BLEEDING DISORDERS—LECTURE 1

Patient Evaluation

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OUTLINE:

- Patient Evaluation
- Medical history and physical examination
- Laboratory testing
    - Primary hemostasis (Platelets)
    - Secondary hemostasis (Coagulation factors)
    - Clot stability / Fibrinolysis
    - Whole blood clotting tests

OBJECTIVES:

- Know and be able to interpret the various tests available to assess platelet number and functionality
- Be able to interpret the results of PT and PTT tests and be able to identify specific factor deficiencies based on these tests
- Understand the results of mixing studies
- Know the use of the D-Dimer test

REFERENCE:

- The current Pathology text provides little information on Bleeding Disorders. In order to supplement information on this topic, extensive NOTES are provided with the study materials form this course

Bleeding Disorders

LECTURE 1

Overview

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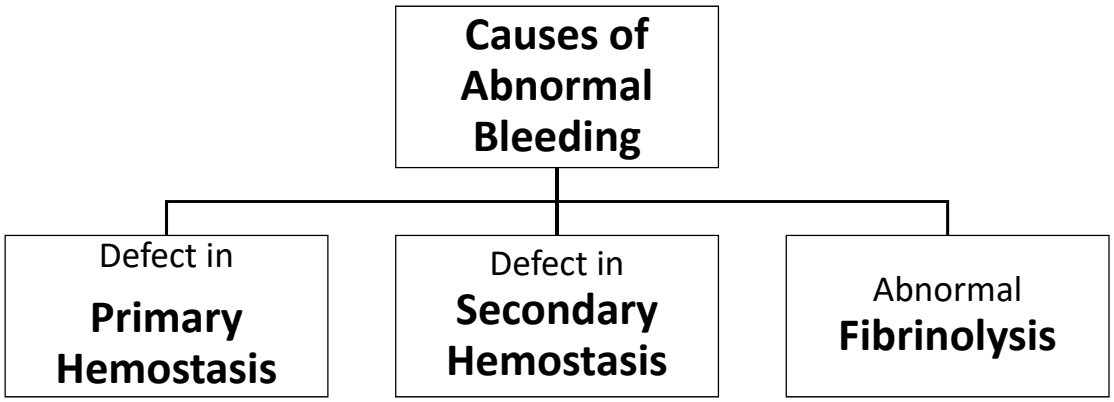
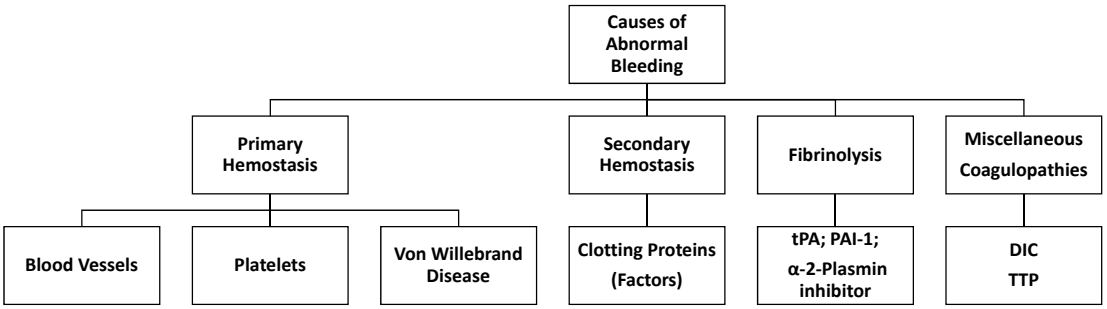
Bleeding Disorders Overview



Bleeding Disorders Overview



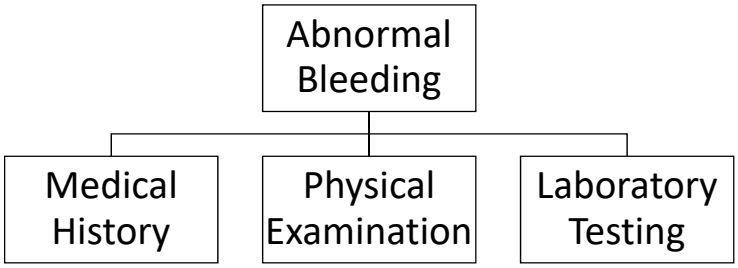
Outline of 4 lectures

- **Lecture 1:**
  - Patient Evaluation
  - Laboratory Testing
- **Lecture 2:**
  - Disorders of Primary Hemostasis
    - Vascular Disorders
    - Platelet Disorders
    - Von Willebrand Disease
- **Lecture 3:**
  - Disorders of Secondary Hemostasis
    - Coagulation Factor Deficiencies
    - Hemophilias
    - Inhibitors
  - Fibrinolysis
- **Lecture 4:**
  - Disseminated Intravascular Coagulation (DIC)
  - Thrombotic Thrombocytopenic Purpura (TTP)
  - Special Cases

Bleeding Disorders
Evaluation of Abnormal Bleeding

Evaluation of Abnormal Bleeding

- Evaluating patients for excessive bleeding occurs in many situations: a personal or family history of bleeding, excess bleeding after surgery, or an abnormal laboratory result.
- The evaluation of these patients has several parts:



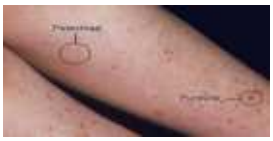
Evaluation of Abnormal Bleeding
MEDICAL HISTORY

Patient Bleeding History	• Extent of bleeding • Location of bleeding • Appearance • Age of onset
Patient Medical History	• Patient medications • Dental and surgical procedures • Liver or Kidney disease
Family History	• Family members with abnormal bleeding • If so, males, females or both • History of consanguinity

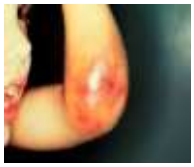
- The medical history can elicit information on:
  - The cause of bleeding
  - Whether bleeding diathesis is inherited or acquired
  - Whether medications, or herbal supplements, could be involved
  - And even if the source of bleeding is related to platelet or coagulation factor abnormalities, as demonstrated in the next slide

Evaluation of Abnormal Bleeding
PHYSICAL EXAMINATION

- This initial patient examination often allows the clinician to begin to categorize the cause of a bleeding disorder, for example:
  - **Defect in Primary hemostasis** is generally due to abnormalities in **platelet count or functionality** and present with:
    - Mucocutaneous bleeding (gums)
    - Superficial bruising
    - Petechiae
    - ↓ Platelet count



  - **Defect in Secondary hemostasis** is usually due to deficiencies or inhibitors of one of more **coagulation factors** and often present with:
    - Deep, soft tissue hematomas
    - Extensive bruising
    - Hemarthroses
    - ↑ PT and/or PTT



Evaluation of Abnormal Bleeding

- At the conclusion of the office visit—the history and examination of the patient, the clinician should:
  - Have some confidence that a bleeding abnormality actually exists, and whether this abnormality is
    - Acquired or inherited and if inherited whether it is sex-linked or autosomally inherited
    - Possibly caused by medication or dietary issues
    - Caused by abnormalities in primary or secondary hemostasis
- The next step in the evaluation of the patient is laboratory testing which often begins with a routine panel of screening tests:
  - CBC, PT, PTT, Platelet Count (sometimes Fibrinogen or Thrombin Time)
- These screening tests can then be supplemented with more specific or specialized tests to arrive at a diagnosis

Bleeding Disorders

Which Laboratory Tests Will Be Discussed?

- **Primary Hemostasis (Platelets):**
  - Platelet count and morphology
  - Bleeding time
  - Aggregometry
  - Verify Now
  - Tests for von Willebrand Disease
- **Secondary Hemostasis (Coag. Factors):**
  - Prothrombin Time (PT)
  - Partial Thromboplastin Time (PTT)
  - Mixing Studies
  - Thrombin Time (TT)
  - Factor Assays
  - Fibrinogen
- **Clot Stability / Fibrinolysis:**
  - D-Dimer
  - Tissue Plasminogen Activator
  - Plasminogen Activator Inhibitor (PAI-1)
  - Factor XIII
- **Whole Blood Clotting Tests:**
  - Viscoelastometry

Primary Hemostasis (Platelet Assays)

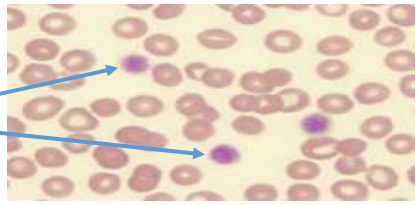
- Tests of Primary Hemostasis (Platelets)
  - Platelet Count
  - Platelet Morphology
  - Bleeding Time
  - Platelet Aggregation
  - "Verify Now"
  - Tests for von Willebrand Disease
    - vWD Antigen
    - Ristocetin Cofactor
    - Multimer Analysis

Primary Hemostasis: Platelet Tests

- Platelet Count
  - Platelet count is usually between 150,000 -400,000/µL
  - Platelet counts below 150,000/µL can be adequate for hemostasis
- Platelet Morphology
  - Mean platelet volume (MPV) is usually between 7-11 fL
  - An elevated MPV may suggest increased peripheral destruction of platelets (e.g. ITP) since platelets newly released from the bone marrow tend to be larger. A decreased MPV can be seen in marrow failure
  - Certain syndromes--such as Bernard-Soulier Syndrome--are associated with large (or giant) platelets

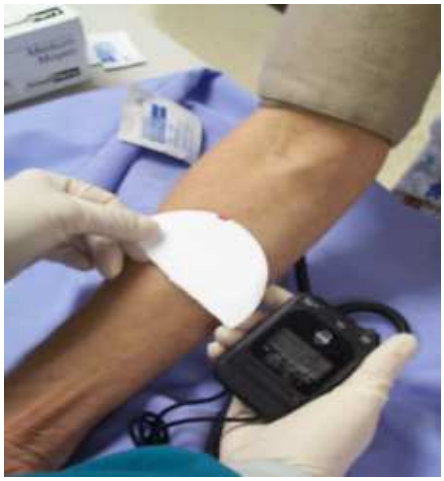
- Normal platelets in a blood smear:

- Giant platelets of Bernard-Soulier:



Primary Hemostasis: Platelet Tests

- **Bleeding Time** (Ivy Bleeding Time)**:**
  - A template guided incision is made in the forearm of the patient
  - The time for bleeding to stop is measured and is supposed to correlate with platelet count and/or function
  - Normal bleeding time is ~9-11 minutes
  - The bleeding time test is subject to considerable variability and is poorly reproducable—***and hence is rarely used in clinical practice*** *(MUSC has discontinued the use of this test)*
  - Platelet counts less than 100,000/μL generally are associated with prolonged bleeding times as do syndromes in which platelets are dysfunctional
  - NOTE: *a slightly prolonged bleeding time does not necessarily indicate a significant risk of spontaneous bleeding*



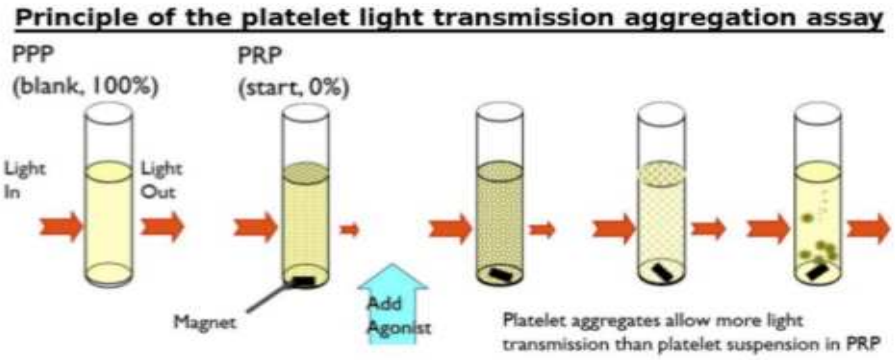
Primary Hemostasis

Platelet Tests-Light Transmission Aggregometry

- This test measures the ability of certain platelet agonists (e.g. collagen, epinephrine, ristocetin, ADP) to cause platelets to aggregate
  - *[Agonist—a chemical that binds to a receptor to produce a biological response]*
- The test is performed by making a platelet suspension known as Platelet-Rich Plasma (PRP) which is turbid due to the large number of platelets in suspension. The turbidity of the suspension blocks the passage of light
- If a particular agonist causes platelets to aggregate, the clumps of platelets fall out of suspension and the suspension clears allowing more light to pass through
- The platelets from various diseases respond differently to the various agonists as demonstrated in the next slide:

Primary Hemostasis
Platelet Tests-Light Transmission Aggregometry

- Diagram of Light Transmission Aggregometry:



Primary Hemostasis:
Platelet Tests LTA (Continued)

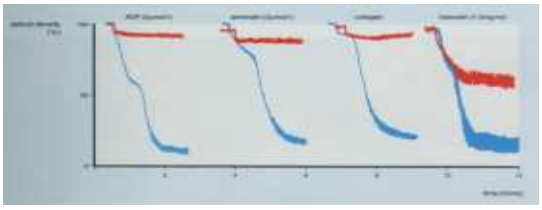
- Light Transmission Aggregometry:
  - Certain agonists are commonly used in aggregometry and the differing responses to these agonists is suggestive of various diseases; see the example below of how LTA helps to identify two platelet functional disorders (Glanzmann and Bernard-Soulier):

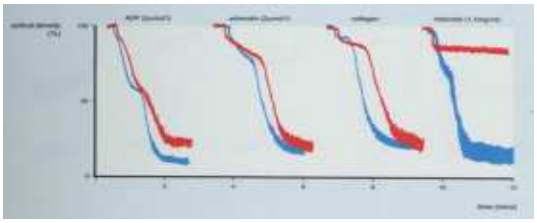
Disease	Agonists			
	ADP	**Collagen**	**Epinephrine**	**Ristocetin (1.0 mg/mL)**
Glanzmann	No aggregation	No aggregation	No aggregation	Normal Aggregation
Bernard-Soulier	Normal aggregation	Normal aggregation	Normal Aggregation	No Aggregation

Primary Hemostasis: Platelet Tests (LTA (Continued)

- The data in the previous chart would actually look like this:
- **Glanzmann's Thrombasthenia:**
  - No aggregation with all agonists except ristocetin
- **Bernard-Soulier:**
  - No aggregation with ristocetin; other agonist result in normal aggregation

(Blue: normal control; Red: patient)

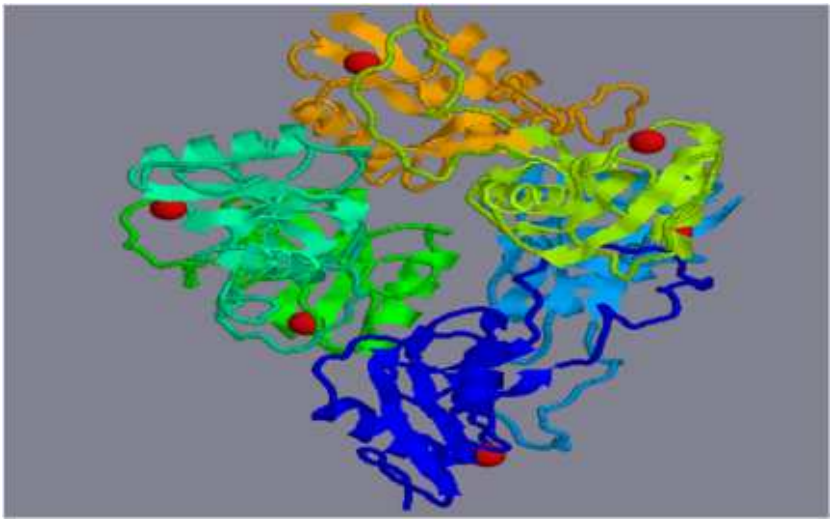




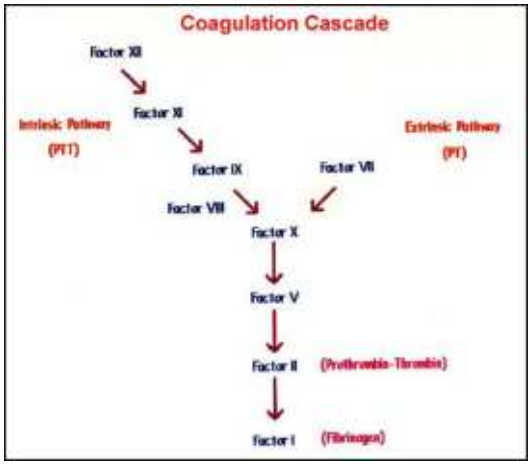
Primary Hemostasis Von Willebrand Factor Tests

- Von Willebrand Tests
  - There are several variants of von Willebrand Disease
  - The tests listed below are used to confirm a diagnosis of von Willebrand Disease as well identify the particular variant:
    - **Von Willebrand Factor Antigen**—this assay measures the total amount of von Willebrand Factor antigen whether functional or not
    - **Ristocetin Cofactor**—this assay measure the amount of "functional" von Willebrand Factor
    - **Multimer Analysis**—von Willebrand Factor is a large multimeric protein. In certain variants of vWD, some of the subunits are missing. This electrophoresis assay can be used to help classify the type of vWD
  - The use of these tests will be considered when von Willebrand Disease is discussed

Secondary Hemostasis: Coagulation Factor tests



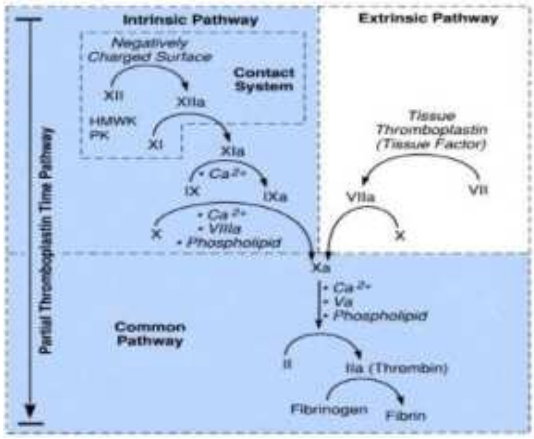
Coagulation Factor Tests



- Notes About the Coagulation Cascade:
  1. This is an "artificial" construction that allows interpretation of certain coagulation tests (PT; aPTT); this diagram does *NOT* reflect the actual mechanism of action in the body
  2. An abnormally prolonged PT or aPTT suggests that there is either a **deficiency** or an **inhibitor** of one or more coagulation factors
     - **Deficiency**—inadequate amount of functional coagulation factor
     - **Inhibitor**—there is an IgG antibody present that blocks the action of a coagulation factor
- More about deficiencies and Inhibitors later!!

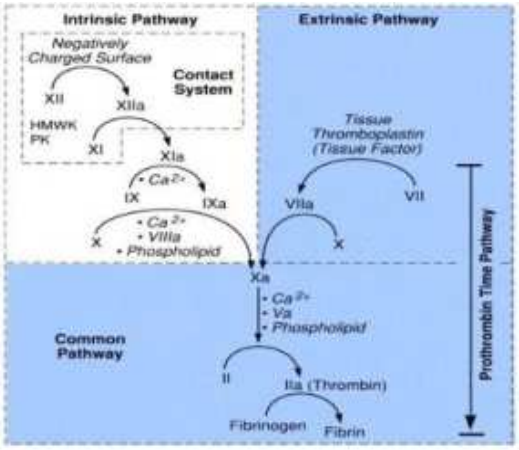
Coagulation Factor Tests
Activated Partial Thromboplastin Time (aPTT)

- The aPTT is a test of the ***intrinsic*** and ***common*** coagulation pathways **(IN BLUE)**
- Normal aPTT = 23–36 seconds
- Clinical Utility:
  - Detect a *deficiency of (or inhibitor to)* Factors **XII, XI, IX VIII, X, V, II, Prekallikrein, HMWK, and Fibrinogen** (*aPTT is usually prolonged if factors <30% of normal*)
    - ***NOTE:*** *aPTT measures* ***all*** *coagulation factors* ***except*** *VII and XIII)*
  - Monitoring heparin therapy
  - May be prolonged in some cases of von Willebrand Disease
  - Useful in detection of lupus anticoagulant
  - **NOTE**: Prekalikrein, HMWK, and Factor XII are all measured by the PTT—but none of these result in a risk of bleeding



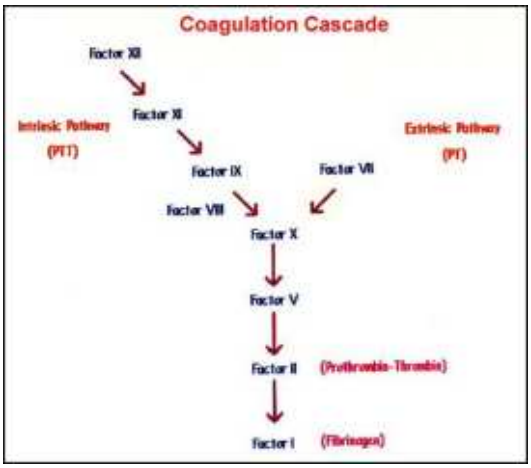
Coagulation Factor Tests:
Prothrombin Time (PT)

- The PT is a general test of the ***extrinsic*** *and* ***common*** coagulation pathways **(IN BLUE)**
- Normal PT = 12.2 – 14.2 seconds
- Clinical Utility:
  - Detect a *deficiency of (or an inhibitor to)* Factors **VII, X, V, II (prothrombin/thrombin), Fibrinogen.** *PT is prolonged when Factor VII, V, II or X is less than 40% of normal—or fibrinogen is < 100 mg/dL*
    - NOTE: ***Even though both the aPTT and PT assess factors in the common pathway, the PT is more sensitive to common pathway deficiencies—thus, mild deficiencies of X, V, II, and fibrinogen may have prolonged PT but normal aPTT***
  - Monitoring oral anticoagulant (warfarin) therapy



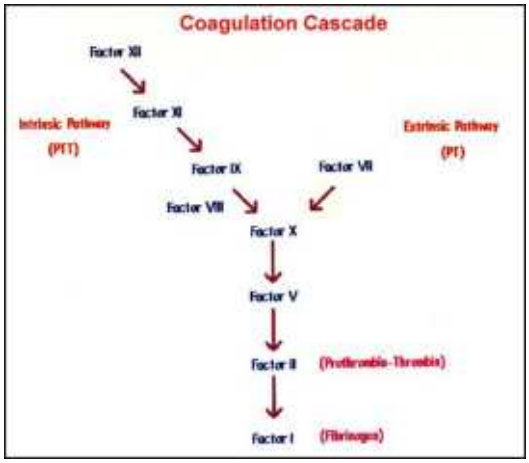
Coagulation Factor Tests
Combining the PT and PTT Results

- Patients will be tested for both PT and PTT:
  - If the PTT is prolonged, that suggests that there is a deficiency in factors XII, XI, IX, VIII, X, V, II, I.
  - *BUT*, the normal PT says that factors X, V, II, I are normal
  - *SO*, combining the PT and PTT results, only **XII, XI, IX, VIII** could be causing the PTT to be prolonged in the setting of a normal PT



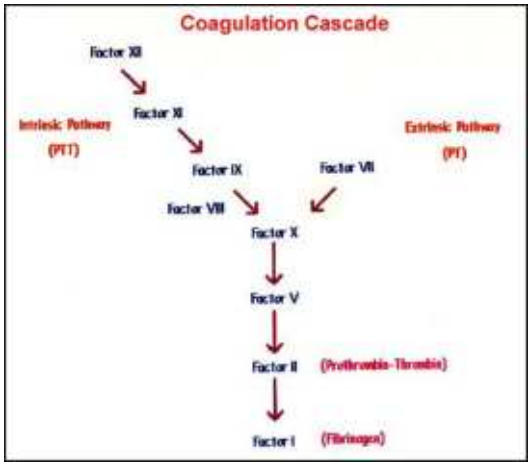
Coagulation Factor Tests
Combining the PT and PTT Results

- A prolonged PT but a normal PTT initially looks pretty simple:
  - The prolonged PT suggests that factors VII, X, V, II, or I might be deficient.
  - *BUT*, the normal PTT says that X, V, II, and I are normal
  - *AND*, that only leaves factor **VII** to be the cause of the prolonged PT
- Here's the exception: a **mild** deficiency of X, V, II, or I might be detected by the PT (more sensitive) but be missed by the PTT
- This means that a **mild** deficiency of X, V, II, or I might yield a prolong PT but a normal PTT



Coagulation Factor Tests
Combining the PT and PTT Results

- When BOTH the PTT and PT are prolonged the two possibilities are:
  - Multiple factor deficiencies (e.g. severe liver disease or ***severe*** Vitamin K deficiency; a ***mild*** Vitamin K deficiency might cause only a prolonged PT but a normal PTT)
    - *Note: Vitamin K is required for the production of Factors II, VII, IX, and X)*
  - A significant deficiency of X, V, II, or I



Coagulation Factor Tests
Prothrombin Time versus the INR

- In the 1980's it was recognized that the ***reactivity*** of different PT reagents (i.e. *thromboplastins*) varied and this caused the monitoring of warfarin therapy to be difficult--particularly from one lab to another
- The **International Normalized Ratio (INR)** was developed to minimize laboratory to laboratory variation in PT results.
- NOTE: PT_{test} *: patient's PT in sec.*
  PT_{normal} *: mean PT from a group of normals*
  ISI: value indicating the reagent sensitivity; supplied by manufacturer

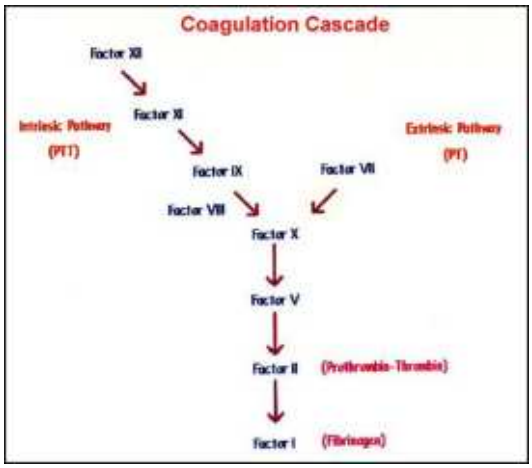
$$INR = \left(\frac{PT_{test}}{PT_{normal}} \right)^{ISI}$$

The INR is a calculated way of standardizing the PT results among laboratories using different PT reagents

PTT and PT---A Little Practice

Patient	PTT / PT Results	Factor Abnormality
1	PTT: 30 sec. PT: 19.3 sec.	??
2	PTT: 42 sec PT: 13.1 sec.	??
3	PTT: 47 sec. PT: 18.9 sec	??

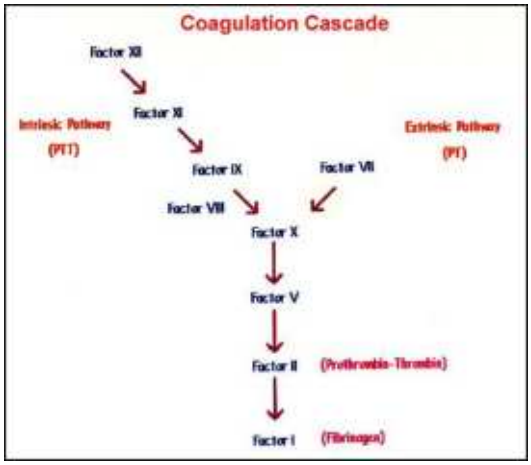
Normal Ranges:
PTT: 23-36 sec.
PT: 12.2-14.2 sec.



PTT and PT---A Little Practice

Patient	PTT / PT Results	Factor Abnormality
1	PTT: 30 sec. PT: 19.3 sec.	VII Maybe mild X, V, II, I
2	PTT: 42 sec PT: 13.1 sec.	XII, XI, IX, VIII
3	PTT: 47 sec. PT: 18.9 sec	X, V, II, I Or multiple factors

Normal Ranges:
PTT: 23-36 sec.
PT: 12.2-14.2 sec.

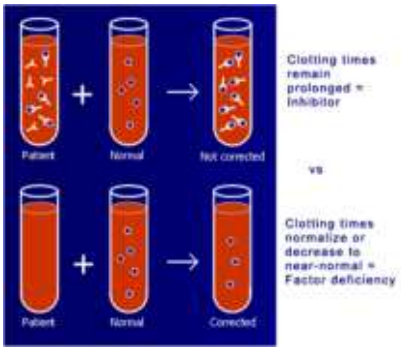


Prolonged PT or aPTT

- A prolonged PT or aPTT can be due to a **deficiency** of a coagulation factor or an **inhibitor** of a specific coagulation factor (which are usually IgG antibodies)
- Which of these alternatives is causing the abnormal PT or aPTT is determined by performing a **MIXING STUDY.**

Mixing Studies—Deficiency or Inhibitor?

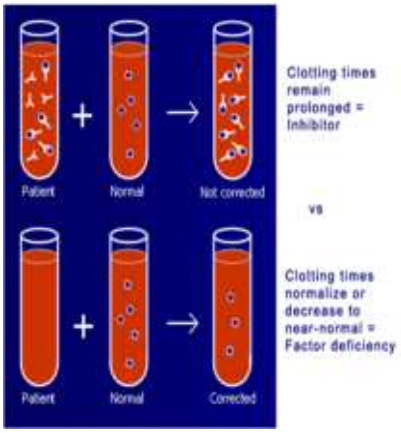
- ***Mixing studies*** **are used in the evaluation of a prolonged PT or aPTT to distinguish between a factor deficiency and the presence of an inhibitor:**
  - Since only 30-40% of normal factor level gives normal PT or aPTT, a 1:1 mix of **deficient** plasma with **normal plasma** should yield normal coagulation result
  - **Inhibitors** are typically present in excess and will inhibit coagulation factors in added normal plasma
  - Some inhibitors are time-dependent (e.g. lupus inhibitors and factor VIII inhibitors) and cause inhibition only after 30-60 minutes of incubation ***(mixing studies should be assessed immediately and after incubation)***



Mixing Studies—Deficiency or Inhibitor

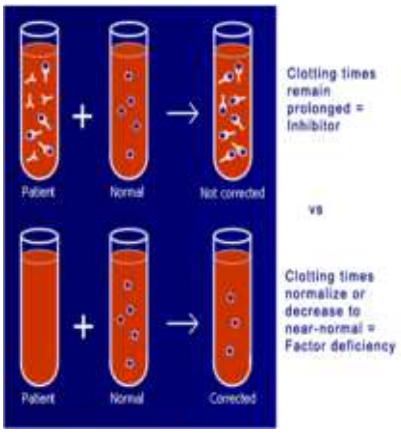
Patient	PTT or PT Pre-Mix	PTT or PT Post-Mix	Deficiency or Inhibitor
1	PTT: 42 sec	PTT: 41 sec.	??
2	PT: 19.2 sec.	13.9 sec.	??

Normal PT: 12.2-14.2 sec.
Normal PTT: 23-36 sec.



Mixing Studies—Deficiency or Inhibitor

Patient	PTT or PT Pre-Mix	PTT or PT Post-Mix	Deficiency or Inhibitor
1	PTT: 42 sec	PTT: 41 sec.	Inhibitor (does not correct)
2	PT: 19.2 sec.	13.9 sec.	Deficiency (corrects)



Coagulation Factor Assays Deficiencies

- If the mixing study indicates that the prolonged PT or PTT is caused by a **factor deficiency**, it is necessary to determine which factor is involved.
  - These tests use modified PT or PTT assays in which a substrate is used that is deficient in the factor being measured.
  - If the patient's plasma replaces some proportion of the factor missing in the substrate, the actual percentage of the factor can be calculated using a standard curve

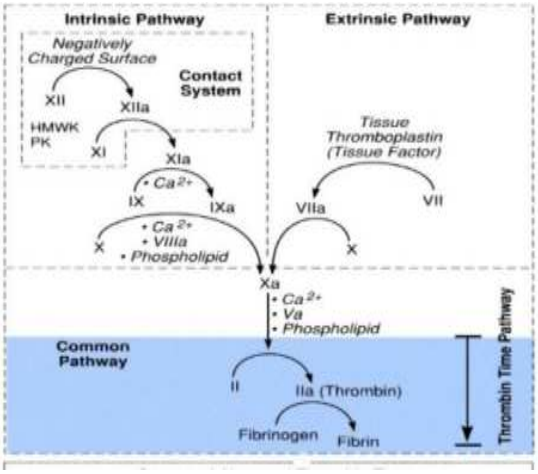
Coagulation Factor Assays Inhibitors

- When the mixing study indicates that an inhibitor is involved, again a modified a PT or PTT test is used, but in this case the ability of serial dilutions of patient plasma (supposedly containing and inhibitor) to prolong the PT or PTT of normal plasma is measured.
  - The strength, or intensity, of the inhibitor is recorded in Bethesda Units
  - 1 Bethesda Unit (BU)is the quantity of inhibitor that neutralizes 50% of the factor
    - A low titer is defined as 0.5 to 5.0 BU; a high titer inhibitor is over 5.0 BU

Coagulation Factor Tests: Thrombin Time

- The TT measures the time of fibrin clot formation after the addition of a standard concentration of thrombin to citrated plasma
- Causes of a **prolonged TT**:

Disorder / Substance	Mechanism of Action
Amyloidosis	Inhibits fibrin monomer polymerization
Direct Thrombin Inhibitors (Argatroban; Bivalirudin) Heparin (Unfractionated) (Low Molecular Weight)	Inhibit thrombin Inhibits thrombin *Low affinity for thrombin- NO EFFECT ON TT*
Fibrin Degradation Products	Inhibits fibrin monomer polymerization
Fibrinogen Abnormalities	Inhibits fibrin monomer polymerization



Coagulation Factor Tests: Fibrinogen Assays

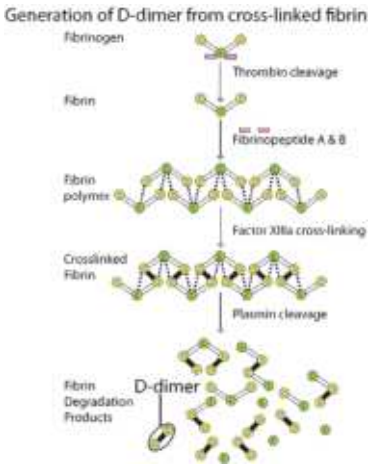
- Fibrinogen can be measured using several different laboratory methods; most commonly used is the "Clauss method" which measures rate of clot formation and hence assesses the amount of <u>functional</u> fibrinogen
  - An immunologic (ELISA) method can also be used to determine the amount of fibrinogen protein whether "functional" or not
- Normal fibrinogen level is typically 170 - 400 mg/dL
- Fibrinogen levels > 100mg/dL are usually adequate for normal coagulation
- Clinical Utility
  - Diagnosis of dysfibrinogenemia or hypofibrinoginemia
  - Diagnosis of DIC, liver failure, or primary fibrinolysis
  - Guiding transfusion therapy with cryoprecipitate (*which is a source of fibrinogen for transfusion*)

Fibrinolysis Testing

- Fibrinolysis is important in the maintenance of blood flow by ensuring that clot formation is localized to the site of vascular injury and the clot is dissolved once the injury is healed
- The fibrinolytic system is regulated by a number of pro- and anti-fibrinolytic enzymes
- Increased or abnormal fibrinolysis (hyperfibrinolysis) can result in excessive bleeding
  - Anytime a patient with a normal PT and aPTT is experiencing non-surgical bleeding, hyperfibrinolysis should be considered.
- There are multiple assays that can be used to assess the fibrinolytic system, for example:
  - PAI-1 activity and antigen *(Plasminogen Activator Inhibitor)*
  - Plasminogen activity and antigen
  - tPA activity and antigen *(Tissue Plasminogen Activator)*
  - D-Dimer assay
- The most commonly used assay is the ***D-Dimer Assay***:
  - The degradation of fibrin by plasmin results in a variety of different fragments ranging from large pieces of fibrin to small fragments of a single fibrin molecule such as the D-Dimer
  - The D-Dimer consists of 2 fibrin "D" domains cross-linked by Factor XIIIa

Fibrinolysis Testing: D-Dimer

- Under usual conditions, very little fibrin is removed by the fibrinolytic system—most of the fibrin in a clot is removed by leukocytes and fibroblasts during the healing process
- When fibrin is broken down by plasmin, multiple different sized fragments are released—fibrin degradation products (FDPs)
- For a D-Dimer to appear, 3 things have to happen:
  - Formation of polymerized fibrin
  - Cross-linking of fibrin by Factor XIII
  - Degradation of the cross-linked fibrin by plasmin



Fibrinolysis Testing: D-Dimer

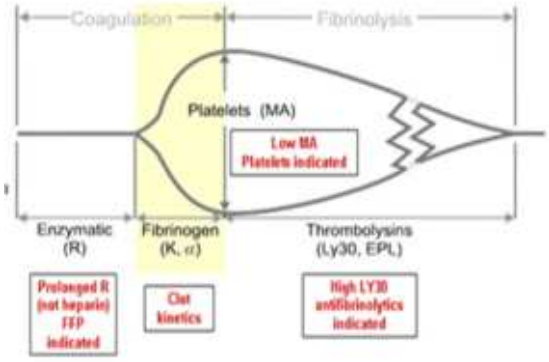
- There are several tests that can be used to detect the presence of the D-Dimer.
- Most commonly, a latex agglutination test is used in which antibodies to D-Dimer are fixed to latex particles which clump in the presence of the antigen (D-Dimer)
- Clinical Use:
  - The D-dimer test is used to diagnoses Disseminated Intravascular Coagulation (DIC)
  - Modifications of this test can also be used to aid in the diagnosis of venous thromboembolism

Whole Blood Clotting Tests

- So far we have been discussing, tests of platelets, tests of coagulation factors, tests of fibrinolysis—but in reality all of these separate parts of the clotting system work together rather than independently.
- More recently techniques have been developed to provide us a more comprehensive view of a patient's coagulation status.
- While initially developed in 1948, the technique has been improved and become more accepted as a way of assessing the coagulation status of patients:

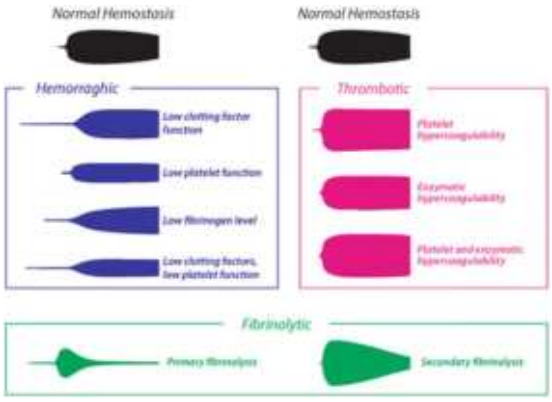
Whole Blood Clotting Tests

- Thromboelastography (TEG)
  - Two versions: **TEG** and **ROTEM** (MUSC uses TEG)
  - Measure clot formation in whole blood samples
  - While there are differences in the basic technologies of TEG and ROTEM, the output is similar yielding results within 10-15 minutes and allowing the clinician to make decisions on therapy in "near-real-time."



Whole Blood Clotting Tests

- Examples of Thromboelastograph Results
  - These TEG Tracings give you an abridged idea of the kinds of information that the clinical staff can obtain from TEG very promptly
  - This, in turn, allows decisions to be made on therapy very quickly



Whole Blood Clotting Tests

Practice And Review

Normal PT: 12.2-14.2 sec.
Normal PTT: 23-36 sec.

Patient A

	Results	Possible Coag Factor(s) Involved
PT	17.5 sec	
PTT	33.0 sec.	

Patient B

	Results	Possible Coag Factor(s) Involved
PT	12.9 sec.	
PTT	39.2 sec	

Patient C

	Results	Possible Coag Factor(s) Involved
PT	18.0 sec	
PTT	49.0 sec.	

Patient D

	Results	Possible Coag Factor(s) Involved
PT	12.6 sec.	
PTT	38.0 sec	

- Patient E:
  - A prolonged PTT in a male patient is thought to be due to a deficiency of either Factor VIII or Factor IX. How would you determine which of these 2 factors is causing the prolonged PTT? _____
- Patient F:
  - A patient presents with a prolonged PTT (41 sec.) as shown below. A mixing study is done. Do the result suggest a deficiency or inhibitor is causing the prolonged PTT?

	Pre-Mix PTT	Post-Mix PTT	Inhibitor or Deficiency
PTT	43 sec.	37 sec.	

ANSWERS:

Patient A: VII (Possible mild deficiency of X, V, II, I)
Patient B: XII, XI, IX, VIII
Patient C: XII, XI, IX, VIII, X, V, II, I, VII
Patient D: XII, XI, IX, VIII
Patient E: Specific factor assays of Factors VIII and IX
Patient F: PTT "corrects" suggesting a DEFICIENCY is causing the prolonged PTT