Platelet Disorders and Secondary Coagulation Disorders

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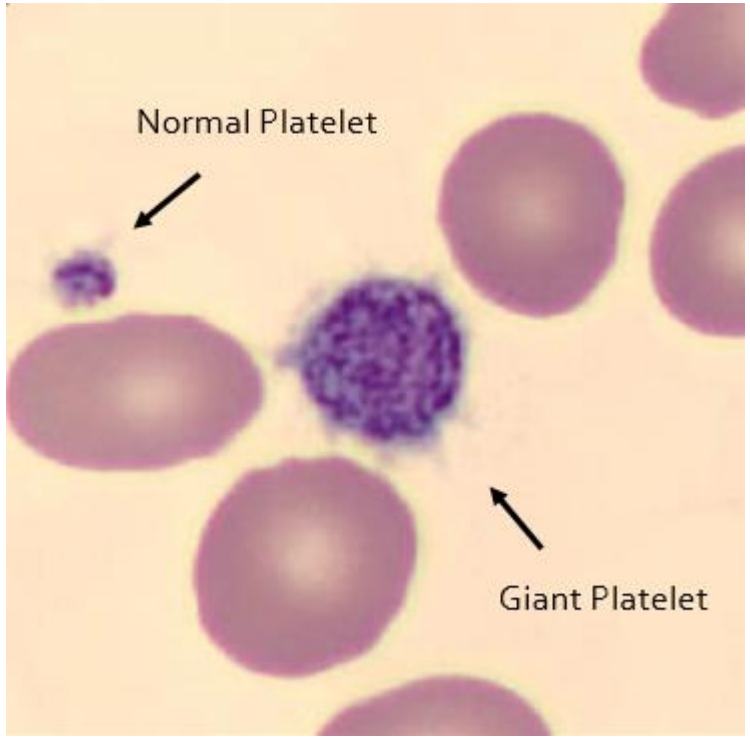
Today's Discussion

- Platelet Disorders
  - Platelet Review
  - Functional Testing for Platelets
  - Qualitative Platelet Disorders
  - Quantitative Platelet Disorders
- Secondary Coagulation Disorders
  - Hemostasis Review
  - Acquired Hemorrhagic Coagulation Disorders
  - Congenital Hemorrhagic Disorders

Platelet Review



- Cells consisting of granular cytoplasm with no nucleus
- On average 2.5 um in diameter
- Normal reference ranges:
  - Platelet count= 150-450 ($x10^3$/uL)
  - Mean platelet volume= 8 to 10 fL
- Turnover of platelets averages 8-9 days in PB

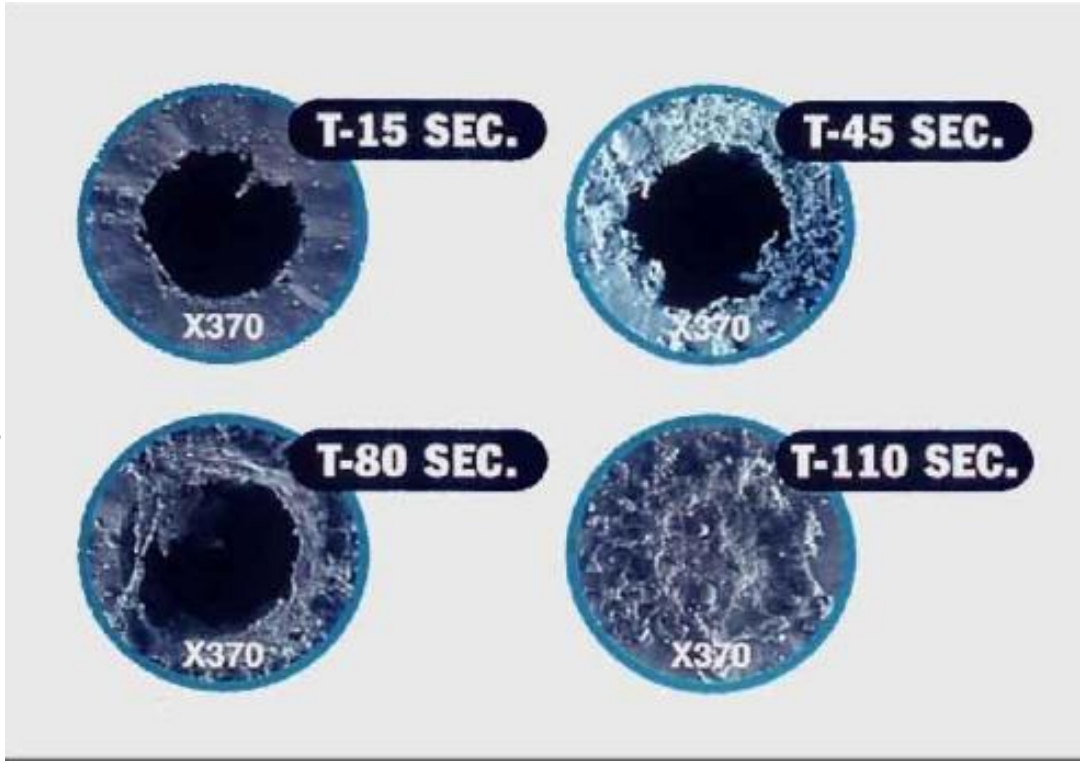
https://doctorlib.info/hematology/rodak-hematology-clinical-principles-applications/14.html

Platelet Function Testing

- Initial work-up of platelet dysfunction is done by using platelet function assays
  - Screening tests
    - Platelet count
    - Platelet Function Screen by PFA (bleeding time)
  - Specific Functional Testing
    - Platelet Aggregation
  - Specific Platelet Activation Tests
    - Release Markers
    - Flow Cytometry
  - Additional Platelet Testing
    - Thromoelastography (TEG)

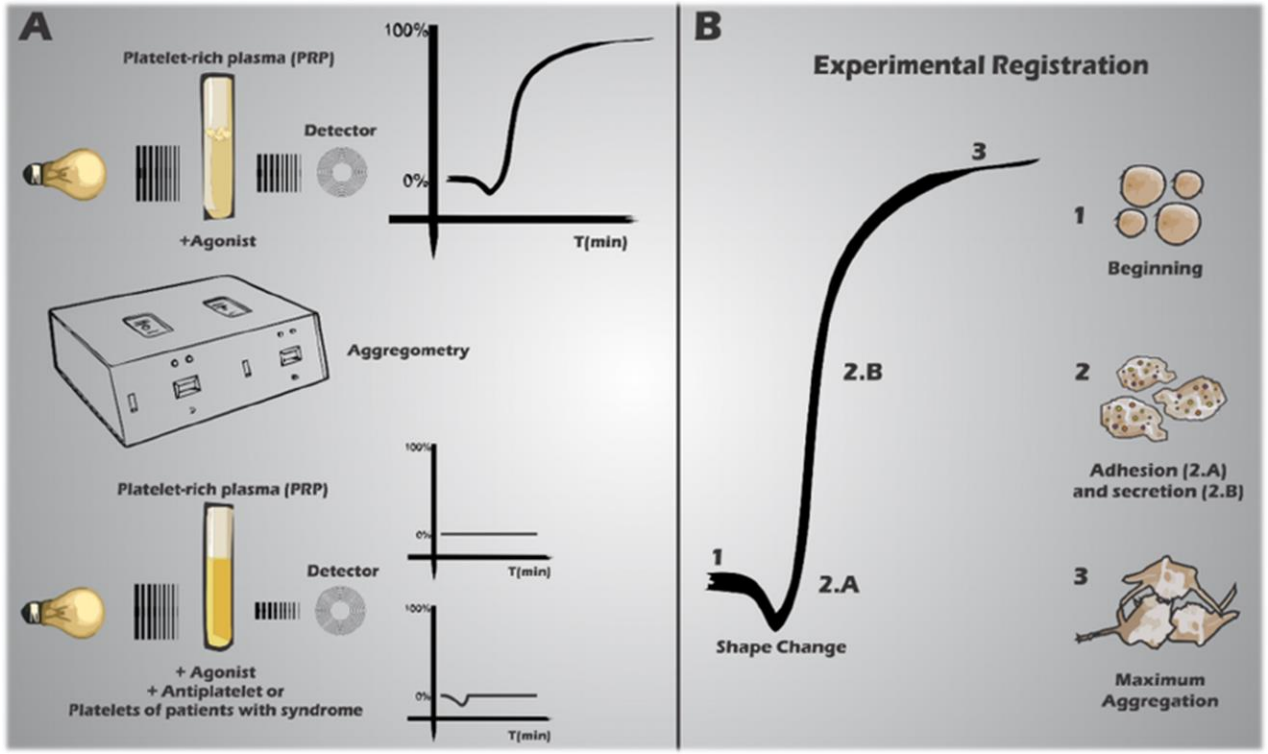
PFA-100

- Whole blood testing under high shear stress
- Agonist-lined cartridges
  - Collagen-ADP
  - Collagen-EPI
- Platelets adhere to the coated membrane and to each other forming aggregates
- The platelet thrombus builds at and on the aperture, arresting blood flow
- Time is measured from the start of testing until the platelets plug the aperture
  - Reported as the closure time
    - Resulted in seconds



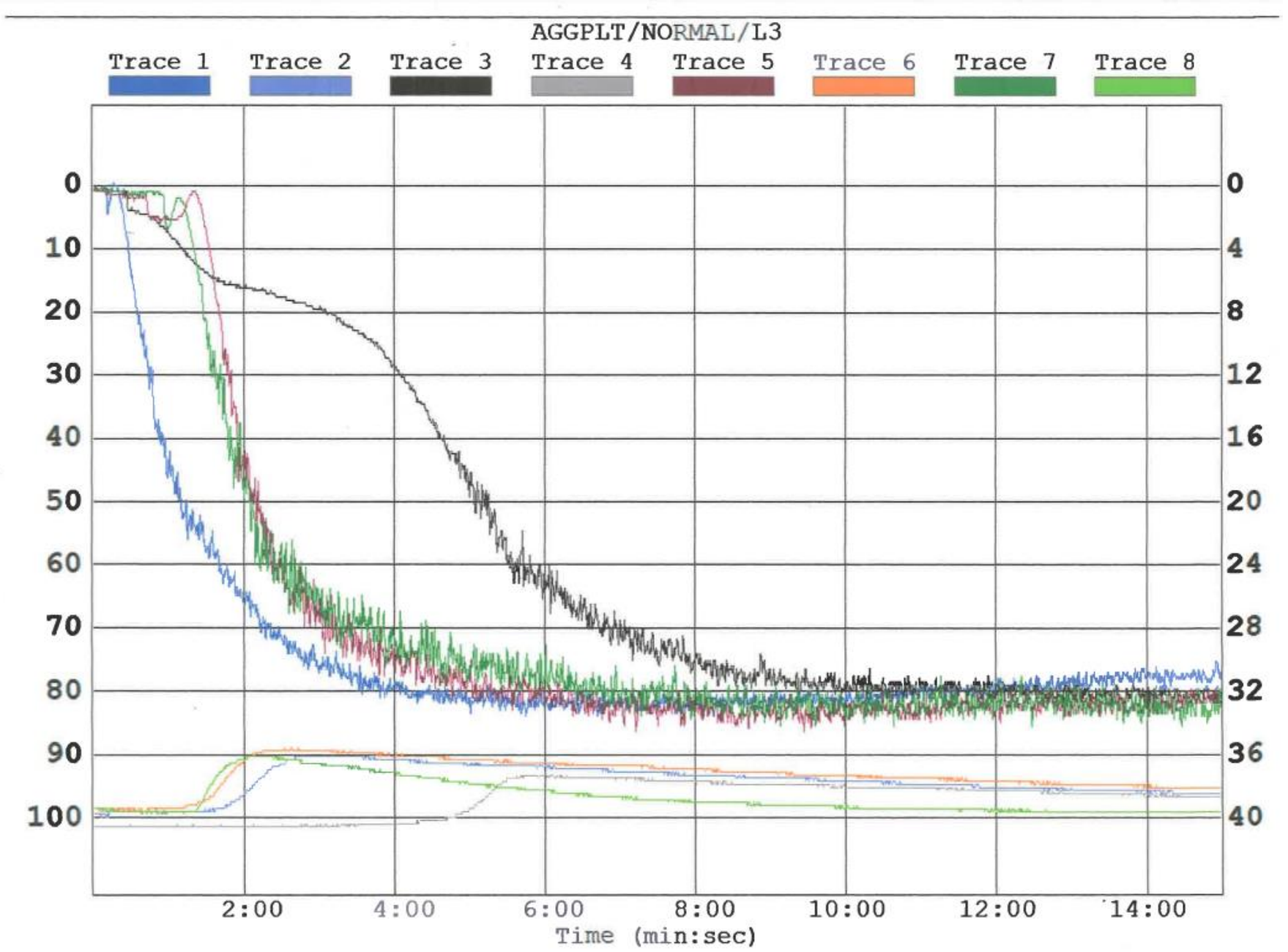
https://www.va.gov/DIAGNOSTICEM/docs/LockhartDiagnosticPltEM102812.pdf

Platelet Aggregation



https://www.mdpi.com/1422-0067/15/10/17901/htm

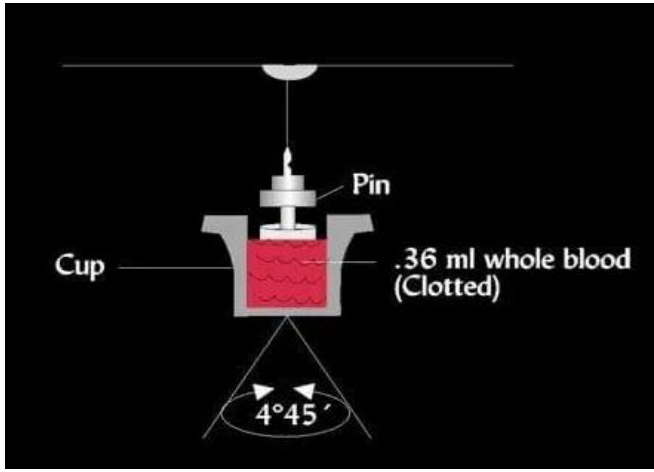
- Both PRP and PPP used from citrated whole blood
- Tested within 3 hours of collection
- Specimen aliquoted into little cuvettes and warmed to 37°C on the machine
- Intensity of light transmitted increases slightly in proportion to degree of shape change upon addition of agonists
  - ADP, EPI, Collagen, Arachidonic Acid, Ristocetin, sometimes Thrombin
- 100% light transmission upon complete aggregate formation



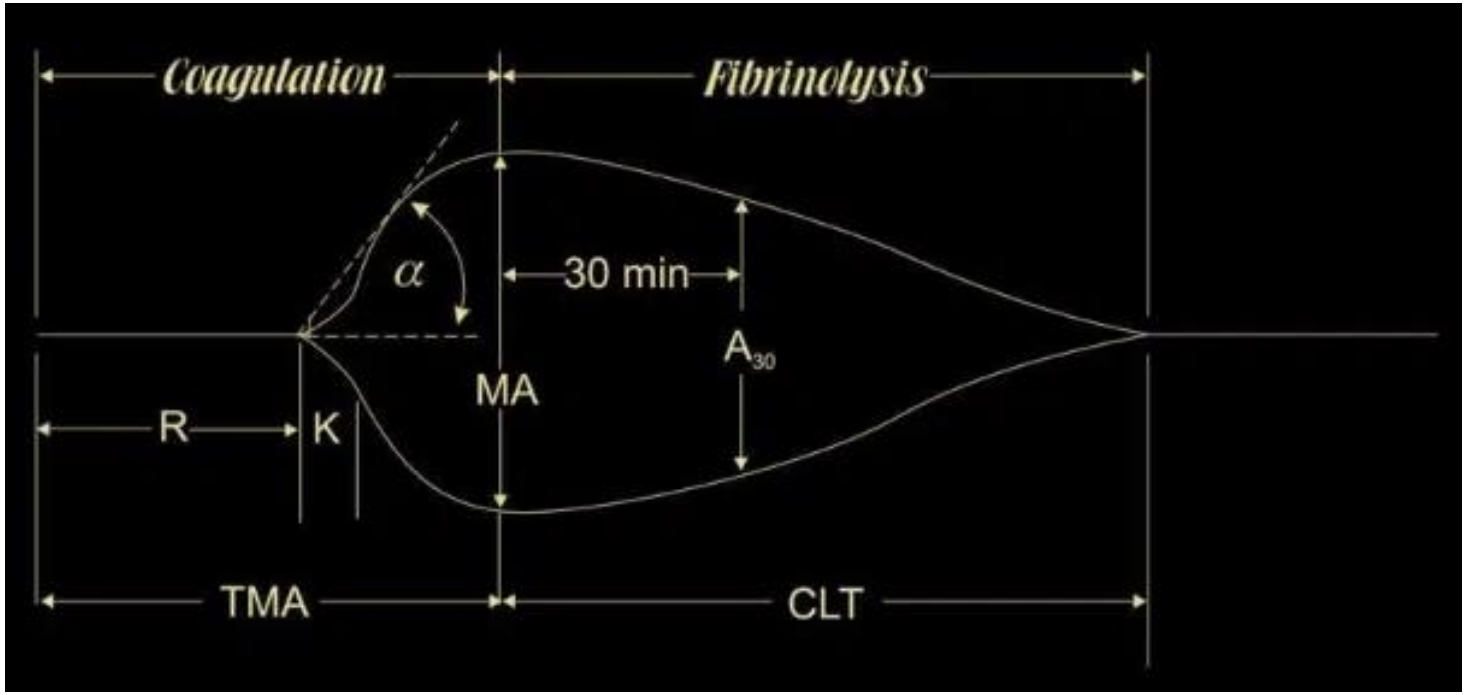
ADP
EPI
Collagen
AA

Thromboelastography (TEG)

- Provides an overall assessment of hemostasis
  - Primary, secondary, and fibrinolysis
- Measures the interaction of platelets with coagulation cascade (aggregation, clot strengthening, fibrin cross-linking and fibrinolysis)
- Helps to predict the need for and to monitor clotting factors administration, platelet transfusion, fibrinolytic therapy and antiplatelet therapy with certain medications (like aspirin)



https://litfl.com/thromboelastogram-teg/



R value (Reaction time): time of latency from the start of the test ot the initial fibrin formation, i.e. initiation

- Measured in seconds

K value (Kinetics): time taken to achieve a certain level of clot strength, i.e. amplification

- Measured on seconds

Alpha (α or angle): slope between R and K, measures the speed at which fibrin build up and cross-linking occurs, i.e. thrombin burst

- Measured in degrees

MA (maximum amplitude): represents the ultimate strength of the fibrin clot

- Measured in mm

LY30 (lysis): amplitude at 30 minutes, percentage decreases in amplitude at 30 minutes post-MA and measures degree of fibrinolysis

- Calculated value

CI (Coagulation Index): overall representative value of hemostasis capabilities

- Calculated value

https://litfl.com/thromboelastogram-teg/

Platelet Disorders

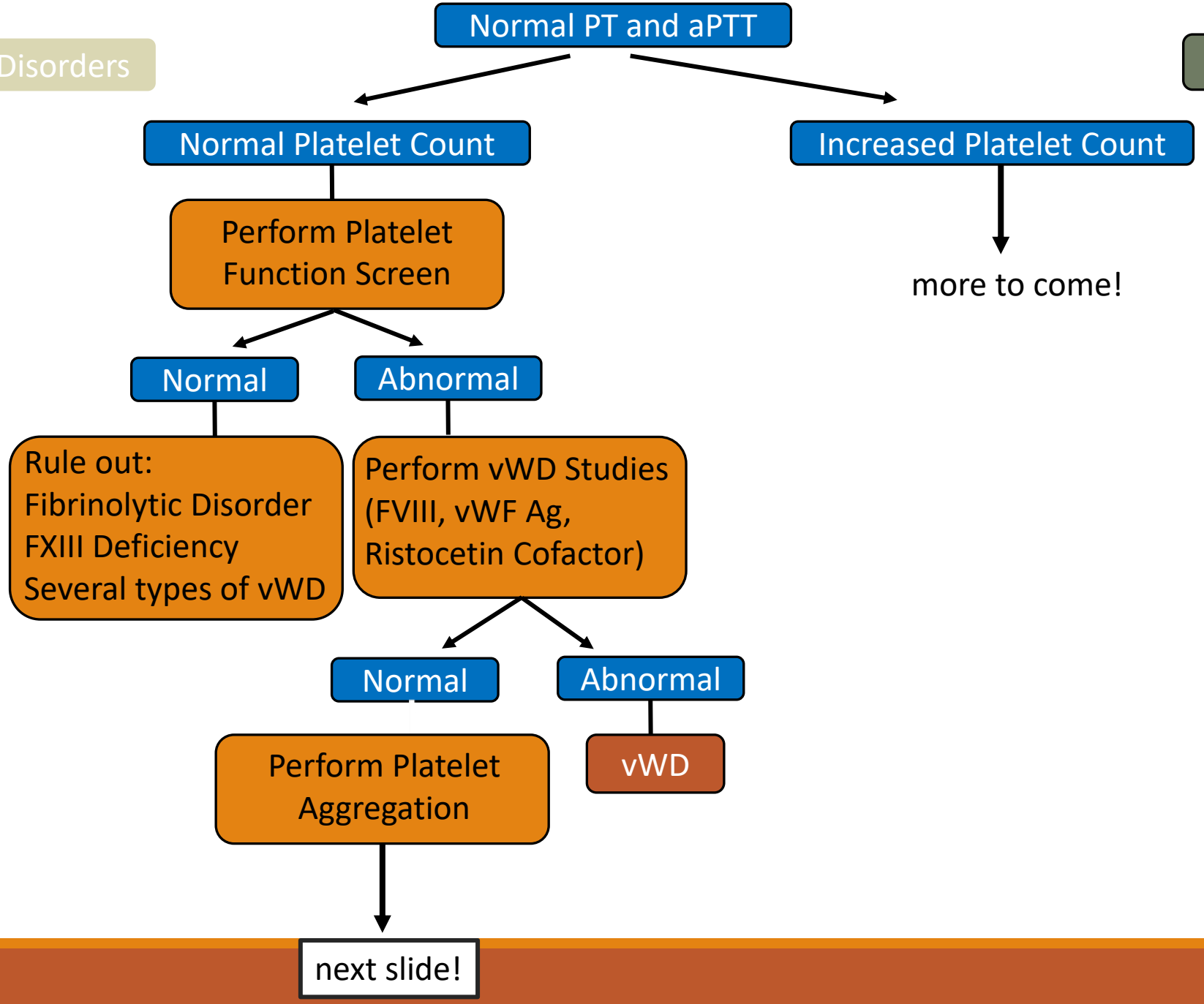
- Individuals primarily present with easy bruising and/or superficial (mucocutaneous) bleeding
  - Symptoms can include: petechiae, purpura, ecchymoses, epistaxis, and gingival bleeding
- Qualitative Platelet Disorders
  - Acquired or congenital disorders of platelet function (normal platelet count)
    - Platelet Adhesion
    - Granule Secretion
    - Platelet Aggregation
- Quantitative Platelet Disorders
  - Acquired or congenital disorders of platelet quantity
    - Thrombocytopenia- decrease in circulating platelets
    - Thrombocytosis- increase in circulating platelets

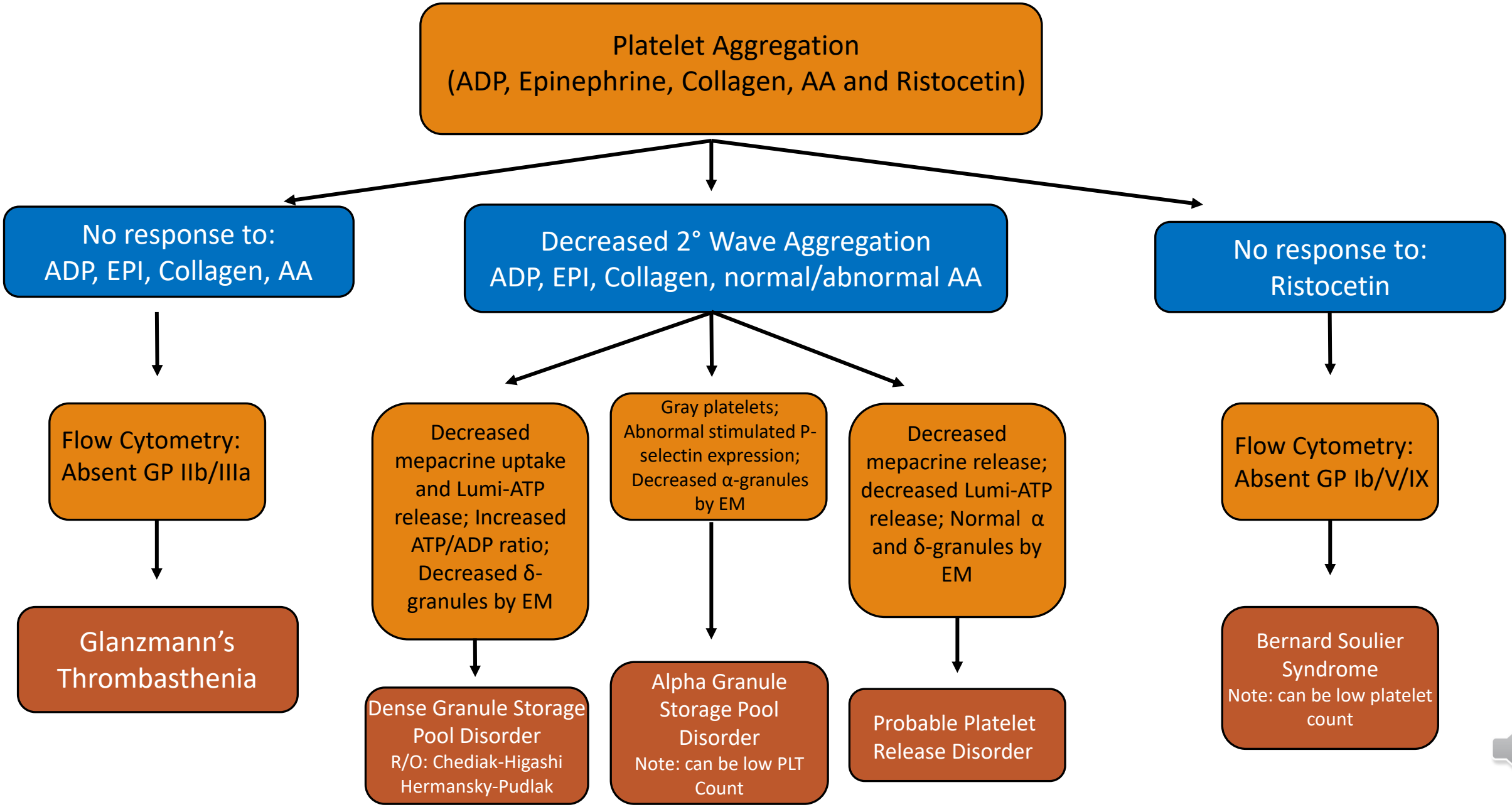
Platelet Disorder Testing

- Basic laboratory work-up on individuals presenting with bleeding tendencies include the following:
  - PT (Prothrombin Time)
  - aPTT (activated Partial Thromboplastin Time)
  - Platelet Count
  - Peripheral Blood Examination of Platelets
- Further laboratory work-up includes:
  - Platelet function assays
  - von Willebrand Disease Testing
  - Bone Marrow Analysis

Qualitative Disorders

Quantitative Disorders




Platelet Aggregation
(ADP, Epinephrine, Collagen, AA and Ristocetin)
No response to:
ADP, EPI, Collagen, AA
Decreased 2° Wave Aggregation
ADP, EPI, Collagen, normal/abnormal AA
No response to:
Ristocetin
Flow Cytometry:
Absent GP IIb/IIIa
Glanzmann's Thrombasthenia
Decreased mepacrine uptake and Lumi-ATP release; Increased ATP/ADP ratio; Decreased δ-granules by EM
Dense Granule Storage Pool Disorder
R/O: Chediak-Higashi Hermansky-Pudlak
Gray platelets; Abnormal stimulated P-selectin expression; Decreased α-granules by EM
Alpha Granule Storage Pool Disorder
Note: can be low PLT Count
Decreased mepacrine release; decreased Lumi-ATP release; Normal α and δ-granules by EM
Probable Platelet Release Disorder
Flow Cytometry:
Absent GP Ib/V/IX
Bernard Soulier Syndrome
Note: can be low platelet count

Qualitative Disorders

Disorders of Adhesion Receptors

- Bernard Soulier Syndrome
- Von Willebrand Disease* (congenital)
- Acquired Defects of Platelet Adhesion
  - Myeloproliferative and lymphoproliferative disorders
  - Antiplatelet antibodies
  - Cardiopulmonary bypass surgery
  - Chronic liver disease
  - Drug-induced membrane modification

Disorders of Platelet Aggregation

- Glanzmann Thrombasthenia
- Hereditary Afibrinogenemia
- Acquired Defects of Platelet Aggregation
  - Acquired von Willebrand disease
  - Acquired uremia

Disorders of Platelet Secretion

- Storage Pool Diseases
  - Dense Granule Deficiency
  - Alpha Granule Deficiency
- Thromboxane Pathway Disorders
- Inherited Disorders of Receptors and Signaling Pathways
- Acquired Defects of Platelet Secretion
  - Renal Failure
  - Cardiopulmonary Bypass Surgery
  - Liver Disease
  - Paraproteinemias
  - Aspirin and NSAIDs

* vWD is special, will have it's own section!

Quantitative Disorders

Thrombocytopenia

- **Impaired/Decreased Production**
  - Congenital
    - Fanconi anemia
    - TAR syndrome
    - Wiskott-Alderich syndrome
    - Bernard Soulier
    - MYH9-related diseases
    - Amegakaryocytic thrombocytopenia
    - Neonatal thrombocytopenia
  - Acquired
    - Drugs
    - Ineffective thrombosis
    - Viruses/Bacteria
    - Malignancy
- **Increased Platelet Destruction**
  - Immunologic responses
    - ITP (Acute or Chronic)
    - Drug induced
      - HIT (Heparin-induced thrombocytopenia)
    - Neonatal auto or alloimmune
    - Post transfusion purpura
  - Nonimmunologic
    - Mechanical Damage
    - Thrombocytopenia in pregnancy and preclampsia
    - Hemolytic Disease of the Newborn
    - TTP (Thrombotic Thrombocytopenic Purpura)
    - HUS (Hemolytic Uremic Syndrome)
    - DIC (Disseminated Intravascular Coagulation)
    - Purpura Fulminans

Thrombocytosis

- Reactive Thrombocytosis
- Thrombocytosis Associated with Myeloproliferative Disorders

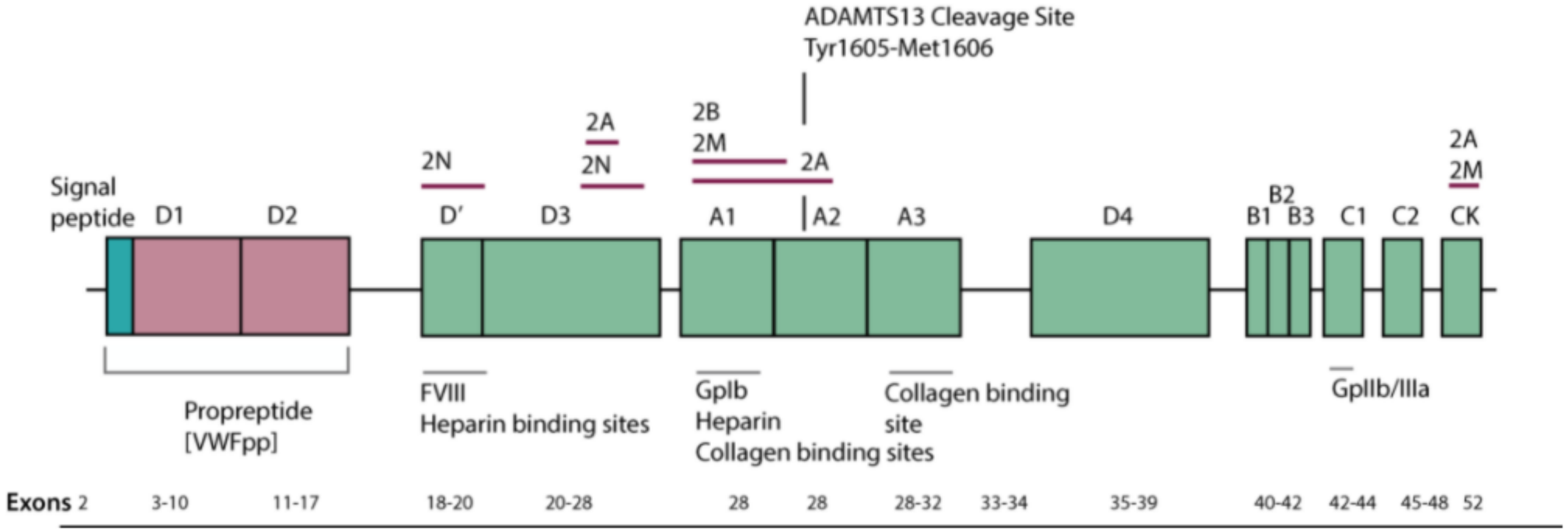
Bernard Soulier

- a.k.a *"Giant Platelet Syndrome"*
- Usually manifested in infancy or childhood
- Clinical characteristics
  - Ecchymoses, epistaxis, gingival bleeding, prolonged bleeding, thrombocytopenia (decreased platelet survival)
- GPIb/IX/V is missing or dysfunctional
  - Most frequently this involves a defect in the Ib synthesis or expression
    - Ineffective binding of vWF and thrombin
- Platelet Aggregation:
  - NO aggregation with Ristocetin
  - Normal response to ADP, EPI, Collagen and Arachidonic Acid
- In contrast to vWD, this abnormality cannot be correct by the addition of normal plasma or cryoprecipitate
  - The defect lies in the platelet!!!

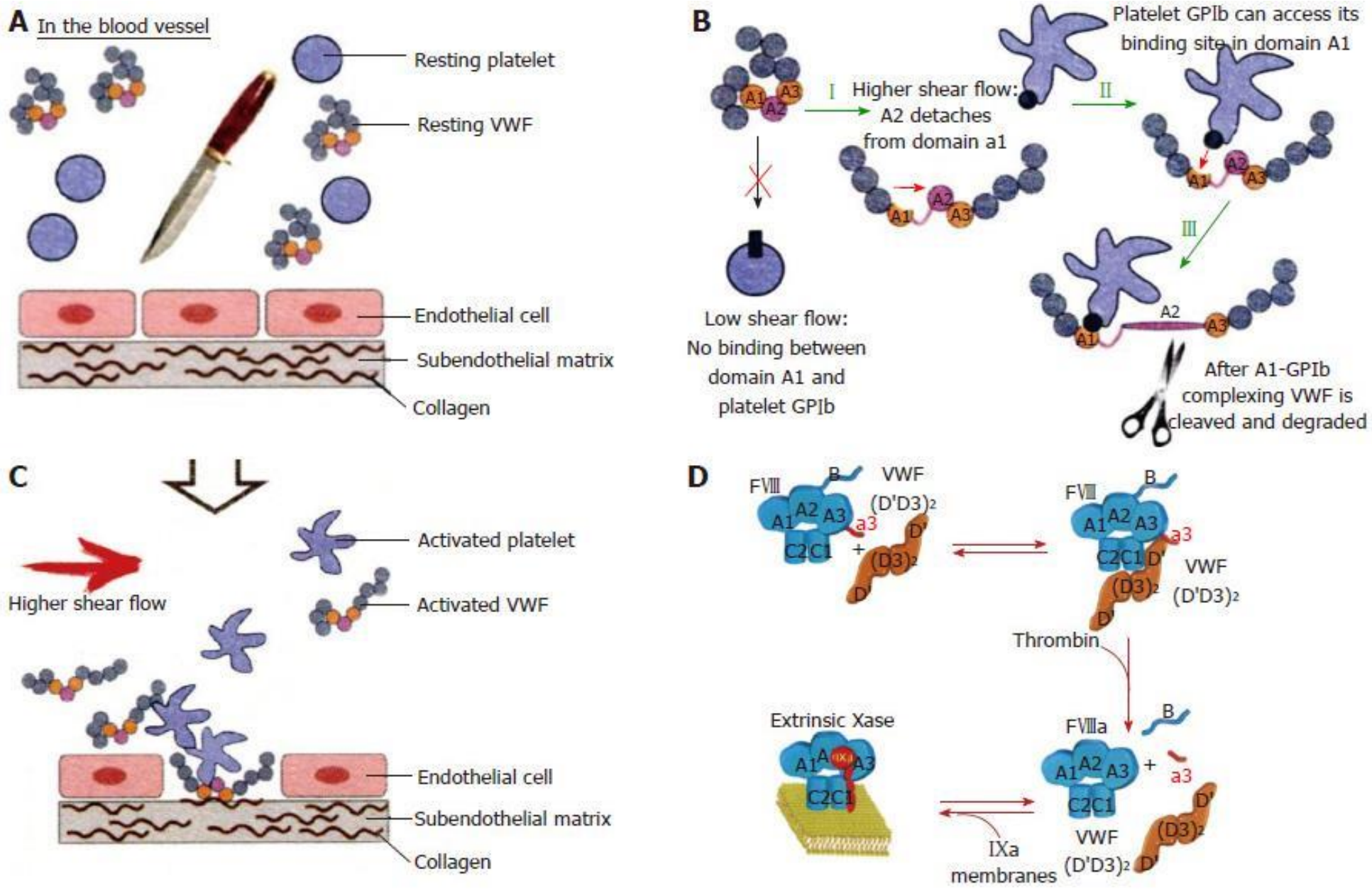
Von Willebrand Disease

- First things first:
  - vWF (von Willebrand factor)
    - Synthesized in the ER of endothelial cells and is stored in the weibel-palade bodies of endothelial cells and platelet α-granules
    - Main functions
      - Platelet adhesion to subendothelial collagen in high shear stress (capillaries and arterioles)
      - Factor VIII carrier molecule
  - vWF in action!
    1. vWF is released from weibel-palade bodies and unfolds to bind subendothelial collagen
       - Collagen is exposed due to vessel injury or desquamation of endothelial cells
    2. Platelets then adhere to vWF through their GPIb portion of the GPIb/IX/V binding site
    3. Platelets become activated and express GPIIb/IIIa which is a receptor site for vWF and Fibrinogen

Molecular breakdown of von Willebrand Factor



https://practical-haemostasis.com/Miscellaneous/adamts13_assays.html



https://www.wjgnet.com/2218-6204/full/v5/i3/WJH-5-61-g004.htm

Von Willebrand Disease

- Most common congenital mucocutaneous bleeding disorder
  - Affects 1-2% of the general population
  - Symptomatic in 1:10,000 individuals
  - Usually characterized as a “hemorrhagic” bleeding disorder
  - Due to either quantitative or qualitative deficiencies of von Willebrand factor (vWF)
    - Abnormality causes decreased platelet adhesion leading to impaired primary hemostasis
    - Decreased FVIII levels can result from quantitative vWF deficiencies (no protection from proteolysis)

- Symptoms can include:
  - Ecchymoses, epistaxis, menorrhagia, hematemesis, GI and surgical bleeding
  - These symptoms can vary depending on the type of vWD
  - Important:
    - When FVIII levels decrease below 30 units/dL, anatomical bleeding into joints and body cavities accompanies mucocutaneous bleeding

Specialized Testing for von Willebrand Disease

Definitive diagnosis depends on 1. Combination of personal family history of mucocutaneous bleeding and 2. The laboratory confirmation of decreased vWF antigen or activity

1st: Physician orders a platelet count and PT/aPTT

- Platelet count rules out bleeding due to thrombocytopenia
- PT/aPTT will assess the coagulation cascade and rule out any factor deficiencies other than vWF

2nd: More specific testing is completed to better assess the variability and complexity of vWD

- VWF:Ag – quantitative assay to measure vWF antigen (immuno-turbidometric assay)
- VWF:RCo – ristocetin cofactor assay to assess the activity of vWF (turbidometric assay measuring platelet agglutination)
  - Ristocetin was a failed attempt as an antibiotic that actually unfolds vWF molecules and allows them to better bind platelet membranes
- FVIII Assay – clot-based assay measuring factor activity
- RIPA – ristocetin induced platelet aggregation

3rd: Ratios are utilized (like vWF:Ag : vWF:RCo) to better differentiate subtypes of vWD

4th: vWF multimer analysis can be done by gel electrophoresis as a confirmatory method

5th: Pathologists interpret the laboratory results and present the findings to the ordering physician

Von Willebrand Disease

Type 1:

- Quantitative vWF deficiency that comprises 40-70% of vWF cases
- Mild to moderate systemic bleeding usually occurring after dental extraction, surgery, or menorrhagia in woman
- Normal PT and PLT count. Decreased FVIII, vWF antigen, and vWF activity
  - Decrease in all 3 analytes are “concordant” and the ratio of vWF activity to vWF antigen approaches 1:1

Type 2:

- Qualitative vWF abnormalities
  - Type 2A- Second most common; moderate bleeding severity; inability to form large vWF multimers
  - Type 2B- Mutations lead to increased binding of vWF to GP Ib “gain of function mutation”; increased ristocetin induced aggregation; platelet coated with vWF are cleared at an increased rate leading to loss of HMW-vWF + ↓ PLT
  - Type 2M- Decreased functionality with normal multimers; impaired ability to bind PLT receptor (GP Ib)
  - Type 2N- “Normandy”- mutation affecting the binding of FVIII; mimics hemophilia A but autosomal pattern of inheritance

Type 3:

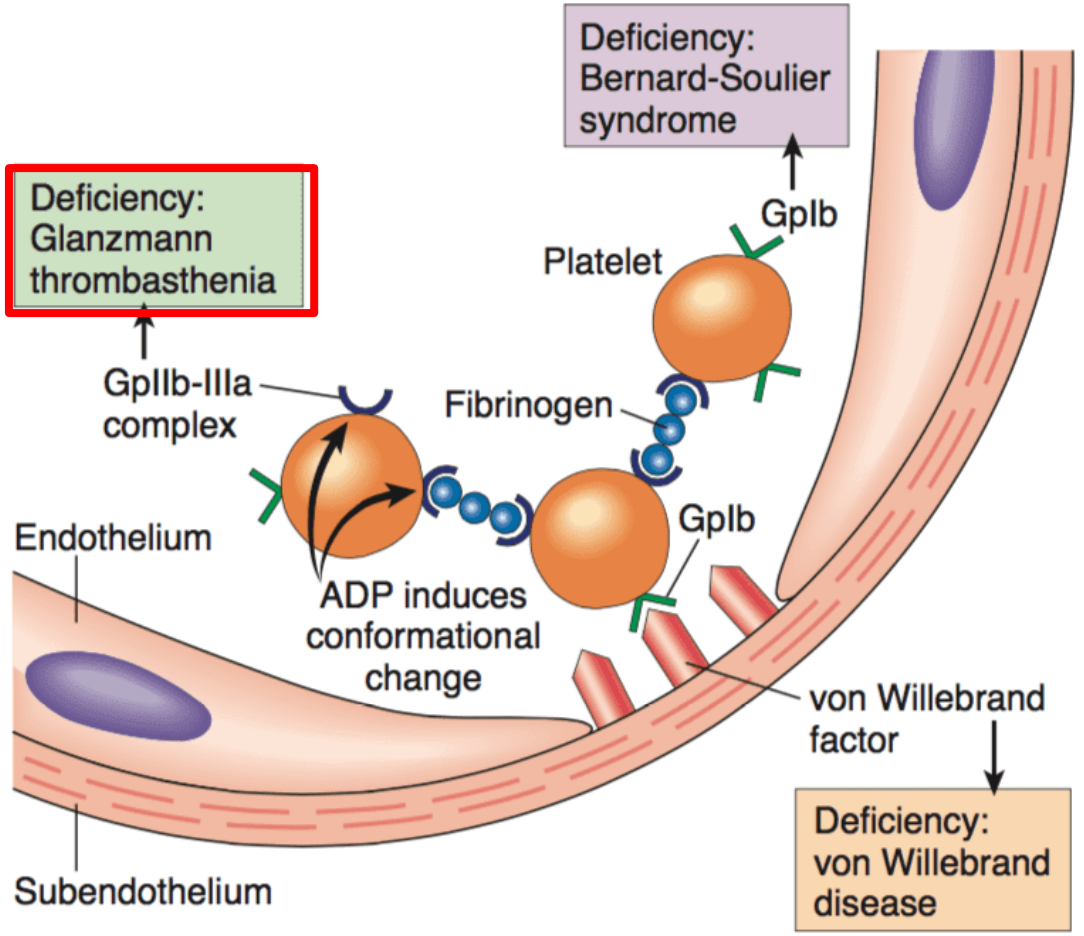
- Qualitative vWF deficiency that is extremely rare’ “null allele” gene translation or deletion mutations occurs in compound heterozygotes or in consanguinity, homozygotes
- Decrease vWF and FVIII

Won Willebrand Disease

	Normal	Type 1	Type 2A	Type 2B	Type 2M	Type 2N	Type 3	PLT-vWD
vWF:Ag	N	↓/↓↓	↓	↓	↓	N/↓	absent	↓
vWF:Rco	N	↓/↓↓	↓↓/↓↓↓	↓↓	↓↓	N/↓	absent	↓↓
FVIII	N	N/ ↓	N/ ↓	N/ ↓	N/ ↓	↓↓	1-9 IU/dl	N/↓
RIPA	N	often N	↓	often N	↓	N	absent	often N
PFA-100® CT	N	N/↑	↑	↑	↑	N	↑↑↑	↑
BT	N	N/↑	↑	↑	↑	N	↑↑↑	↑
Pl-Count	N	N	N	↓/N	N	N	N	↓
vWF multimers	N	N	abnormal	abnormal	N▪	N▪	absent	abnormal
								

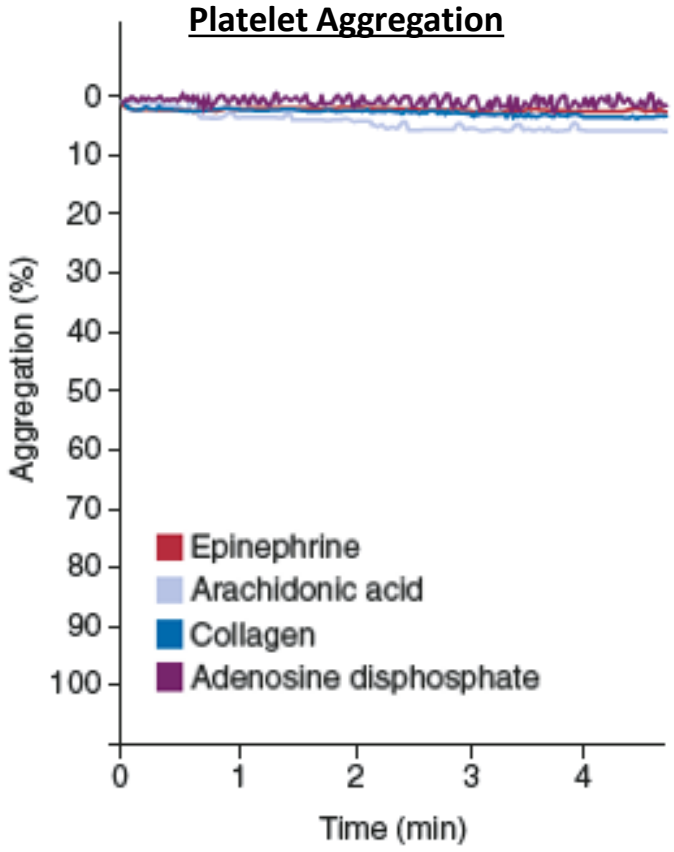
Glanzmann Thrombasthenia

- Rare, autosomal recessive disorder
- Homozygotes experience severe bleeding problems
- Manifestations include: epistaxis, gingival bleeding, easy bruising, menorrhagia, and GI bleeding
- Deficiency or abnormality of GP IIb/IIIa
  - No fibrinogen binding
  - Defect in platelet plug formation
- Laboratory Findings:
  - Normal PT, PTT and platelet count
  - No Platelet aggregation to ADP, epinephrine, AA, and collagen
  - Aggregation to ristocetin normal

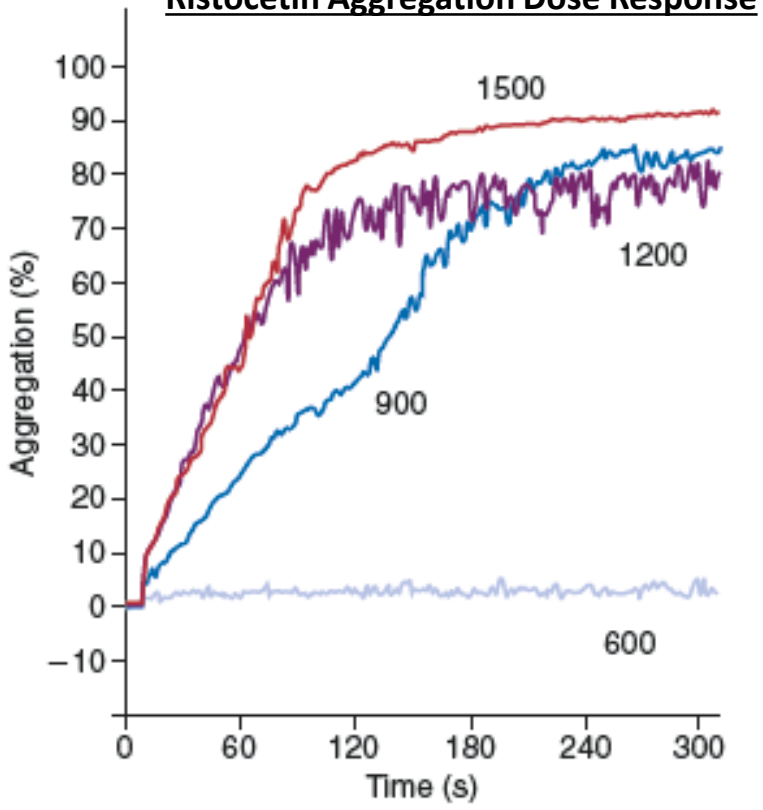


Glanzmann Thrombasthenia

Platelet Aggregation



Ristocetin Aggregation Dose Response



Storage Pool Disorders

A disorder characterized by a low secretion of substances that are stored in platelet granules

Type	Decrease In
δ-SPD	dense granules
α-SPD	alpha granules
αδ-SPD	alpha and dense granules

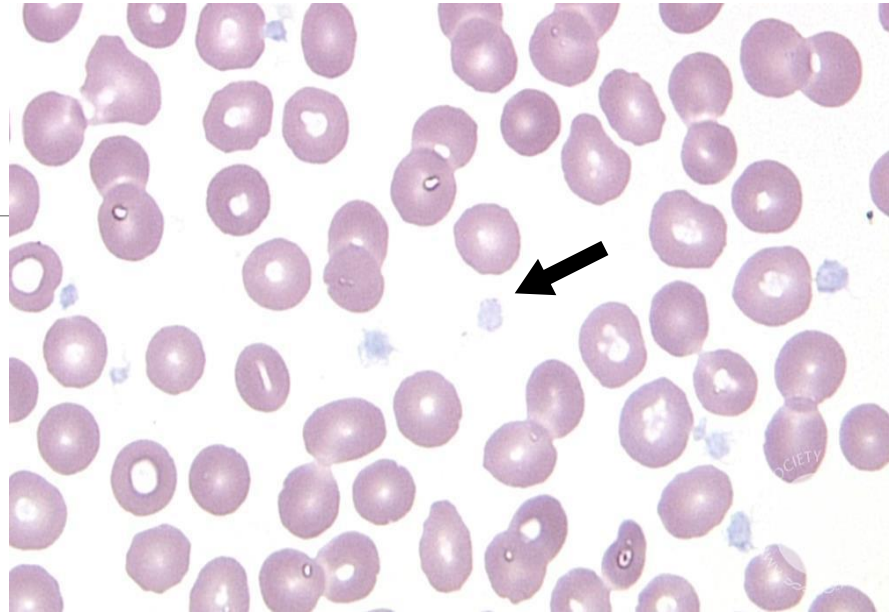
Dense Granule Deficiency (Δ-SPD)

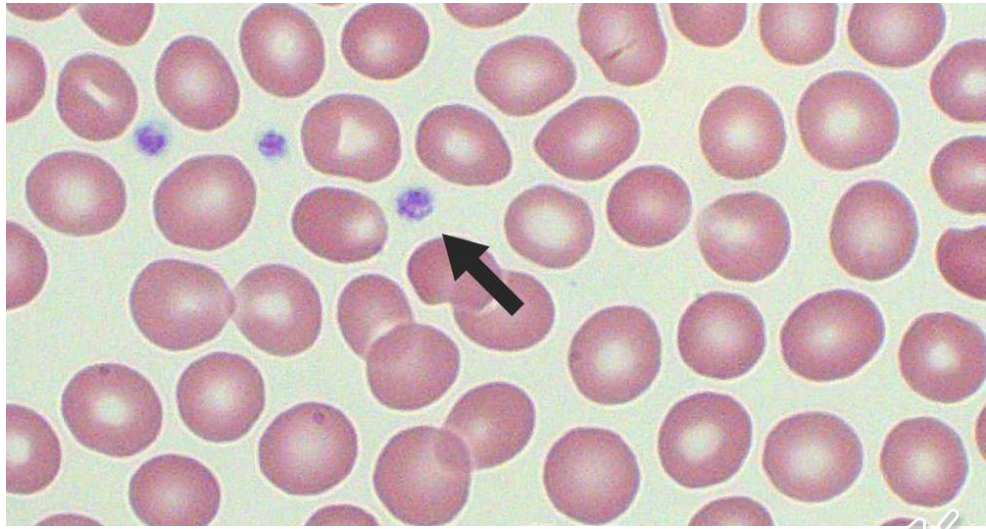
- Lack of aggregation caused by lack of ADP secretion
  - Syndromes associated with δ-SPD
    - Hermansky-Pudlak
    - Chediak-Higashi
    - Wiskott Alderich
    - TAR (Thrombocytopenia with Absent Radii Syndrome)

- Lab findings:
  - Normal PT/aPTT
  - Morphologically normal platelets on PB smear
  - Decreased dense granules by Electron microscopy
  - Prolonged PFA
  - Abnormal aggregation with ADP, epinephrine and collagen in the 2nd wave
    - Normal 1° wave but blunted 2° wave

Alpha Granule Deficiency (α-SPD)

- "Gray platelet syndrome"
- Lab findings:
  - Absence of α-granules causes the platelets to appear agranular on PB smear
  - Platelet aggregation can be normal or decreased
  - Moderate thrombocytopenia
  - Electron microscopy shows absence or low levels of α-granules
  - Increased plasma levels of PF4 and β-thromboglobulin
    - Deficiency or deficient packaging of alpha granule contents
      - Released into circulation instead of being stored





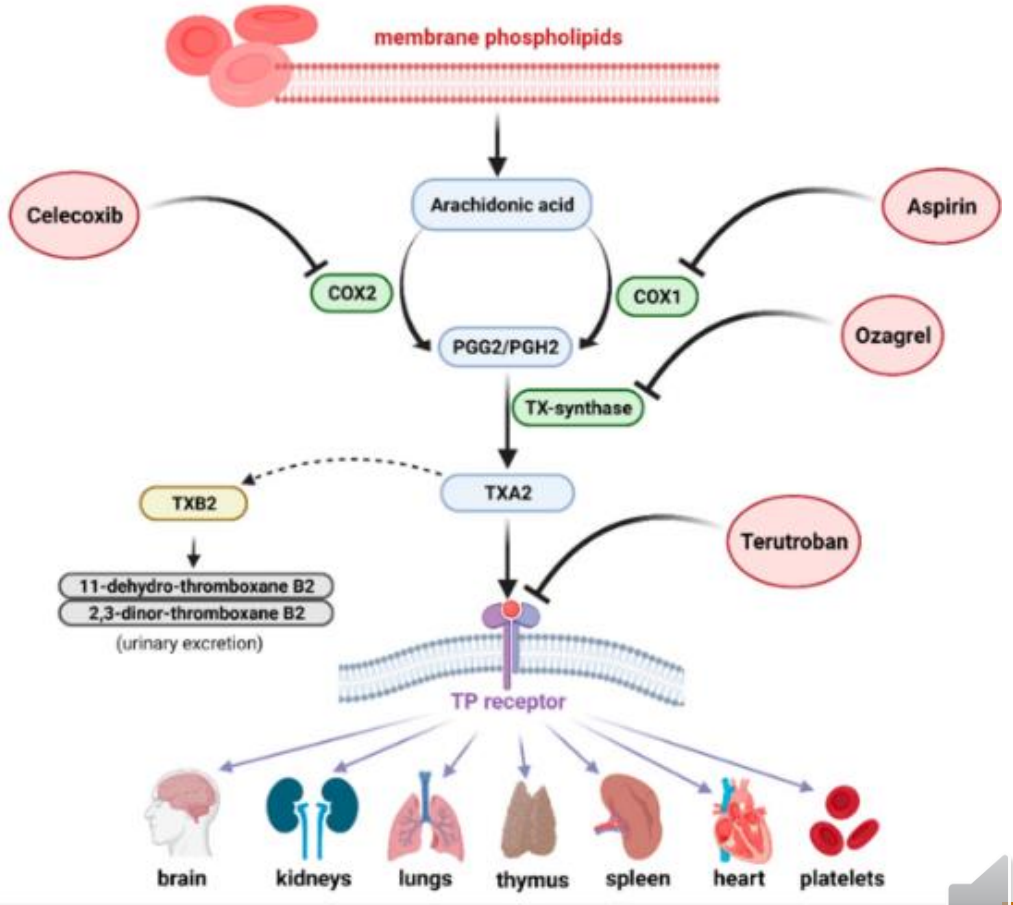
https://imagebank.hematology.org/image/61243/grayplatelet-syndrome https://medschool.co/tests/blood-film/platelet-morphology

Secretion

Thromboxane A2 Pathway Disorders

- Hereditary absence or abnormalities of components of thromboxane pathway
- Thromboxane Pathway
  - Series of phospholipases catalyze the release of arachidonic acid and other compounds from membrane phospholipids
  - Arachidonic acid is converted to intermediate prostaglandins by cyclooxygenase
  - Those intermediate prostaglandins are converted to thromboxane A_2 by thromboxane synthase
- Thromboxane A_2 (with other compounds) mobilizes calcium from internal stores into the cytoplasm initiating events leading to secretion and aggregation of platelets

Figure 2. The scheme of action of thromboxane antagonists: aspirin is an inhibitor of cyclooxygenase 1 (COX-1) and celecoxib inhibits COX-2. Ozagrel blocks the conversion of PGH2 to TXA2 by inhibiting thromboxane synthase (TXS). Terutroban (TP antagonists) block the activation of the thromboxane receptor (TP receptor) (created with BioRender.com).

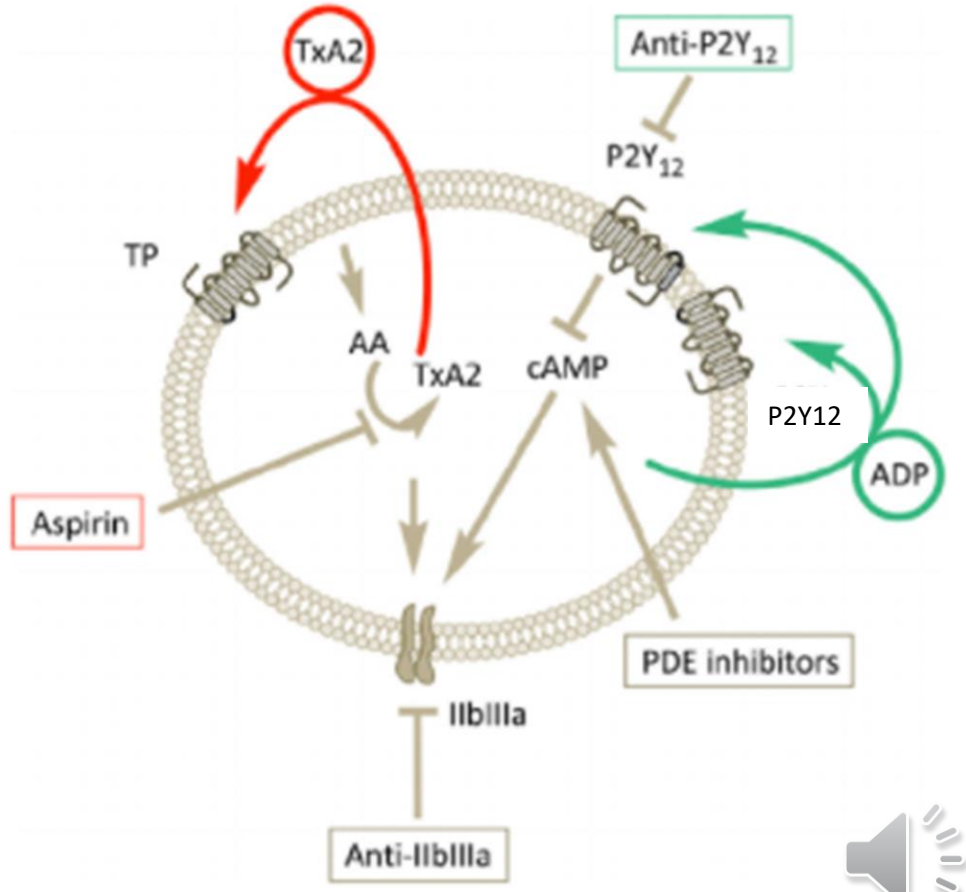


https://www.mdpi.com/1422-0067/22/21/11644

Anti-platelet Drugs

- Platelets play a major role in atherosclerosis and thrombus formation
- Anti-platelet drugs target two important amplification pathways of platelet activation
  - Thromboxane A2 (TxA2) production and the action of adenosine diphosphate (ADP)

- **Aspirin**
  - Irreversibly acetylates platelet cyclooxygenase-1 (COX-1) inhibiting TxA2 production thus impairing platelet activation
- **Clopidogrel**
  - Pro-drug that acts on the ADP receptor P2Y12 preventing the amplification of platelets
- **Tirofiban**
  - Antagonist to integrin αIIbβ3 and is used in more acute clinical situations



Thrombocytopenia

- Platelet count < 100,000/uL (reference range 150,000 – 450,000/uL)
- Most common cause of clinically significant bleeding
- Spontaneous bleeding occurs at <20 x10^3/uL
- Life-threatening at <10 x10^3/uL

Quantitative Disorders

Normal PT and aPTT

Decreased Platelet Count

Platelet Clumping

Pseudo thrombocytopenia
Clumping in EDTA, not citrate or heparin (+/- satellitism)
H/O abciximab

Heparin-induced thrombocytopenia
Clumping in EDTA and Heparin

Macrothrombocytes (MPV >12.5 fL)

Neutrophil Inclusions?

NO

YES

Disorder	Abnormal	Abnormal Aggregation
Bernard-Soulier	GPIb/V/IX	Ristocetin
Velocardiofacial syndrome	GP Ibβ	NL
Mitral Valve insufficiency	GP Ia, Ic, IIa	ADP, AA, Thr
Gray platelet syndrome (αSPD)	P-selectin	Rist, Thr, Col
Quebec Platelet Syndrome	Multimerin/fV	Epinephrine
Montreal platelet syndrome	calpain	Thr
Mediterranean macrothrombocytopenia	Unknown	Unknown

Abnormal neutrophil localization of NMMHCA; MYH9 mutations

MYH9 Disorders
Fechtner syndrome
- hereditary nephritis, deafness, cataracts (Alport's syndrome)

Epstein syndrome
- nephritis, deafness.

May-Hegglin anomaly and Sebastian syndrome
- macrothrombocytopenia only

Small Platelets (MPV 3.8-5.0 fL)

Wiskott-Aldrich Syndrome and X-Linked Thrombocytopenia
X-linked immune deficiency
WASP Gene mutations
May have associated storage pool disorder

Quantitative Disorders

Normal PT and aPTT

↓

Decreased Platelet Count

↓

Normal Platelet Size and Morphology

↓

Perform Bone Marrow Analysis or Platelet Turnover Studies (mRNA) by Flow Cytometry

Adequate to Increased Megakaryocytes or Platelet mRNA

Increased **Destruction**

Disorder	***Features***
ITP	Antibodies to Surface GP
Post-Transfusion Purpura	H/O transfusion; PLA-1 Negative
NAIT	Mother PLA-1 Negative
Drug-Induced	Identify drug therapy
HIT	Heparin PF4 Ab, positive heparin-induced platelet agg.
TTP	Renal Failure, Mental changes, Hemolysis, Fever, ADAMTS13
DIC	Schistocytes, Abnormal coagulation tests
HELLP	Hemolysis, increased liver enzymes, Pre-eclampsia
Splenic Sequestration	Splenic enlargement

Decreased Megakaryocytes Or Platelet mRNA

Decreased **Production**

Hereditary *Disorder*	***Features***
Tel-AML-1	CBFA2 mutations; AML
CAMT	c-Mpl mutations
CTRUS	HOXA11 gene mutations
TAR	Absent radii
XLT-dyserythropoiesis	GATA-1 mutations
XLT-thalassemia	GATA-1 mutations

Acquired Disorder

Aplastic anemia; AATP; Myelophthisic disorders; Myelodysplasia; Drug-induced aplasia; Alcohol-induced; viral-induced; PNH; Cyclic thrombocytopenia

Ineffective Thrombopoiesis

Congenital

- **Fanconi anemia** (pancytopenia)
- **TAR syndrome** – "thrombocytopenia with absent radius"
  - Rare autosomal recessive disorder
  - Severe Neonatal thrombocytopenia (platelet count actually increases with age)
  - Hypoplasia of radial bones of forearms
  - Markedly Elevated WBC
- **Wiskott-Alderich** – X-linked
- **Bernard-Soulier** – autosomal recessive
- **MYH9-Related Diseases** (non-muscle myosin heavy chain)
- Cytoskeletal protein in platelets
  - **May Hegglin anomaly**
  - More rare: **Sebastian syndrome**, **Fechtner syndrome** and **Epstein syndrome**
- **Amegakaryocytic thrombocytopenia**
  - Autosomal recessive reflecting BM failure
  - Infants < 20,000 platelets at birth
  - Petichiae
  - Likely develop aplastic anemia before 1 year old
  - Reduced megakaryocyte progenitors and ↑ TPO (TPO receptor function is lost)
- **Neonatal Thrombocytopenia**
  - Many types
  - Causes:
    - TORCH (**t**oxoplasmosis, **o**ther (*Treponema pallidum*, varicella-zoster virus, parvovirus B19), **r**ubella, **c**ytomegalovirus [CMV], **h**erpes)
    - Drug exposure in utero (sulfonamides)
    - Decrease or absence of megakaryocytes in neonates

Acquired

- Drugs
  - Chemotherapeutic agents
  - Ethanol ingestion (months to years of excessive use)
  - Interferon therapy

Megakaryocyte hypoplasia in BM

Immunologic Responses

- Immune Thrombocytopenic Purpura (ITP)
  - Acute
  - Chronic
- Drug-induced
  - Quinidine/quinine/sulfonamide derivatives
  - Hapten- dependent
  - Drug induced autoantibodies
  - HIT (Heparin-induced thrombocytopenia)
- NAIT – Neonatal Alloimmune Thrombocytopenia
- Neonatal Autoimmune Thrombocytopenia
- PTP – Post Transfusion Purpura

Immune Thrombocytopenic Purpura (ITP)

- Previously termed “idiopathic”
- Autoimmune disorder characterized by immune-mediated destruction of platelets and/or impaired platelet production
- Autoantibodies are mostly Ig(G)- directed against GPIIb/IIIa, GPIb/IX, GPV
- One of the most common disorders causing severe isolated thrombocytopenia
- Labs:
  - Abnormal platelet function testing
  - Diagnosis of exclusion
    - antiplatelet antibodies, BM examination and clinical presentation

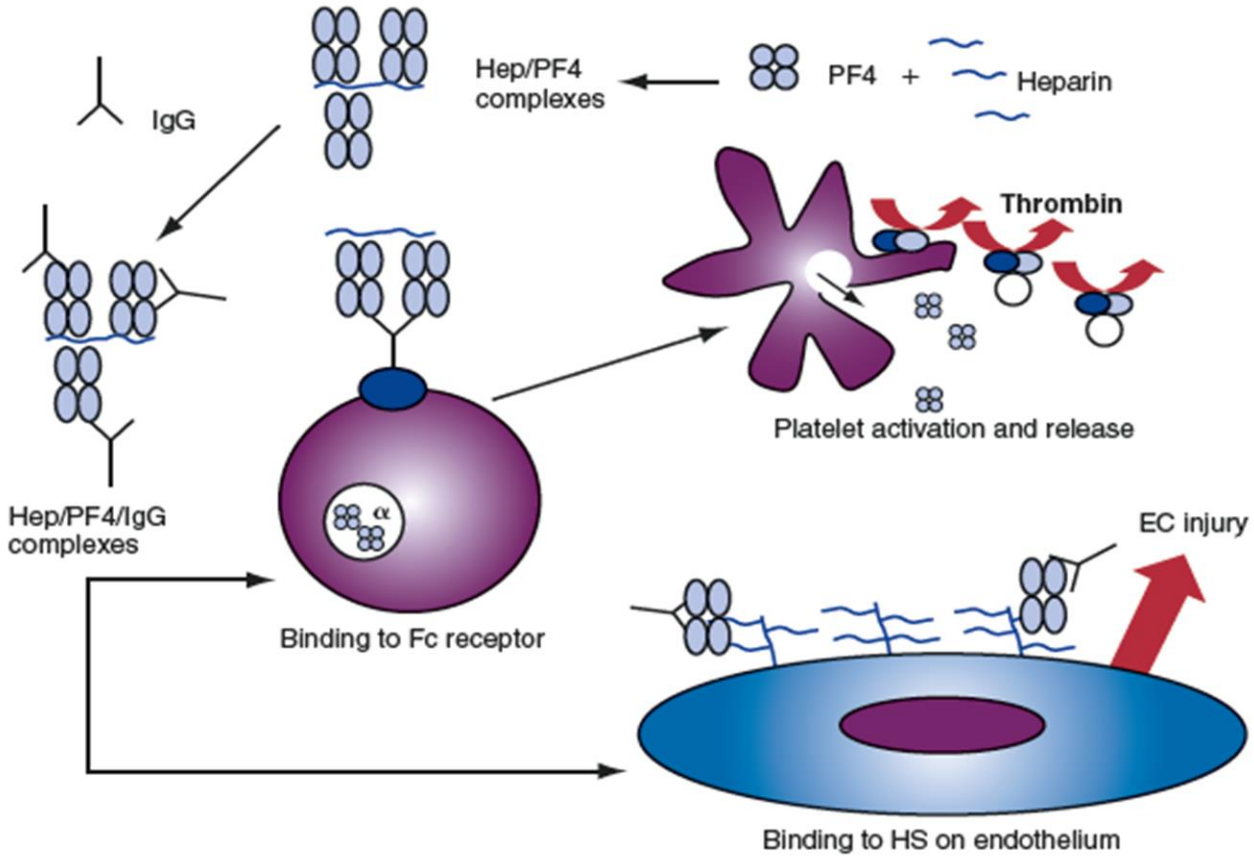
Chronic ITP

- Most prevalent in Adults (Women) 20-50 years
- Insidious onset: mucocutaneous bleeding, menorrhagia, epistaxis, ecchymoses
- Platelet Count: <30-80 x10^3/uL
- Caused by autoantibodies attached to platelets→ shortened platelet lifespan
- Spontaneous remission is rare as course of disease fluctuates

Acute ITP

- Children 2-5 years
- Abrupt onset: bruising, petechiae, mucosal bleeding
- Platelet Count: <20 x 10^3/uL
- 1-3 weeks after infection (upper respiratory or GI virus) or vaccination
- Binding of antibodies from previous infections to platelets
- Spontaneous remission in ~93% of cases

Drug-Induced Thrombocytopenia - Heparin Induced Thrombocytopenia (HIT)



- Consequence of an immune response to UFH and LWMH that is reflected in a lowered platelet count
- HIT can progress to venous or arterial thrombosis
- Hematologists routinely define HIT-related thrombocytopenia as a 30%-50% drop in platelet count from the pre-heparin level
- HIT patients rarely experience bleeding, thrombosis is more concerning

Picture courtesy of Dr. Kandice-Kotke Marchant

Diagnosis of HIT

TABLE 2-3

Pretest Scoring System for Heparin-Induced Thrombocytopenia (4T)

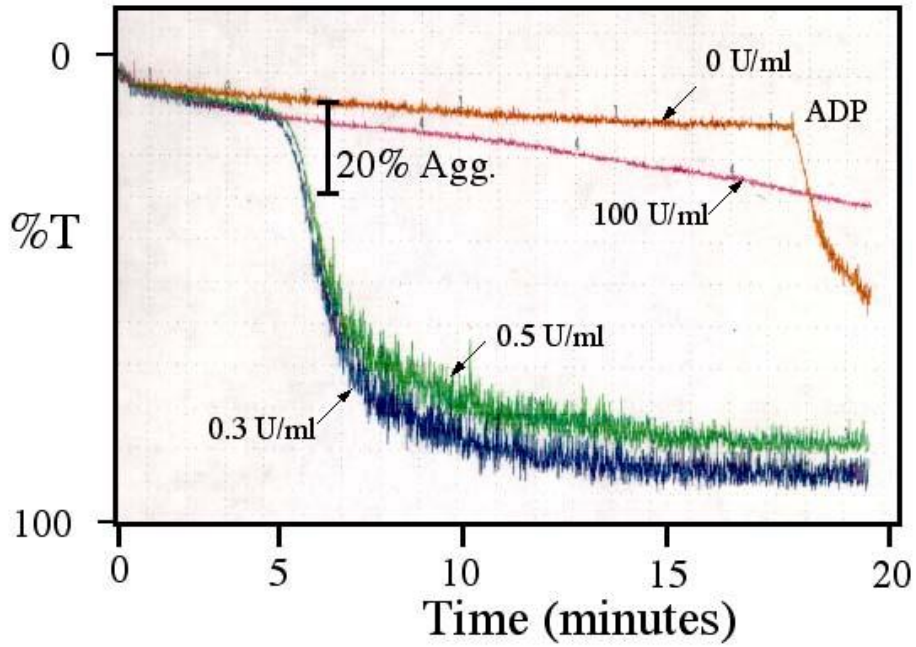
4 *Ts*	2 Points	1 Point	0 Points
Thrombocytopenia	Platelet count fall >50% and platelet nadir >20 × $10^3/\mu L$	Platelet count fall 30%-50% or platelet nadir 10 to 19 × $10^3/\mu L$	Platelet count fall <30% or platelet nadir <10 × $10^3/\mu l$
Timing of platelet count fall	Clear onset between days 5 and 10 or platelet fall ≤1 day (prior heparin exposure within 30 days)	Consistent with days 5-10 fall, but not clear; onset after day 10 or fall ≤1 day (prior heparin exposure 30-100 days ago)	Platelet count fall <4 days without recent heparin exposure
Thrombosis or other sequelae	New thrombosis (confirmed); skin necrosis; acute systemic reaction postintravenous UFH bolus	Progressive or recurrent thrombosis; nonnecrotizing skin lesions; suspected thrombosis (not proven)	None
Other causes for thrombocytopenia	None apparent	Possible	Definite

From Greinacher A, Althaus K, Krauel K, Selleng S: Heparin-induced thrombocytopenia, *Haemostaseologie* 30:17–28, 2010.
UFH, Unfractionated heparin.

Low probability: 0-3
Intermediate: 4-6
High: 7-8

Laboratory Results in HIT

- Immunologic Assay: Anti-PF4 Ab
  - ELISA
- Heparin Induced Platelet Aggregation (HIPA)
  - Confirmatory test
- Serotonin Release
  - Golden standard test
- Treatment
  - Stop heparin
  - Switch to DTI



Picture courtesy of Dr. Kandice-Kotke Marchant

Other Drug-Induced Reasons for Thrombocytopenia

- Quinidine/quinine/sulfonamide derivatives
  - Abrupt onset of bleeding symptoms
  - Drug combines with antibody and binds platelets by Fab regions
  - Fc regions of immunoglobulin still available to bind to Fc receptors of phagocytic cells
  - Platelet count drops rapidly and often may be <10,000/uL

- Hapten- dependent
  - Drug combines with a carrier molecule (usually plasma protein) to then act as a complete antigen
  - Penicillin and penicillin derivatives
  - Platelet count rapidly declines and can be as low as <1,000/uL

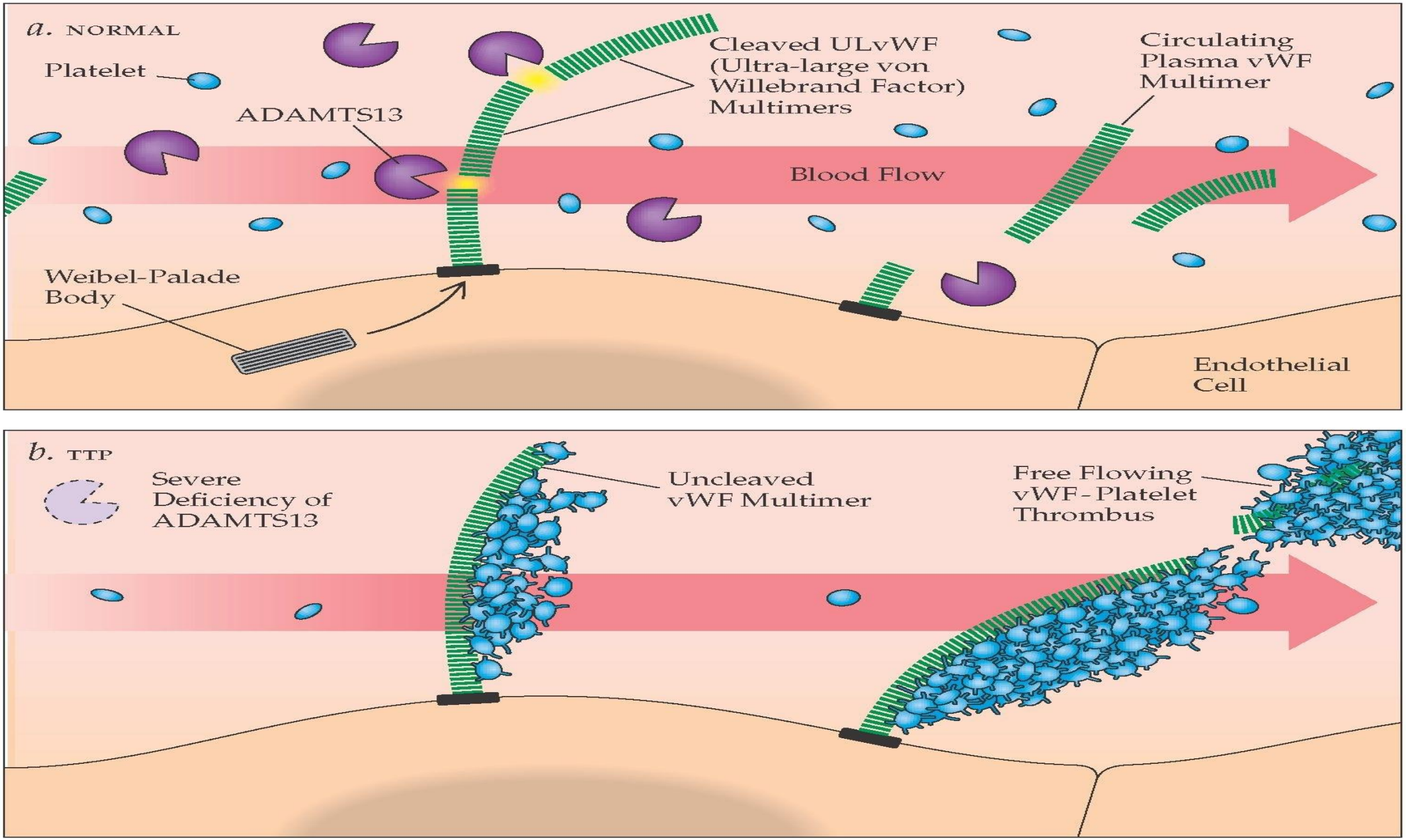
- Drug induced autoantibodies
  - Drugs stimulate formation of autoantibody that binds platelet in absence of drug

Nonimmunologic Responses

- Mechanical damage
- Thrombocytopenia in pregnancy and preclampsia
- Hemolytic Disease of the Newborn
- TTP (Thrombotic Thrombocytopenic Purpura)
- HUS (Hemolytic Uremic Syndrome)
- DIC (Disseminated intravascular coagulation)
  - Acute
  - Chronic
- Purpura Fulminans

Thrombotic Thrombocytopenic Purpura (TTP)

- *Moschcowitz syndrome*
- *Upshaw-Schulman Syndrome*
- Characterized by the triad of
  - Microangiopathic hemolytic anemia (MAHA)
  - Thrombocytopenia (severe)
  - Neurologic abnormalities
- Hemolysis usually severe (less than 10 mg/dL Hgb)
  - Directly related to accumulation of ultralarge von Willebrand factor (UL-VWF) multimers in the plasma
  - In normal plasma the UL-VWF multimers are rapidly cleaved into smaller VWF multimers by the VWF-cleaving protease ADAMTS13
  - Treatment: Therapeutic plasma exchange (TPE) with FFP
    - 1st: some of UL-VWF removed by apheresis
    - 2nd: plasma supplies the deficient ADAMTS13 protease
- Before 1990: TTP was fatal in more than 90% of cases
- Now: 80% of patients who are treated early can be expected to survive

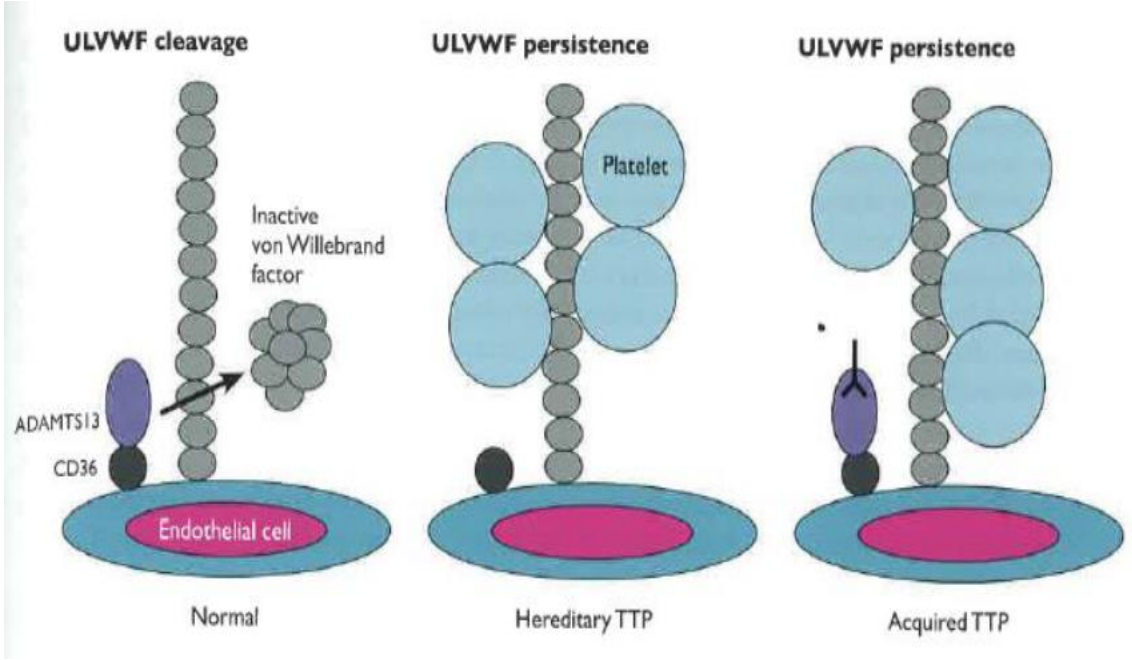


http://what-when-how.com/acp-medicine/platelet-and-vascular-disorders-part-2/

ADAMTS13

A **D**isintegrin **A**nd **M**etalloprotease with a **T**hrombo**S**pondin type 1 motif, member 13)

- ADAMTS13 seems to be more effective when VWF multimers are partially unfolded by high shear stress
- UL-VWF multimers bind platelet GPIb/IX or GPIIb/IIIa complexes much better than smaller VWF multimers
  - Spontaneous binding of these UL-VWF multimers to platelets form aggregates within arterial and capillary vasculature



HUS (Hemolytic Uremic Syndrome)

- Microangiopathic hemolytic anemia
- More common than TTP
- 90% of cases caused by *Shigella dysenteriae* serotypes or entrohemorrhagic *E. coli* OH serotypes (E. coli O157)
- Toxins enter the bloodstream and attach to renal glomerular capillary endothelial cells
  - Become damaged/swollen and release UL-VWF
- Can also be caused by certain drugs
- Cardinal signs of HUS:
  - Hemolytic anemia, renal failure, and thrombocytopenia
    - Thrombocytopenia more mild in comparison to TTP

DIC (Disseminated Intravascular Coagulation)

DIC (Disseminated intravascular coagulation)

- activation of coagulation cascade (many causes) resulting in a consumptive coagulopathy that entraps platelets in intravascular fibrin clots
- Simultaneous formation of thrombin and plasmin
- similar to TTP including MAHA and deposition of thrombi in arterial circulation of most organs
- DIC = red clots whereas TTP = white clots

Acute DIC

- Compensatory hemostatic mechanisms overwhelmed → severe consumptive coagulopathy → hemorrhage
- Severe thrombocytopenia with decreased FV, FVIII and fibrinogen
- D-dimer is positive

Chronic DIC

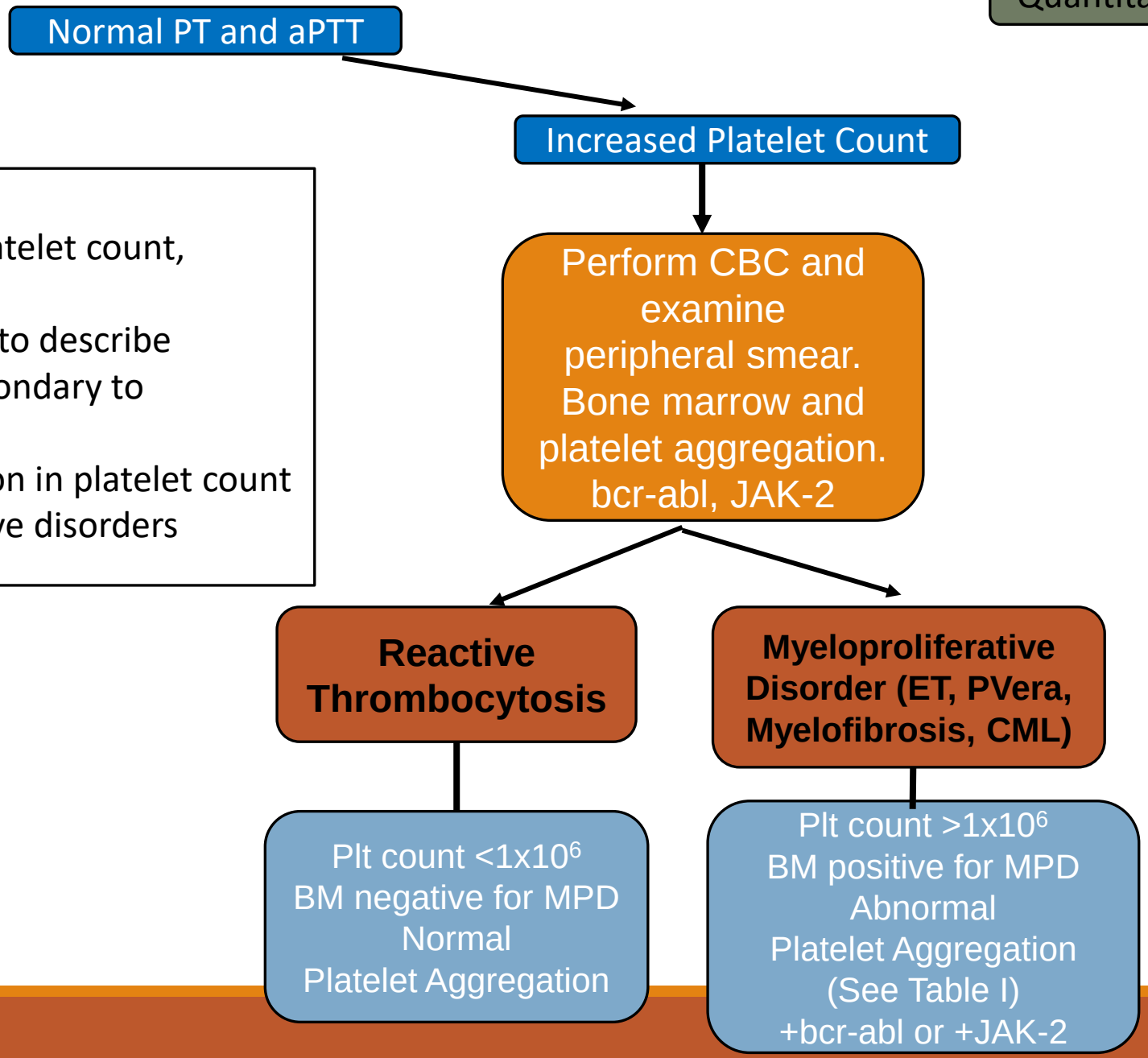
- Liver and bone marrow compensate with coagulation factors and platelets
- Clotting factors may be slightly reduced or normal and compensatory thrombocytopoiesis results in lower to normal platelet counts
- D-dimer not usually elevated but can be slightly increased

DIC

- Treatment
  - No specific treatments- supportive therapy
    - Plasma and platelet substitution therapy, anticoagulants, physiologic coagulation inhibitors, FFP (factors, fibrinogen, inhibitors, and platelets), possibly heparin to turn off coagulation (may require antithrombin to be effective)
  - Acute DIC
    - Therapies that slow the clotting process and therapies that replace missing platelets and coagulation factors
      - Plasma (coag factors and replaces blood volume), prothrombin complex concentrate, fibrinogen concentrate, factor VIII concentrate
  - Platelet transfusions necessary when thrombocytopenia is severe
  - Red cells administered to treat the resulting anemia

Thrombocytosis

- Defined as abnormally high platelet count, typically >450,000/uL
- Reactive thrombocytosis used to describe elevation of platelet count secondary to inflammation
- Marked and persistent elevation in platelet count is hallmark of myeloproliferative disorders



Reactive Thrombocytosis

- Platelet counts between 450,000 to 800,000/uL with no change in platelet function

  - Reactive Thrombocytosis Associated with Hemorrhage or Surgery
    - Platelet count can be low for 2-6 days but rebounds to slightly elevated levels
    - Normal levels by 10-16 days after the blood loss
  - Postsplenectomy Thrombocytosis
    - Platelet count can exceed 1 million/uL
    - Spleen normally sequesters 1/3 of circulating platelets at any given time
  - Thrombocytosis Associated with Iron Deficiency Anemia
    - Believed iron plays a role in regulating thrombopoiesis
    - Platelet count as high as 2 million have been seen in IDA with return to normal when iron treatment is administered
  - Thrombocytosis Associated with Inflammation and Disease
    - Similar to elevation in C-reactive protein, fibrinogen and VWF and other acute phase reactants
    - *Kawasaki disease*
      - Disorder caused by inflammation of the walls of small and medium-sized arteries throughout the body
  - Exercise-Induced Thrombocytosis
  - Rebound Thrombocytosis

Thrombocytosis Associated with Myeloproliferative Disorders

- Common finding in four chronic myeloproliferative disorders including:
  - Polycythemia Vera (PV)
  - Chronic myelogenous leukemia (CML)
  - Myelofibrosis with myeloid metaplasia (primary myelofibrosis)
  - Essential thrombocythemia (ET)
    - ET is a chronic myeloproliferative neoplasm
    - Most common cause of thrombocytosis when reactive thrombocytosis can be excluded
    - Platelets of 1 million/uL and proliferation of marrow megakaryocytes
    - Persistent elevation of platelet count is an absolute requirement for diagnosis
      - ET presents with hemorrhage, platelet dysfunction and thrombosis
      - Thrombosis in microvasculature or larger vasculature can occur
      - Lab Findings:
        - Platelet size is heterogeneous and platelets may be notable clumped on smears
        - Platelets may look agranular or hypogranular
        - Giant or misshapen platelets is common finding
        - Aggregation usually absent in response to EPI and ADP

Secondary Coagulation Disorders

Review: Primary Hemostasis

- Interaction of vasoconstriction and platelet adhesion/aggregation
- Refers to the role of blood vessels and platelets in formation of the platelet plug (white clot) in response to vascular injury
- Response either to blood vessel injury or commonplace desquamation of dying or damaged
- Endothelial cells - release of procoagulant substances

1. Blood vessel response -> will contract to seal wound or reduce blood flow
2. Platelets response -> activation of platelets, adherence of platelets to exposed collagen, and secretion of granular contents
3. Leads to aggregation with other platelets to form primary platelet plug

Review: Secondary Hemostasis

- Activation of plasma proteins to form a fibrin clot which reinforces the primary platelet plug – (red clot)
- Plasma transports at least 16 glycoproteins (mostly trypsin-like enzymes – serine proteases)
  - Serine proteases = inactive zymogens
  - Once activated, these zymogens for complexes activating other zymogens
  - Ex) Thrombin converts fibrinogen into fibrin monomer
    - **Thrombin** is key to secondary hemostasis
- Also present are a number of cofactors and control proteins
  - (Ca^{2+}, phospholipid, vit k., etc)

Clotting Factors: Nomenclature	
I*	Fibrinogen
Ia*	Fibrin
II*	Prothrombin
IIa*	Thrombin
III*	Thromboplastin
IV*	Calcium
V	Labile Factor; parahemophilia
VII	Proconvertin; stable factor
VIII	Anti-hemophilic factor (AHF); hemophilia A
IX	Christmas factor; hemophilia B
X	Stuart-Prower Factor
XI	Plasma Thromboplastin Antecendent (PTA); hemophilia C
XII	Hageman Factor
XIII	Fibrin Stabilizing Factor

* Indicates factors more commonly referred to as full name

Review: Secondary Hemostasis

Secondary hemostasis can be broken down into an intrinsic pathway and extrinsic pathway

- Intrinsic pathway – activation of factor XII culminating in fibrin polymerization
- Extrinsic pathway – tissue factor:VIIa formation proven to be in vivo initiation of coagulation
- Common pathway – both intrinsic and extrinsic include factor X, V, prothrombin and fibrinogen

- Complexes within Coagulation Cascade
  - Extrinsic Tenase = VIIa, TF, phospholipid and Ca^{2+} -> activates IX and X
  - Intrinsic Tenase = IXa, VIIIa, phospholipid and Ca^{2+} -> activates X
  - Prothrombinase = Xa, Va, phospholipid and Ca^{2+} -> activates prothrombin

- Control mechanisms are in place to control and inhibit coagulation (naturally occurring & artificially)

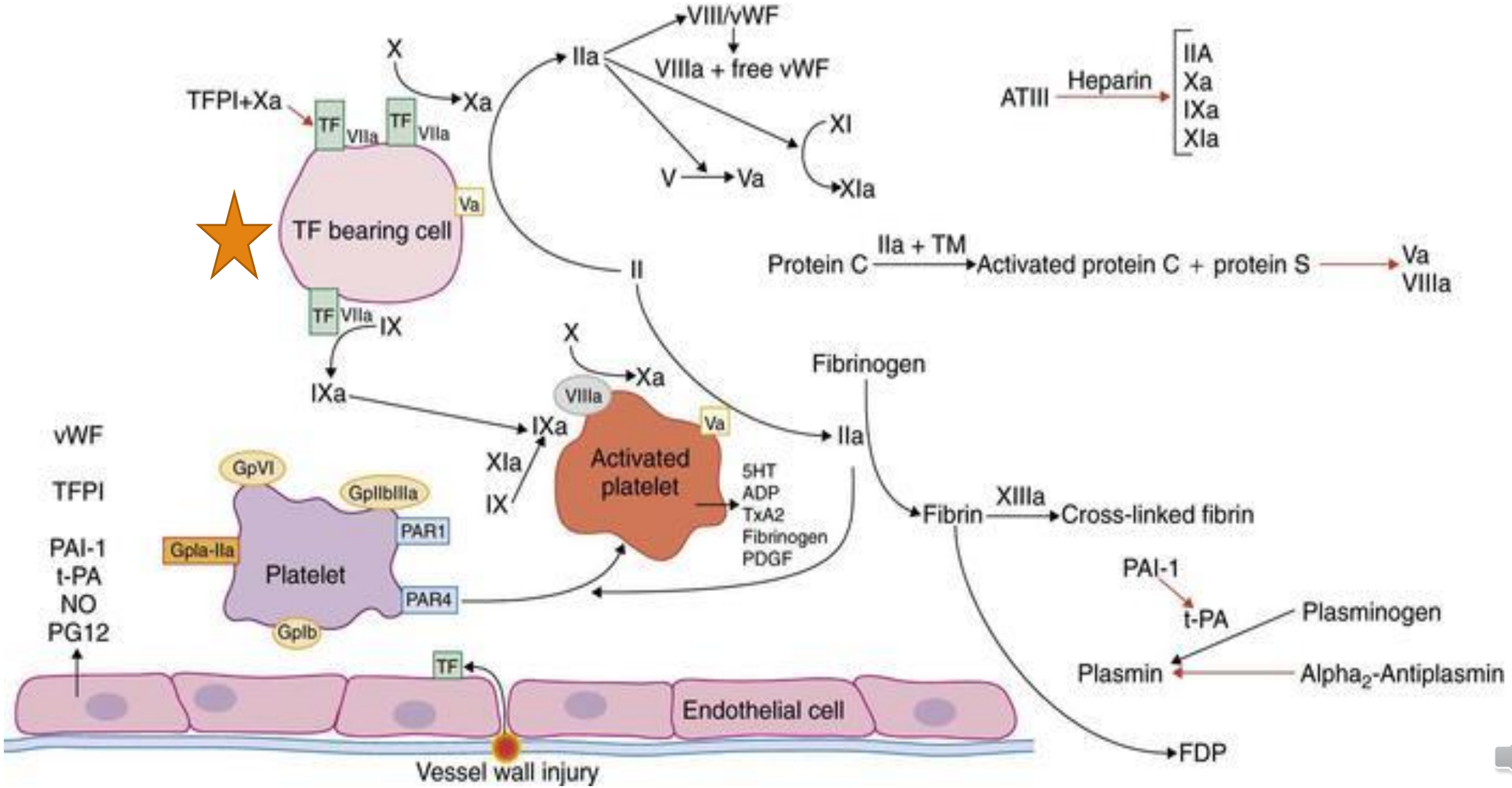


Image courtesy of Dr. Rogers, Hematology and Coagulation Staff Pathologist

Basic Coagulation Workup for Hemorrhagic Patient

- Platelet Count
- aPTT
- PT
- TT
- Fibrinogen

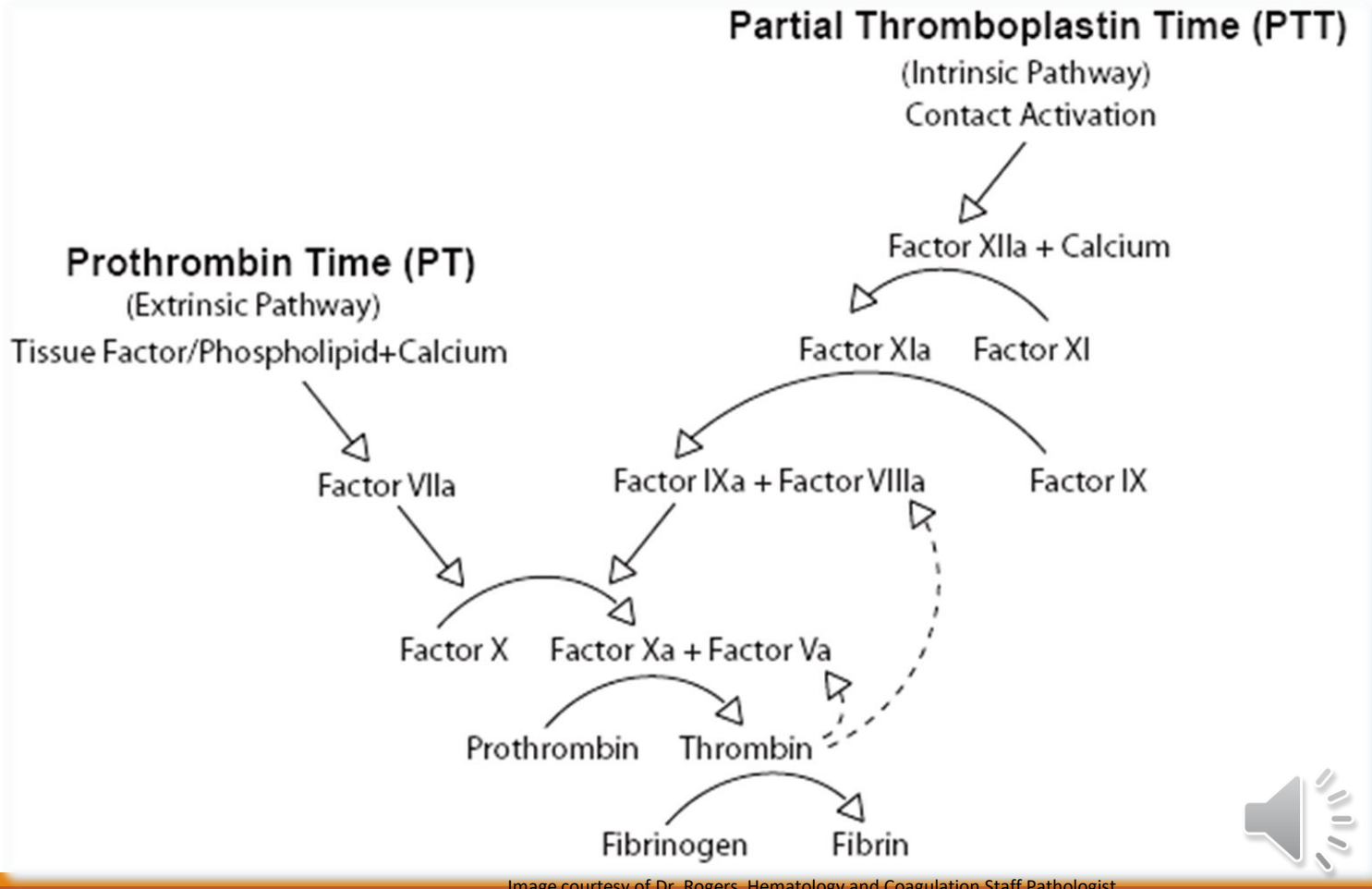
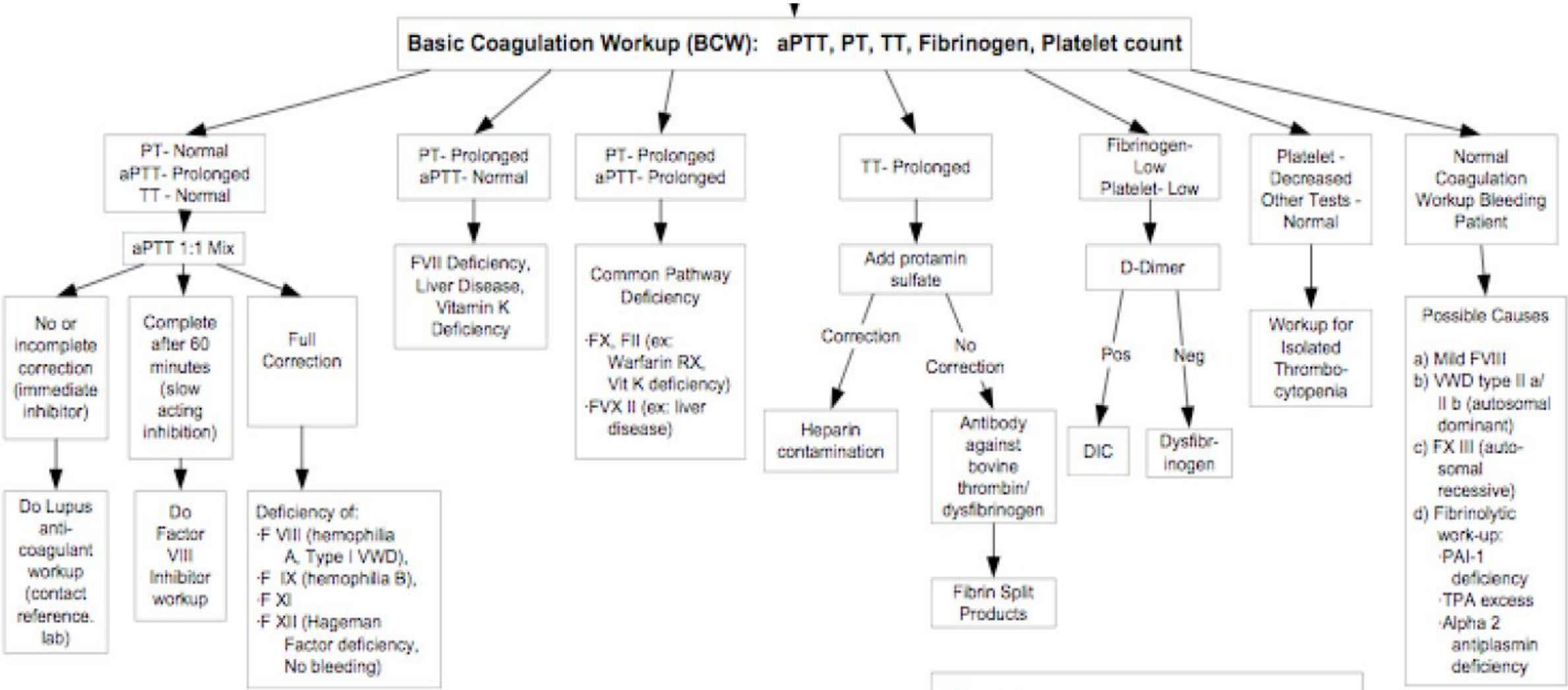


Image courtesy of Dr. Rogers, Hematology and Coagulation Staff Pathologist



NOTE: Bleeding time or platelet function assay maybe useful as an additional diagnostic tool for familial or acquired platelet disorders such as Von Hildebrand's disease or Ticlipod medication. In general, it is not a predictor of bleeding for surgical procedures.

REFERENCES: Work up extracted from literature and modified by University of Washington Department of Laboratory Medicine.

Abbreviations:
aPTT: Activated Partial Thromboplastin Time
DIC: Dessiminated Intravascular Coagulation
F: Factor
PAI: Plasminogen Activator Inhibitor
PT: Prothrombin Time
TPA: Tissue Plasminogen Activator
VWD: Von Willebrand's Disease

Algorithms courtesy of Dr. Andrew Schade MD, PhD

Hemorrhagic Coagulation Disorders

Hemorrhage is excessive bleeding that requires medical or physical intervention

- Bleeding can be *local* or *general*, *mucocutaneous* or *anatomic*, *acquired* or *congenital*
  - Localized: bleeding indicates injury, infection, tumor or an isolated blood vessel defect
  - Generalized: bleeding from multiple sites, spontaneous and recurring, or hemorrhage requiring physical intervention
  - Mucocutaneous: usually skin or at body orifices; purpura, petechiae, ecchymoses, menorrhagia, bleeding from gums, epistaxis; tends to be defects in primary hemostasis
  - Anatomic: in soft tissue, muscles, joints, or deep tissue; **acquired** or **congenital** defects in secondary hemostasis

To further determine cause of bleeding the physician orders a complete coagulation workup

Acquired Hemorrhagic Disorders

Trauma Induced Coagulopathy

Liver disease

Renal Failure with Hemorrhage

Vitamin K Deficiency

Acquired Anti-VIII inhibitor and Hemophilia

Acquired VWF

Trauma Induced Coagulopathy (TIC)

Coagulopathy is defined as any hemostasis deficiency (coagulation or platelet deficiency)

93,000 deaths a year are due to trauma of some sort

- 20,000 of initial survivors die within 48 hours from hemorrhage

ACOTS (Acute coagulopathy of trauma-shock) accounts for most fatal hemorrhages

- Triggered by acute inflammation, platelet activation, TF release, hypothermia, acidosis and hypoperfusion (poor blood distribution due to low blood pressure) all of which are elements of systemic shock

Systemic shock can lead to:

- Acute reduction of ADAMTS13
- TF release
- Coagulation factor activation
- Loss of coagulation control proteins
- Hyperfibrinolysis

TIC Management

Massive Transfusion

- Massive hemorrhage defined as:
  - Blood loss exceeding total blood volume within 24 hours
  - Loss of 50% of blood volume within a 3-hour period
  - Blood loss exceeding 150mL/min
  - Blood loss that necessitates plasma and platelet transfusion

Plasma donation

- BE mindful of TACO and TRALI

Platelet Concentration

- Administered usually only when platelet count fall bellows 50,000/uL

Concentrates

- Examples include: Activated prothrombin complex concentrate, cryoprecipitate and fibrinogen concentrates, and recombinant activated factor VII

Liver Disease Coagulopathy

Liver

Produces nearly all plasma coagulation factors and regulatory proteins

Causes of suppression of Liver production

- Hepatitis
- Cirrhosis
- Obstructive jaundice
- Disorder of bilirubin metabolism

Suppression of hepatocytes which will reduce concentration or function of plasma coagulation factors to less than hemostatic levels (<40 units/dL)

Affects production of vitamin K dependent factors (II, VII, IX, X, Protein C, S, Z)

- VII first to show decreased activity
  - ½ life= 6 hours

Liver

Declining factor V is specific marker of liver disease

- Differentiates vitamin K deficiency from liver disease (FV is not Vit. K Dependent)

Decrease of fibrinogen <100 mg/dL is a mark of liver failure

Dysfibrinogenemia is seen in moderate liver disease

- Prolonged PT and exceptionally prolonged reptilase time (RT)
- vWF, VIII, XIII can be normal or elevated
- Thrombocytopenia occurs in 1/3 of the cases of disease

Alcohol toxicity suppresses platelet production

DIC in Liver Disease

- Significant complication of liver disease
- Decreased production of AT, protein C, or protein S and release of procoagulants
- Liver does not clear these procoagulants
- Acute
  - PT, PTT, TT prolonged
  - Fibrinogen < 100 mg/dL
  - Increased FDPs
- Chronic and compensated
  - Abnormal D-dimer

Renal Failure and Coagulopathy

Chronic renal failure associations

- Platelet dysfunction
- Mucocutaneous bleeding
- Acute GI bleeding

Seen in the lab

- Decreased PLT adhesion/aggregation
- Decreased RBC mass and thrombocytopenia

Bleeding may be corrected with dialysis, EPO, RBC transfusion and Interleukin-11 therapy

Renal Failure Continued…

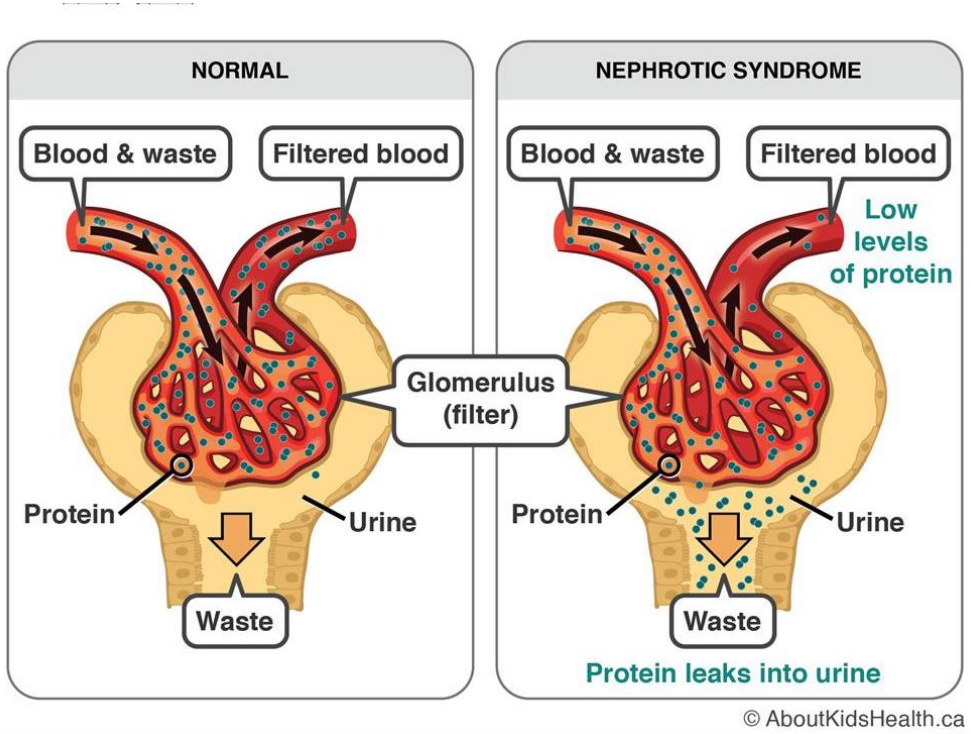
Hemostasis Activation Syndromes

- Deposits fibrin into renal microvasculature which reduces glomerular filtration
  - DIC
  - HUS
  - TTP
- Cause thrombocytopenia → bleeding
- PT and PTT are expected to be normal
- Platelet function assay may be prolonged

More Renal Failure…

Nephrotic Syndrome and Hemorrhage

- Increased glomerular permeability
- Associations
  - Amyloidosis
  - Diabetic glomerulosclerosis
  - SLE
  - Glomerulonephritis
  - Renal vein thrombosis
- LMW proteins and procoagulants found in urine
- Coagulation factors II, VII, IX, X, XII, antithrombin and protein C been found in the urine



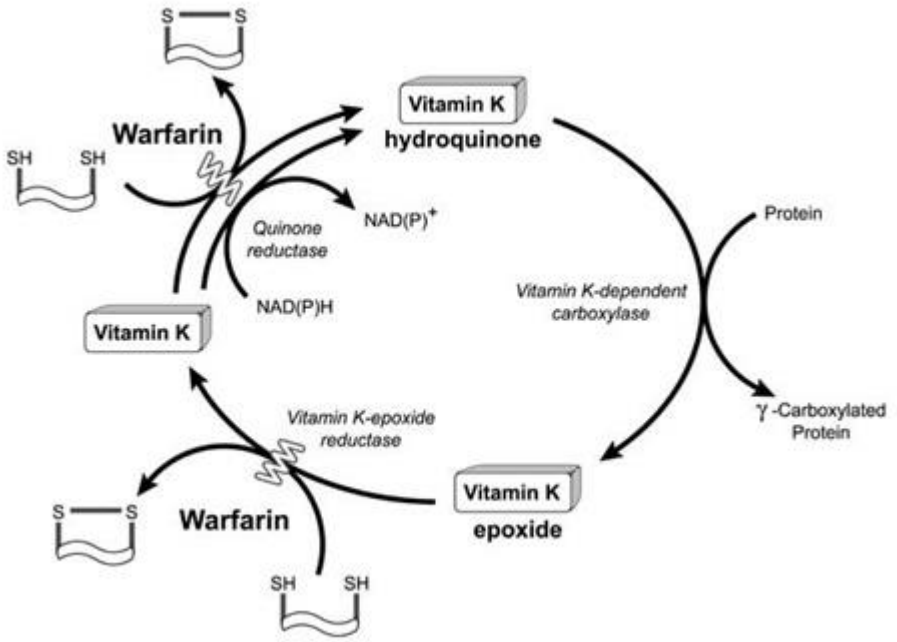
Vitamin K-Deficiency

γ-carboxylation cycle is interrupted

Causes
- Biliary duct obstruction
- Fat malabsorption
- Chronic diarrhea
- Broad spectrum antibiotics that disrupt gut flora

Hemorrhagic Disease of Newborn
- Breast feeding prolongs deficiency

Antagonists
- Coumadin (Warfarin)
- Disrupt vitamin K epoxide reductase and quinone reductase reactions → release of dysfunctional *des-gamma-carboxyl prothrombin* (VII, IX, X, C, S)
  - These inactive forms are called PIVKA factors
    - *Proteins Induced by Vit. K Antagonists*



https://step1.medbullets.com/hematology/114068/vitamin-k-deficiency

Lab Findings
- Prolonged PT
- PTT can be normal or prolonged
- Mixing study yields normal results
- Decreased factor VII (followed by IX, X and II)

Other Acquired Hemorrhagic Disorders to be Discussed Further Elsewhere

Acquired Anti-VIII Inhibitor and Hemophilia

- Anti-VIII is most common acquired autoantibody
  - Highest risk when > 60 years of age or women 2-5 months pregnant
- Lab findings in acquired hemophilia
  - Prolonged PTT w/likely normal PT, TT
  - Mixing study
    - Corrects on initial
    - Can be prolonged with incubation at 37 C
      - IgG isotype (time and temp dependent)
- Type I Kinetics: Linear in-vitro neutralization over 1-2 hours
  - Complete inactivation
- Type II Kinetics: Early rapid loss with residual activity
  - Intermediate equilibrium
- Quantified by Bethesda titer
- Treated with DDVAP or rFVIIIa

Acquired VWD

- Manifests w/moderate to severe mucocutaneous bleeding and no family history of bleeding
- Associations
  - Hypothyroidism
  - Lymphoproliferative or myeloproliferative disorders
  - Wilms tumor (nephroblastoma)
  - Congenital heart disease
  - HUS
  - Pesticide exposure
- Prolonged PTT is severe (↓VWF and FVIII)
- Diminished ristocetin cofactor/VWF activity/VWF antigen

Congenital Hemorrhagic Disorders

Von Willebrand Disease (discussed previously: Platelet Disorders)

Hemophilia A

Hemophilia B

Hemophilia C

Other Congential Single-Factor Deficiencies

Hemophilia A (Factor VIII Deficiency)

a.k.a classic hemophilia

Congenital single factor deficiency marked by anatomic soft tissue bleeding

85% of all hemophiliacs

Factor VIII deficiency
- Factor VIII deteriorates ~5% per hour at RT in vitro

X chromosome abnormality

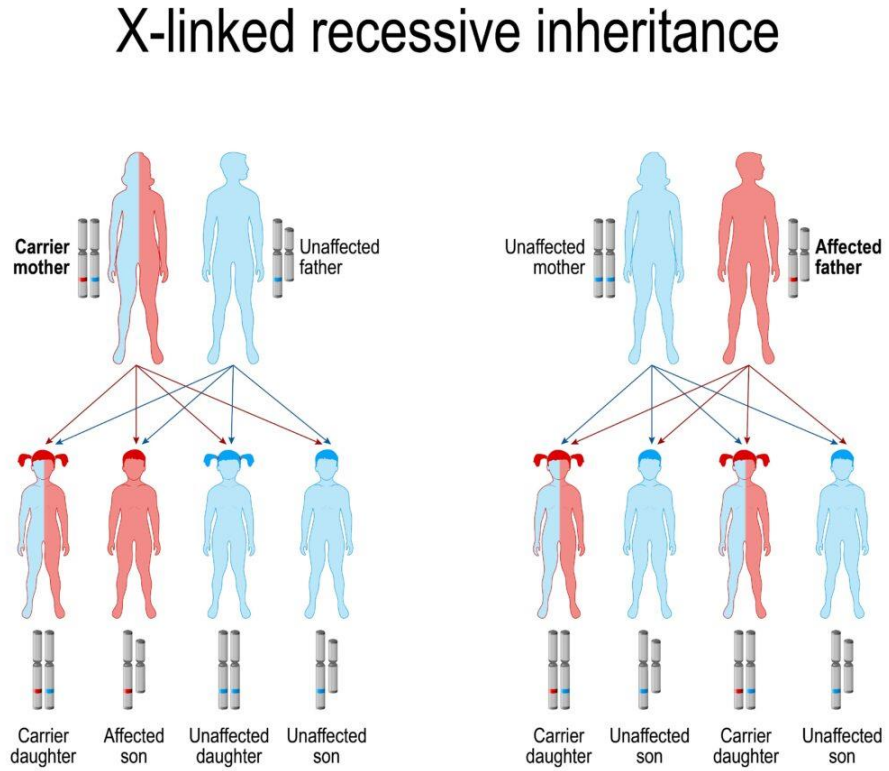
Male hemizygotes experience anatomic bleeding
- Females are carriers

All sons of hemophiliac men are normal if non-carrier mom
- Daughters are carriers

30% arise from spontaneous germline mutations (no family history)

Rare symptomatic females
- True homozygosity or double heterozygosity
- Extreme lyonization
  - Disproportional inactivation of X chromosome with normal gene

Factor VIII inhibitor in about ~30% of severe hemophilia cases (3% in moderate cases)



https://haemophilia.org.uk/bleeding-disorders/haemophilia-a-and-b/what-causes-haemophilia/

Hemophilia A (factor VIII Deficiency)

Clinical manifestations

- Deep muscle and joint hemorrhage
- Hematomas
- Wound oozing
- Bleeding into CNS, GI, kidneys
- Inflammation with chronic joint bleeds
- Cranial bleeds → neurological symptoms

Severity is *inversely* proportional to factor VIII activity

- <1% activity: severe
- 1-5%: moderate
- 5-40%: mild
  - hemorrhage follows significant trauma

70% cases treated before 1984 were HIV (+) or died from AIDS

Laboratory Findings in Hemophilia A

Lab Findings

- Prolonged PTT
- 90% of female carriers are detected using the ratio of factor VIII activity to vWF antigen (VWF unaffected by ↓ FVIII)
  - ratio below normal lower limit → carrier
- If FVIII level is >30%, no inhibitor is likely
- If FVIII level is <30%, mixing study is needed

Bethesda Assay (Nijmegen-Bethesda)

- If inhibitor is suggested
- Normal plasma providing 100 units/dL factor activity mixed at increasing dilutions (decreasing conc.) in a series of tubes with full-strength patient plasma
- The FVIII assays are performed and the results of the dilutions are expressed as titer (BU)

Treatment of Hemophilia A

Recombinant Factor VIII (rFVIIIa)

Desmopressin acetate (DDAVP)

Intravenous FVIII concentrates

Main goal: keep patients Factor VIII activity >75%

- Dependent on threat of bleeding
- May not need this high a level if risk of bleeding is lower

Hemophilia B (Factor IX Deficiency)

a.k.a. Christmas Disease

Approximately 14% of hemophiliacs

X-linked recessive

Laboratory Findings:

- PTT prolonged with a normal PT
- Factor IX assay ran even if PTT is normal range
- Factor IX inhibitor is approximately 3% of cases

Hemophilia C (Factor XI Deficiency)

a.k.a Rosenthal Syndrome

Autosomal dominant disorder with mild to moderate bleeding

Greater than 50% of cases in Ashkenazi Jews

Frequency and severity of bleeding episodes do not correlate with Factor XI levels

References

Rodak's Hematology 6th Edition

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