: hairy cells
 - Lymphocytes
 - Hair-like cytoplasm projections



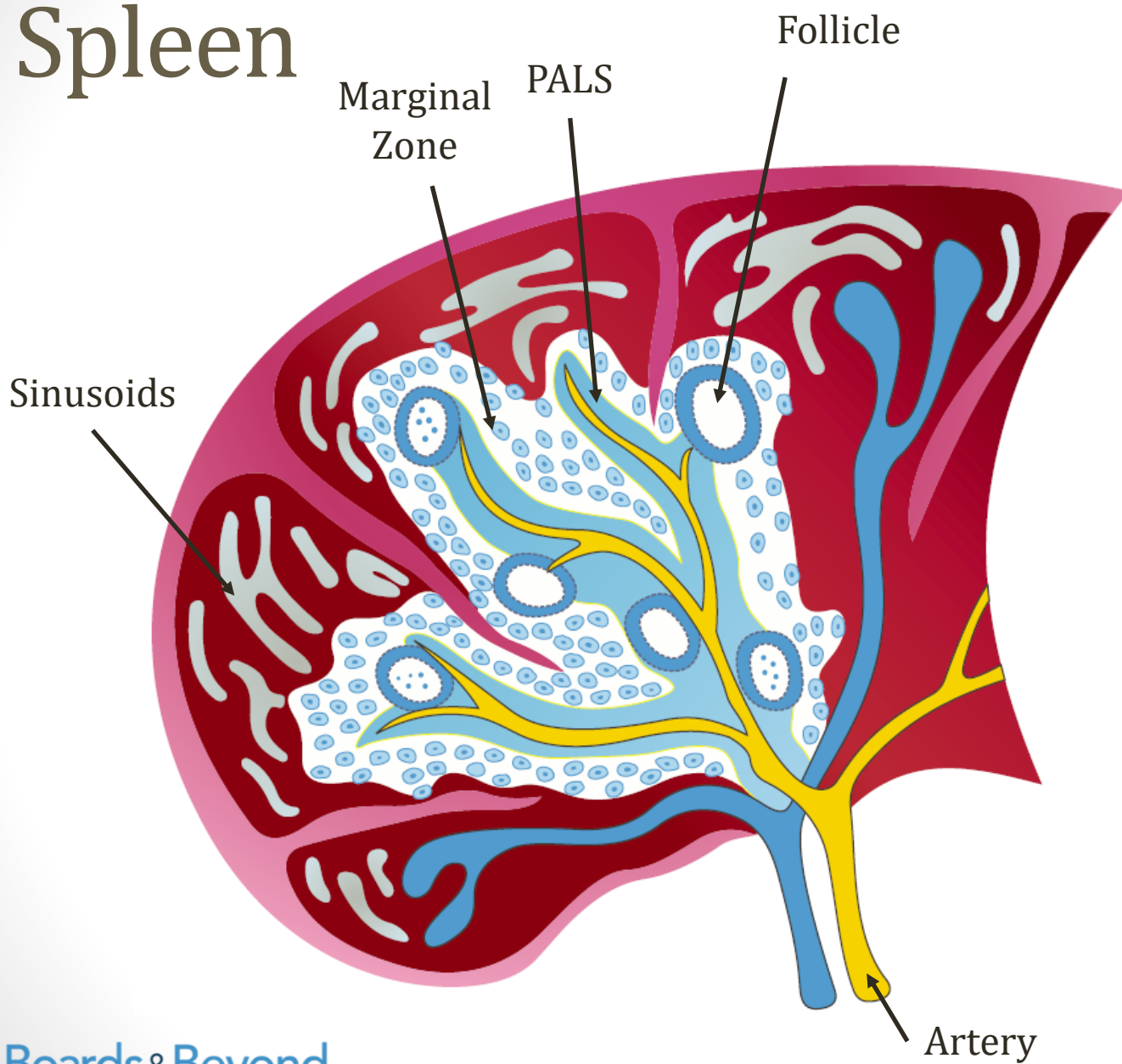
Paulo Henrique Orlandi Mourao/Wikipedia

Hairy Cell Leukemia

Unique Features

- Massive **splenomegaly**
 - **Red pulp** engorged
 - Atrophy or obliteration of white pulp
- **“Dry tap”** on bone marrow biopsy
 - Hairy cells induce marrow fibrosis
- **Tartrate-resistant acid phosphatase (TRAP)**
 - Cellular enzyme
 - Hairy cells: strong positivity for TRAP staining

Spleen



Hairy Cell Leukemia

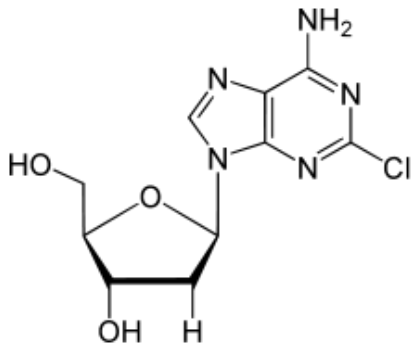
Clinical features

- Median age: 52
- Presenting feature often **abdominal pain**
- Fatigue, weakness
- Splenomegaly
- Bone marrow suppression (anemia, ↓platelets)

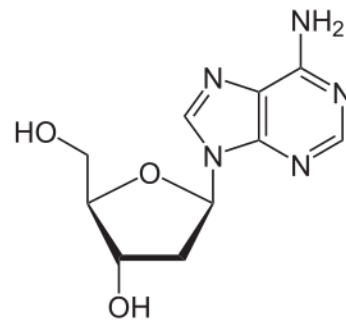
Cladribine

2-chlorodeoxyadenosine (2-CdA)

- Preferred initial therapy for HCL
- Excellent clinical response
- Similar to adenosine (“**purine analog**”)
- Highly toxic to leukemic cells in HCL



Cladribine



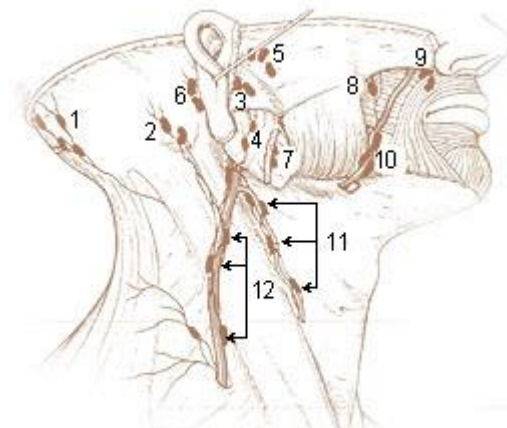
Deoxyadenosine

Hodgkin Lymphoma

Jason Ryan, MD, MPH

Lymphomas

- Malignancies of lymphocytes (B cells, T cells)
- Often involve **lymph nodes**
- Also “extranodal” (skin, GI tract)



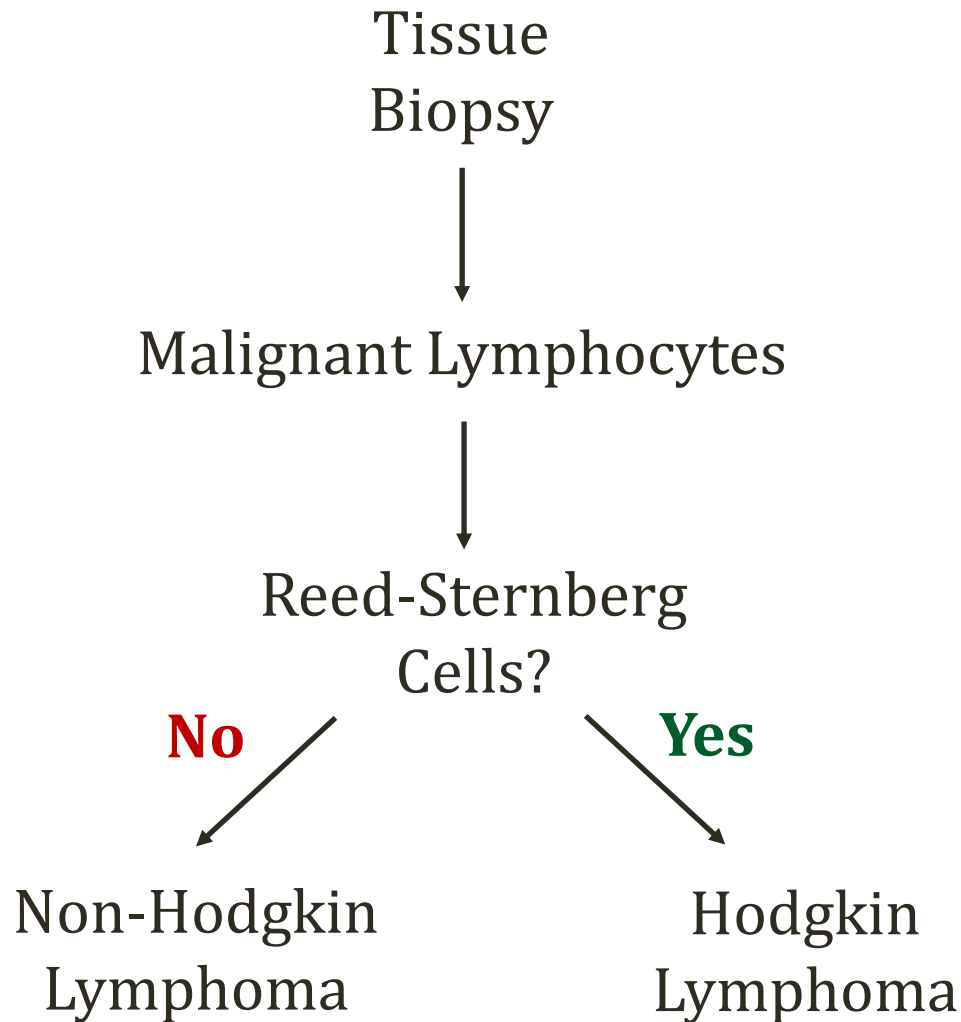
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Lymphomas

Signs and Symptoms

- Enlarged, **painless** lymph nodes
- “B symptoms”
 - Systemic symptoms
 - Fever, chills, night sweats

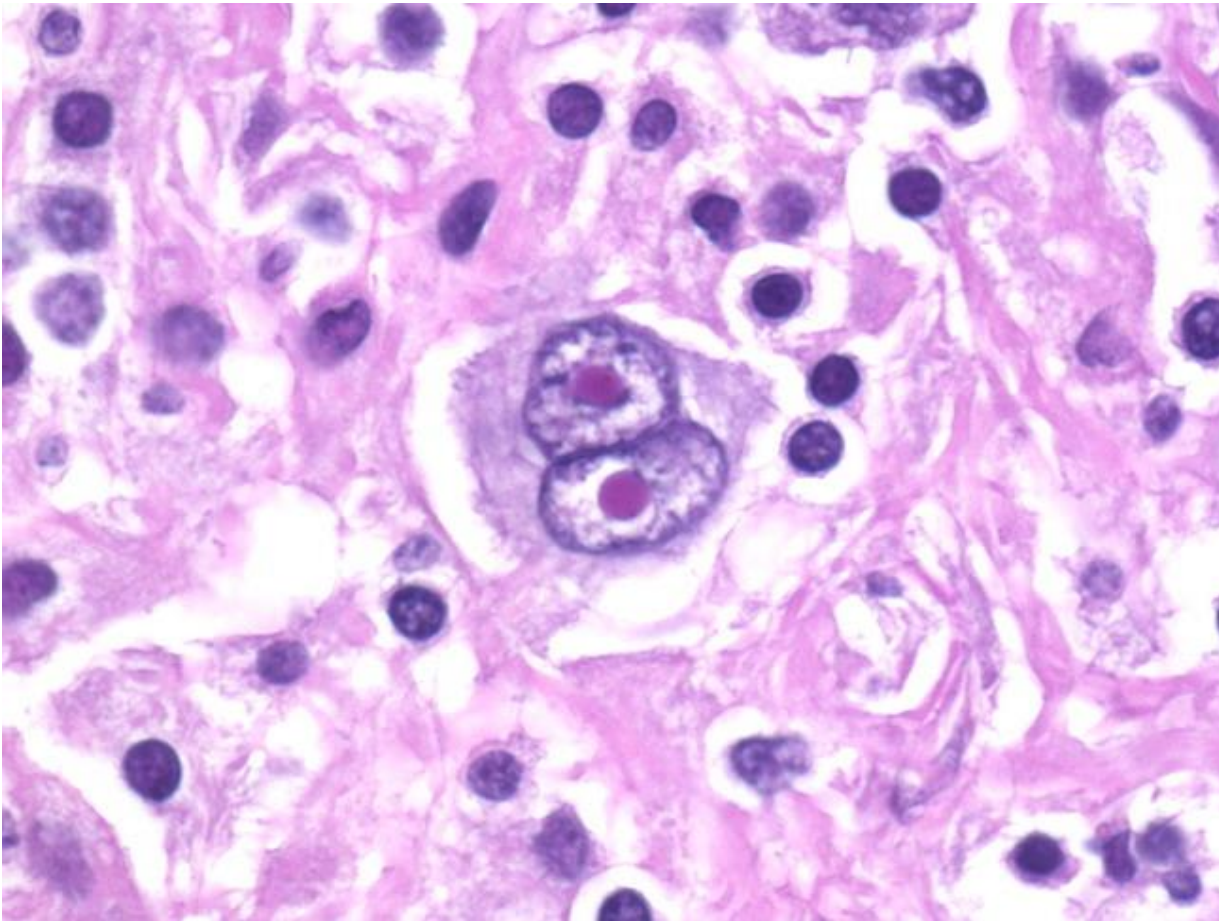
Lymphomas



Reed-Sternberg Cells

- Large cell
- Multi-lobed nucleus
 - Two halves; often mirror images (“owl-eyed”)
- Usually derive from **B cells** (rarely from T cells)
- Usually **CD15+ and CD30+**
- Usually NOT positive for B cell markers
 - CD19, CD20, CD21, CD22
- Sometimes seen in other disorders

Reed-Sternberg Cells



Nva1991/Wikipedia

Hodgkin Lymphoma

- Malignant cell: Reed-Sternberg cell
 - A **minority** of cells in enlarged nodes (~1 to 5%)
- Release cytokines → generate **reactive cells**
 - Majority of cells in node are reactive
 - B symptoms common (more than non-Hodgkin lymphoma)
 - Macrophages may activate → hypercalcemia



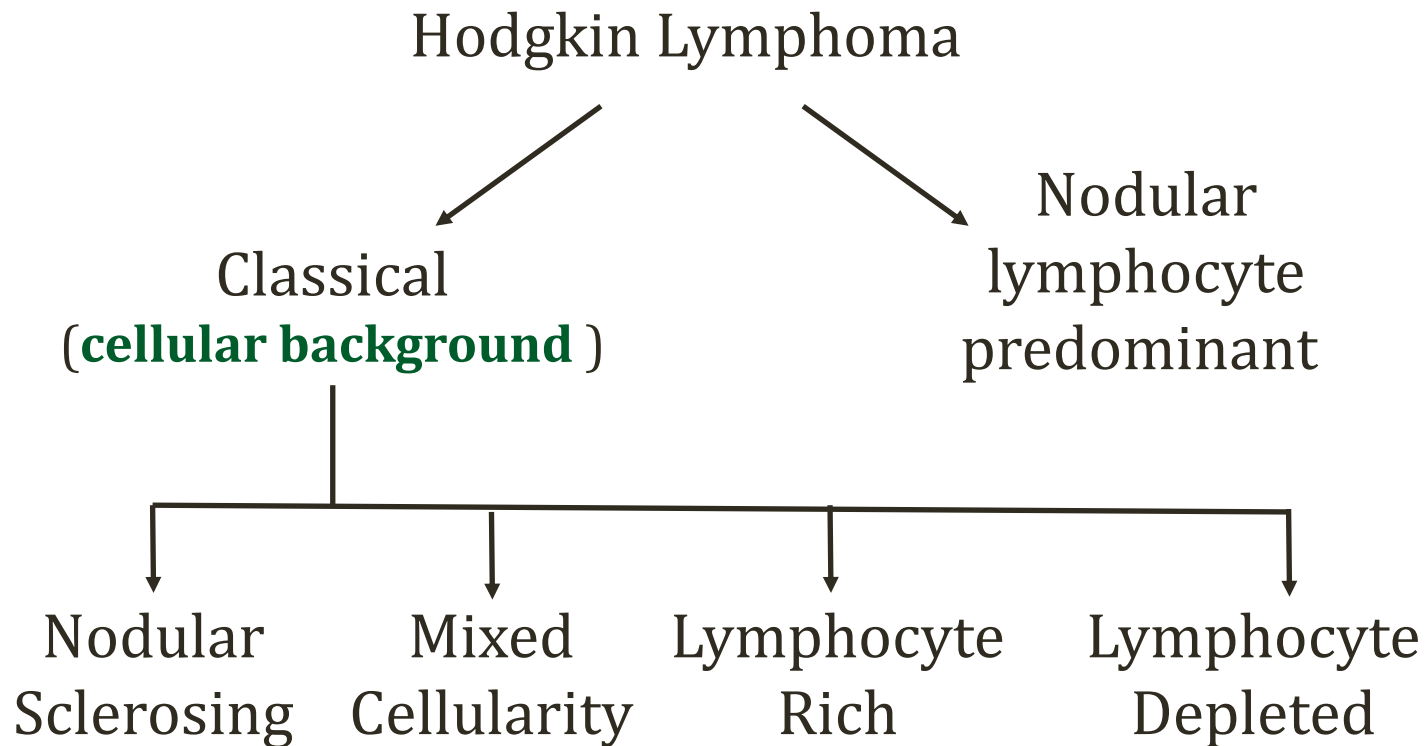
Hodgkin Lymphoma

- Commonly presents with **cervical lymphadenopathy**
 - Often with B symptoms
- Spreads in a very predictable manner
- Limited disease highly curable
- **Stage** is strongest predictor of prognosis

Hodgkin Lymphoma

- **Bimodal** age distribution
 - Peaks at age 20 and 65
- Risk factors
 - Prior EBV infection (virus infects B cells)
 - Immunosuppression (HIV, transplant)
 - Autoimmune disease: Rheumatoid arthritis and lupus
- Treatment: chemotherapy and radiation

Classification

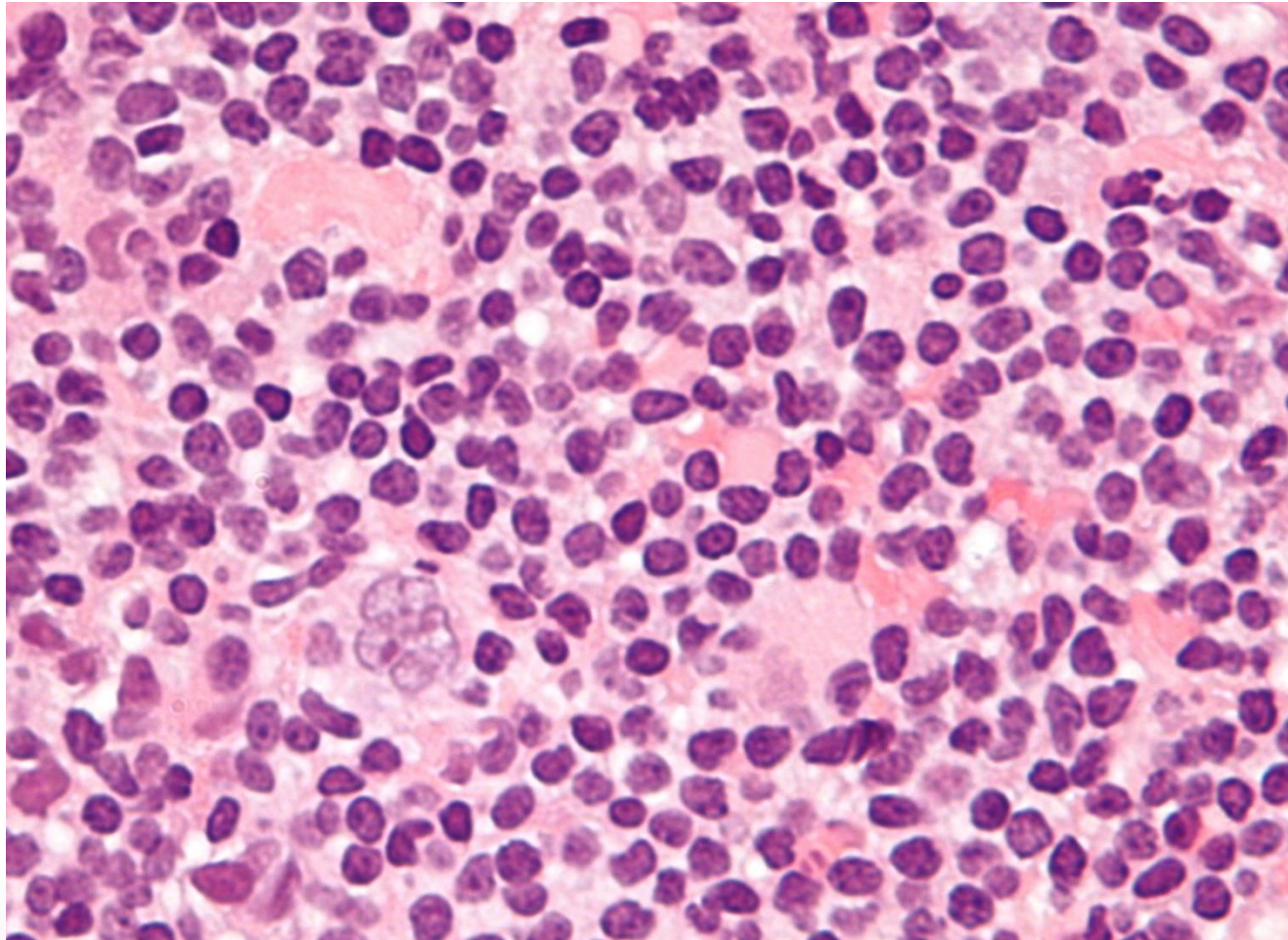


Lymphocyte Predominant

Nodular Lymphocyte Predominant

- Rare variant of Hodgkin lymphoma
- Malignant cell: LP cells
 - Lymphocyte predominant
 - Sometimes called “**popcorn cells**”
- Unusual surface marker expression
 - Usually lack CD15 and CD30
 - **Express CD20**

Popcorn Cells



Nephron/Wikipedia

Classical Hodgkin Lymphoma

- Nodular sclerosing
 - Most common type HL: 60% to 80% of all cases
 - More common in women (most HL more common men)
 - Often presents with a **mediastinal mass on CXR**
 - Reed-Sternberg cells seen in clear space (“lacunar variant”)
 - Slow growing (“indolent”)
 - Good long-term survival

Classical Hodgkin Lymphoma

- Mixed cellularity
 - Eosinophils, neutrophils, macrophages, plasma cells
- Lymphocyte rich
 - Excellent prognosis
- Lymphocyte depleted
 - Poor prognosis

Hodgkin versus. Non-Hodgkin

Clinical Features

Hodgkin

- Often localized
- Orderly spread from node to node
- Extranodal involvement rare

Non-Hodgkin

- Often multiple peripheral sites
- Noncontiguous spread
- Extranodal involvement common
 - GI (thickened bowel wall)
 - Skin

Hodgkin Lymphoma

Treatment

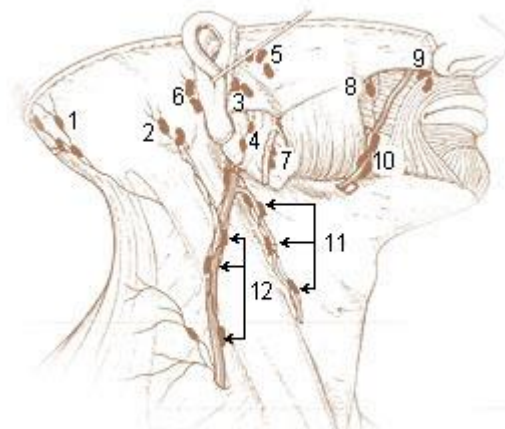
- Many different regimens
- ABVD
 - Adriamycin (doxorubicin) - cytotoxic antibiotics
 - Bleomycin - cytotoxic antibiotics
 - Vinblastine – microtubule inhibitor
 - Dacarbazine – alkylating agent

Non-Hodgkin Lymphoma

Jason Ryan, MD, MPH

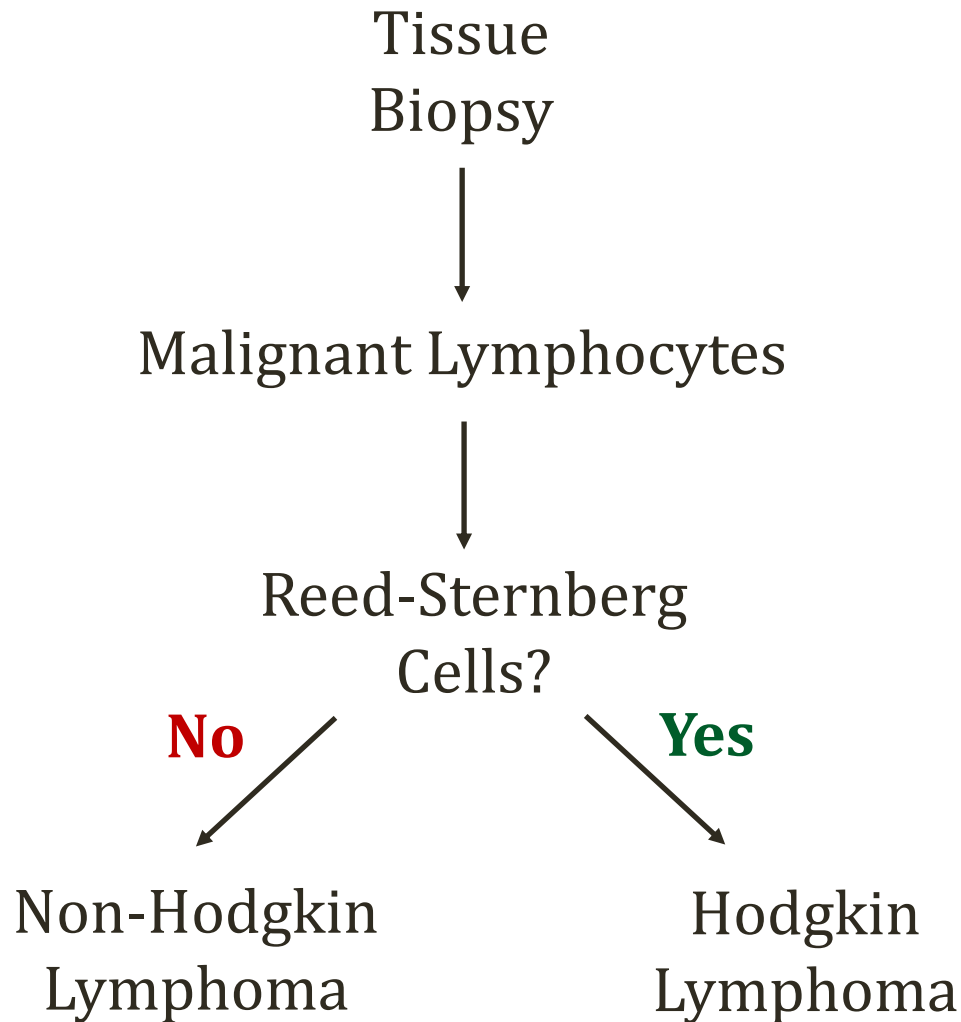
Lymphomas

- Malignancies of lymphocytes (B cells, T cells)
- Often involve **lymph nodes**
- Also “extranodal” (skin, GI tract)



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Lymphomas



Hodgkin versus. Non-Hodgkin

Clinical Features

Hodgkin

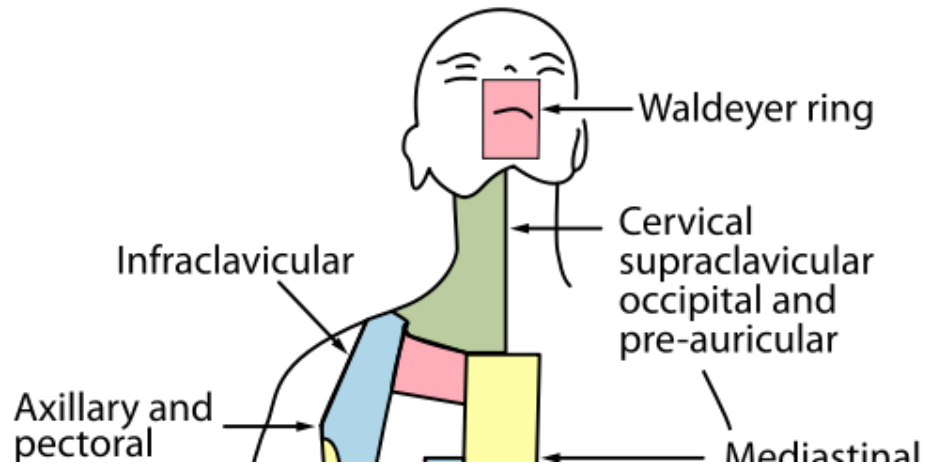
- Often localized
- Orderly spread from node to node
- Extranodal involvement rare

Non-Hodgkin

- Often multiple peripheral sites
- Noncontiguous spread
- Extranodal involvement common
 - GI (thickened bowel wall)
 - Skin

Waldeyer's Ring

- Lymphoid tissue in the pharynx
- Often involved in **non-Hodgkin lymphoma**
 - Rare in Hodgkin lymphoma



Wikipedia/Public Domain

Lymphocyte Antigens

Primarily T-Cell Associated	Primarily B-cell Associated
CD1	CD10
CD2	CD19
CD3	CD20
CD4	CD21
CD5	CD22
CD7	CD23
CD8	

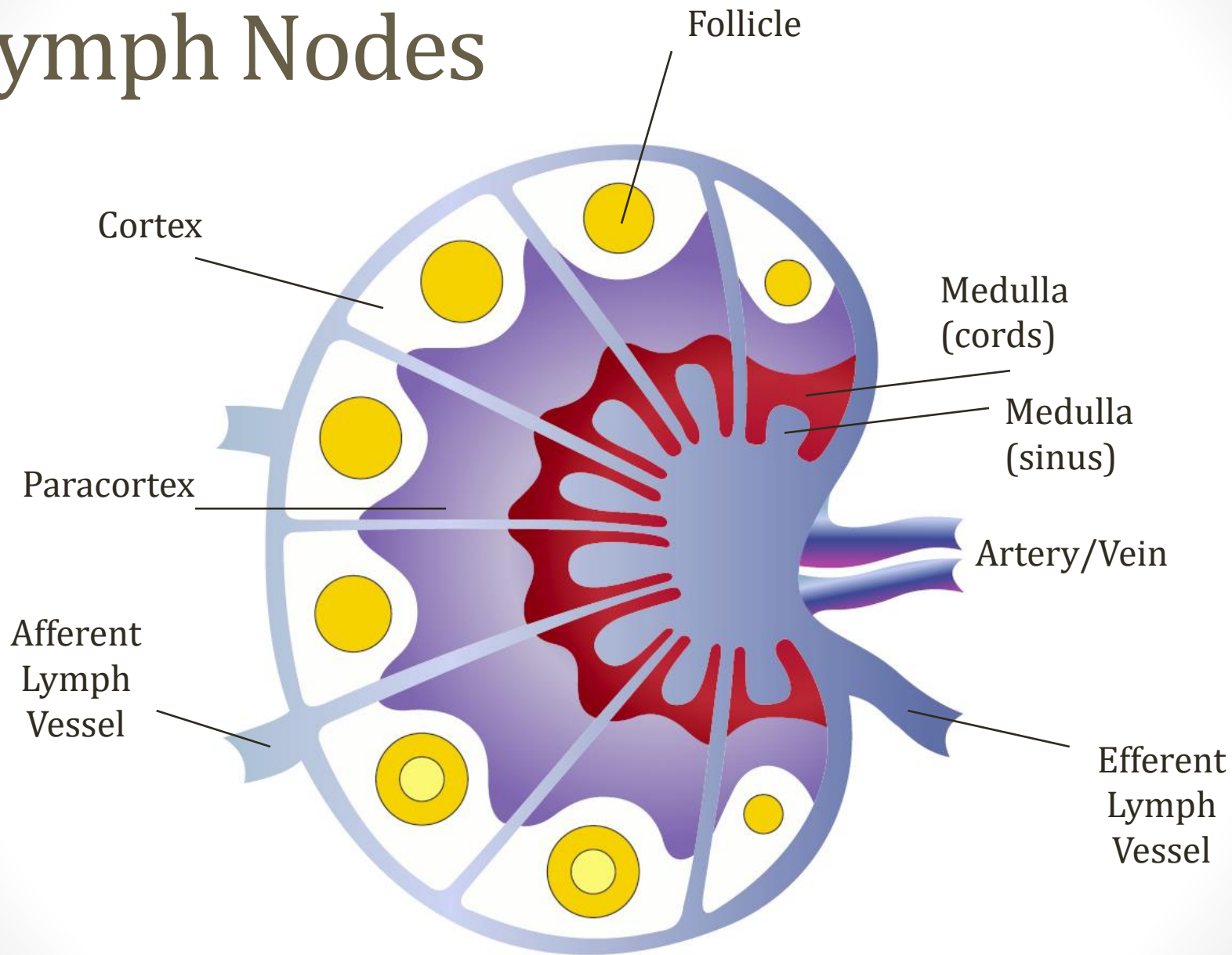
Non-Hodgkin Lymphoma

- B and T cell malignancies
 - Most are B cell disorders
 - Malignant cells obliterate lymph node architecture
- More than two dozen subtypes per WHO
- Classified by:
 - B versus T cell
 - Cell size (small versus large)
 - Histologic appearance
 - Expression of markers (“immunophenotype”)
 - Genetics

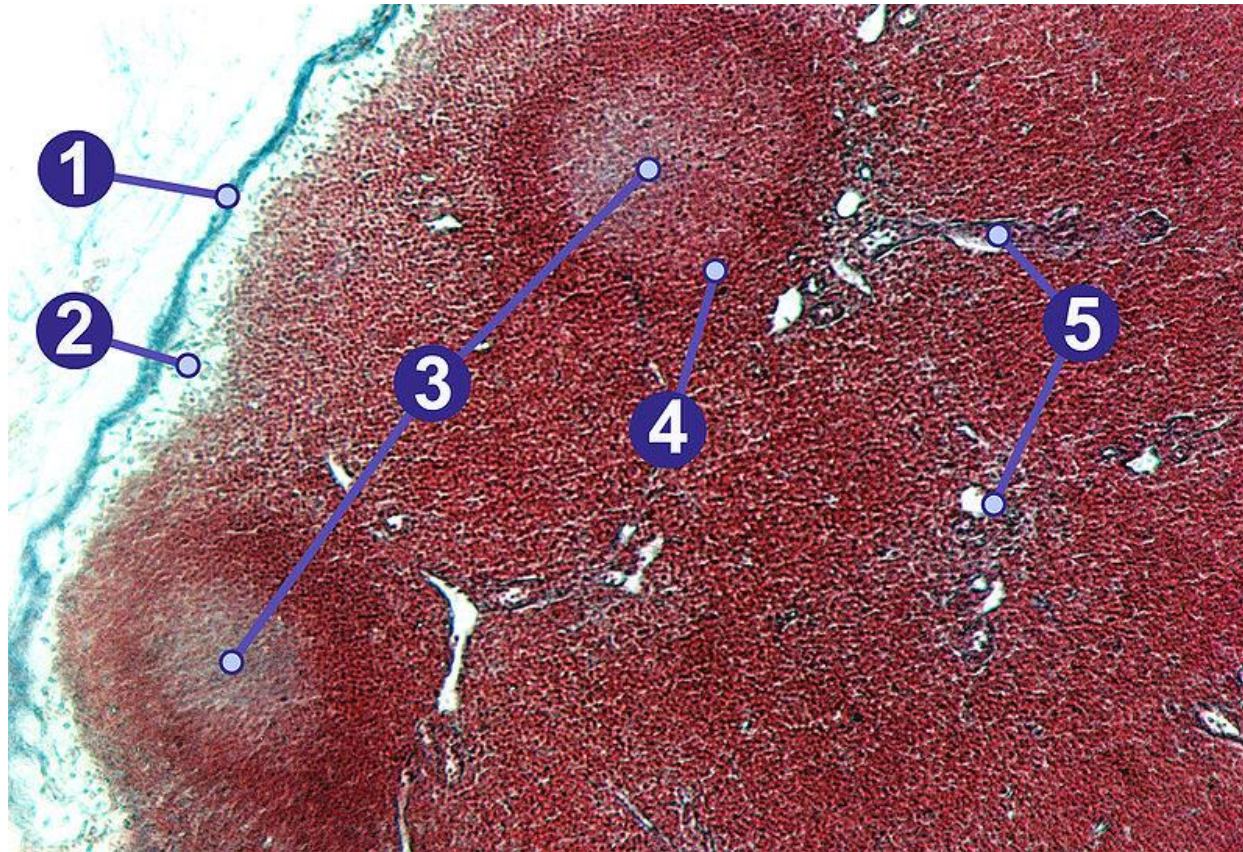
Non-Hodgkin Lymphoma

- Follicular
- Marginal cell
- Mantle zone
- Diffuse Large B Cell
- Small lymphocytic lymphoma
- Burkitt's
- Adult T-cell Leukemia/Lymphoma
- Cutaneous T-cell Lymphomas

Lymph Nodes



Lymphoid Follicles

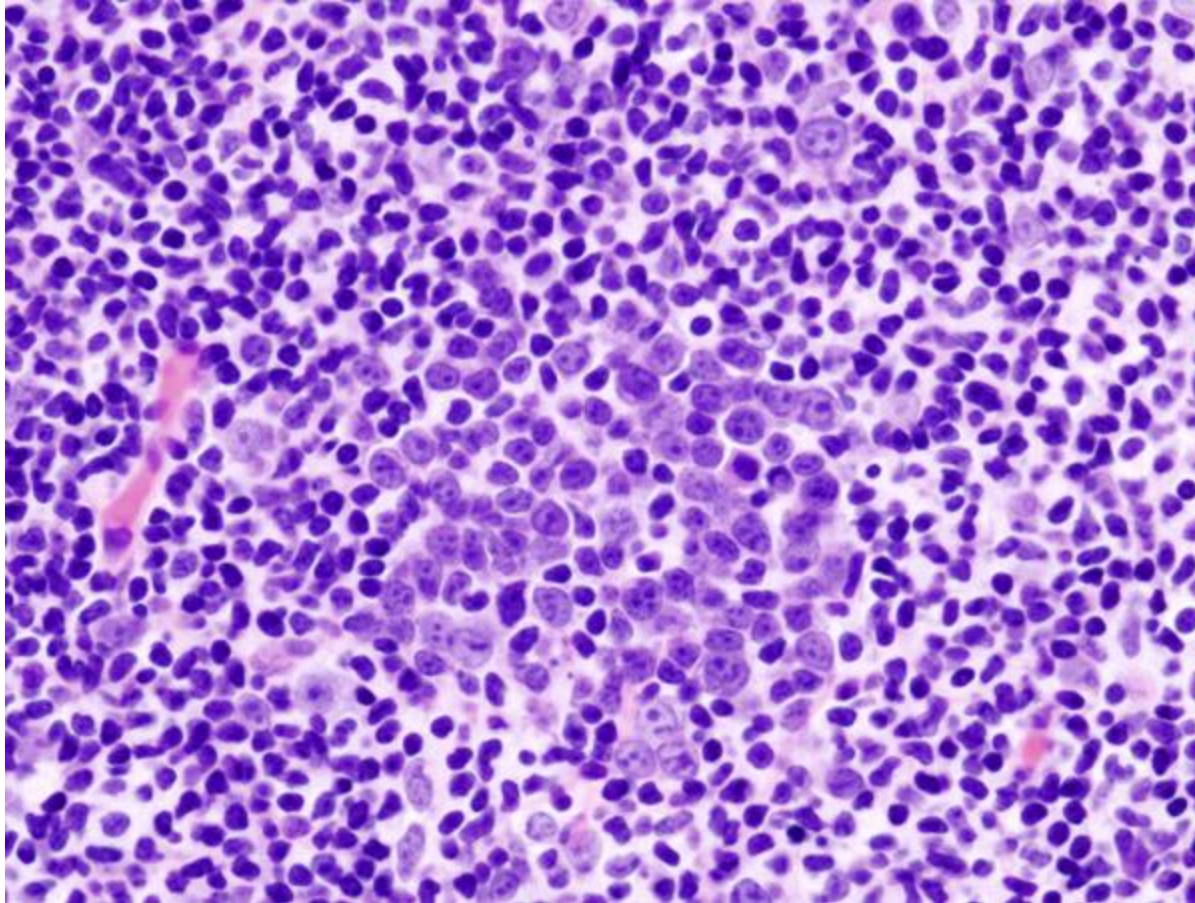


Gleiberg/Wikipedia

Diffuse Large B-cell Lymphoma

- **Most common NH lymphoma**
- B cell malignancy
 - Express CD19, CD20
 - Most cells express surface immunoglobulin

Diffuse Large B-cell Lymphoma



KGH/Wikipedia

Diffuse Large B-cell Lymphoma

- Median age at presentation is 64 years
- Occurs in HIV
 - AIDS defining malignancy

Diffuse Large B-cell Lymphoma

Prognosis

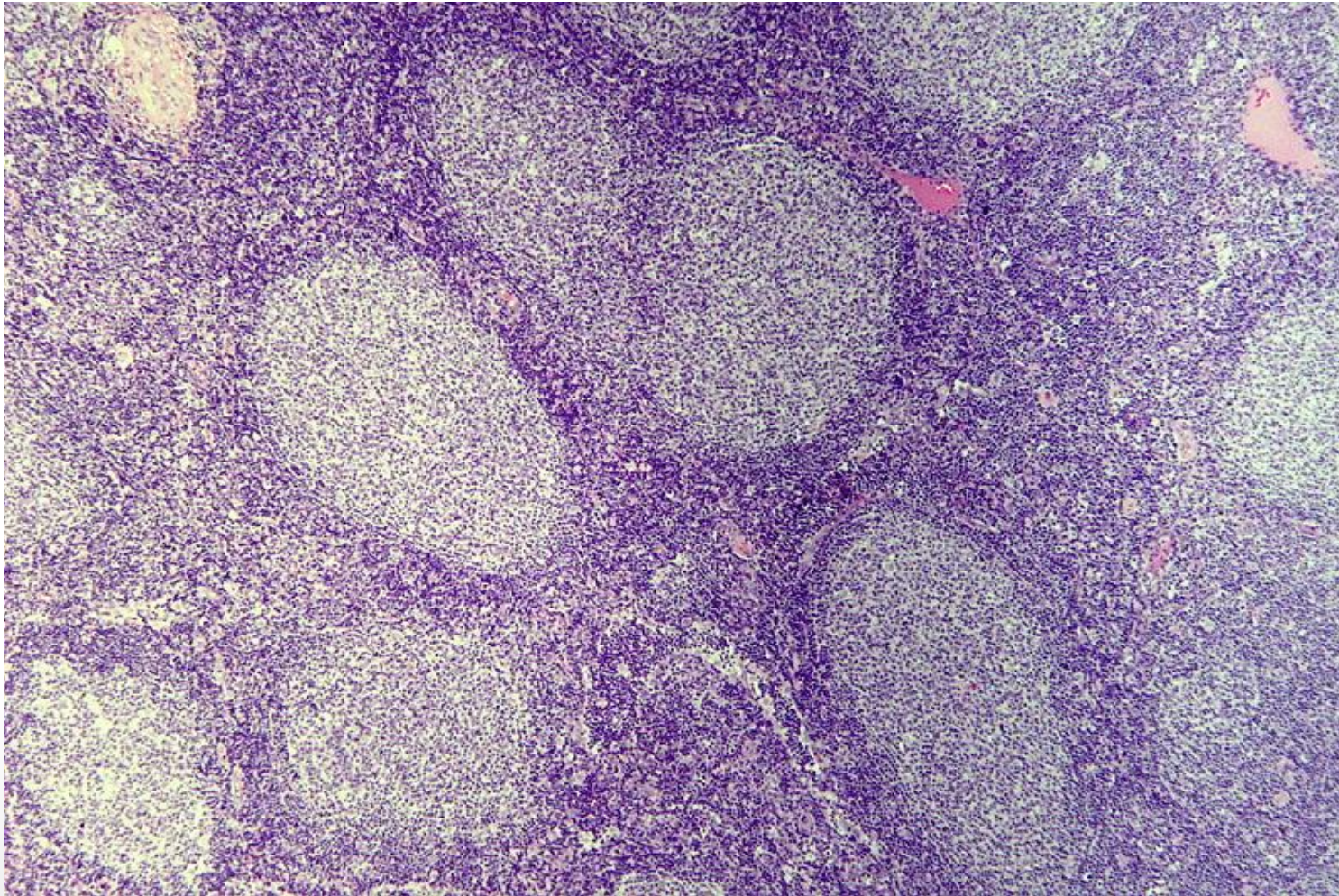
- Variable prognosis
- International Prognostic Index (IPI) score
 - Age >60 years
 - Increased LDH
 - Patient functional status
 - Clinical stage
 - Number of extranodal sites

Rituximab

- Monoclonal CD20 antibody
- Used in CD20+ B cell lymphomas
 - Diffuse large B cell
 - Follicular



Follicular Lymphoma



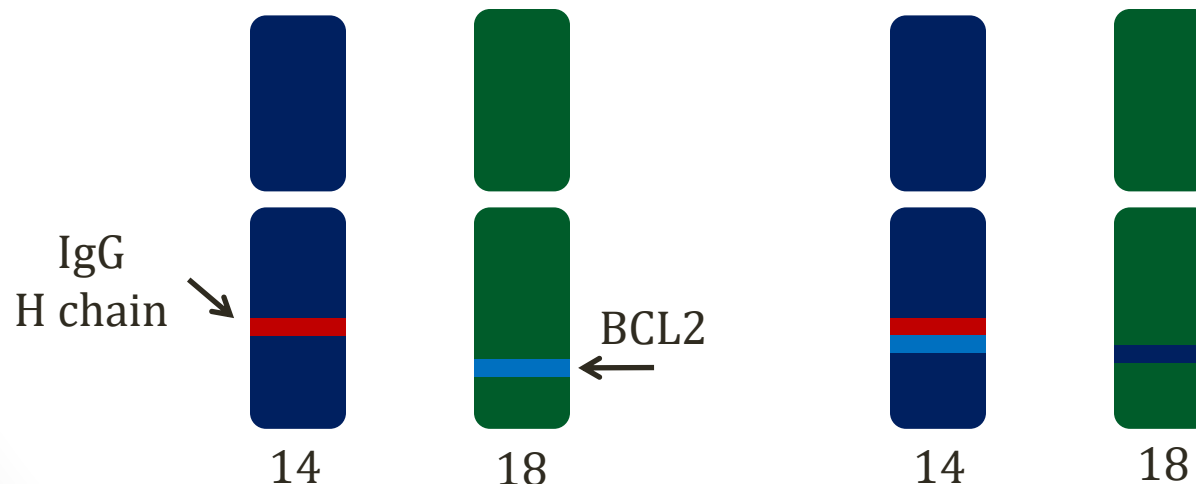
Patho/Wikipedia

Follicular Lymphoma

- B cell malignancy
 - Usually express CD19, CD20
 - Most cells express surface immunoglobulin

Follicular Lymphoma

- Genetic hallmark: **14;18 translocation**
- Overexpression of **BCL2**
 - Blocks apoptosis (“antagonist” of apoptosis)
 - Germinal center B cells usually lack BCL2
 - Undergo apoptosis unless selected by somatic hypermutation



Follicular Lymphoma

- Median age at diagnosis: **65 years**
- **Indolent course:** waxes/wanes for years
 - Not all patients require treatment
 - Difficult to cure
- Diffuse large B cell lymphoma (DLBCL)
 - Histologic transformation: 10 to 70% cases over time
 - Poor prognosis

Lymphoma vs. Reactive

- Follicular lymphoma vs. reactive lymphadenopathy
 - Both have ↑ follicle growth
 - Must distinguish in diagnosis of lymphoma
- Reactive lymphadenopathy (LAD)
 - Somatic hypermutation of B cells
 - Apoptosis of many B cells
 - B cell death → debris → macrophages

Lymphoma vs. Reactive

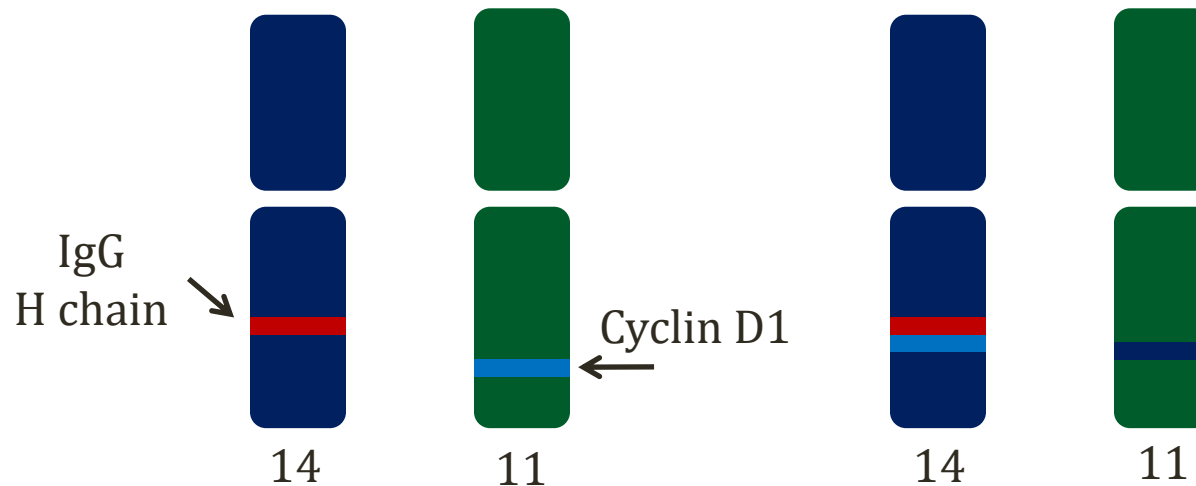
Lymphoma	Reactive LAD
Similar size/shape follicles	Varying size/shape follicles
Relative absence of macrophages	Tingible body (debris-laden) macrophages
++ BCL2 Staining	-- BCL2 Staining

Mantle Cell Lymphoma

- B cell malignancy
 - Follicle mantle or germinal center
 - Usually express CD19,CD20
 - Most cells express surface immunoglobulin
 - **Express CD5** (“Co-express CD20 and CD5”)
- Median age at diagnosis: 68 years
- Median overall survival: 3 to 4 years (poor prognosis)

Mantle Cell Lymphoma

- 50 to 65%: **11;14 translocation**
- Overexpression of **cyclin D1**
 - Promotes cell cycle transition from G1 to S phase



Marginal Zone Lymphoma

- B cell malignancies
- Marginal zone forms from inflammation
- Often extranodal
- Lymphoma in **chronic inflammatory disorders**
 - Salivary glands in Sjogren's
 - Thyroid gland in Hashimoto's thyroiditis
 - Stomach in chronic H. Pylori infection (MALToma)

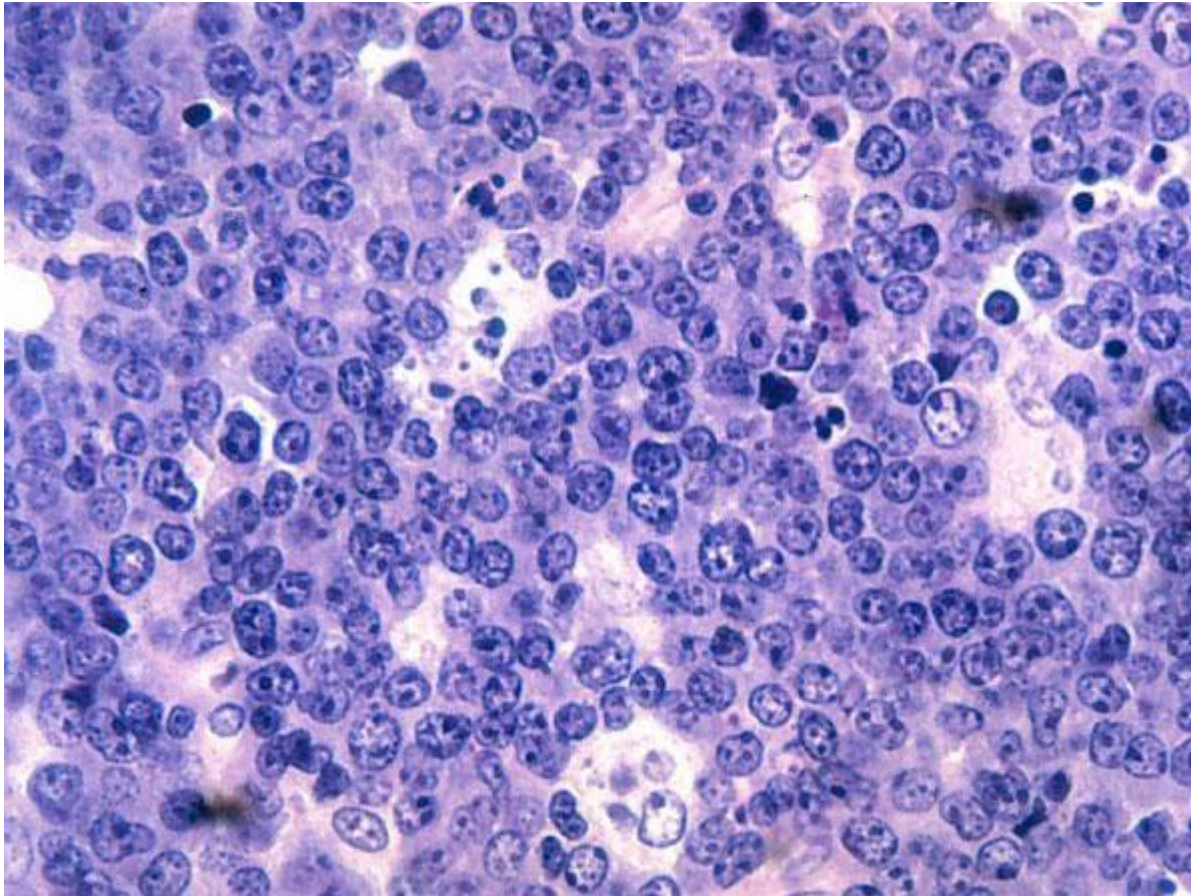
Small Lymphocytic Lymphoma

- CD5+ B cells
 - “Co-express CD20 and CD5”
 - Similar markers to mantle cell lymphoma
 - Typically negative for cyclin D1
- Same malignant cells as CLL
 - Only difference is degree of lymphocytosis (↑WBC)
 - Peripheral blood: normal or mild lymphocytosis
- SLL definition: lymphocyte count of <5000
 - If >5000 → CLL

Burkitt's Lymphoma

- B cell malignancy
 - Usually express CD19, CD20
 - Most cells express surface immunoglobulin
- Very aggressive – rapid proliferation
- Key distinctions:
 - “Starry sky” morphology
 - Epstein-Barr virus (EBV)
 - C-myc translocation

Burkitt's Lymphoma

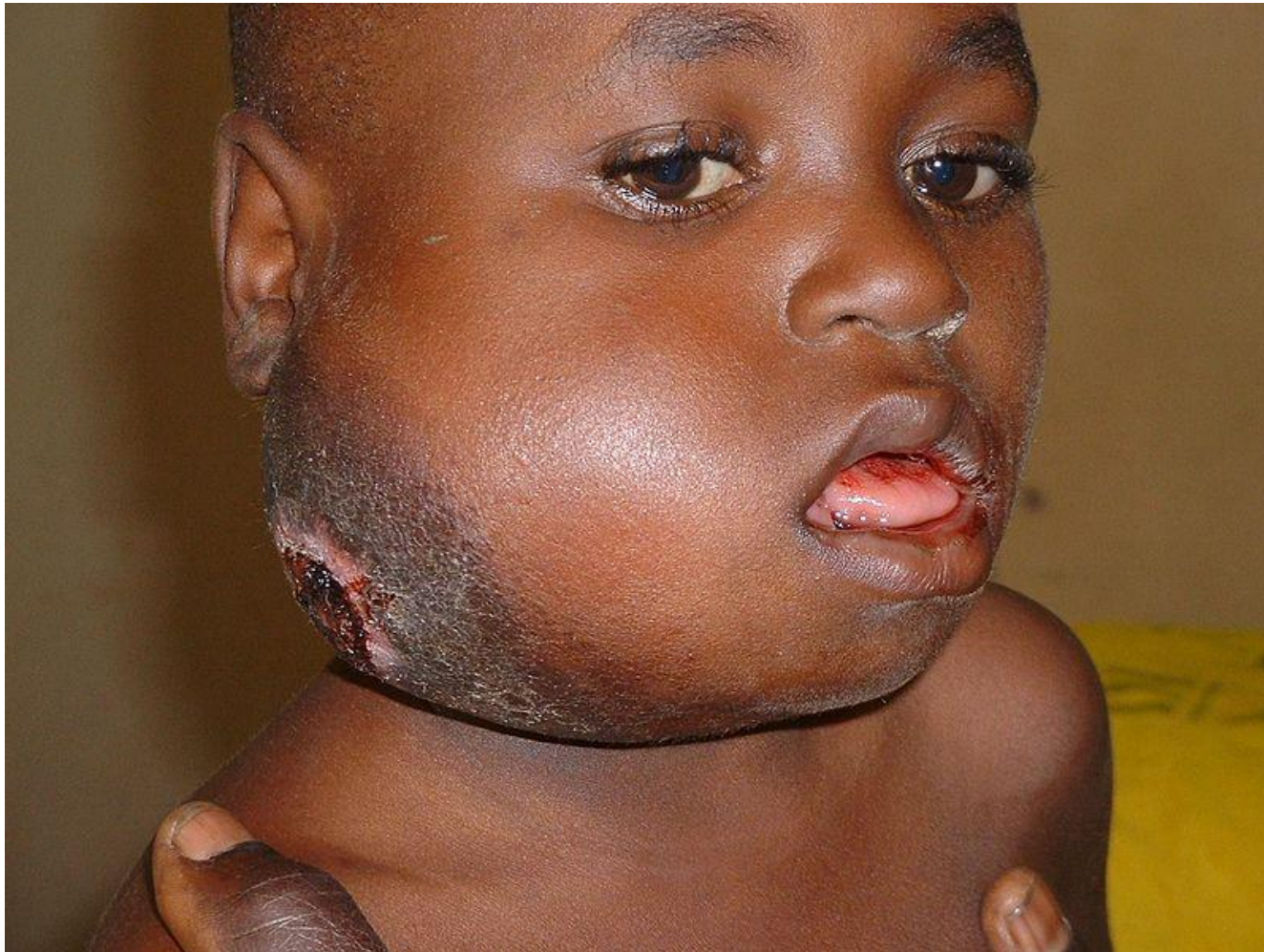


Wikipedia/Public Domain

Burkitt's Lymphoma

- Endemic form
 - Found in Africa and New Guinea
 - 30 to 50% of childhood cancer in some regions
 - **Children** four to seven years old
 - Male to female ratio ~ 2:1
 - Commonly presents as mass in the **mandible**
- Sporadic form
 - Also occurs in children
 - **Abdominal mass**: ileocecum or peritoneum

Burkitt's Lymphoma



Mike Blyth/Wikipedia

Burkitt's Lymphoma

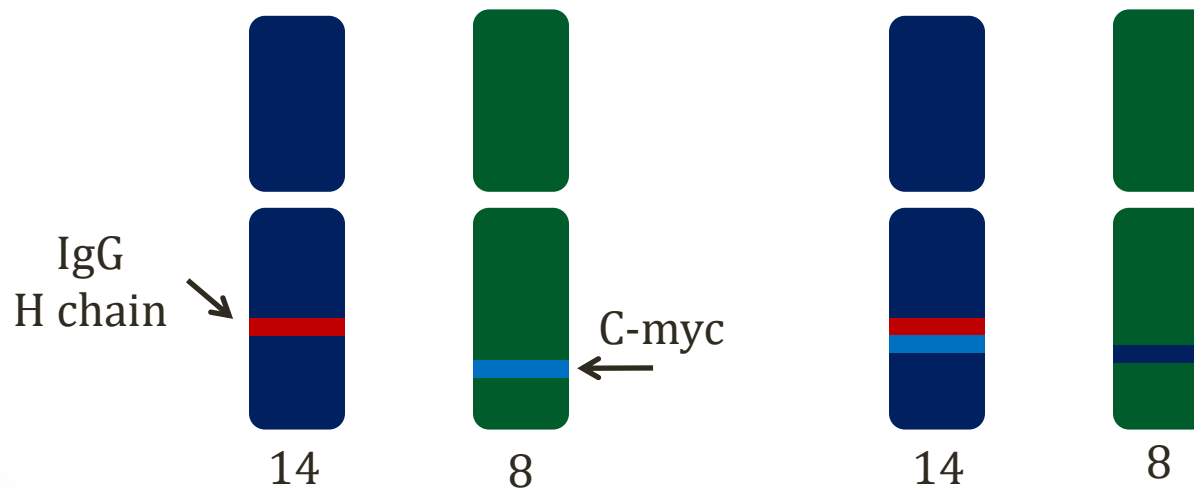
Associations

- Epstein Barr virus (EBV) infection
 - Nearly all endemic tumors associated with latent infection
 - Express CD21 (EBV receptor)

Burkitt's Lymphoma

Associations

- C-myc translocation
 - Growth promoter
 - Activates transcription



T-Cell Leukemia/Lymphoma

- CD4+ T cell malignancy
- Occurs with HTLV-1 infection
 - RNA Virus
 - Infects CD4+ T cells
- Key diagnostic test: anti-HTLV1 antibodies

T-Cell Leukemia/Lymphoma

- Clinical scenario
 - Patient from Japan, Caribbean, West Africa (endemic regions)
 - Lymphocytosis
 - Lymphadenopathy
 - Skin lesions (ulcers, nodules, papular rash)
 - Rapidly progressive symptoms → usually fatal in months
- Lytic bone lesions with ↑ calcium
 - Don't confuse with multiple myeloma

Cutaneous T-cell Lymphoma

CTCL

- Skin disorder of malignant T-cells
 - Variable expression of CD markers
- Presents with skin lesions
 - Localized disease: Mycosis Fungoides
 - Diffuse systemic disease: Sezary syndrome

Cutaneous T-cell Lymphoma

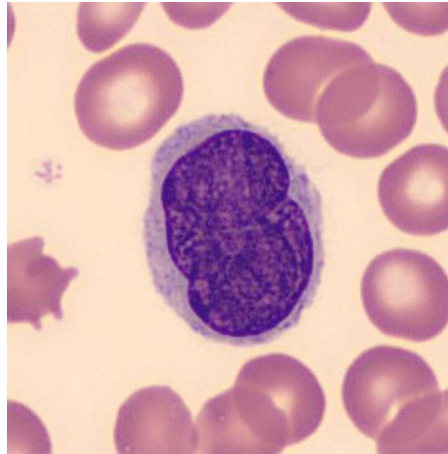
CTCL

- Mycosis Fungoides
 - Patches, plaques, tumors
 - Varying size/shape
 - Lesions progress slowly changing size/shape/appearance
 - “Indolent”: Slowly developing
 - Classically in a “bathing trunk” distribution
- Diagnosis: Skin biopsy shows lymphoid cells
 - Upper dermis
 - Epidermal aggregates (Pautrier microabscesses)

Cutaneous T-cell Lymphoma

CTCL

- Sezary syndrome
 - T-cell lymphoma affecting skin of entire body
 - Widespread erythema (skin bright red)
 - Lymphadenopathy
 - Malignant cells in blood (Sezary cells)



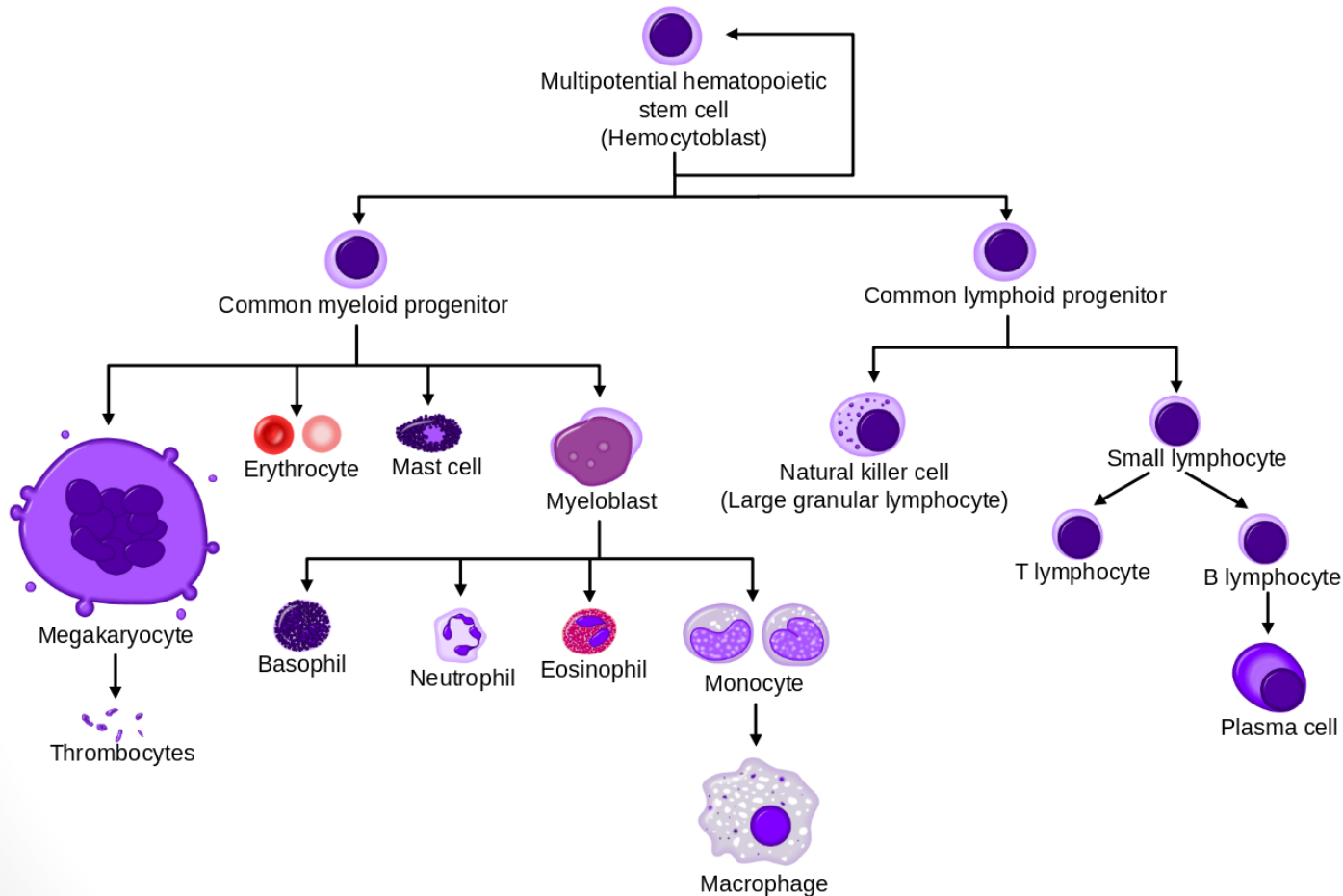
El*Falaf

Multi-lobed nucleus
“Cerebriform”

Plasma Cell Disorders

Jason Ryan, MD, MPH

Hematopoiesis



Multiple Myeloma

- Malignancy of plasma cells
 - Dependent on **IL-6**
 - Required for myeloma cell proliferation
- Excess production of **immunoglobulin**
- Disorder of older patients (median age: 66)

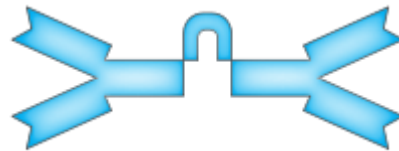
IL-6

Multiple Myeloma

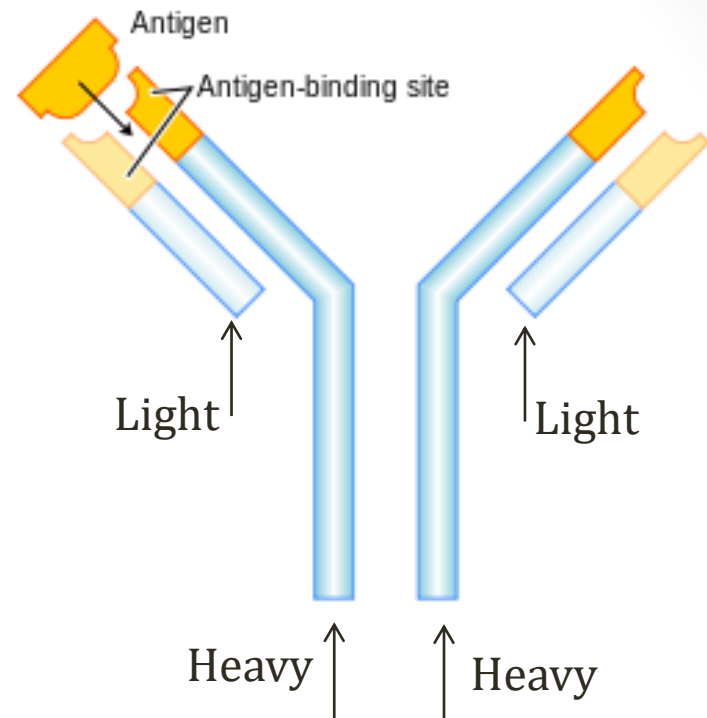
- IgG (~50%)
- IgA (~20%)
- Light chains only (~15%)
- “Paraproteins”



IgG



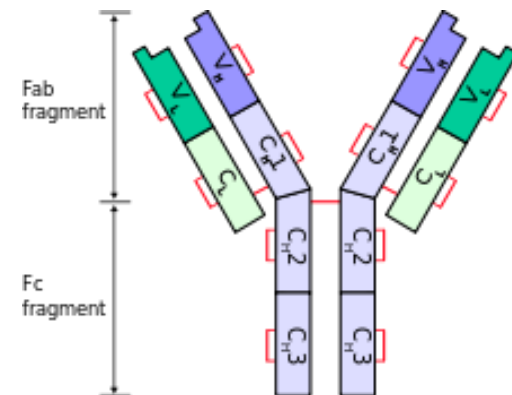
IgA



Fvasconcellos /Wikipedia

Light Chains

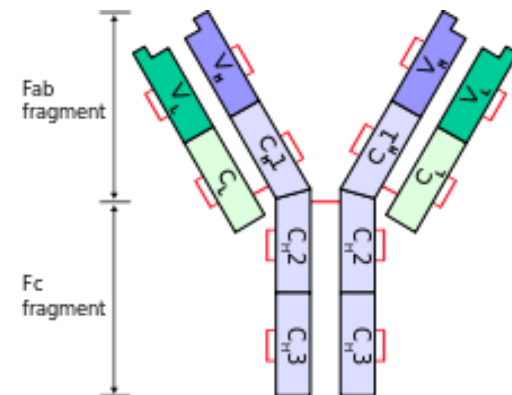
- Two types: Kappa (κ) or lambda (λ)
 - Each antibody: two identical light chains
 - Heavy chain type determines antibody type: G, A, E, etc.
- Slight excess of light chains produced normally
- Filtered by glomerulus \rightarrow reabsorbed proximal tubule



Yohan/Wikipedia

Light Chains

- Excess light chains can occur in multiple myeloma
- Excess light chains leads to pathology:
 - Renal damage
 - AL amyloidosis

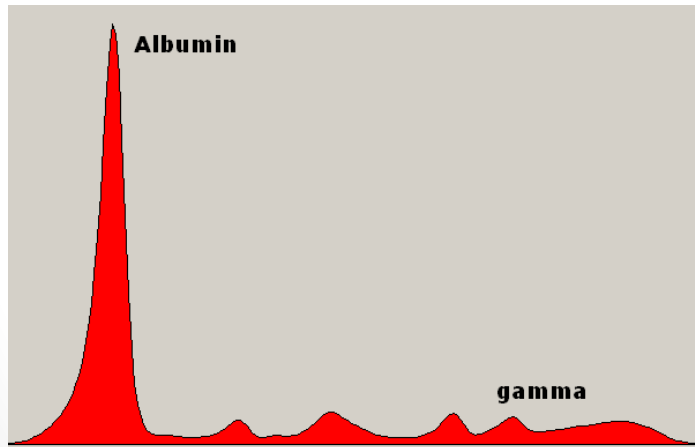


Yohan/Wikipedia

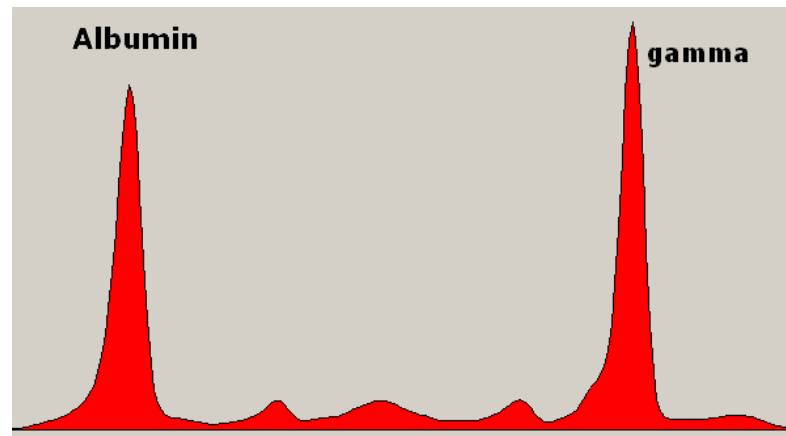
SPEP

Serum protein electrophoresis

- Electrical current separates serum proteins
 - Based on size and charge
- Gamma fraction contains immunoglobulin
- Multiple myeloma: “**M spike**”



Normal SPEP



Multiple Myeloma

Multiple Myeloma

Clinical Features

- Bone pain/fractures
- Hypercalcemia
- Renal failure
- Anemia
- Infections

Multiple Myeloma

Bone/hypercalcemia

- **Osteoclast-mediated** bone resorption
- Caused by **cytokines** from myeloma cells
- “Lytic lesions” on x-ray (“punched out”)
- Pathologic fractures, especially vertebral column
- Elevated serum **calcium**



Utsav Agrawal

Multiple Myeloma

Renal Failure

- Caused by **light chains** and **hypercalcemia**
- Light chains (“myeloma kidney”)
 - Small amount of light chains normally filtered/reabsorbed
 - MM: proximal tubular capacity exceeded
 - Light chains reach distal tubule
 - Combine with Tamm–Horsfall mucoprotein (THP)
 - Form obstructing **casts**
 - Light chains in urine = “Bence Jones” proteins

Multiple Myeloma

Renal Failure

- **Hypercalcemia**
 - Impairs renal ability to concentrate urine
 - Polyuria → volume contraction
 - Decreased GFR

Multiple Myeloma

Renal Failure

- Urine dipstick negative for protein
 - Mostly detects albumin
 - Poor detection of light chains
- **Urine protein electrophoresis (UPEP)**
 - Similar to SPEP (“SPEP/UPEP”)
 - Detects light chains (“Bence Jones proteins”)



J3D3

Multiple Myeloma

Renal Failure

- Serum free light chain
 - Antibody-based system
 - Sensitive test for *serum* kappa/lambda light chains
 - Alternative to UPEP

Multiple Myeloma

Anemia

- Normocytic, normochromic
- Multifactorial
 - Bone marrow replacement by plasma cells
 - Renal failure (low EPO)
- Weakness, pallor often present at diagnosis

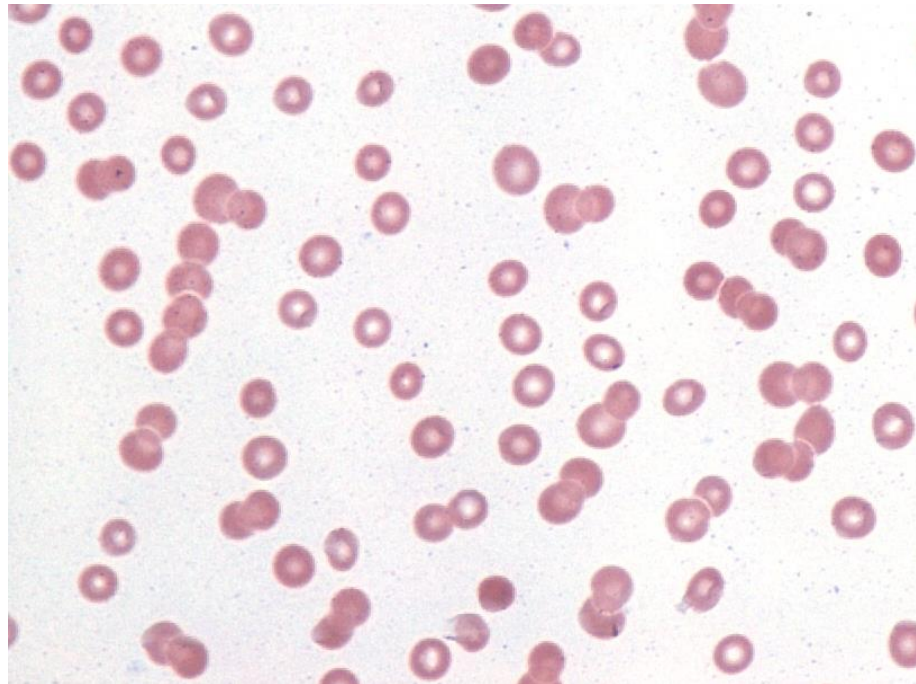
Multiple Myeloma

Infections

- Infection is leading cause of death
- Decreased production of normal immunoglobulins
 - Depressed humoral immunity
- Recurrent **bacterial** infections
 - Strep Pneumoniae
 - Staph Aureus
 - E. Coli

Rouleaux

- RBCs form a stack of coins
- Caused by elevated protein levels in plasma



Gabriel Caponetti /Wikipedia

Multiple Myeloma

Diagnosis

- SPEP: Monoclonal protein
- Diagnostic criteria
 - Bone marrow biopsy: clonal bone marrow plasma cells
 - End-organ damage

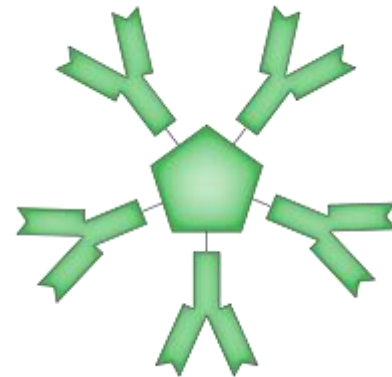
MGUS

Monoclonal gammopathy of undetermined significance

- Asymptomatic plasma cell disorder
- Abnormal SPEP (presence of M protein)
- No end-organ damage
- Can progress to multiple myeloma
- Often detected in workup of another problem
 - Anemia
 - Hypercalcemia
 - Bone pain

Waldenstrom Macroglobulinemia

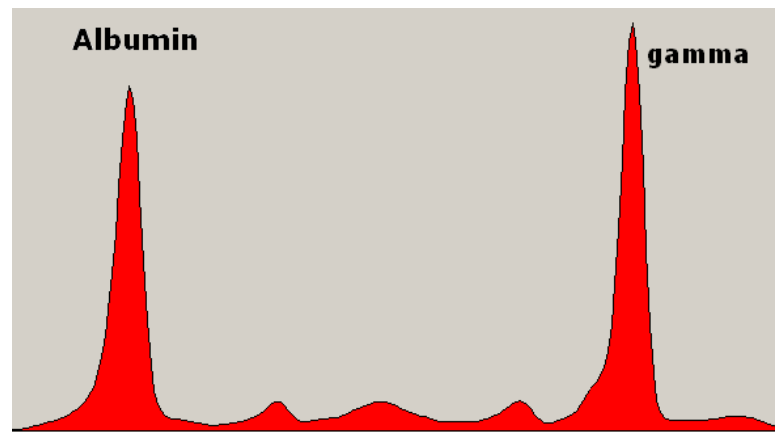
- Also called lymphoplasmacytic lymphoma
- **B-cell lymphoma**
- Tumor cells differentiate into plasma cells
- Produce **IgM antibodies**
- Leads to hyperviscosity symptoms



Martin Brändli /Wikipedia

Waldenstrom Macroglobulinemia

- Weakness, fatigue, weight loss
- Lymphadenopathy (25% of patients)
 - Sometimes splenomegaly, hepatomegaly
- **No osteolytic bone lesions**
- SPEP: M spike from IgM



Steven Fruitsmaak

Hyperviscosity Syndrome

- IgM increases viscosity of blood
- Sluggish blood flow and sludging
- **CNS**: Headache, dizziness, coma
- **Visual impairment**
- Medical emergency: emergent plasmapheresis

Poiseuille's Law

$$R = \frac{8 \eta \text{ (viscosity)} L \text{ (length)}}{\pi r \text{ (radius)}^4}$$

Amyloidosis

Jason Ryan, MD, MPH

Amyloid

- Amyloid: Pathologic aggregate of amyloid proteins
- “Pathologic:” Damages tissues
- Accumulates in **extracellular** space of tissues
- Amyloid proteins:
 - More than 20 proteins form amyloid
 - Different proteins = different diseases

Amyloid

- Localized amyloid deposition
 - Alzheimer's: Beta Amyloid
 - Cerebral amyloid angiopathy: Beta Amyloid
 - Type II diabetes: Amylin deposits in pancreas
- Diffuse amyloid deposition = **amyloidosis**

Systemic Amyloidosis

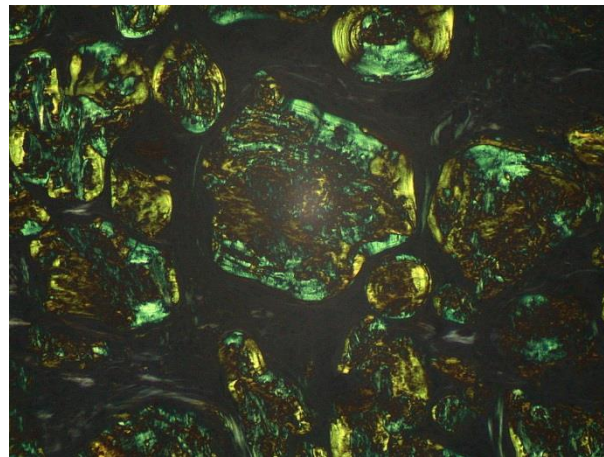
- Primary (AL) amyloidosis: Light chains
- Secondary (AA) amyloidosis: Serum amyloid A
- Dialysis-related amyloidosis: Beta-2 microglobulin
- Age-related systemic amyloidosis: : Transthyretin
- Familial
 - Many types
 - Most common: Abnormal transthyretin gene

Transthyretin

- Formerly called prealbumin
- Transports thyroid hormone and retinol (vitamin A)
- Amyloidosis: Amyloid transthyretin (ATTR)
- Mutant form seen in **hereditary** amyloidosis
- Normal transthyretin seen in **age-related** amyloidosis

Amyloid

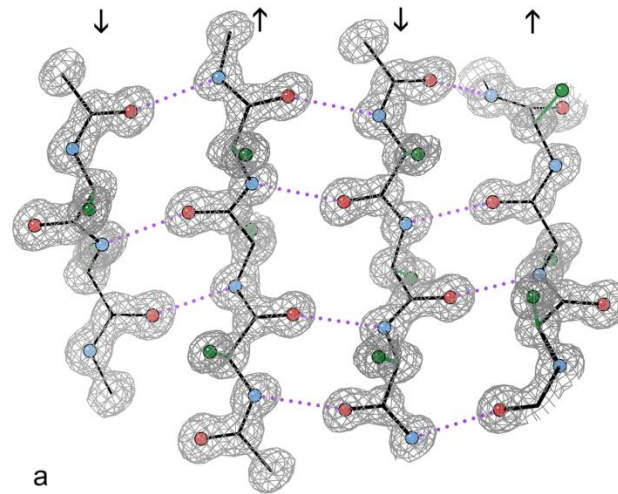
- Pink on standard biopsy
 - Similar to collagen, fibrin, other proteins
- Specialized stain for detection
 - Congo red
 - Pink under ordinary light
 - Shows apple-green birefringence under polarized light



Ed Uthman, MD

Amyloid

- Forms **beta-pleated sheets**
 - Secondary protein structure
 - Detected by crystallography and spectroscopy
- Responsible for Congo Red staining



Dcrjrs

AL Amyloidosis

Primary Amyloidosis

- Plasma cell malignancy (“dyscrasia”)
 - Amyloid formed from **light chains**
 - Can occur alone
 - Also in association with MM, Waldenstrom’s, lymphoma
- Bone marrow biopsy: monoclonal plasma cells
- Can be treated with stem cell transplantation

AA Amyloidosis

Secondary Amyloidosis

- Occurs in chronic inflammatory conditions
- Rheumatoid arthritis, ankylosing spondylitis, IBD
- Amyloid: **serum amyloid A (SAA) proteins**
 - SAA proteins: acute phase reactants
 - Apolipoproteins
 - Many roles in inflammatory response

Familial Mediterranean Fever



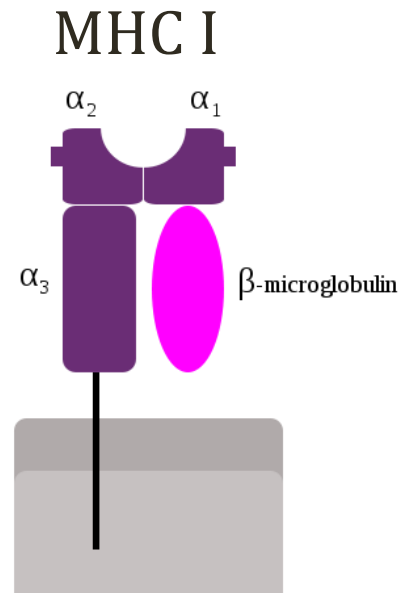
Neutrophil

Mikael Häggström

- Rare hereditary disorder
- Inflammatory disease
- Involves neutrophils
- Recurrent episodes of fever and inflammatory pain
- “Serosal” inflammation
 - Abdominal pain; pericarditis
- Secondary (AA) amyloidosis: **major cause of death**
- Treatment: **Colchicine** (inhibits neutrophils)

Dialysis-related Amyloidosis

- **β 2 microglobulin**
- Complication of renal failure
- Dialysis does not effectively remove β 2 microglobulin
- Bones, joints, tendons
- **Shoulder pain**
- **Carpal tunnel syndrome**



atropos235

Age-related Amyloidosis

Senile Amyloidosis

- **Wild-type (normal) transthyretin**
- Usually develops >70 years old
- Predominantly occurs in the heart
- Rarely other significant organ involvement

Familial Amyloidosis

- **Mutant transthyretin**
- Produced by **liver**
- Can be treated with liver transplant
- Symptoms in adulthood

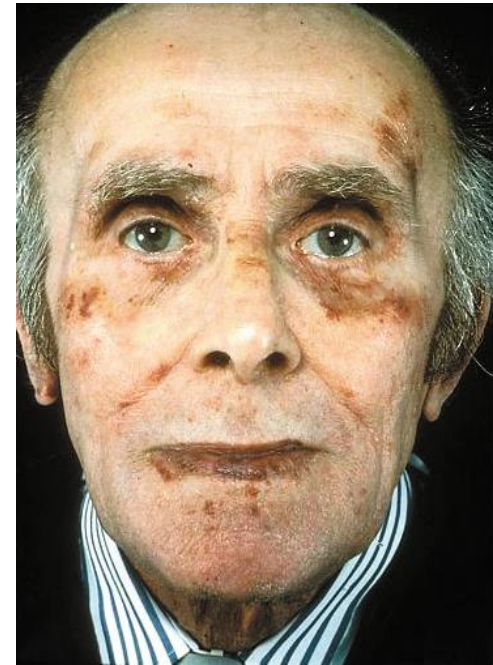


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Amyloidosis

Clinical Features

- May involve almost any tissue/organ
- Skin: Periorbital purpura (raccoon eyes)
- Muscles: Enlarged tongue
- Nerves: Peripheral neuropathy
- Liver: Hepatomegaly
- Bowel: Malabsorption
- Blood vessels: Bleeding



Professor P N Hawkins

Amyloidosis

Clinical Features

- Kidneys
 - Most commonly involved organ
 - Leads to proteinuria and the **nephrotic syndrome**

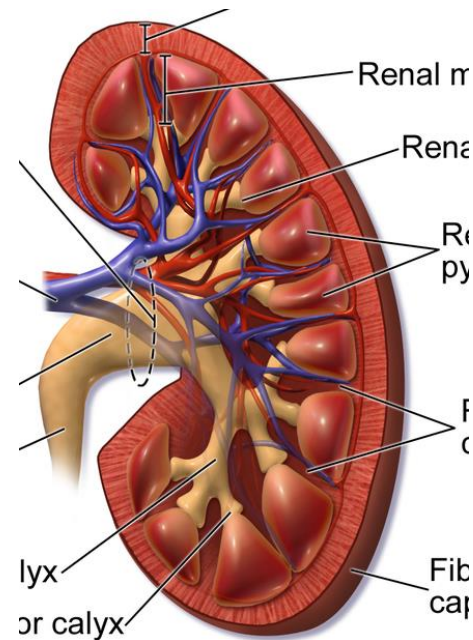
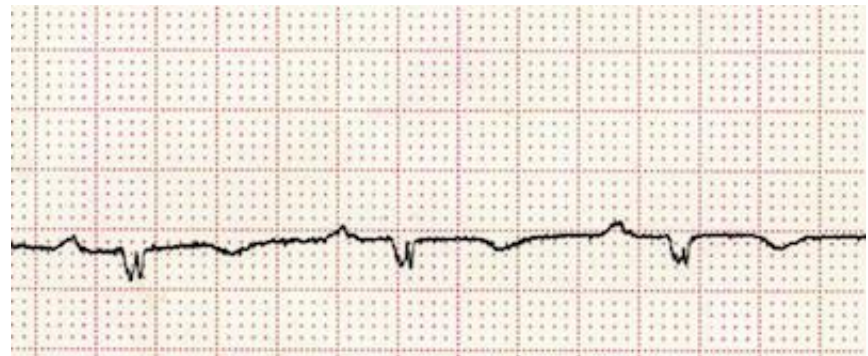


Image courtesy of BruceBlaus

Amyloidosis

Clinical Features

- Heart
 - Can cause a **restrictive** cardiomyopathy
 - Common with light chains and transthyretin amyloidosis
 - Increased wall thickness, diastolic heart failure
 - **Arrhythmias**, sudden death



Amyloidosis

Diagnosis

- Biopsy: tissue infiltration of amyloid
- **Abdominal fat pad** preferred
 - Easy to access (low risk procedure)
 - Good sensitivity



Wikipedia/Public Domain

Amyloidosis

Signs/Symptoms

Biopsy

+ Amyloid

Primary (AL)
Light Chains

Secondary (AA)
Serum Protein A

Dialysis
 β 2 Microglobulin

Senile
TTR

Hereditary
TTR

Myeloproliferative Disorders

Jason Ryan, MD, MPH

Myeloproliferative Disorders

- Disorders of myeloid **proliferation**
 - Granulocytes, red cells, platelets
- Often leads to increased peripheral cell counts
 - Chronic myeloid leukemia: granulocytes
 - Essential thrombocytosis: platelets
 - Polycythemia vera: red blood cells

Myeloproliferative Disorders

Major Types

- Chronic myeloid leukemia (granulocytes)
- Essential thrombocytosis (platelets)
- Polycythemia vera (red blood cells)
- Myelofibrosis

Myeloproliferative Disorders

Major Types

- Chronic myeloid leukemia (granulocytes)
- Essential thrombocytosis (platelets)
- Polycythemia vera (red blood cells)
- Myelofibrosis

Myeloproliferative Disorders

Major Types

Disorder	Genetics
Chronic Myelogenous Leukemia	Philadelphia Chromosome t 9;22; BCR-ABL
Polycythemia Vera	JAK2 (~100%)
Essential Thrombocytosis	JAK2 (~60%)
Myelofibrosis	JAK2 (~60%)

JAK2 Mutation

- Gene for cytoplasmic **tyrosine kinase**
 - Chromosome 9
- Mutation → ↑ tyrosine phosphorylation
- Progenitor cells: **Hypersensitivity to cytokines**
- More growth; longer survival

Polycythemia Vera

- Elevated red blood cell mass

Measurement	Normal	P. Vera
Hgb (g/dL)	15	20
Hct (%)	45	60

Polycythemia Vera

- Must exclude other causes
 - Hypoxia (lung disease)
 - EPO secreting tumor (renal cell carcinoma)

Measurement	Hypoxia	RCC	P. Vera
PaO ₂	↓	Normal	Normal
EPO	↑	↑	↓

Polycythemia Vera

Symptom Mechanisms

- Increased **RBC mass**
 - Leads to increase in **blood volume**
 - Causes hypertension, flushing
- Thrombosis
 - Increased viscosity of blood
 - Also increased platelets

Poiseuille's Law

$$R = \frac{8 \eta \text{ (viscosity)} L \text{ (length)}}{\pi r \text{ (radius)}^4}$$

Polycythemia Vera

Symptoms

- Many patients asymptomatic (routine CBC)
- Red, puffy skin (“facial plethora”)
- **Aquagenic pruritus**
 - “Unbearable” pruritus after warm bath or shower
- **Deep vein thrombosis**
 - Classically Budd Chiari syndrome (hepatic vein)

Polycythemia Vera

Treatment

- Phlebotomy
- Hydroxyurea
 - Inhibits **ribonucleotide reductase**
 - Blocks formation of deoxynucleotides for DNA



Public Domain

Polycythemia Vera

Complications

- Spent phase (~15% of patients)
 - Progression to **myelofibrosis**
- **Leukemia**
 - Usually acute myeloid leukemia (AML)
 - Rarely chronic myeloid leukemia (CML)
- **Gout**
 - Excess DNA turnover from ↑ RBC production
 - Increased **purine** metabolism → ↑ uric acid
 - Also seen in CML

Essential thrombocytosis

Essential thrombocythemia

- Malignant proliferation of myeloid cells
- Predominantly affects **megakaryocytes/platelets**

Essential thrombocytosis

Essential thrombocythemia

- “Diagnosis of exclusion”
- Must exclude a **reactive thrombocytosis**
 - Iron deficiency anemia
 - Acute bleeding or hemolysis
 - Infections/inflammation
 - Metastatic cancer
- Key blood test: acute phase reactants
 - C-reactive protein, fibrinogen, ESR, ferritin
 - Increased levels suggest occult inflammatory process

Essential thrombocytosis

Symptoms

- Abnormal platelet function
- **Bleeding**
- **Thrombosis**

Essential thrombocytosis

Prognosis and Treatment

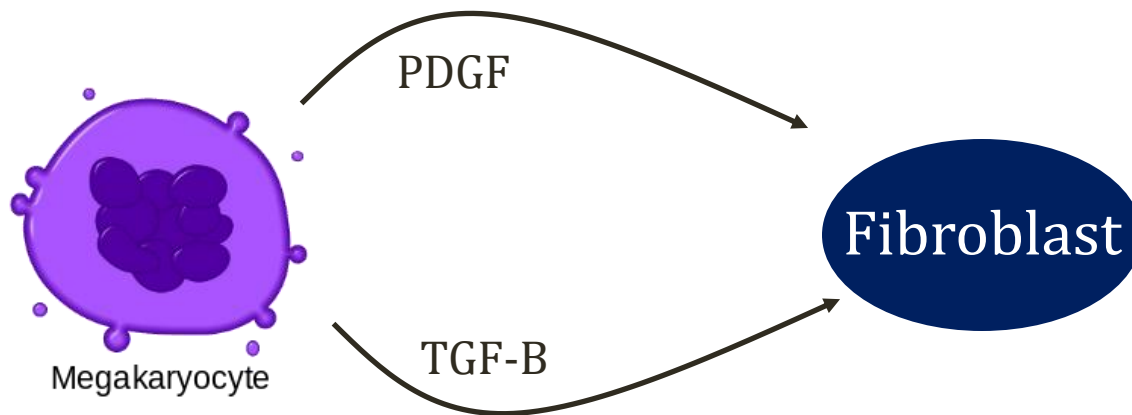
- Most patients have no disease-related complications
- Polycythemia vera complications unusual
 - AML, myelofibrosis, hyperuricemia
- High risk patients treated with:
 - Hydroxyurea
 - Aspirin

Myelofibrosis

- Primary myelofibrosis
 - Myeloproliferative disorder
- Secondary myelofibrosis
 - Polycythemia vera, chronic leukemia, other causes

Primary Myelofibrosis

- Excess collagen from fibroblasts → marrow fibrosis
- Stimulation by growth factors of **megakaryocytes**
 - Platelet-derived growth factor (PDGF)
 - Transforming growth factor beta (TGF-B)



Primary Myelofibrosis

Pathophysiology

- Marrow failure → **extramedullary hematopoiesis**
- Spleen, liver, lymph nodes
- Can be seen in CNS, lungs, bladder, even in skin!

Primary Myelofibrosis

Clinical Features

- Occurs in older patients (median age 67)
- Fatigue, weight loss, night sweats
 - Increased metabolism

Primary Myelofibrosis

Clinical Features

- Massive splenomegaly
 - Spleen: principle site of **extramedullary hematopoiesis**
 - Left upper abdominal pain
 - Early satiety (compression of stomach)
- May also see enlarged liver, lymph nodes
- Leukoerythroblastosis
 - Inappropriate release of cells from marrow
 - Immature erythroid and granulocyte precursors in blood

Primary Myelofibrosis

Clinical Features

- Normocytic, normochromic anemia
 - Severe
 - Hemoglobin often less than 10g/dL
- WBC and platelets variable
 - Elevated, normal, or reduced
 - Immature neutrophils seen
 - Myeloblasts
- Hyperuricemia (gout)
 - High cell turnover → increased metabolism
- Treatment: Stem cell transplant

Tear Drop Cells

Dacrocytes

- Classic finding of myelofibrosis
- Red blood cells deformed leaving fibrotic marrow



Paulo Henrique Orlandi Mourao/Wikipedia

Langerhans Cell Histiocytosis

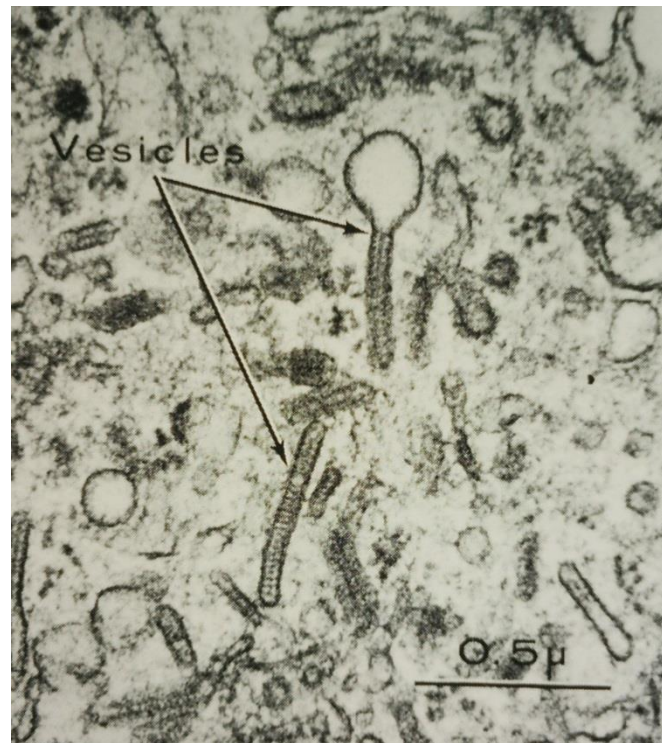
- Histiocyte = connective tissue macrophage
- Histiocytosis = proliferation of histiocytes
- Langerhans Cell = dendritic cells
 - Common in skin, connective tissue
 - Consume antigens
 - Migrate to lymph nodes
 - Present antigens → activate T-cells

Langerhans Cell Histiocytosis

- Clonal proliferation of dendritic cells
- Cells of myeloid origin
- Express CD1a, S100, CD207
 - Same as Langerhans cells

Birbeck Granules

- Found in cytoplasm of Langerhans cells
- Seen on electron microscopy



Yale Rose/Wikipedia

Langerhans Cell Histiocytosis

Clinical Features

- Most common in **children**
- Can involve any organ system
- Often involves **bone** and **skin**

Langerhans Cell Histiocytosis

Clinical Features

Letterer-Siwe Disease

Occurs in child (~2 years old)

Diffuse skin rash

Cystic bone lesions

Multi-system failure

Rapidly fatal

←→
Spectrum

Eosinophilic Granuloma

Adolescents

No skin involvement

Pathologic bone fracture

NOT osteosarcoma

Langerhans cells/eosinophils

Hand-Schuller-Christian Disease

Triad: skull, diabetes insipidus, exophthalmos

Scalp lesion

Posterior pituitary (DI)

Protrusion of eye

Langerhans Cell Histiocytosis

Clinical Features



Countincr/Wikipedia



Madhero88/Wikipedia

Antimetabolites

Jason Ryan, MD, MPH

Antimetabolites

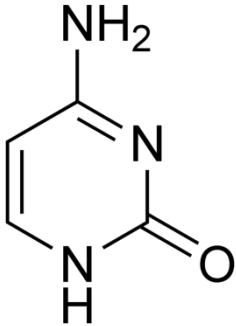
- Chemotherapy drugs used to treat malignancy
- **Block formation of components of DNA**
- Cell cycle specific
- Toxic effects in **S phase** of cell cycle

Antimetabolites

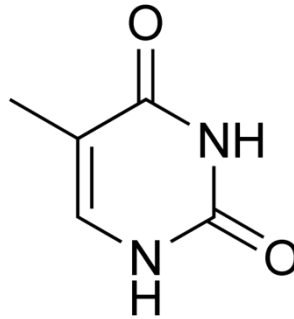
- Cytarabine
- Cladribine
- Methotrexate
- 5-fluorouracil
- Azathioprine
- 6-mercaptopurine
- 6-thioguanine
- Hydroxyurea

Nitrogenous Bases

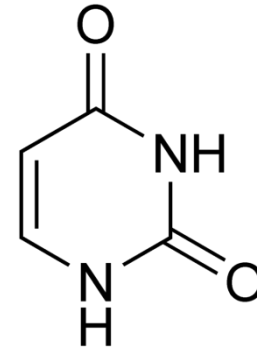
Pyrimidines



Cytosine

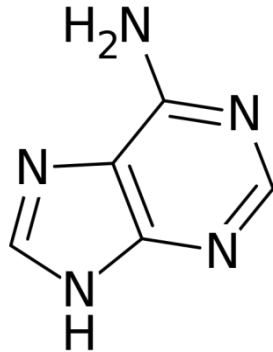


Thymine

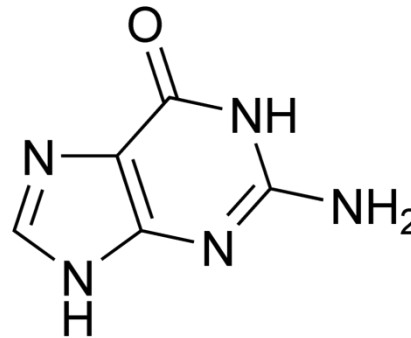


Uracil

Purines



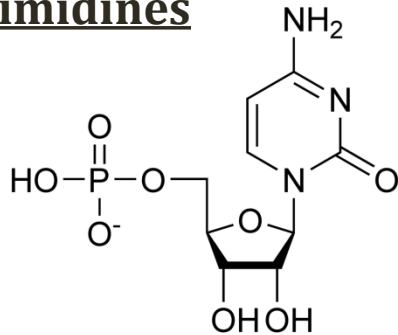
Adenine



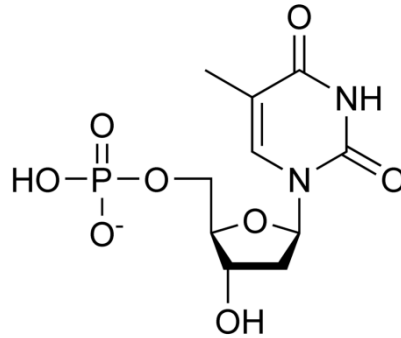
Guanine

Nucleotides

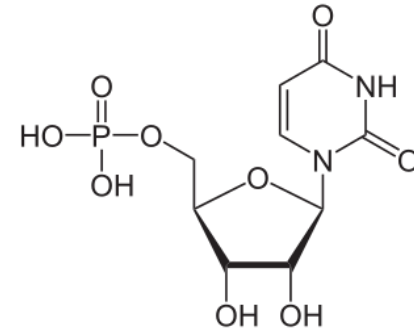
Pyrimidines



Cytidine

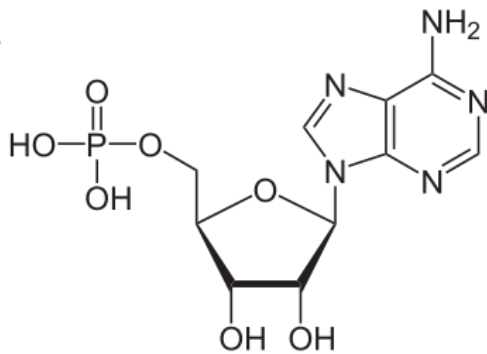


Thymidine

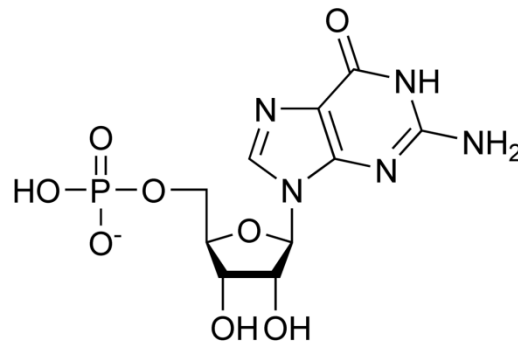


Uridine

Purines

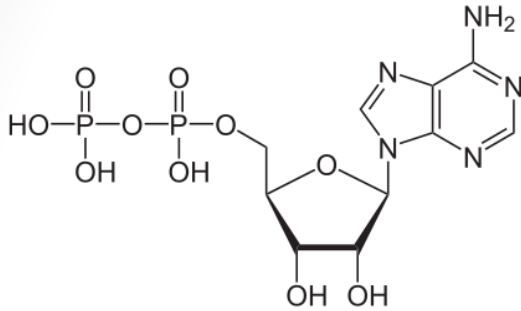


Adenosine

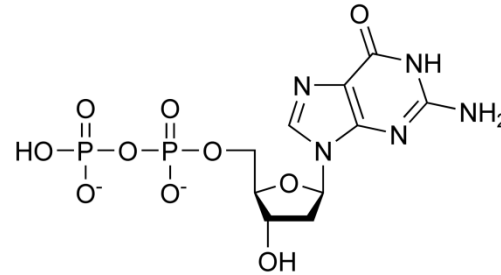


Guanosine

Deoxyribonucleotides

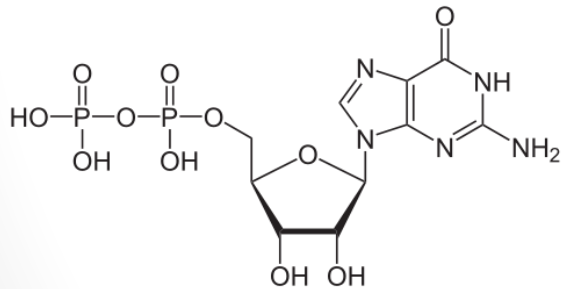


ADP

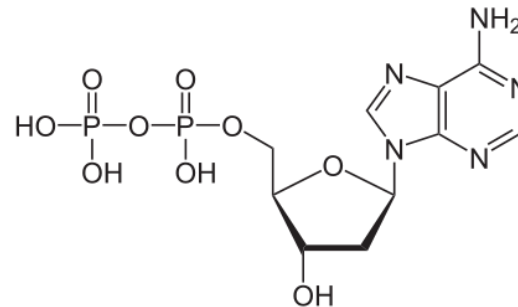


dADP

**Ribonucleotide
Reductase**



GDP



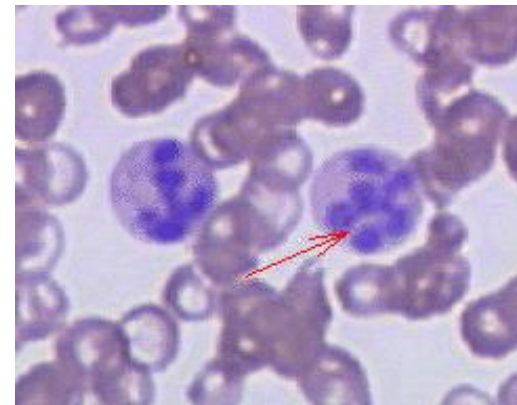
dGDP

Common Side Effects

- Drugs target rapidly dividing cells (DNA synthesis)
- Bone marrow precursors cells: rapidly dividing
- **Myelosuppression**
 - Megaloblastic anemia
 - Thrombocytopenia
 - Leukopenia
- Absolute neutrophil count (ANC)
 - Less than 500 cells/ μ L = neutropenia
 - High risk of infections

Megaloblastic Anemia

- Anemia (\downarrow Hct)
- Large RBCs (\uparrow MCV)
- Hypersegmented neutrophils
- Commonly caused by defective DNA production
 - B12/Folate deficiency
 - Chemotherapy drugs (MTX, 5-FU, hydroxyurea)

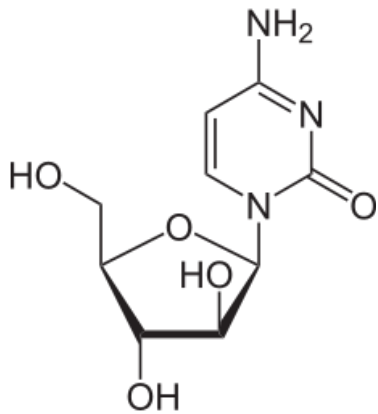


Wikipedia/Public Domain

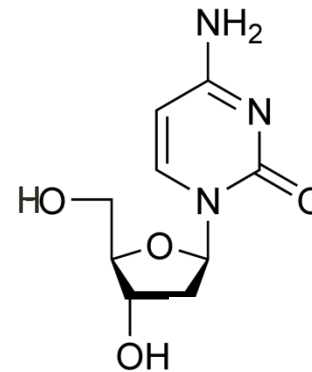
Cytarabine

Ara-C or cytosine arabinoside

- **Pyrimidine** analog
- Mimics cytidine
- Inhibits DNA polymerase



Ara-C



dCytidine

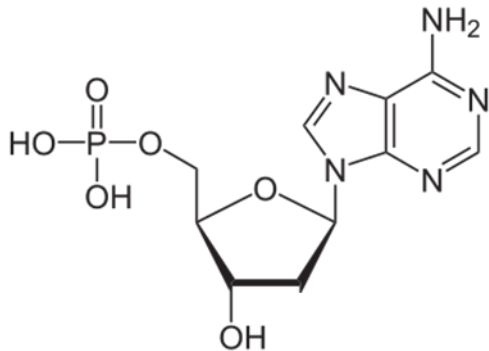
Cytarabine

Ara-C or cytosine arabinoside

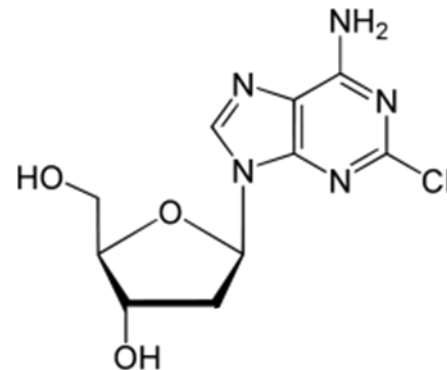
- Only effective in leukemia and lymphomas
- Adverse effects
 - Myelosuppression
 - Nausea/vomiting
 - High doses: Neurotoxicity
 - Peripheral neuropathy, confusion, cerebellar ataxia

Cladribine

- **Purine** analog
- Mimics adenosine
- Highly toxic to lymphocytes
- Drug of choice in **hairy cell leukemia**
- Main adverse effect is myelosuppression



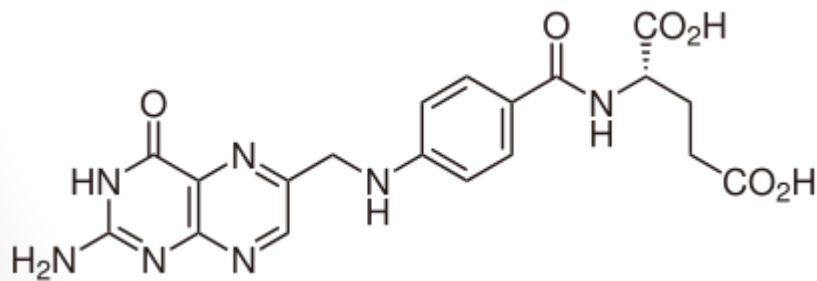
Adenosine



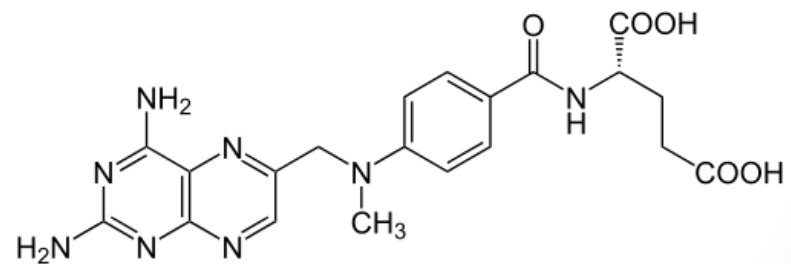
Cladribine

Methotrexate

- Mimics of folate
- Inhibits **dihydrofolate reductase**
- Blocks synthesis of **tetrahydrofolate**
- Required for DNA, RNA, some proteins
- Blocks synthesis of **thymidine** (dTMP)

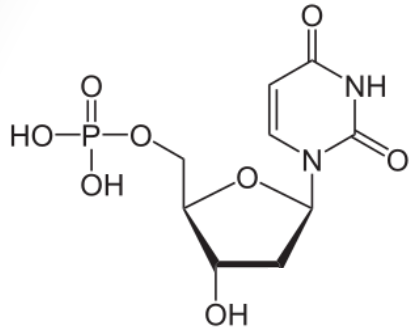


Folate



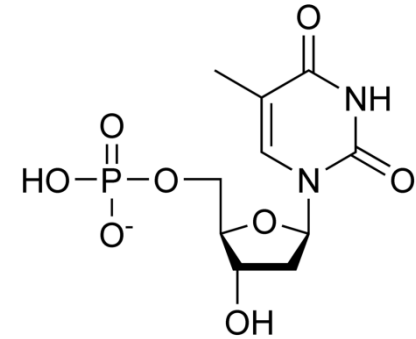
Methotrexate

Thymidine



dUridine-MP

Thymidylate
Synthase



Thymidine-MP

N5, N10 Tetrahydrofolate

DHF ← Folate



**Dihydrofolate
Reductase**

THF

Methotrexate

Clinical Uses

- Oral or intravenous
- Many malignancies
 - Solid tumors
 - Leukemia/Lymphomas
- Immunosuppression
 - Autoimmune diseases
 - “Steroid sparing” agents
 - Used to wean/eliminate need for long-term steroid use
- Pregnancy abortion
 - Ectopic/tubal pregnancies

Methotrexate

Side Effects

- Myelosuppression
 - More common with high doses
 - Reversible with **leucovorin (folinic acid)**
 - Converted to THF
 - Does not require dihydrofolate reductase
 - “Leucovorin rescue”

Methotrexate

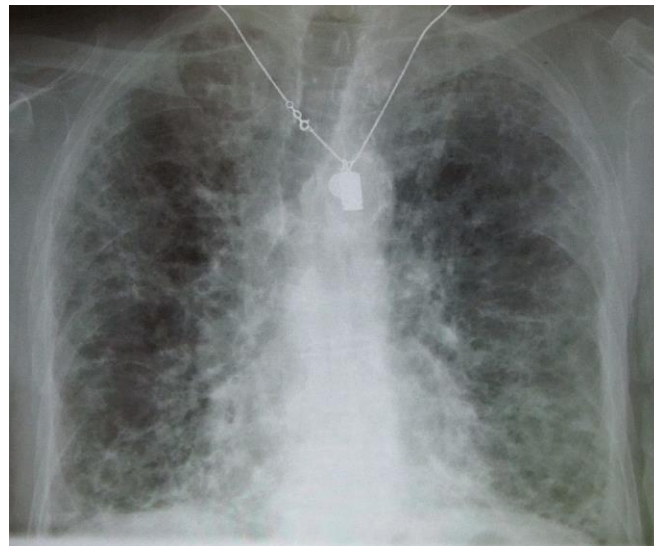
Side Effects

- Mucositis (mouth soreness)
 - Occurs with many chemo agents
 - Common with methotrexate
 - GI epithelial cell damage
 - Loss of mucosal integrity → pain, bacterial growth
- Abnormal LFTs, GI upset

Methotrexate

Side Effects

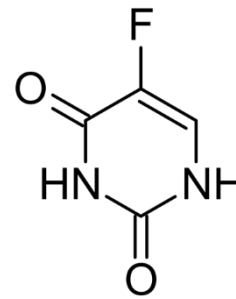
- Rarely causes **methotrexate-induced lung injury**
 - Often after week/months of low-dose therapy
 - Usually a hypersensitivity reaction
 - Lymphocytes, eosinophils
 - Can progress to pulmonary fibrosis
 - Usually resolves on discontinuation of drug



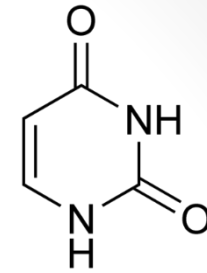
5-Fluorouracil

5-FU

- Mimics **uracil** (**pyrimidine**)
- Converted to 5-FdUMP (abnormal dUMP)
- Inhibition thymidylate synthase
- Blocks dTMP synthesis (“thymineless death”)
- Effects **enhanced** by leucovorin



Fluorouracil

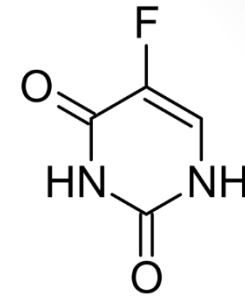


Uracil

5-Fluorouracil

5-FU

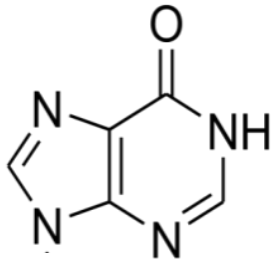
- Commonly used in **colorectal cancer**
- Other solid tumors: breast, pancreas
- Topical therapy for basal cell skin cancer
- Adverse effects
 - Myelosuppression
 - Nausea/vomiting/diarrhea
 - Mucositis
 - Cerebellar ataxia and encephalopathy (rare)
 - Coronary vasospasm



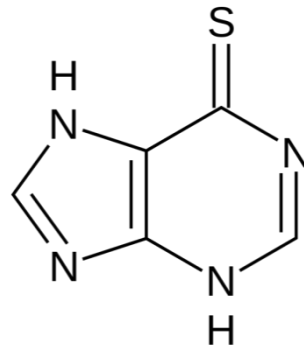
Fluorouracil

6-Mercaptopurine

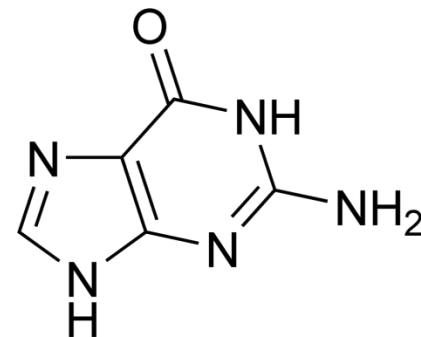
- Mimics hypoxanthine/guanine (**purines**)
- Added to PRPP by HGPRT → Thioinosinic acid
- Inhibits multiple steps in purine salvage
- ↓IMP/AMP/GMP



Hypoxanthine

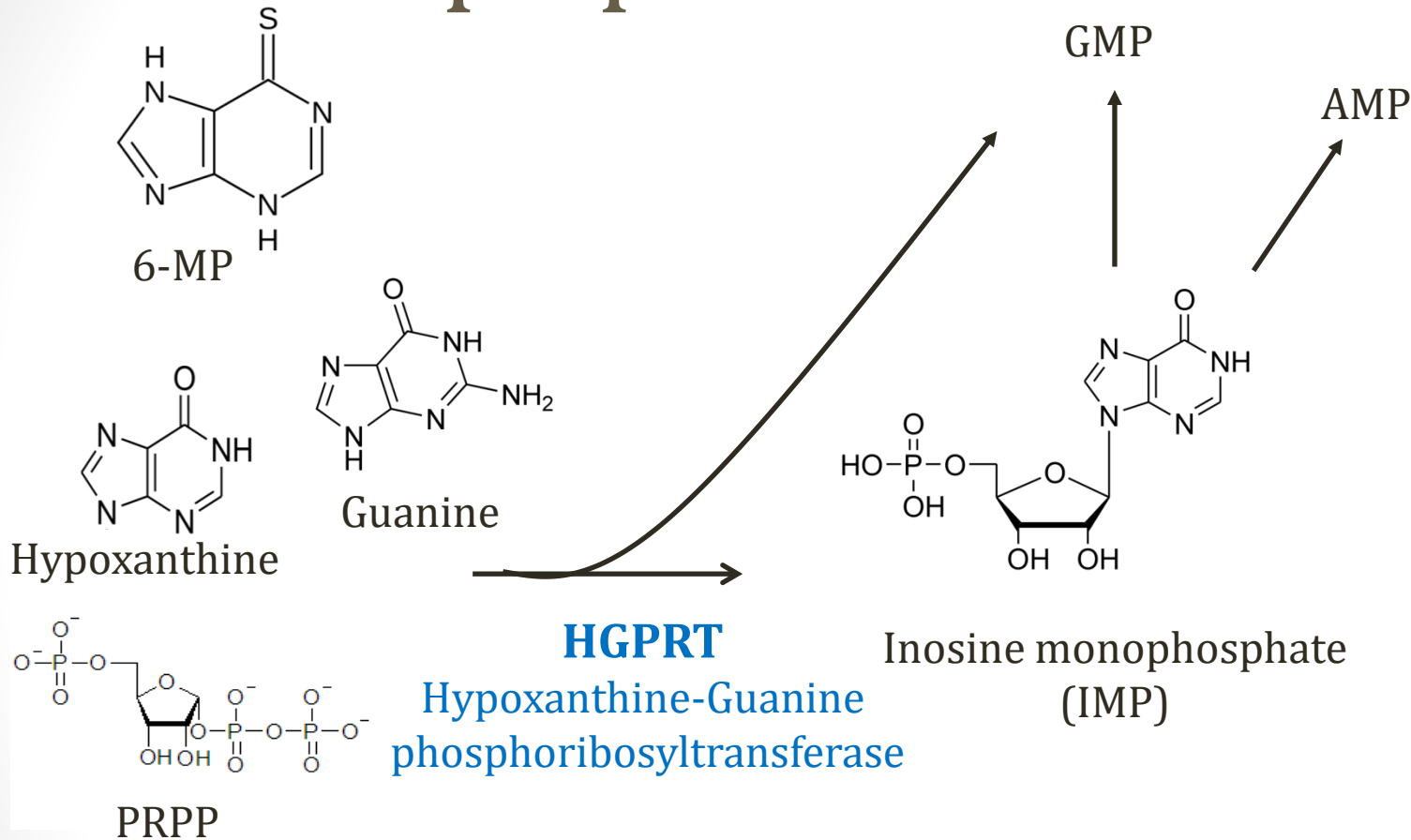


6-MP



Guanine

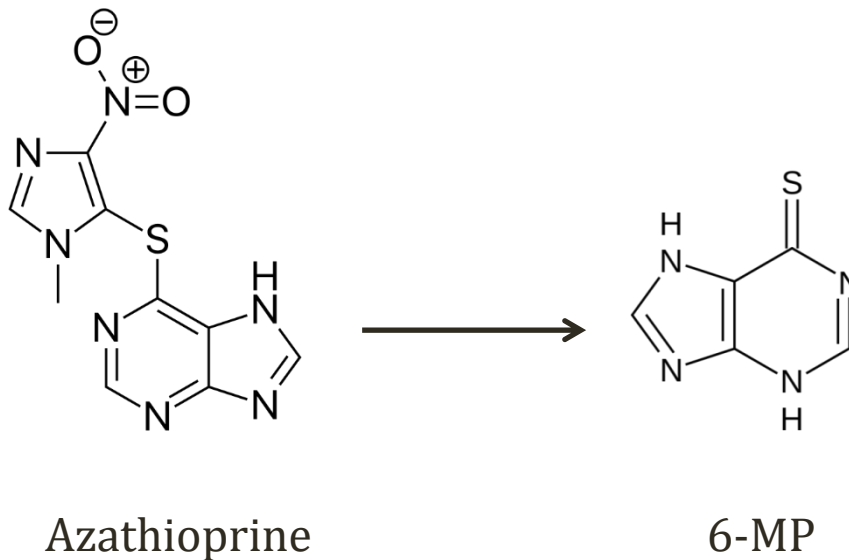
6-Mercaptopurine



Purine Salvage Pathway

Azathioprine

- Pro-drug
- Converted by the body to 6-MP



Azathioprine/6-MP

Clinical Uses

- Immunosuppression
 - Steroid sparing agents
 - Inflammatory bowel disease
 - Solid organ transplant
 - Autoimmune diseases

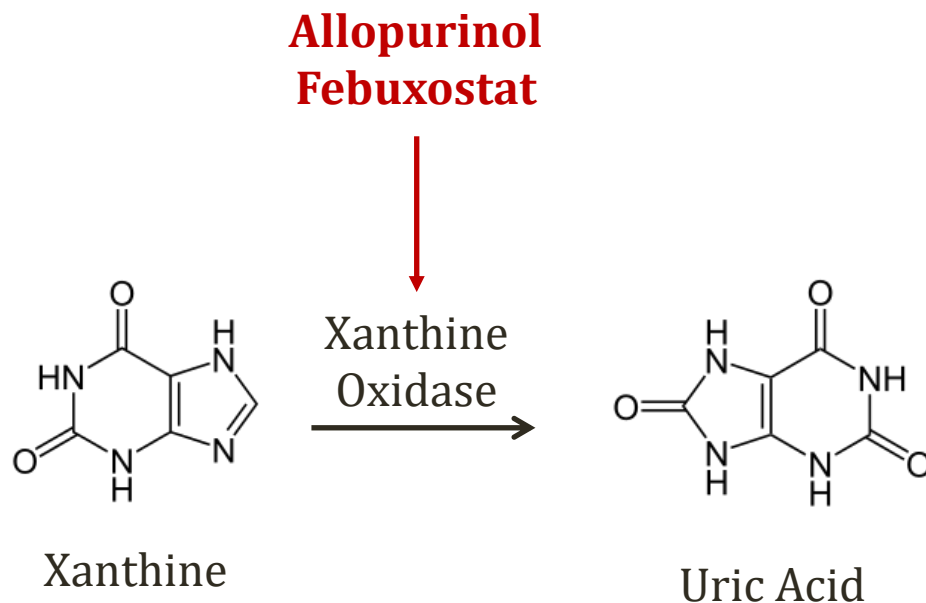
Azathioprine/6-MP

Adverse Effects

- Myelosuppression
- Abnormal LFTs
- Anorexia/nausea/vomiting

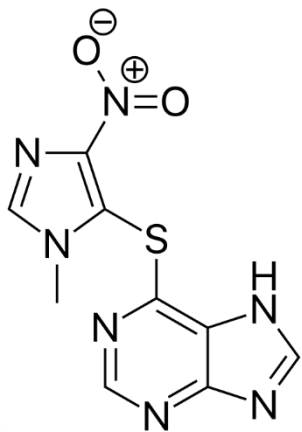
Xanthine Oxidase

- Purine metabolism enzyme
- Converts xanthine into uric acid
- Inhibited by allopurinol and febuxostat (gout)

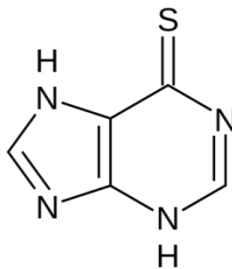


Azathioprine/6-MP

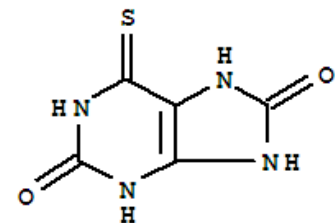
- Also metabolized by xanthine oxidase
- Converts 6-MP to inactive metabolite
- Caution with allopurinol/febuxostat



Azathioprine



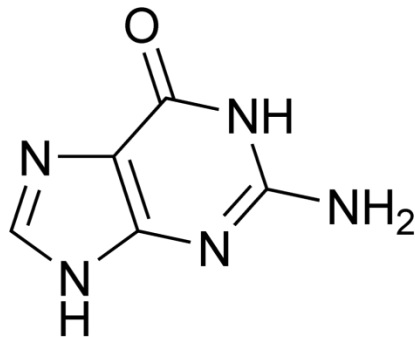
6-MP



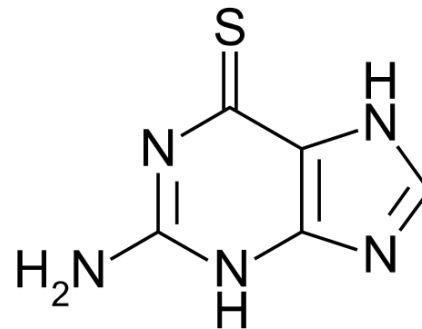
6-thiouric acid
(inactive)

6-Thioguanine

- Also mimics hypoxanthine/guanine (**purines**)
- Similar mechanism to 6-MP
- ↓IMP/AMP/GMP



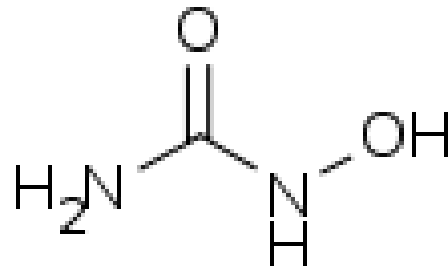
Guanine



6-TG

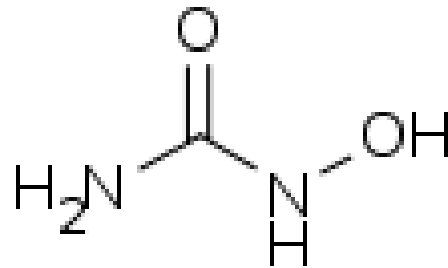
Hydroxyurea

- Inhibits **ribonucleotide reductase**
- Blocks formation of deoxynucleotides
- Good oral bioavailability – can be used PO
- Main adverse effect is myelosuppression

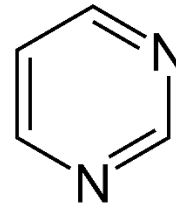
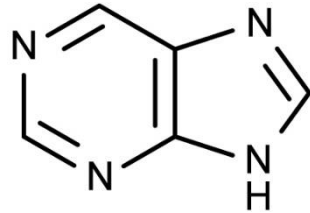


Hydroxyurea

- Rarely used for malignancy
- Used for polycythemia vera, essential thrombocytosis
- Used in sickle cell anemia
 - Increases fetal hemoglobin levels (mechanism unclear)



Antimetabolites



Folate	Purines	Pyrimidines	Other
Methotrexate	Cladribine 6-MP Azathioprine 6-TG	Cytarabine 5-FU	Hydroxyurea

Alkylating Agents

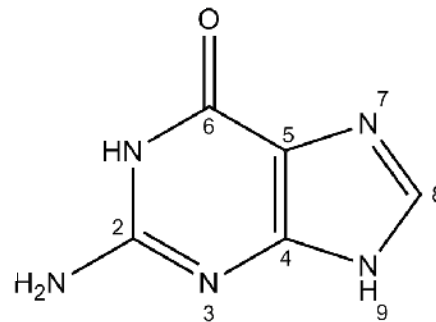
Jason Ryan, MD, MPH

Alkyl Groups

- Molecular groups with formula: C_nH_{2n+1}
- Methyl group: $-CH_3$
- Ethyl group: $-CH_2CH_3$
- Propyl group: $-CH_2CH_2CH_3$

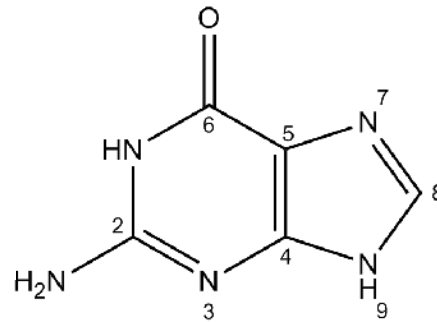
Alkylating Agents

- Add alkyl groups to nucleotide bases
- Most commonly **N7 nitrogen of guanine**
- **DNA strands cross link**
- Inhibit DNA replication and cause DNA damage
- Cell cycle non-specific

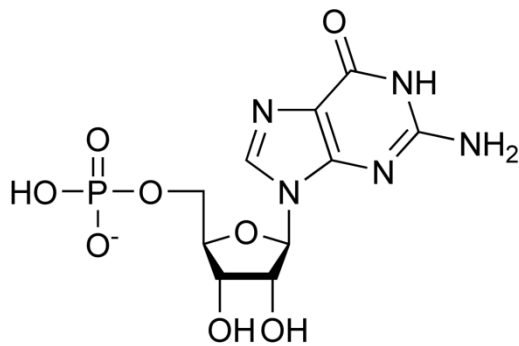


Guanine

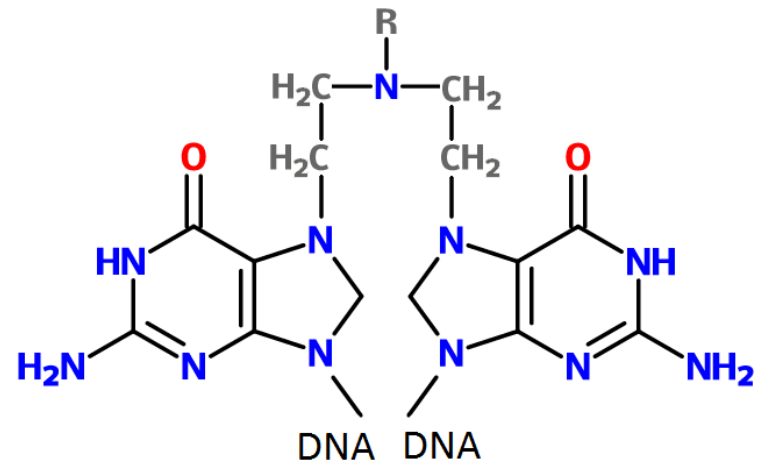
Alkylating Agents



Guanine



Guanosine



N7 interstrand crosslinked DNA

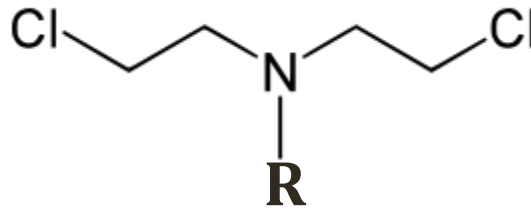
Alkylating Agents

- Nitrogen mustards
- Nitrosoureas
- Busulfan
- Dacarbazine

Nitrogen Mustards

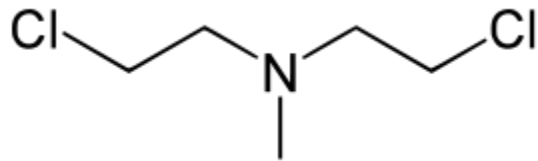
Alkylating Agents

- Alkylating agents similar to mustard gas
- Contain nitrogen and two chlorine atoms

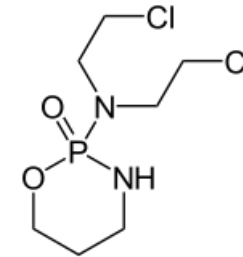


Nitrogen Mustards

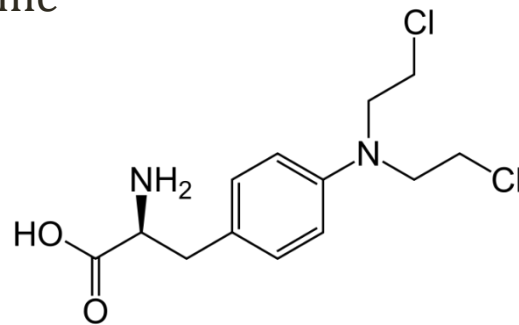
Alkylating Agents



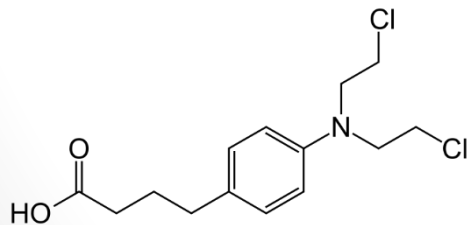
Mechlorethamine



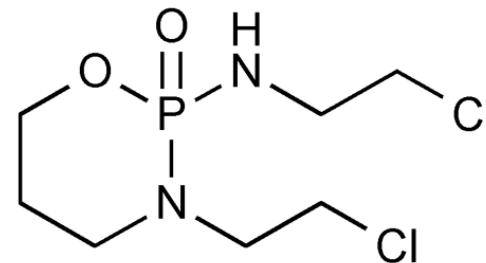
Cyclophosphamide



Melphalan



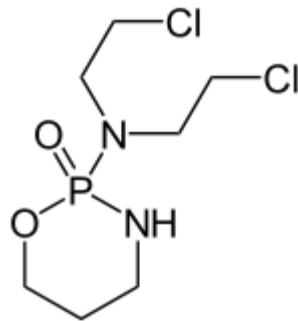
Chlorambucil



Ifosfamide

Cyclophosphamide

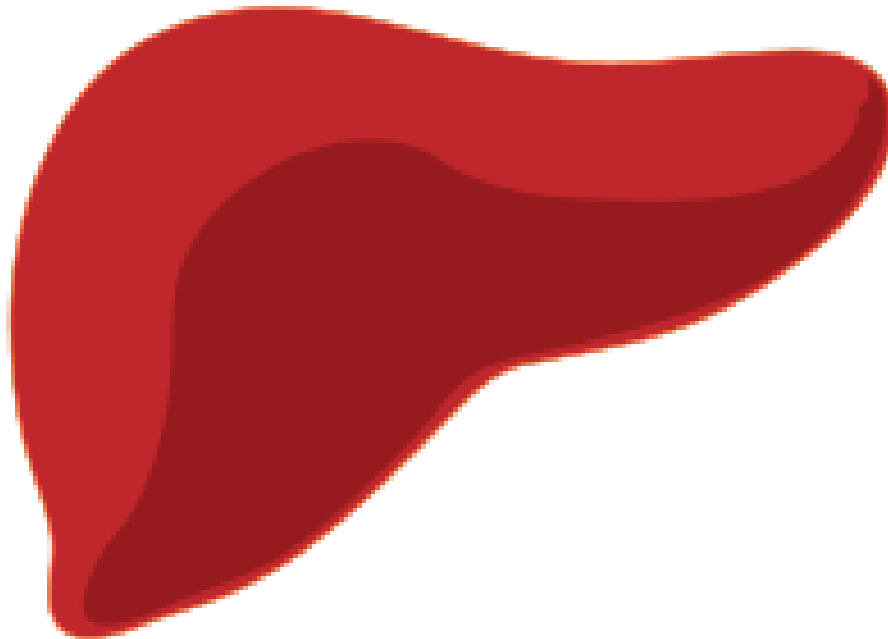
- Intravenous or oral forms
 - Good bioavailability when given orally
- Powerful immunosuppressant
 - Used in vasculitis, glomerulonephritis (oral)
- Solid tumors, lymphomas, leukemia



Cyclophosphamide

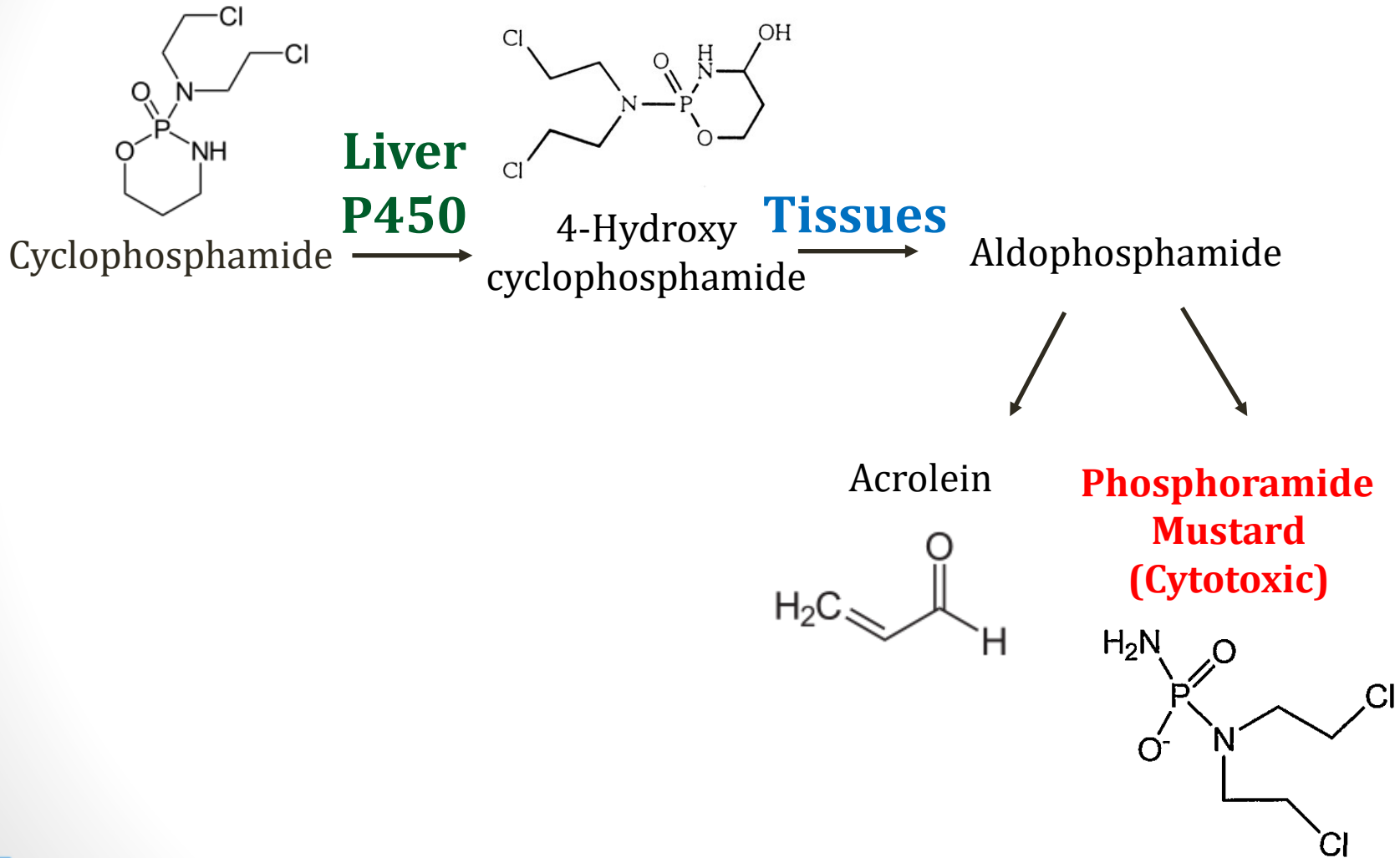
Cyclophosphamide

- Prodrug: Requires bioactivation by **liver**
 - Converted to **phosphoramidate mustard**
 - Metabolized by **liver P450 system**



Wikipedia/Public Domain

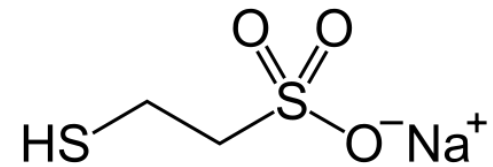
Cyclophosphamide



Cyclophosphamide

Side Effects

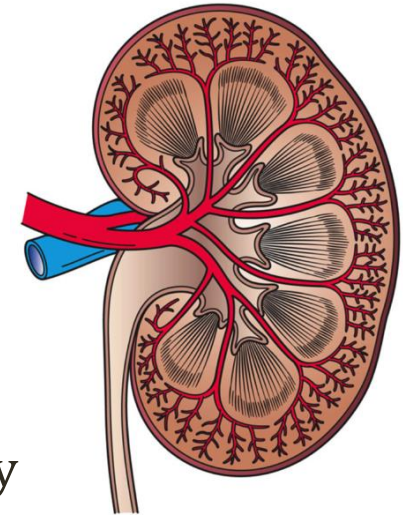
- Myelosuppression
 - ↓WBC, ↓Hct, ↓Plt
- Hemorrhagic cystitis
 - **Acrolein** metabolite toxic to bladder
 - Hematuria +/- dysuria
 - Lower risk with hydration and **mesna**
 - Mesna: sodium 2-mercaptoethane sulfonate
 - Mesna binds and inactivates acrolein in the urine



Mesna

Cyclophosphamide

Side Effects

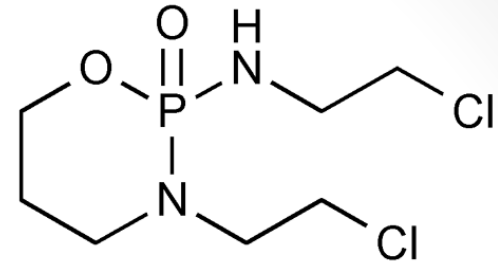


Holly Fischer/Wikipedia

- SIADH
 - Drug has antidiuretic effects
 - Usually occurs with IV dosing for chemotherapy
 - **Hyponatremia**; possible **seizures**
 - Compounded by IVF
 - Complex mechanism: More ADH release, less renal response

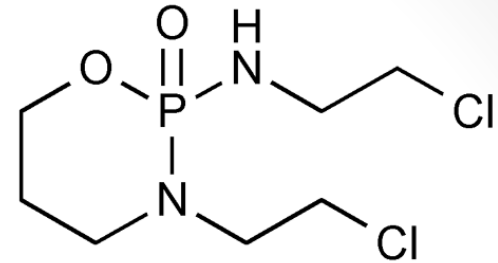
Ifosfamide

- Isomer of cyclophosphamide
- Used in germ cell cancer and sarcomas
- May also cause **hemorrhagic cystitis**



Ifosfamide

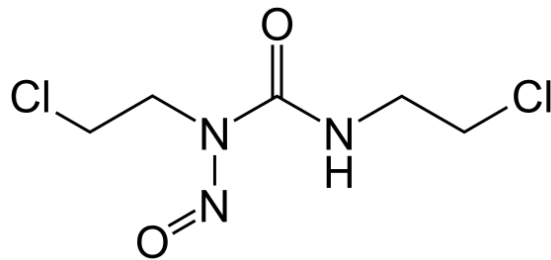
Ifosfamide



Ifosfamide

- Special side effect: **nephrotoxicity**
 - Toxic to proximal tubular cells
 - May cause **Fanconi syndrome**
 - Polyuria
 - Electrolyte losses: Hypokalemia, hypophosphatemia
 - Metabolic acidosis (loss of bicarb in urine)
- Special side effect: **encephalopathy**
 - 10-30% of patients

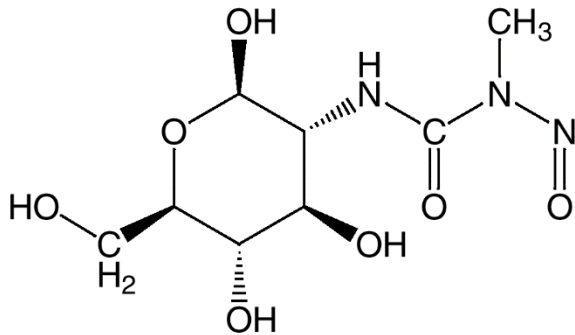
Nitrosoureas



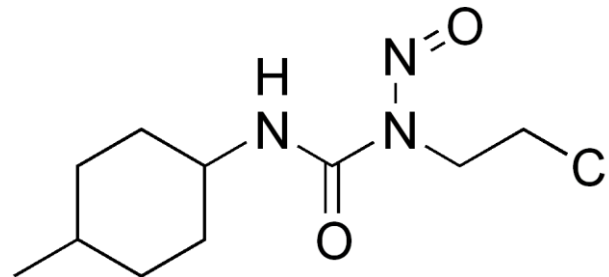
Carmustine
bis-chloroethylnitrosourea
BCNU



Lomustine
chloroethylnitrosourea
CCNU



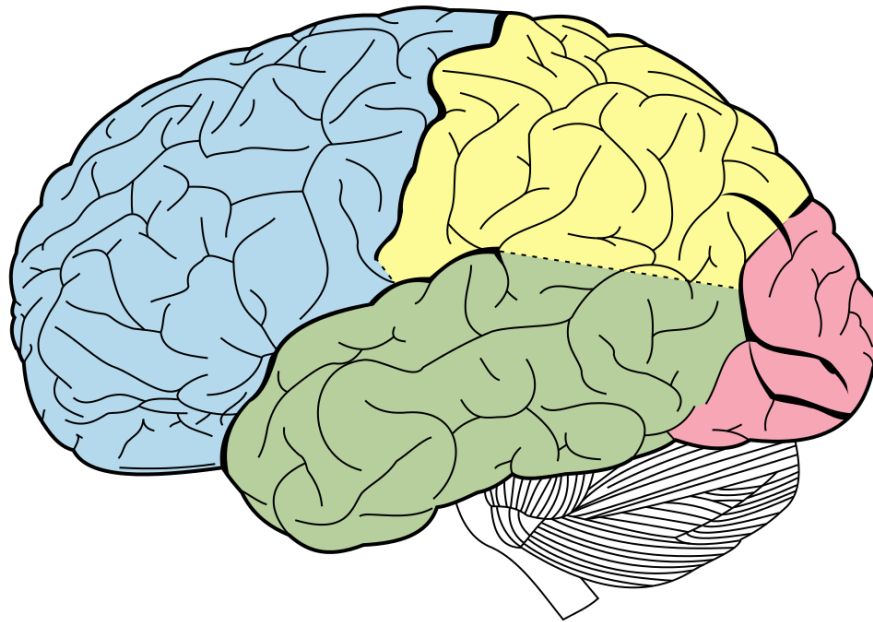
Streptozotocin



Semustine

Nitrosoureas

- Bioactivated in **liver**
- Highly lipid soluble → cross blood-brain barrier
- Used for **brain tumors** (glioblastoma multiforme)

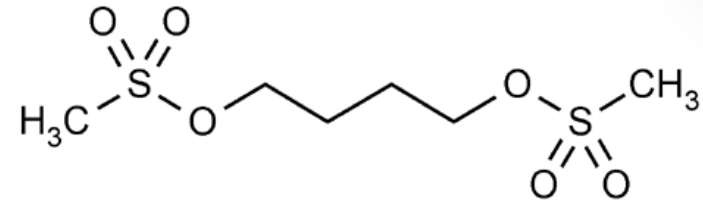


Nitrosoureas

Toxicity

- Myelosuppression
- Rarely leads to **pulmonary fibrosis**
- Rarely chronic interstitial nephritis (renal failure)
- **Encephalopathy and seizures**
 - Very high dosages (BCNU for bone marrow transplant)

Busulfan



- **Myeloablation**
 - Single, high-dose of Busulfan
 - Results in severe pancytopenia (bone marrow ablation)
 - Preparation for stem cell transplant
- Chronic myeloid leukemia (CML)

Busulfan

Toxicity

- Myelosuppression
- Skin hyperpigmentation
 - Also occurs with other chemotherapy (Bleomycin)
- Seizures (high dosages)

Busulfan

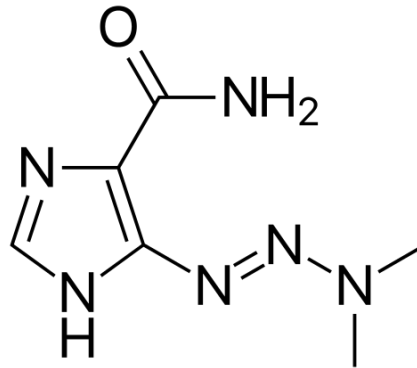
Toxicity

- **Pulmonary toxicity**
 - Cough, dyspnea
 - Can progress to pulmonary fibrosis
 - Ground glass opacities
 - Restrictive PFTs
 - Reduced DLCO



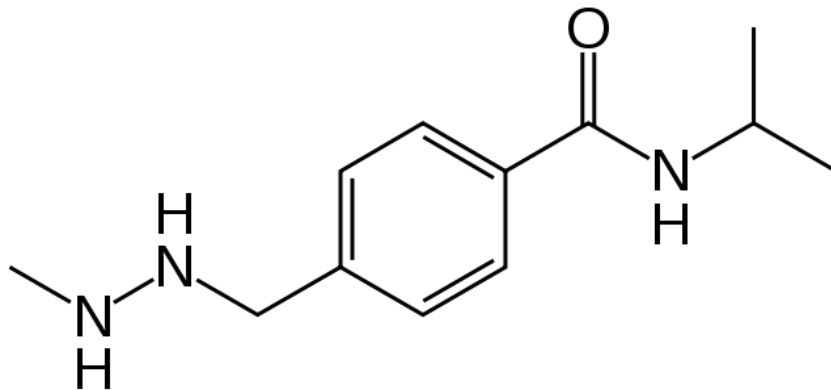
Dacarbazine

- Part of ABVD protocol for Hodgkin lymphoma
 - Adriamycin (doxorubicin) - cytotoxic antibiotics
 - Bleomycin - cytotoxic antibiotics
 - Vinblastine – microtubule inhibitor
 - **Dacarbazine** – alkylating agent



Procarbazine

- Part of MOPP protocol for Hodgkin lymphoma
 - Mechlorethamine – Mustard agent
 - Oncovin (Vincristine) – Microtubule drug
 - **Procarbazine**
 - Prednisone



Antitumor Antibiotics

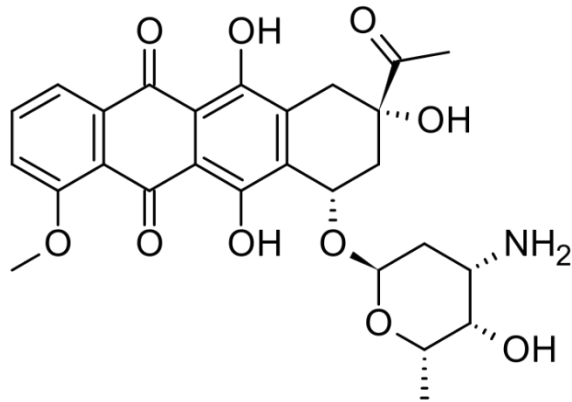
Jason Ryan, MD, MPH

Antitumor Antibiotics

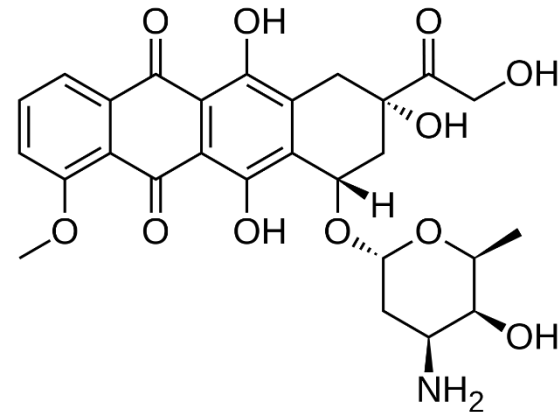
- Drugs derived from *Streptomyces* bacterial strains
- Anthracyclines
- Dactinomycin
- Bleomycin

Anthracyclines

- Key drugs: daunorubicin and doxorubicin
- Others: idarubicin, epirubicin, mitoxantrone



Daunorubicin



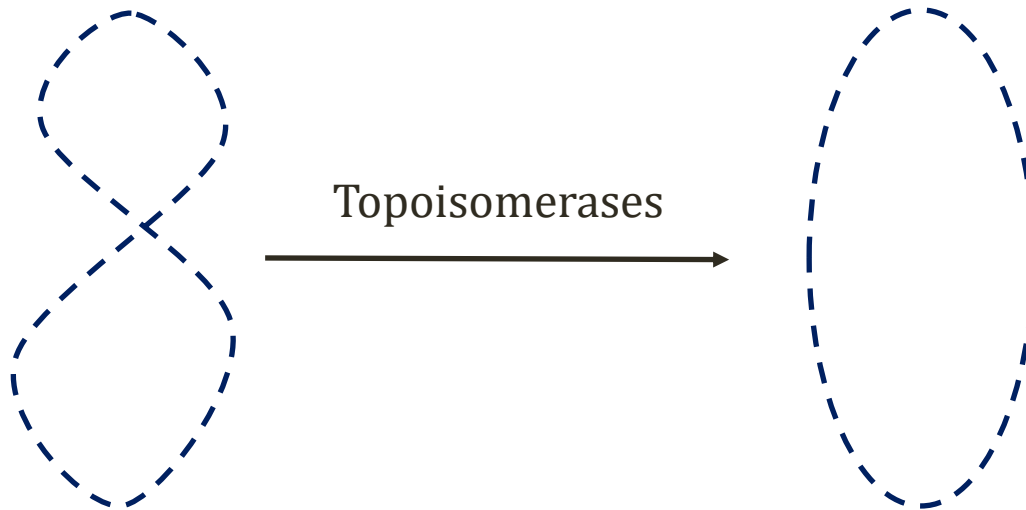
Doxorubicin
(Adriamycin)

Anthracyclines

- Multiple toxic mechanisms
 - Inhibition of topoisomerase II → DNA breaks
 - Intercalation of DNA → blocks synthesis of DNA/RNA
 - Generation of free radicals
- Cell cycle non-specific

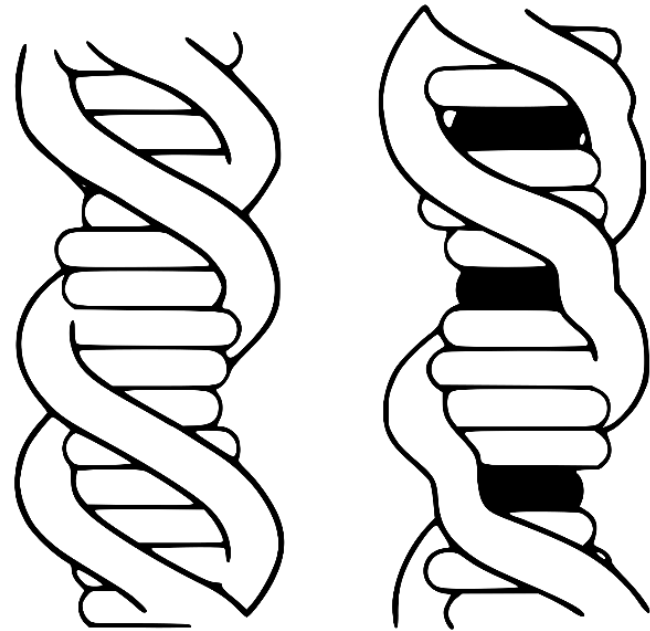
Topoisomerase II

- Cuts both strands of DNA helix then reseals
- Relieves tangles and supercoils
- Anthracycline inhibition → breaks with no resealing
- Result: DNA damage



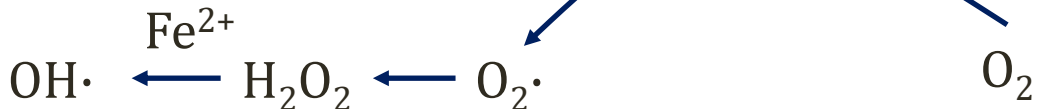
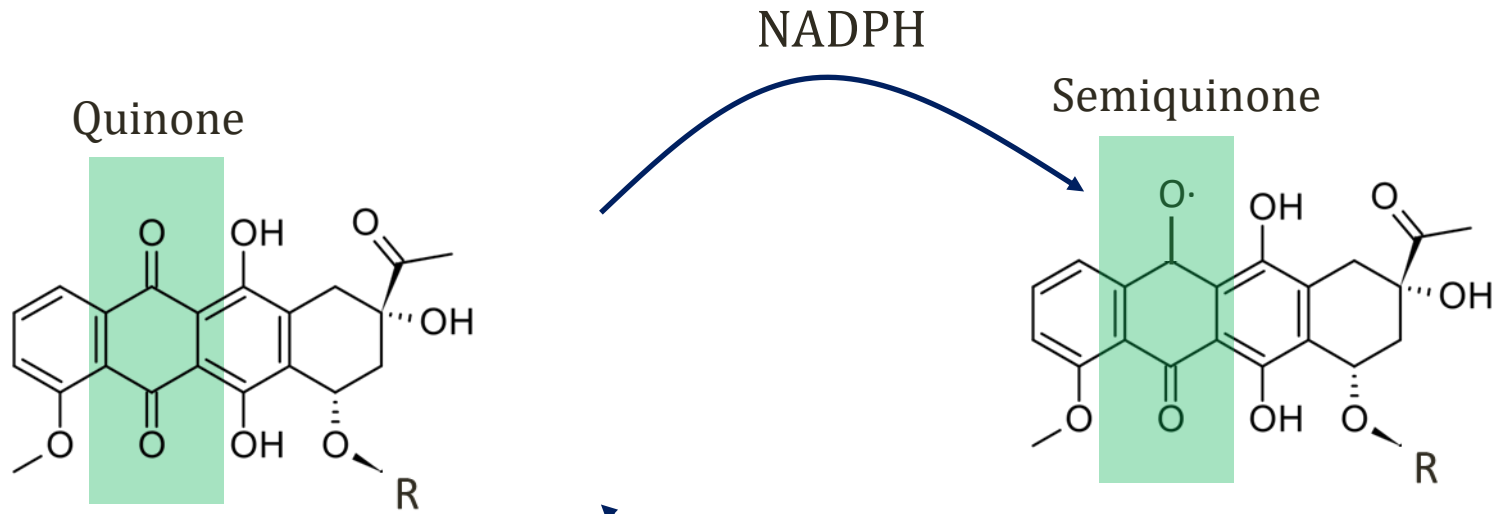
DNA Intercalation

- Binds to DNA
- Inserts between base pairs
- Inhibits replication/transcription



Wikipedia/Public Domain

Free Radicals



**DNA
Oxidative
Damage**

Anthracyclines

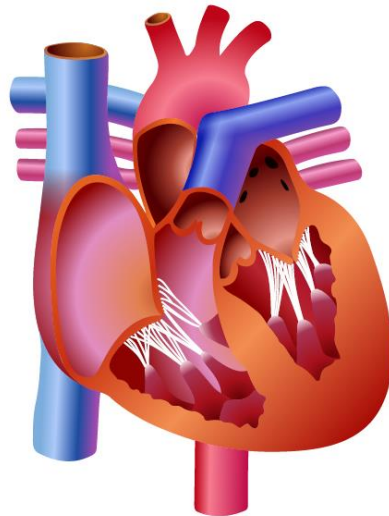
Clinical Uses

- **Doxorubicin (Adriamycin)**
 - Widely used anticancer drug
 - **Breast cancer**
 - Many solid tumors
 - Childhood cancers: neuroblastoma, Ewing's, osteosarcoma
 - Leukemia/lymphoma

Anthracyclines

Cardiotoxicity

- **Systolic heart failure (\downarrow LVEF)**
- **Free radical damage** to myocytes \rightarrow necrosis
- Can present with dyspnea, fatigue, edema
- Screening: echocardiogram after infusions



Anthracyclines

Cardiotoxicity

- Rarely seen with lower total dosages
- **Dexrazoxane**
 - Iron chelating agent
 - Limits anthracycline-induced cardiotoxicity

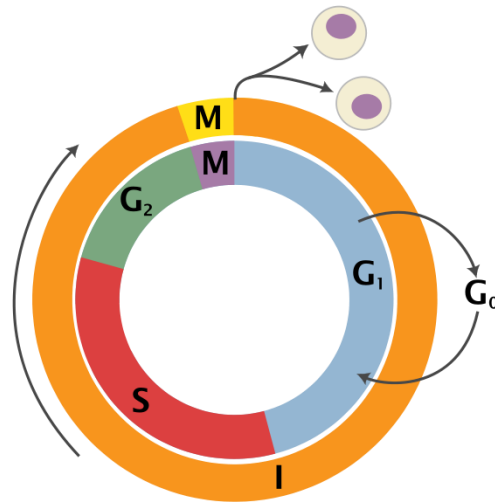
Dactinomycin

Actinomycin D

- Several mechanisms
 - Intercalates in DNA
 - Inhibits RNA synthesis (transcription)
 - Double strand breaks
- **Childhood cancers**
 - Neuroblastoma, Ewing's sarcoma, osteosarcoma
- Major adverse effect: myelosuppression

Bleomycin

- Binds to DNA
- **Free radical formation** (oxygen, iron)
- Single and double strand breaks
- Cell cycle-specific drug: accumulates in **G2 phase**



Zephyris /Wikipedia

Bleomycin

Clinical Uses

- Lymphomas
- Germ cell tumors
- Head and neck cancer
- Squamous cell cancer of skin
- Cancers of cervix and vulva

Bleomycin

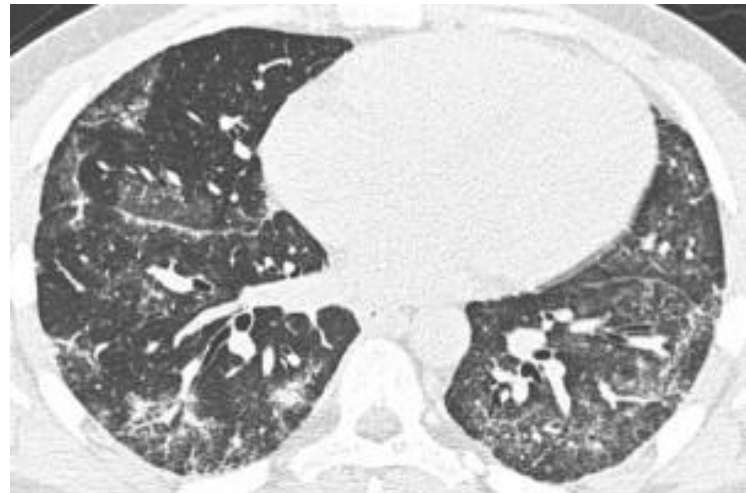
Toxicity

- Inactivated by enzyme bleomycin hydrolase
- Lower enzyme activity in skin and lungs
- **Skin toxicity**
 - Many skin changes described
 - Also seen with other chemotherapy drugs (Busulfan)
 - “Flagellate erythema”: Red/dark streaks on skin

Bleomycin

Pulmonary Toxicity

- Dose-limiting adverse effect
- Usually presents as **pneumonitis**
 - Cough, dyspnea, crackles
 - Infiltrates on chest X-ray
- Risk factors
 - Older patients (>70)
 - Prior pulmonary disease

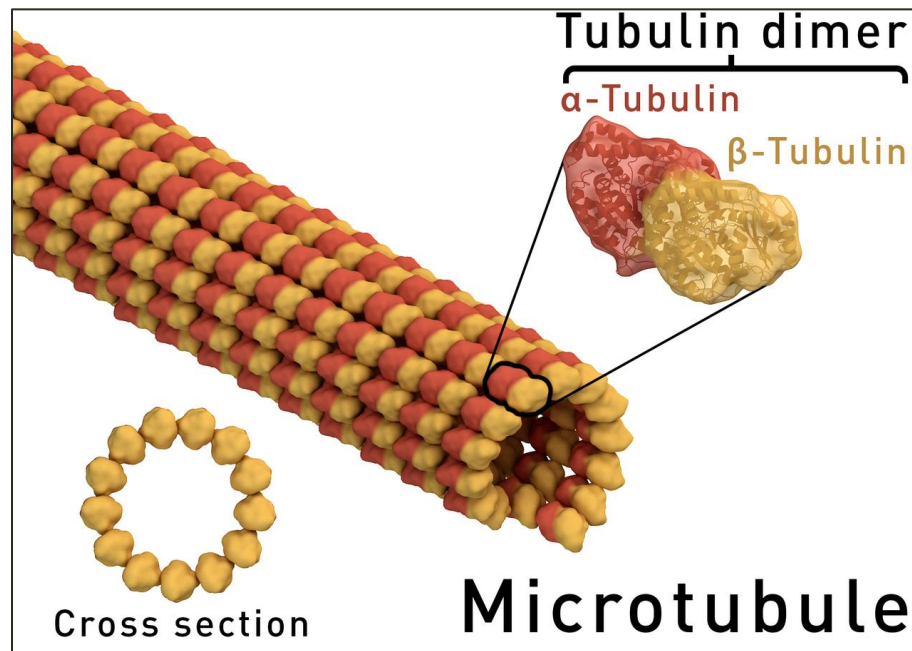


Microtubule Inhibitors

Jason Ryan, MD, MPH

Microtubules

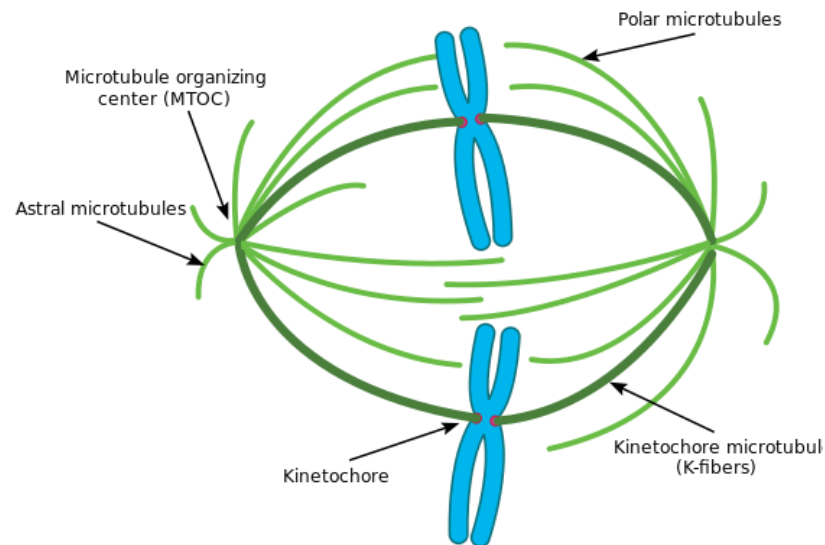
- Polymers of **α and β tubulin**
- Can grow/collapse
- Flagella, cilia, cellular transport (axons)



Thomas Spletstoeser (www.scistyle.com)

Mitosis

- Part of cell cycle
- Separation of chromosomes for cell division
- Depends heavily on **microtubules** (mitotic spindle)
- Followed by cytokinesis: cell divides

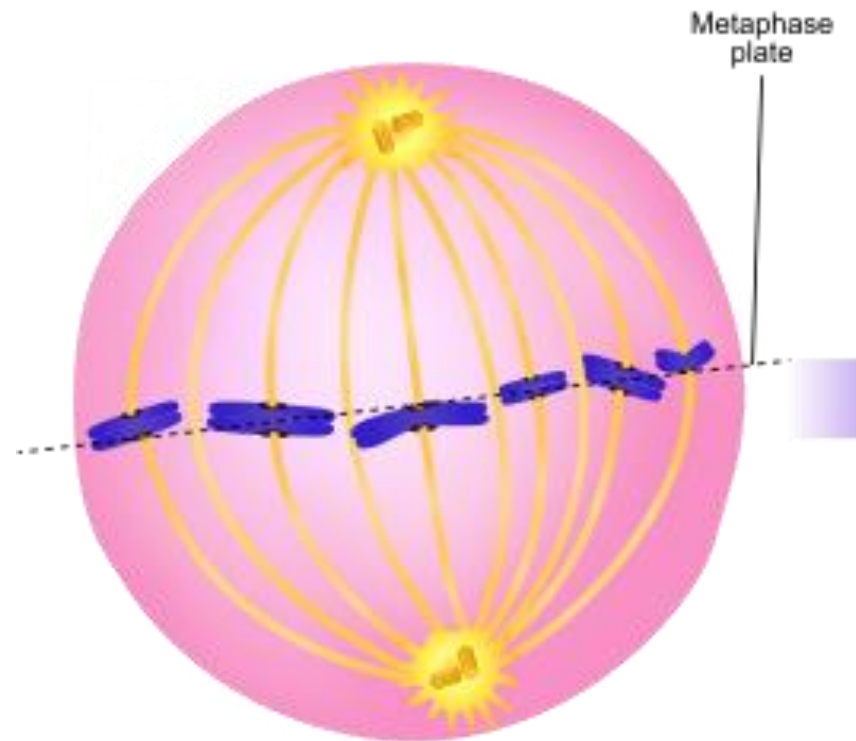


Lordjuppiter /Wikipedia

Mitosis

Metaphase

- Chromosomes line up on metaphase plate

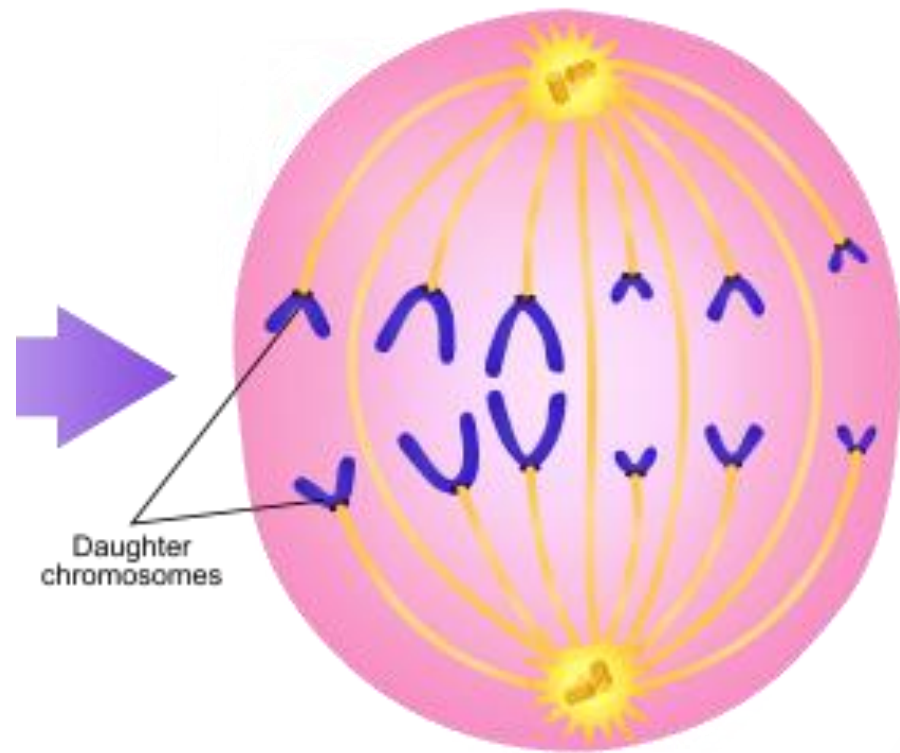


Ali Zifan/Wikipedia

Mitosis

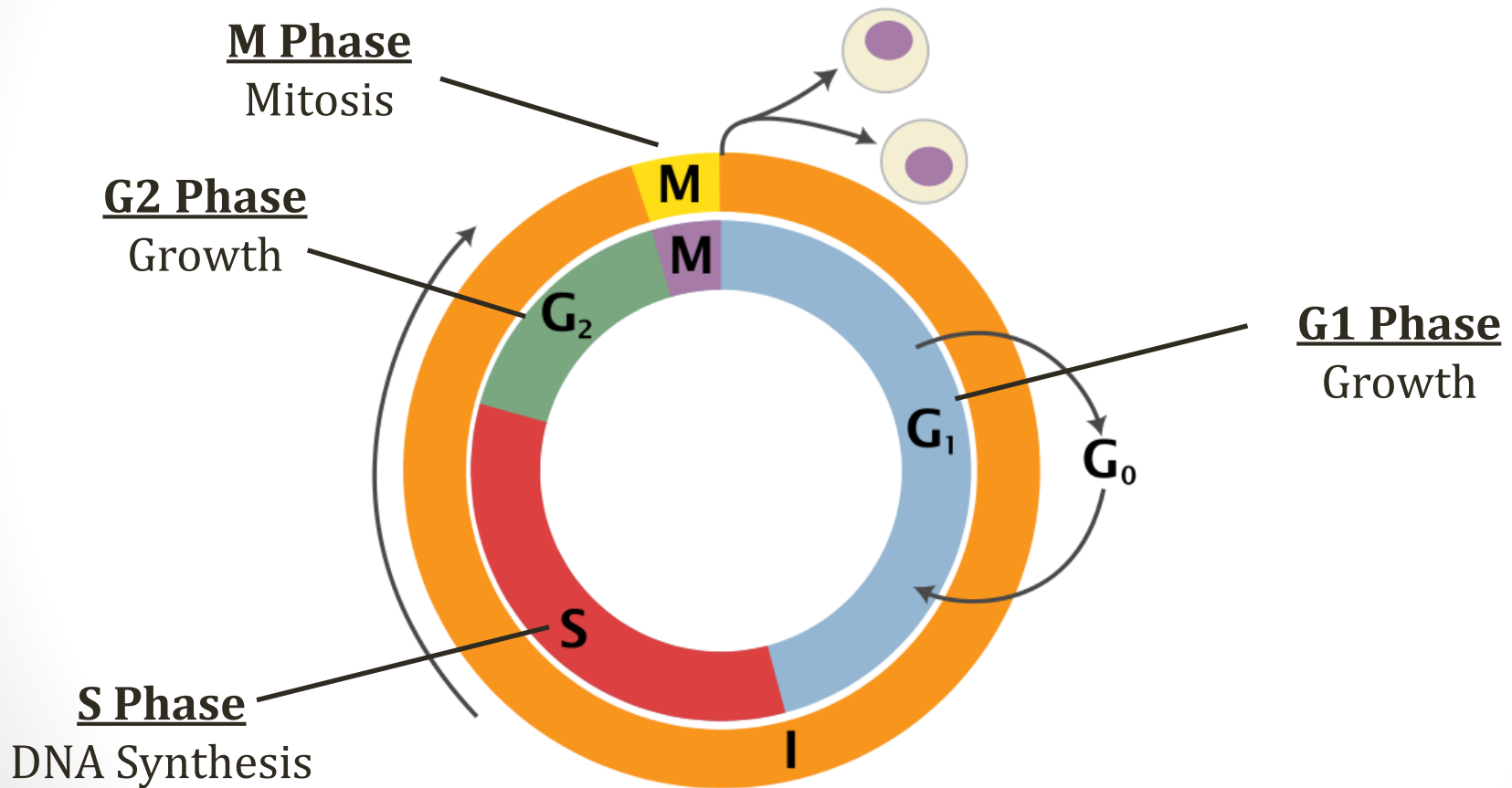
Anaphase

- Chromosomes separate



Ali Zifan/Wikipedia

Cell Cycle



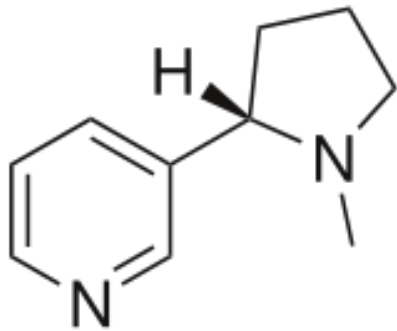
Zephyris /Wikipedia

Microtubule Inhibitors

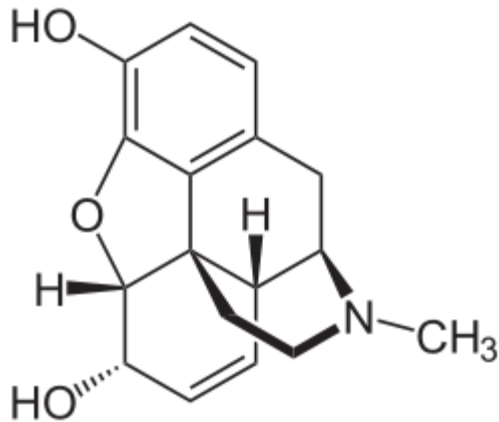
- Taxols
- Vinca alkaloids

Vocabulary

- Alkaloids
 - Naturally occurring substances
 - Nitrogen-containing bases
 - Usually derived from plants or trees



Nicotine
(Tobacco)



Morphine
(Opium)

Taxols

Paclitaxel, Docetaxel

- Alkaloids from yew trees
- Mitotic spindle poisons
- Bind microtubules
- **Enhance tubulin polymerization**
- Microtubules **cannot break down**
- Blocks cell cycle at metaphase/anaphase transition
- Anaphase cannot occur

Taxols

Clinical Use

- Solid tumors
 - Ovarian and breast cancer
 - Non-small cell and small cell lung cancer
 - Head and neck cancers
 - Prostate and bladder cancer

Taxols

Toxicity

- **Hypersensitivity** reactions (up to 30% patients)
 - Dyspnea/wheezing
 - Urticaria
 - Hypotension
- **Premedication** often used for prevention
 - Glucocorticoids and antihistamines
- **Nabpaclitaxel** (Abraxane)
 - Albumin-bound paclitaxel
 - Lower risk hypersensitivity reactions
 - Premedication not required



James Heilman, MD

Taxols

Toxicity

- Myelosuppression
- **Neuropathy**
 - Sensory nerves
 - Usually burning paresthesias of hands/feet

Vinca Alkaloids

Vincristine, vinblastine

- Derived from periwinkle plant (*Vinca rosea*)
- Bind **β -tubulin**
- **Inhibit polymerization**
- Prevent spindle formation
- Mitotic arrest in metaphase

Vinca Alkaloids

Clinical Uses

- Breast cancer
- Germ cell cancer
- Lymphomas
- ABVD Protocol (Hodgkin lymphoma)
 - Adriamycin (doxorubicin) - cytotoxic antibiotics
 - Bleomycin - cytotoxic antibiotics
 - **Vinblastine**
 - Dacarbazine – alkylating agent

Vinca Alkaloids

Toxicity

- Myelosuppression
- SIADH (rare)
- Vincristine: **Neurotoxicity**
 - Dose-limiting toxicity
 - Loss of axonal transport
 - Sensory and motor
 - Paresthesias/pain in fingers and feet
 - Distal weakness

DNA Drugs

Jason Ryan, MD, MPH

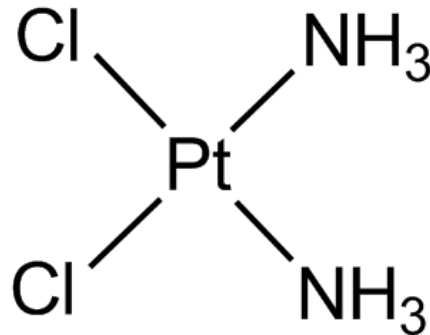
DNA Drugs

- Antitumor Antibiotics
- Alkylating agents
- Platinum agents
- Topoisomerase I and II inhibitors

Platinum Agents

Cisplatin, carboplatin, oxaliplatin

- Cross link DNA similar to alkylating agents
 - Most commonly at **N7 nitrogen of guanine**
 - “Alkylating like” drugs
 - Cell cycle nonspecific (like alkylating agents)



Cisplatin

Platinum Agents

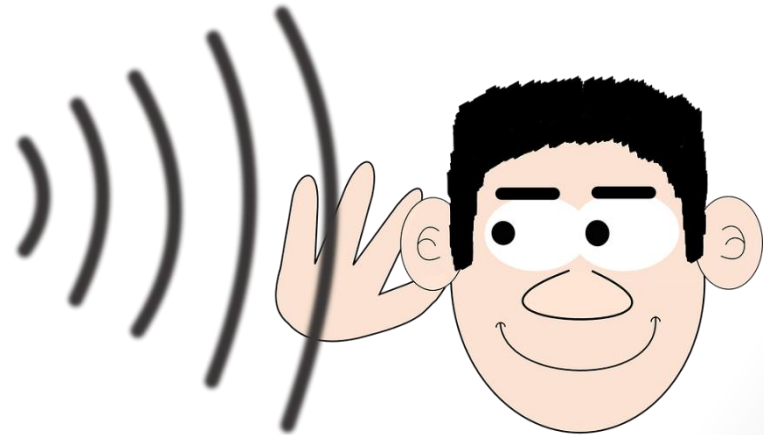
Clinical Uses

- Solid tumors
 - Non-small cell and small cell lung cancer
 - Esophageal and gastric cancer
 - Head and neck cancers
 - Testicular cancer
 - Ovarian cancer

Platinum Agents

Toxicity

- All can cause **neuropathy**
 - Usually a peripheral sensory neuropathy
 - Pain, burning, tingling
 - Often in feet or hands
- May also cause **ototoxicity** (hearing loss)
- GI distress (nausea/vomiting) up to 90% patients

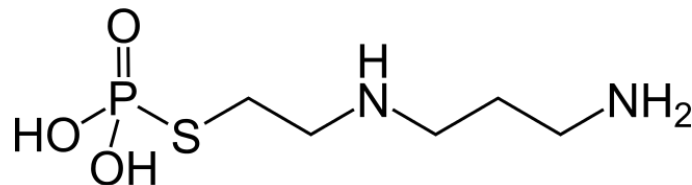


Pixabay/Public Domain

Platinum Agents

Nephrotoxicity

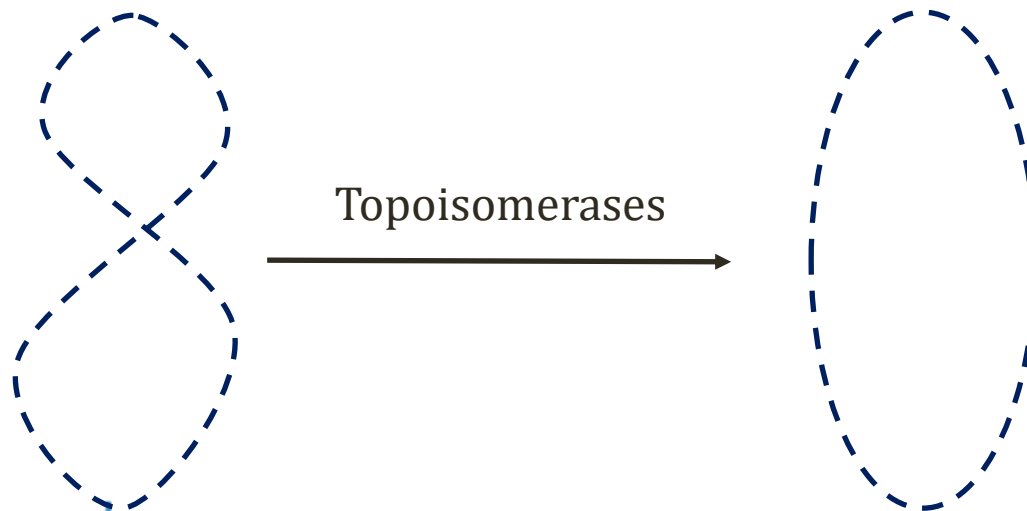
- Main, dose-limiting side effect of **cisplatin**
 - Often presents as acute kidney injury (\uparrow BUN/Cr)
 - Prevented with IV fluids (normal saline)
 - Increase urine output (cause diuresis)
 - **Amifostine**: Free radical scavenger
 - Used in ovarian cancer with repeated cisplatin doses
- Carboplatin: less renal toxicity



Amifostine

Topoisomerases

- Relieve tangles and supercoils in DNA
- Cuts strands of DNA helix then reseal
- Chemotherapy inhibition → breaks with no resealing
- Result: DNA damage
- Affect S/G2 phase (during/after DNA synthesis)



Topoisomerases

- Topoisomerase I
 - Breaks single strands of DNA then reseals
- Topoisomerase II
 - Breaks double strands of DNA then reseals

Topoisomerase I Inhibitors

Irinotecan, topotecan

- “Camptothecins”
- From *Camptotheca* (“happy tree”) tree in China



Geographer/Wikipedia

Topoisomerase I Inhibitors

Clinical Uses

- Irinotecan
 - Colon Cancer
- Topotecan
 - Ovarian cancer
 - Small cell lung cancer

Topoisomerase I Inhibitors

Toxicity

- Myelosuppression
- Severe diarrhea
 - Risk of volume depletion



Elya/Wikipedia

Topoisomerase II Inhibitors

Etoposide, teniposide

- Synthesized from Podophyllotoxins
- Derived from May apple plant (*Podophyllum*)



Wikipedia/Public Domain

Topoisomerase II Inhibitors

Clinical Use

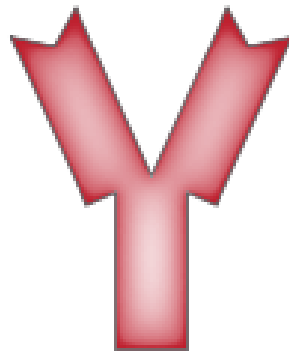
- Intravenous and oral
- Germ cell cancers
- Small cell and non-small cell lung cancer
- Lymphomas
- Main toxicity: Myelosuppression, nausea/vomiting

Other Cancer Drugs

Jason Ryan, MD, MPH

Monoclonal Antibodies

- Laboratory-produced antibody
- Derived from cloned cells in culture
- Designed to bind a specific antigen
- Administered by intravenous infusion



Infusion Reactions

- Usually occur after 1st or 2nd infusion
- Antibody-antigen binding → cytokine release
- Most are mild to moderate
- Fever/chills
- Flushing and itching
- Skin rashes
- Nausea, vomiting, and/or diarrhea
- Treatment/prevention: antihistamines, steroids

Bevacizumab

Avastin



- Monoclonal antibody to **VEGF-A**
- Prevents VEGF-A from binding VEGF receptors
- Used in many solid tumors
 - Colorectal cancer
 - Breast
 - Renal cell carcinoma

VEGF

Vascular endothelial growth factor

- Family of signal proteins
- Several forms (VEGF-A/B/C/D)
- VEGF-A: Stimulates **angiogenesis**
- Secreted by tumors → vascular growth
- VEGF Inhibitors
 - Bevacizumab (cancer)
 - Ranibizumab (retinopathy)

Bevacizumab

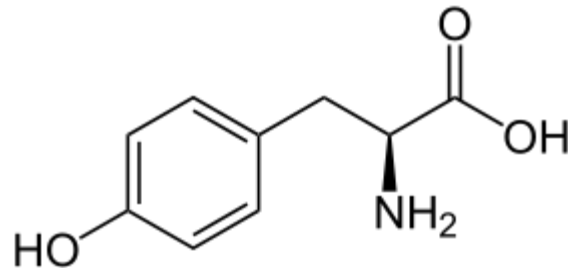
Toxicity

- VEGF mediates **vasodilation** via **nitric oxide**
- Inhibition → vasoconstriction
- **Cardiovascular** adverse effects
 - Hypertension
 - Increased risk of arterial thromboembolism
 - Myocardial infarction/stroke/TIA
- Other effects
 - Delayed wound healing
 - Bleeding

EGF

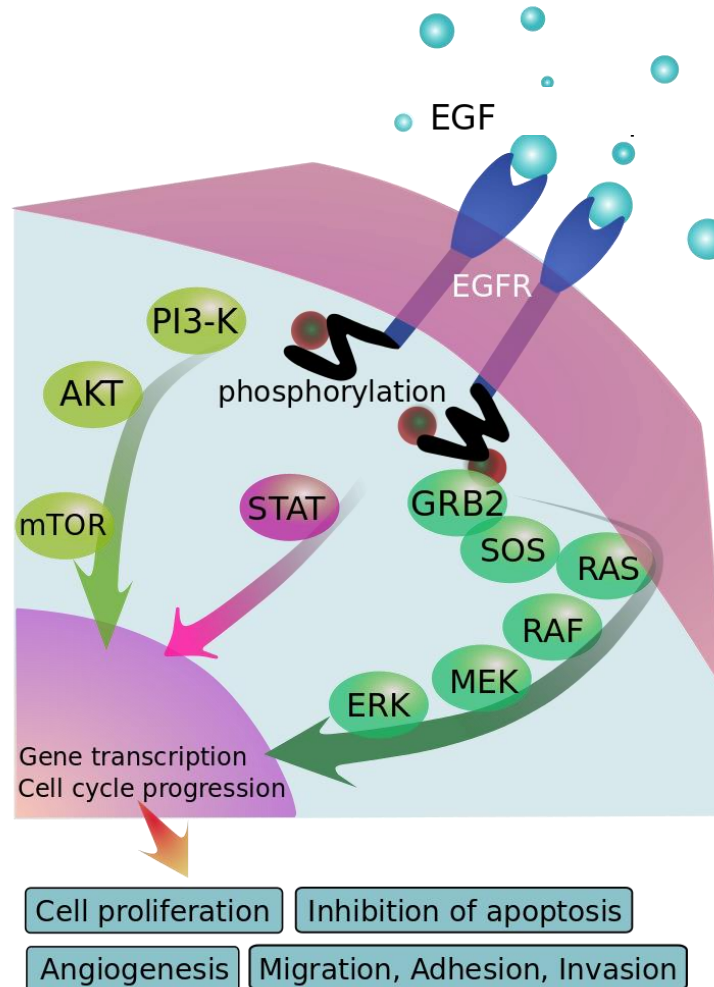
Epidermal growth factor

- Stimulates cell growth and differentiation
- Binds to **EGFR**
 - Tyrosine kinase receptor
 - EGF-EGFR binding → phosphorylation of tyrosine residues
 - Phosphorylated EGFR → downstream effects
- EGFR overexpressed in many tumors



Tyrosine

EGFR



Wikipedia/Public Domain

Cetuximab



- Monoclonal antibody
- Binds **extracellular domain** of EGFR
- Blocks binding of EGF-EGFR
- Solid tumors
 - Non-small cell lung cancer
 - Colon cancer
 - Head and neck cancer
- Side effects in clinical trial:
 - Rash (acne)
 - Diarrhea

KRAS Mutation

Colorectal Cancer

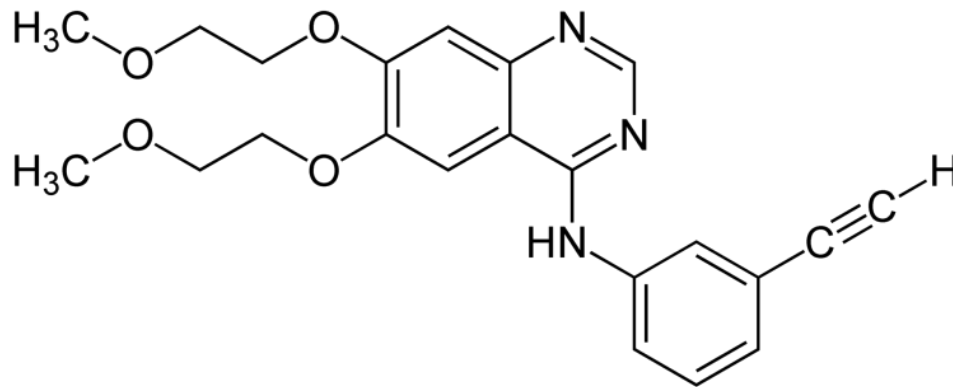
- K-ras
 - G-protein
 - Downstream of EGFR
 - Can acquire activating mutations in colon cancer
 - Mutations isolate tumor cells from effect of EGFR
- Mutated K-ras: **No benefit** from cetuximab
- Wild-type K-ras: Cetuximab beneficial

K-ras mutations and benefit from cetuximab in advanced colorectal cancer.
N Engl J Med. 2008 Oct 23;359(17):1757-65.

Erlotinib

Tarceva

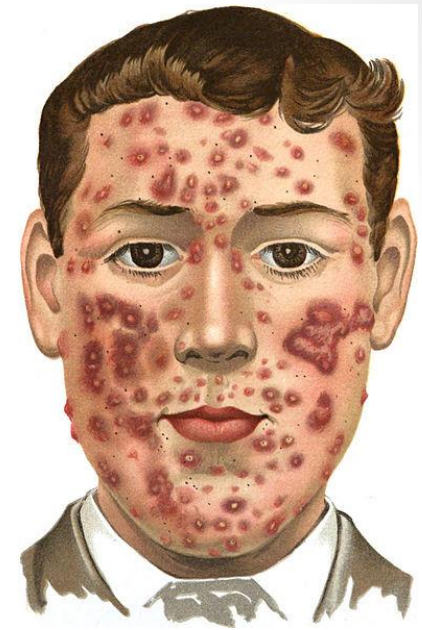
- EGFR **tyrosine kinase inhibitor** (oral)
- Major use: non-small cell lung cancer



Erlotinib

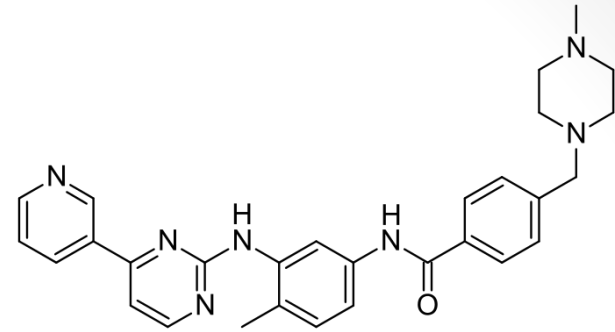
Tarceva

- Main adverse effect: **Skin rash**
 - Acne-like eruption
 - Upper torso, face, neck
 - May be an indicator of drug effect
 - Seen with all EGFR-blocking drugs (Cetuximab)



Imatinib

Tyrosine Kinase Inhibitor



- **Chronic myeloid leukemia**
 - Philadelphia chromosome/BCR-ABL fusion gene
 - Tyrosine kinase protein
 - Treatment: Imatinib
 - Also other TKIs: dasatinib, nilotinib, bosutinib

Imatinib

Tyrosine Kinase Inhibitor

- **Gastrointestinal stromal tumors (GIST)**
 - Rare stomach and small intestine tumors
 - Associated with c-KIT mutations
 - Treatment: surgery +/- Imatinib

KIT Mutations

- KIT (c-KIT) protein
 - Found on cell surface (CD117)
 - **Tyrosine kinase receptor**
 - Binds KIT ligand (stem cell factor)
 - Stimulates growth
- KIT mutations → cancer (proto-oncogene)
- KIT gain-of-function mutations in 95% GIST
 - CD117 positive cells

Imatinib

Adverse Effects

- **Fluid retention**
 - Usually peripheral or periobital edema
 - Sometimes pulmonary edema
- Rash



Klaus D.Peter,



James Heilman, MD/Wikipedia

Rituximab



- Monoclonal **CD20** antibody
- Leads to depletion of B cells
- Used in B-cell malignancy and autoimmune diseases

Rituximab

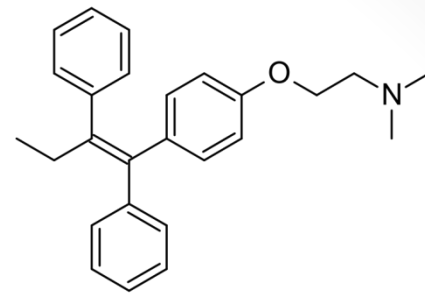
- **B-cell malignancies**
 - Non-Hodgkin lymphoma
 - Chronic lymphocytic leukemia
- **Rheumatoid arthritis**
- **Immune thrombocytopenia (ITP)**
 - First line therapy: IVIG and steroids
 - 2nd line therapy: splenectomy
 - Alternative: Rituximab

Rituximab

Toxicity

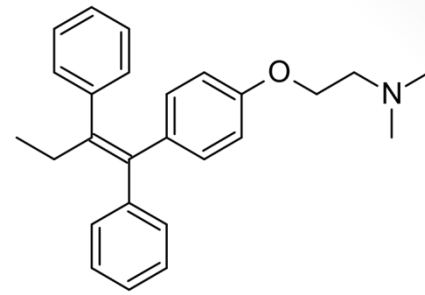
- Rare cases of **opportunistic infections**
 - Pneumocystis jirovecii pneumonia
 - Cryptococcal meningitis
 - Cytomegalovirus colitis
 - **Progressive multifocal leukoencephalopathy (JC virus)**
- **Hepatitis B reactivation**

Tamoxifen



- Selective estrogen receptor modulator (SERM)
- Oral drug
- **Competitive antagonist** of breast estrogen receptor
 - Used in ER positive (ER+) breast cancer
 - Used as part of primary therapy
 - Also used for prevention

Tamoxifen



- Estrogen **agonist** in other tissues (bone/uterus)
- Bone: Estrogen increases bone density
- Uterus: Estrogen promotes endothelial growth

Tamoxifen

Toxicity

- **Hot flashes** (estrogen blockade)
- Increased risk of DVT/PE
 - Estrogen effects → increased clotting factors

DVT/PE

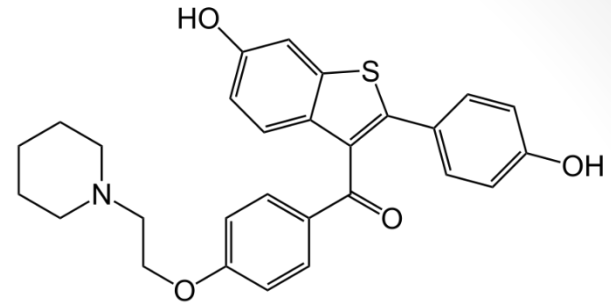
Tamoxifen

Toxicity

- Partial agonist to endometrium
 - Endometrial proliferation
 - Hyperplasia
 - Polyp formation
- May cause **endometrial cancer**
 - Associated with invasive carcinoma and uterine sarcoma
- Major risk in postmenopausal women

Raloxifene

Evista

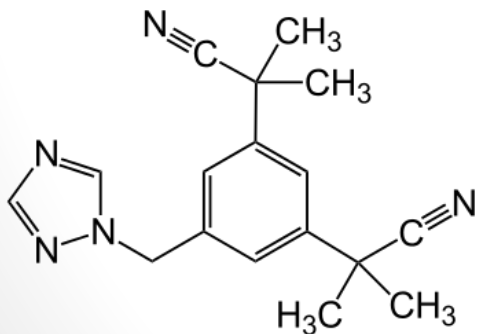


- Also a SERM
- Estrogen actions on bone
- Anti-estrogen in breast/uterus
- **Osteoporosis** (postmenopausal women)
- Also used for prevention of breast cancer
- May cause hot flashes
- Associated with DVT/PE

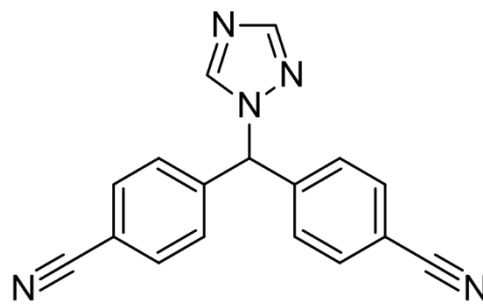
Aromatase Inhibitors

Anastrozole, Letrozole, Exemestane

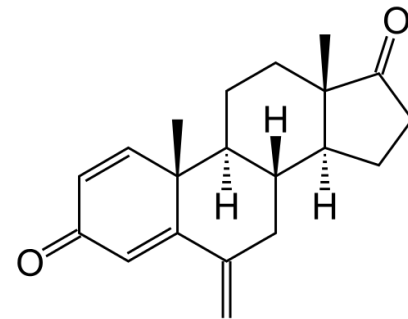
- ER+ breast cancer – postmenopausal women
- Block estrogen production
 - Peripheral tissues
 - Also occurs in breast cancer cells
- Inhibit aromatase enzyme
 - Androstenedione → estrone
 - Testosterone → estradiol



Anastrozole



Letrozole

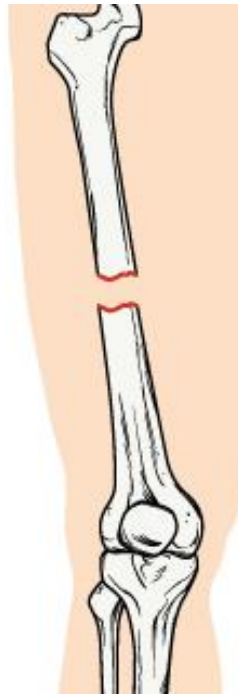


Exemestane
(steroid)

Aromatase Inhibitors

Adverse Effects

- Osteoporosis (loss of estrogen)
- Increased risk of fracture



Open Stax College

Trastuzumab

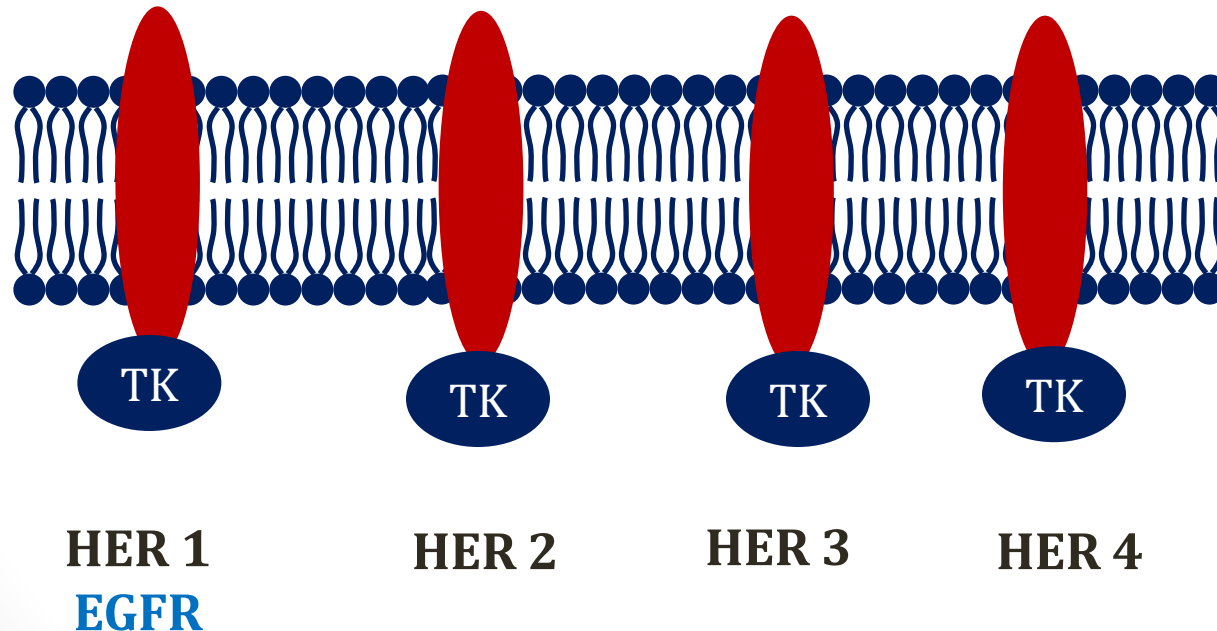
Herceptin



- Monoclonal antibody to **HER-2**
 - Surface receptor
 - Activation → cell growth and proliferation
 - Overexpressed by cancer cells
 - Expressed in 25 to 30% of **breast cancers**
- Many names for HER2
 - HER2/neu
 - ERB2
 - CD340

HER Family

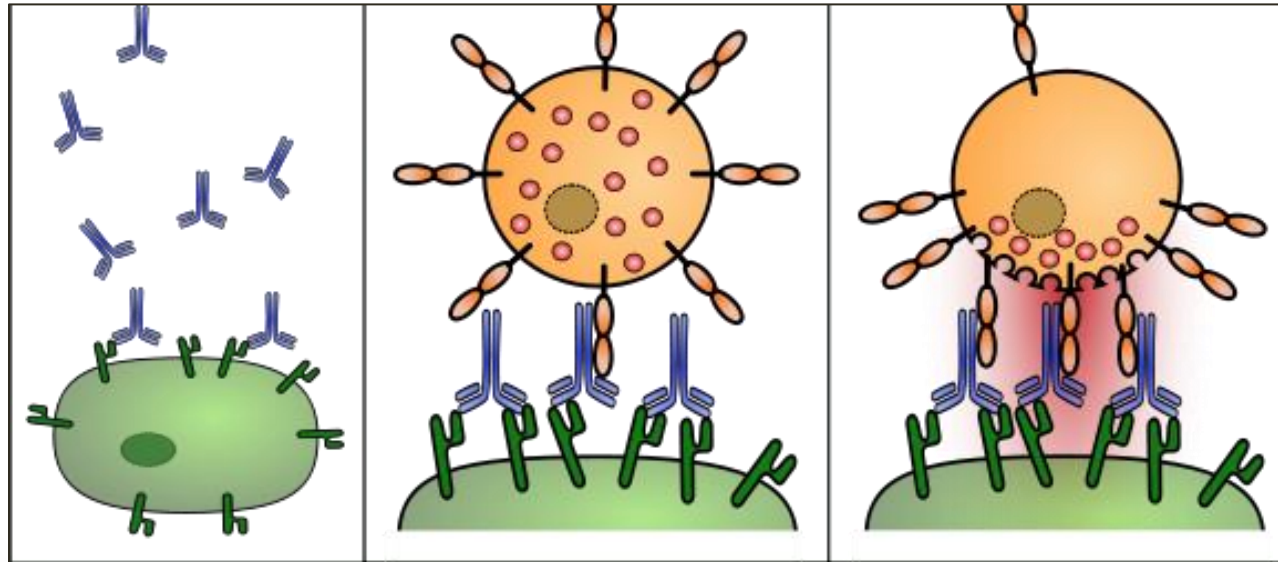
- Human epithelial receptors
- All have inner tyrosine kinase domain
- Activation → signaling cascade → growth



Trastuzumab

Herceptin

- Improves survival in breast cancer
 - Inhibits proliferation of tumor cells
 - **Antibody-dependent cell-mediated cytotoxicity**



Trastuzumab

Toxicity

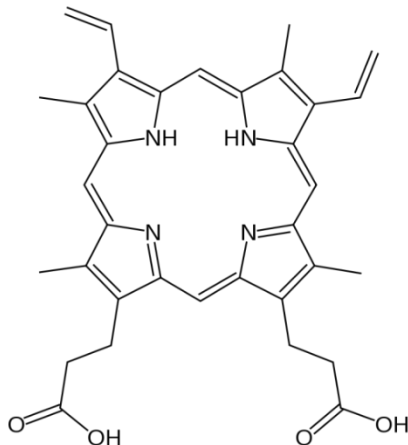
- **Cardiomyopathy**
 - Usually asymptomatic ↓ LVEF
 - May lead to heart failure (dyspnea, fatigue, edema)
- Most likely due to blockade of HER2 in myocytes
 - May lead to stunning of myocardium
- Different from anthracycline cardiotoxicity
 - Not dose dependent
 - **Often reversible** when drug discontinued
 - Re-challenge often tolerated after LVEF recovery
 - Biopsy: No necrosis

Porphyrias

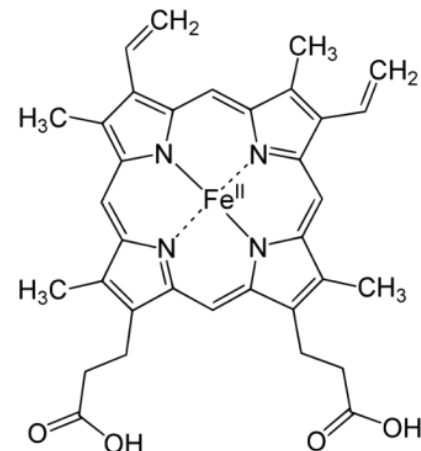
Jason Ryan, MD, MPH

Porphyrins

- Large nitrogen-containing molecules
- Porphrias = disorders of porphyrin synthesis
- All from **deficient enzymes** in heme synthesis
- Rarely cause anemia
- Symptoms from accumulation of porphyrins



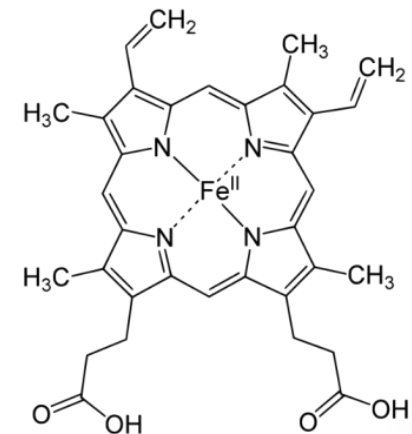
Protoporphyrin



Heme

Heme

- Component of hemoglobin
- Mostly produced in **bone marrow and liver**
- Bone marrow: 80% of heme production
 - Used in red blood cells as hemoglobin
- **Liver:** 20% heme production
 - Used in **cytochrome P450 enzymes**

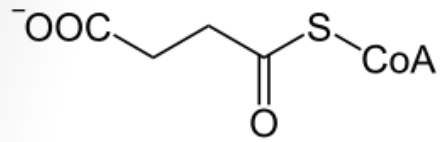


Heme

Heme Synthesis

- Begins in **mitochondria**
 - Initial ingredients: **Succinyl CoA and glycine**
 - Combined to form δ -ALA (delta-aminolevulinic acid)
 - Enzyme: **δ -ALA synthase**
 - Rate limiting step (inhibited by heme)
- Middle pathway in cytosol
- Final steps in mitochondria

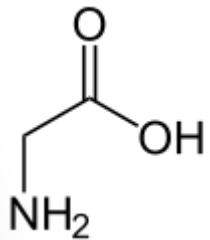
Heme Synthesis



Succinyl-CoA

δ -ALA Synthase

Glycine



Sideroblastic Anemias

δ -ALA

δ -ALA dehydratase



Porphobilinogen



Protoporphyrin

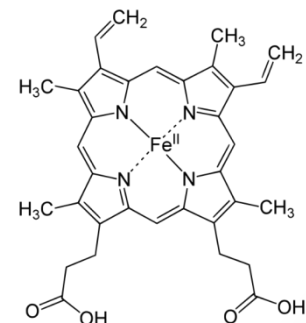
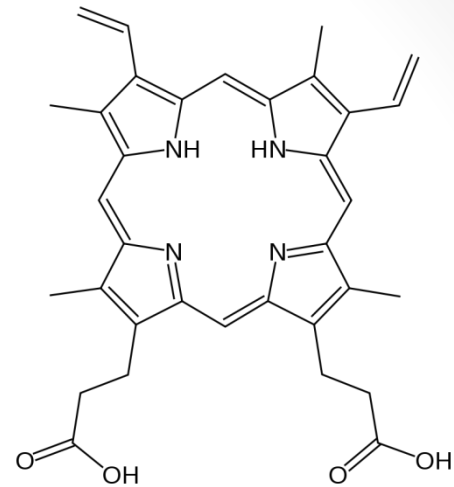
Fe^{2+}

Ferrochelatase



Heme

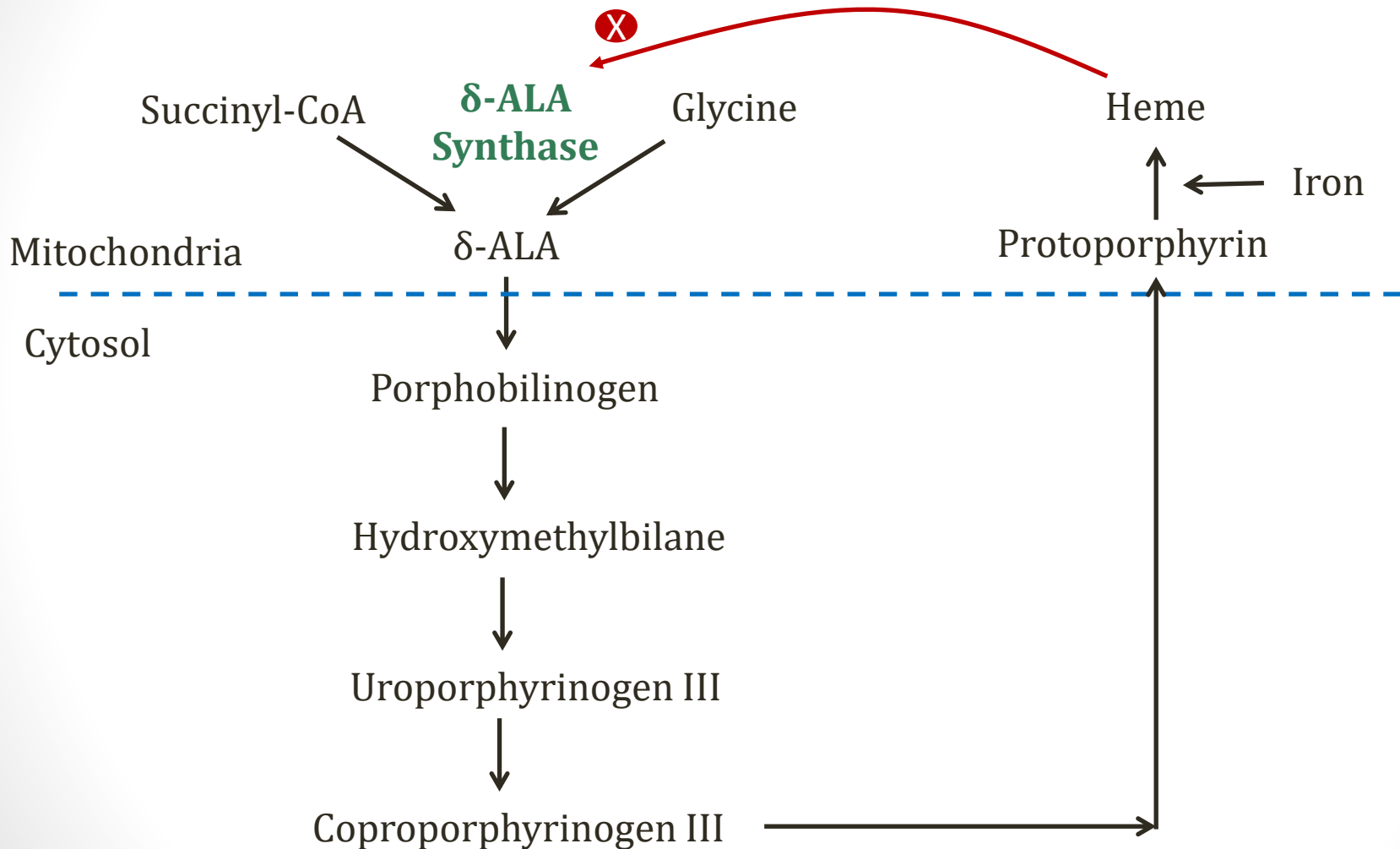
Lead Poisoning



Lead Poisoning



Heme Synthesis



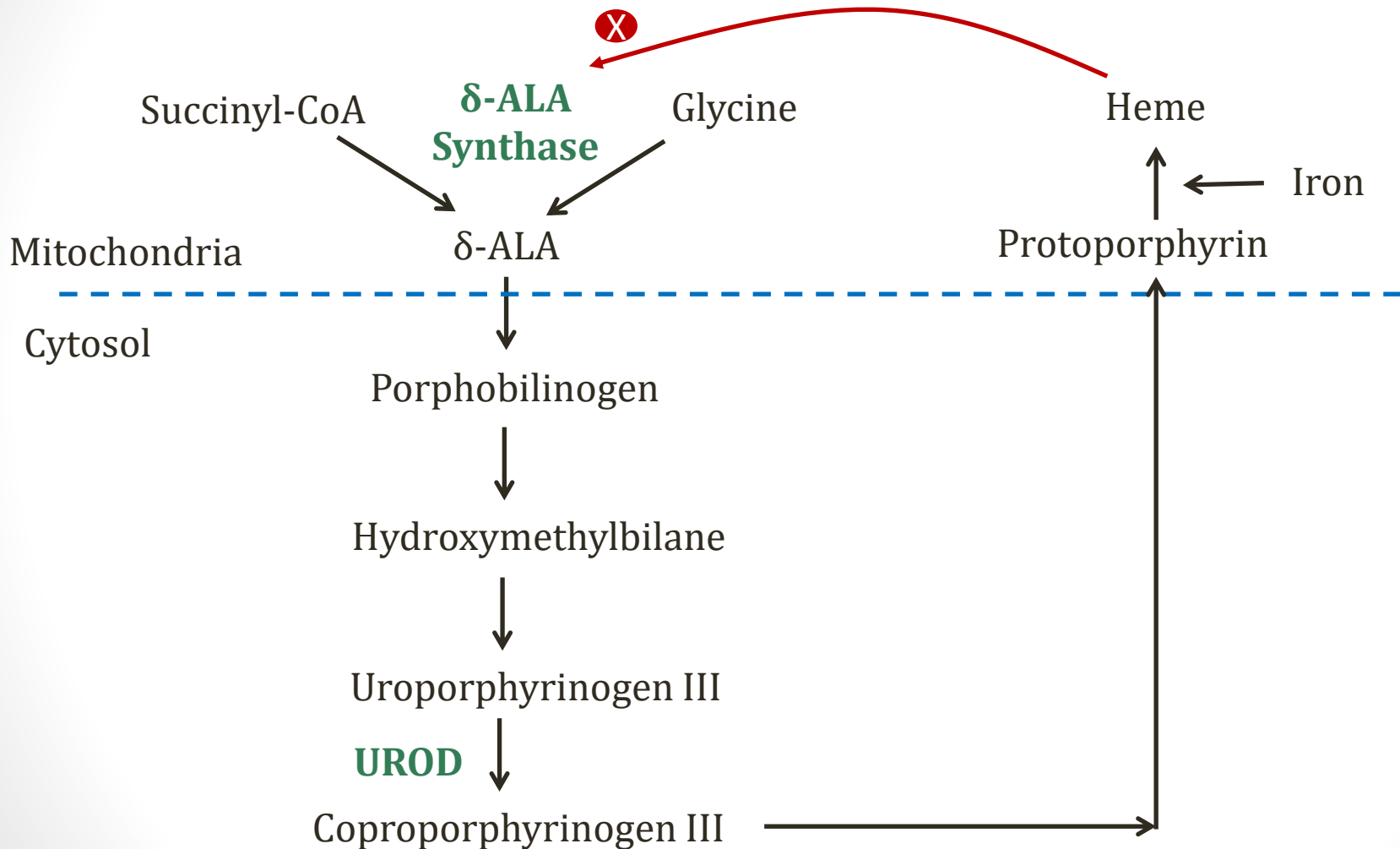
Porphyrias

- More than eight different types described
- Two most common (all rare):
 - Porphyria cutanea tarda
 - Acute intermittent porphyria
- Both do not cause anemia (no ↓ hemoglobin)
- Symptoms from accumulation of intermediates

Porphyria Cutanea Tarda

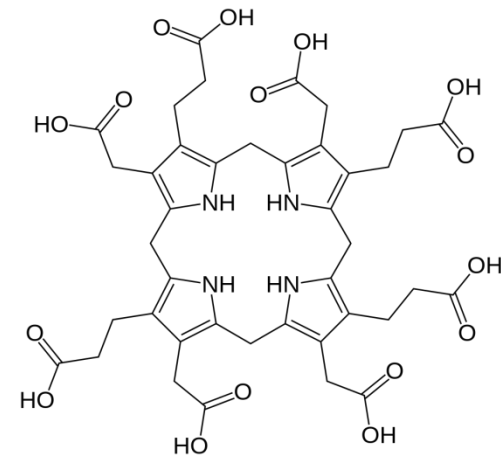
- Most common porphyria
- Deficient activity of UROD
 - **Uroporphyrinogen decarboxylase**
 - 5th enzyme in heme synthesis pathway

Porphyria Cutanea Tarda



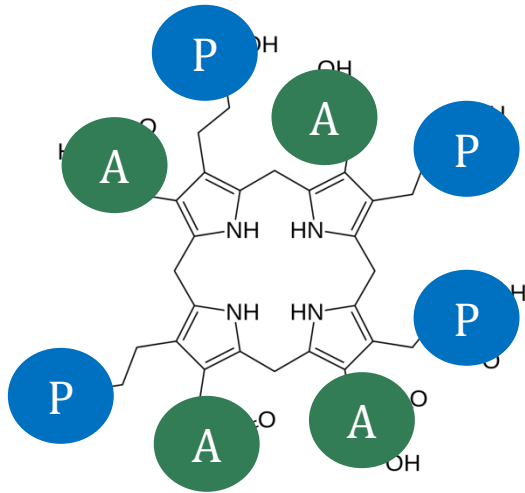
Vocabulary

- Uroporphyrinogens:
 - Four propionic acid groups (3 carbons)
 - Four acetic acid groups (2 carbons)
- Different arrangements in I versus III
- Uroporphyrinogen III more common form
- Forms II, IV do not occur naturally

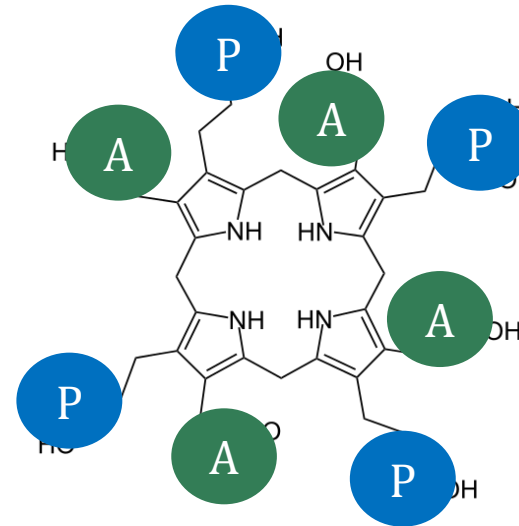


Uroporphyrinogen III

Vocabulary



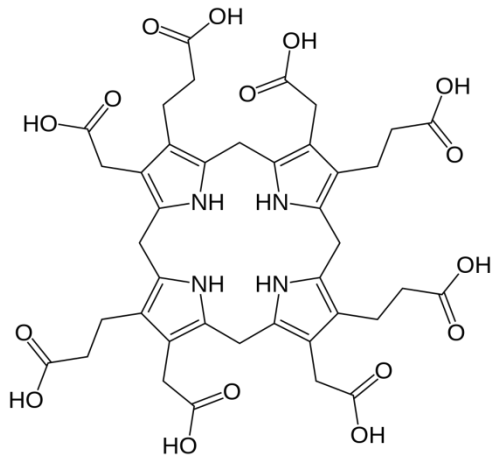
Uroporphyrinogen III



Uroporphyrinogen I

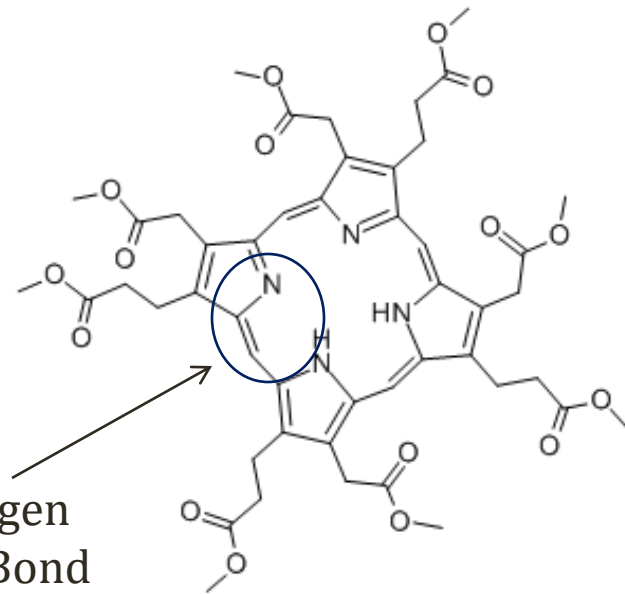
Vocabulary

- “Porphyrinogen” = non-oxidized molecule
- “Porphyrin” = oxidized molecule (loss of hydrogen)



Uroporphyrinogen III

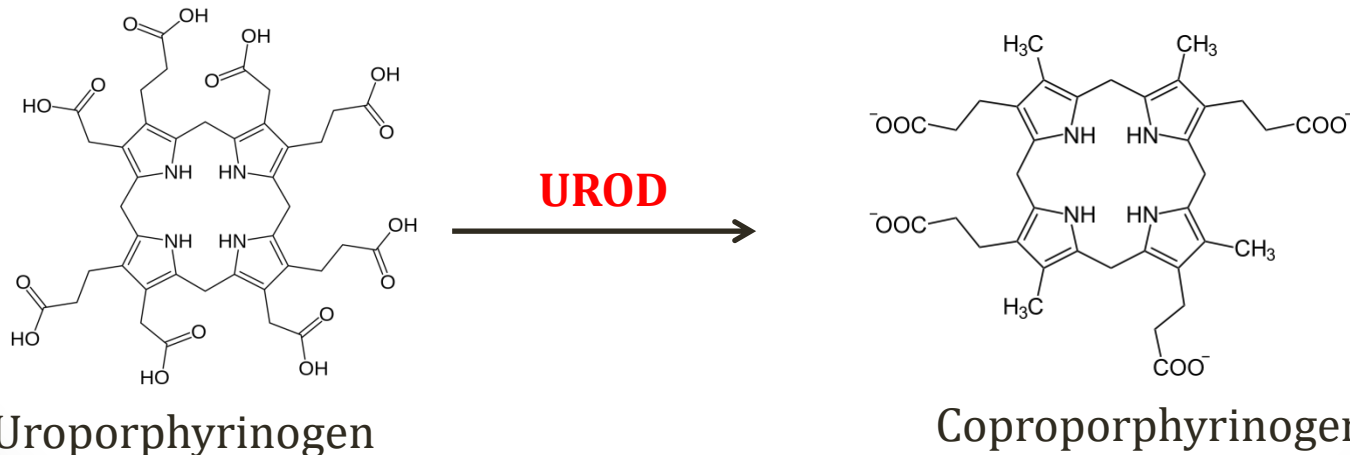
↓ Hydrogen
Double Bond



Uroporphyrin III

Porphyria Cutanea Tarda

- Accumulation of uroporphyrinogen
- Uroporphyrinogen oxidized → **uroporphyrin**
- Appears in plasma, excreted in urine
- Transported to skin
- Causes skin damage on exposure to light



Porphyria Cutanea Tarda

Symptoms

- Chronic **blistering skin lesions**
- Often accompanied by \uparrow AST/ALT
- **Urine appears “tea colored”**

Porphyria Cutanea Tarda

- Decreased UROD activity is usually **acquired**
 - “Type 1” PCT has normal gene function
 - Unknown environmental trigger → ↓ UROD activity
- **Excess iron** plays central role
 - ↑ hepatic iron found on liver biopsy
 - Phlebotomy used for treatment (reduces iron stores)
 - PCT is an “iron overload syndrome”

Porphyria Cutanea Tarda

- Susceptibility factors (worsen disease):
 - Alcohol
 - Hepatitis C virus
 - HIV

Porphyria Cutanea Tarda

Diagnosis

- Measurement of **plasma or urine porphyrins**
 - Done for screening
 - Will be elevated
- Fractionation of porphyrins: **↑ uroporphyrin**

Porphyria Cutanea Tarda

Treatment

- Phlebotomy
- Hydroxychloroquine
 - Malaria drug
 - Mechanism unclear

Porphyria Cutanea Tarda

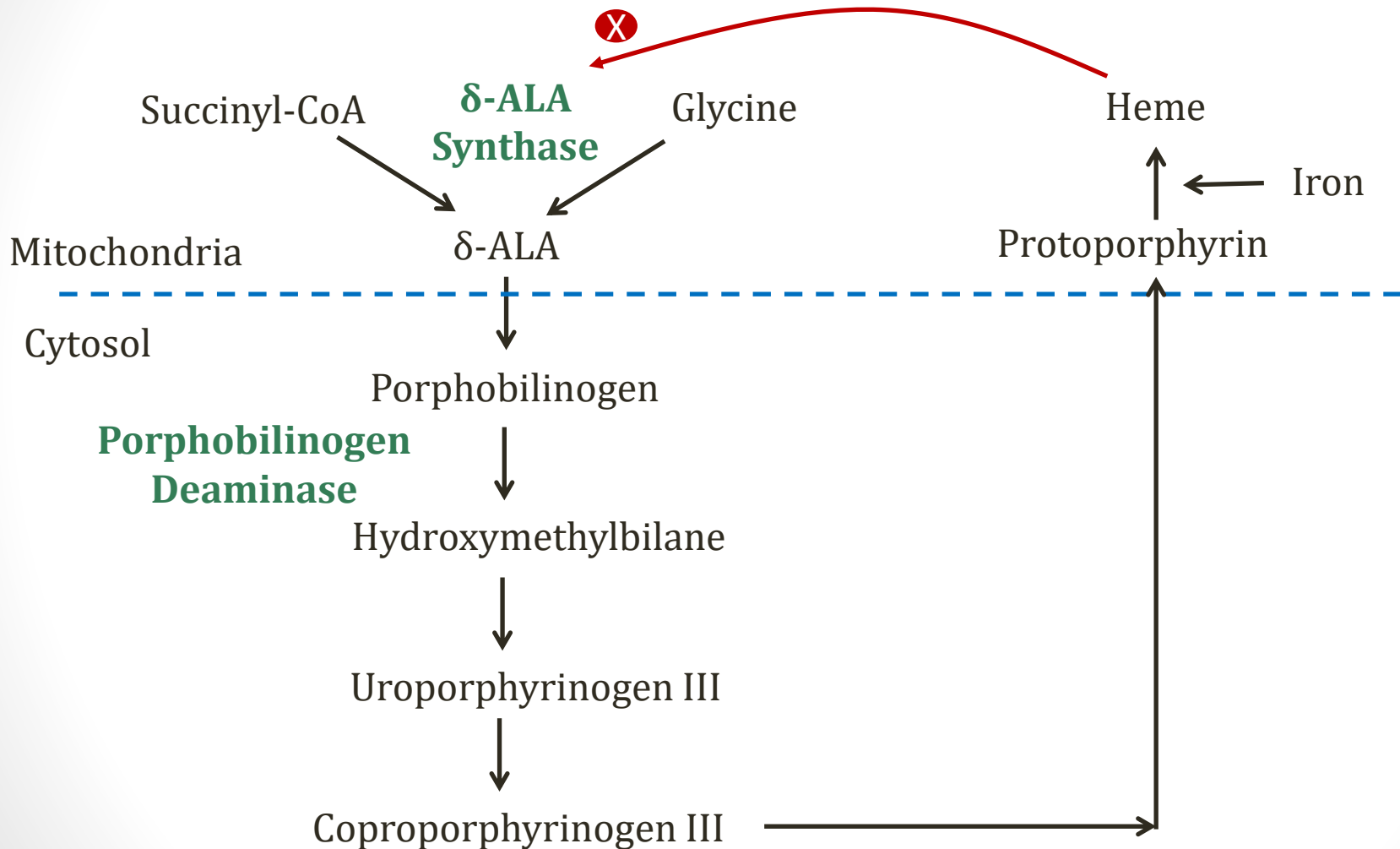


Nosfposter2/Flickr

Acute Intermittent Porphyria

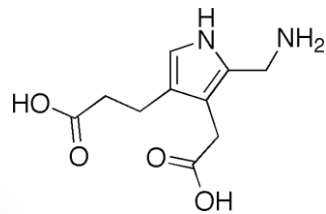
- Deficiency of enzyme PBGD
 - **Porphobilinogen deaminase**
- 3rd enzyme in heme synthesis pathway
- **Autosomal dominant** disorder
 - Low penetrance
 - Symptoms variable

Acute Intermittent Porphyria

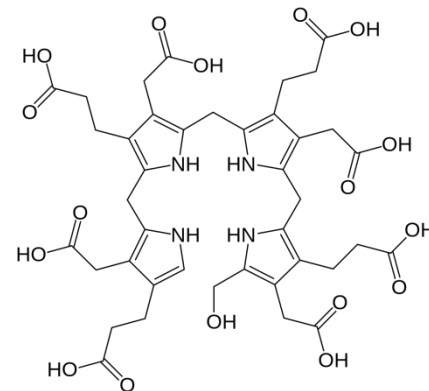


Acute Intermittent Porphyria

- Symptoms occur as **acute, intermittent attacks**
- Occur when porphobilinogen levels rise
- δ -ALA synthase: Rate limiting enzyme
 - Feedback inhibition by heme
- Activation δ -ALA synthase \rightarrow acute attack



Porphobilinogen



Hydroxymethylbilane

Acute Intermittent Porphyria

Triggers

- **Medications**
 - Long list of meds can induce P450 system
 - Leads to ↑ heme synthesis in liver
 - Griseofulvin (antifungal)
 - Phenobarbital (seizures)
- Alcohol and smoking
- Starvation and/or reduced intake of calories
 - Low glucose activates ALA synthase

Acute Intermittent Porphyria

Mechanisms of Symptoms

- δ -ALA and Porphobilinogen: Neurotoxins
- Cerebral dysfunction
- Neuropathy
- Abdominal pain

Acute Intermittent Porphyria

Symptoms

- Five P's:
 - **Abdominal pain**
 - **Port-wine colored urine**
 - Polyneuropathy
 - Psychological disturbances
 - Precipitated by drugs

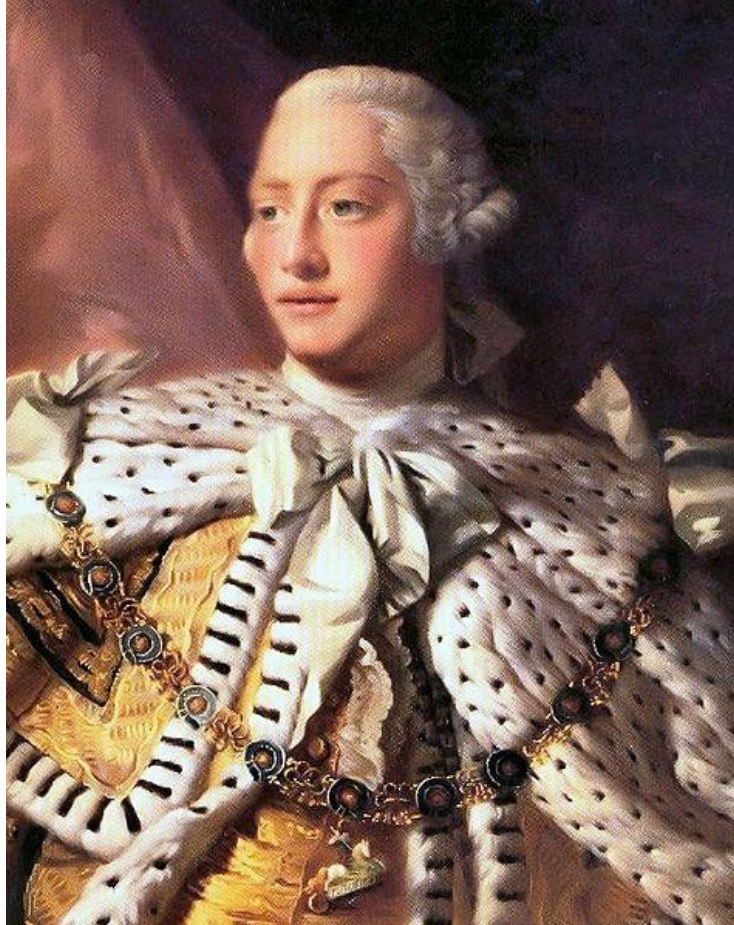
Acute Intermittent Porphyria

Symptoms

- Classic case:
 - Adult (30s-40s)
 - **Recurrent unexplained abdominal pain attacks**
 - **Abnormal color of urine**
 - Confusion and/or neuropathy

Acute Intermittent Porphyria

King George III



Wikipedia/Public Domain

Acute Intermittent Porphyria

Diagnosis

- **Best to test during an attack**
- ↑ Porphobilinogen (PBG)
- ↑ δ -ALA

Acute Intermittent Porphyria

Treatment

- Inhibition of heme production
 - **Hemin** (synthetic heme)
 - **Glucose**
- Inhibit hepatic **δ -ALA synthase** activity