BLEEDING DISORDERS—LECTURE 3

Secondary Hemostasis

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OUTLINE:

- Secondary hemostasis
  - ↑PT; Normal PTT
  - Normal PT; ↑PTT
    - Hemophilia A, B, C
    - Inhibitors
  - ↑PT; ↑PTT
- Fibrinolysis

OBJECTIVES:

- Be able to identify specific coagulation factor deficiencies (or inhibitors) based on PT and PTT results
- Be familiar with the clinical features of hemophilia A, B, and C
- Be familiar with the inhibitors of coagulation factors: alloantibodies, autoantibodies, and "lupus" inhibitors
- Be aware of the clinical effects of fibrinolytic bleeding disorders

REFERENCES:

- The current pathology textbook has limited information on bleeding disorders, therefore, extensive notes have been provided to supplement student information on this topic.

Bleeding Disorders
LECTURE 3
Secondary Hemostasis

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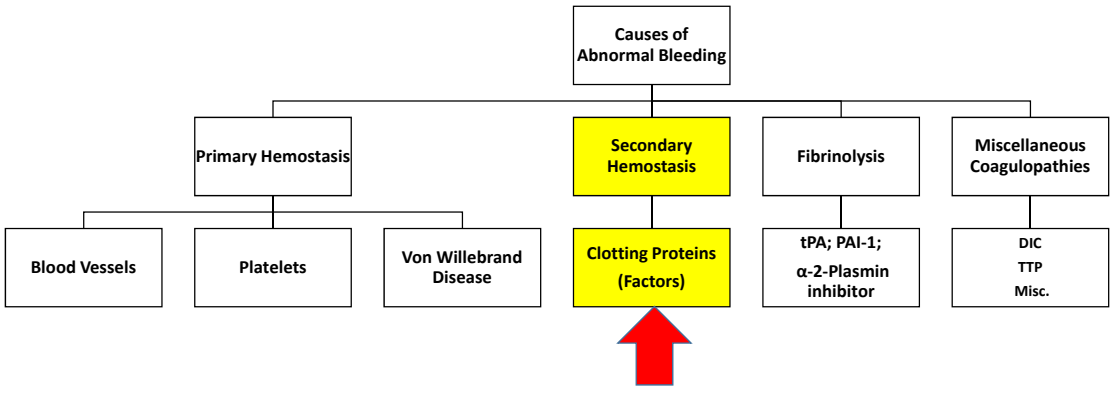
Bleeding Disorders



Primary to Secondary Hemostasis

- Primary hemostasis, discussed in the previous lecture, is the result of both vasoconstriction and, most importantly, the formation of a "platelet plug"
  - This "platelet plug" is formed rapidly, but is ***fragile***
- Secondary hemostasis essentially involves reinforcing the "platelet plug" with a fibrin mesh which provides a more stable and long-term solution to bleeding
  - Fibrin is formed as a result of the action of a series of serine proteases or coagulation factors ultimately resulting in the formation of fibrin and stabilization of the clot
  - This lecture will focus on deficiencies and inhibitions of these coagulation factors that can lead to bleeding.

Tests of Secondary Hemostasis

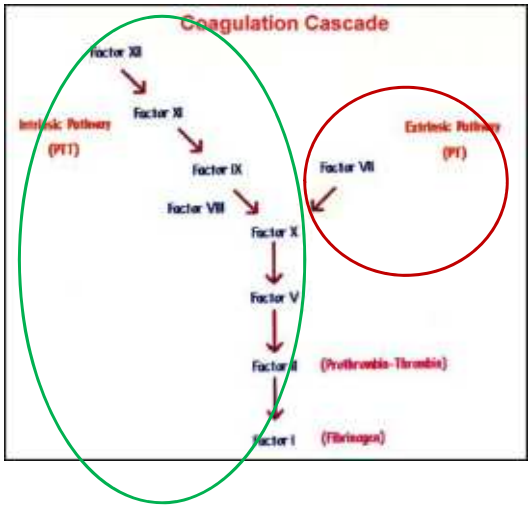
- As you recall from our first lecture, The PT (Prothrombin Time) the Partial Thromboplastin Time (PTT) are basic tests that are generally used to identify deficiencies (or inhibitors) of coagulation factors that might be associated with abnormal bleeding in patients.
- In addition, more specialized tests such as mixing studies and specific coagulation factor assays are also often employed to specifically identify the cause of abnormal PT and / or PTT test results

Secondary Hemostasis

- If we only consider the results of both the PT and PTT, there are 4 possible outcomes:
  - PT ↑; PTT normal
  - PT normal; PTT ↑
  - PT ↑; PTT ↑
  - PT normal; PTT normal
- In the next few slides, we will identify which coagulation factors could be causing the abnormal test results—and spend some time discussing some specific diseases / syndromes characteristically associated with each of these categories of test results

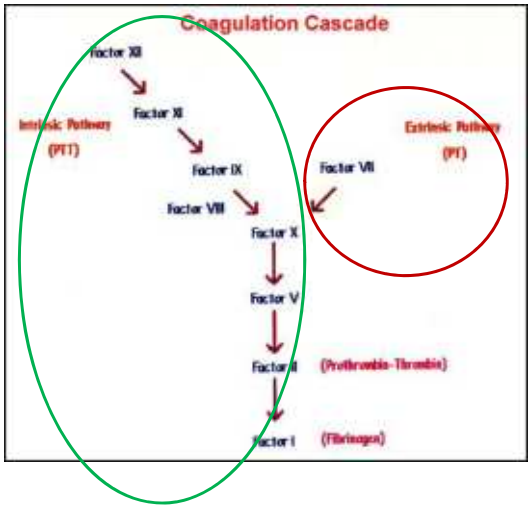
↑ PT; Normal PTT

- With this pattern of testing:
  - **Normal PTT**: (within the green oval) suggests that the coagulation factors in both the Intrinsic and Common pathways are normal (*XII, XI, IX, VIII, X, V, II, I*)
  - **↑PT:** suggests that factor VII is abnormal (*even though the PT measures the common pathway factors, the PTT says they are normal* ***EXCEPT…***)
  - PT is more sensitive than PTT, so a **mild** deficiency/inhibitor of X, V, II, or I could also cause a ↑PT with a "normal" PTT
- Differential Diagnosis???



↑ PT; Normal PTT

- Differential Diagnosis:
  - VII (deficiency or inhibitor)
  - Mild deficiency or inhibitor:
    - Common Pathway (X, V, II, I)—that would be detected by the more sensitive PT and missed by the PTT)
- Also it will be necessary to determine if the prolonged PT is due to a **deficiency** of an implicated coagulation factor or an **inhibitor** which will be assessed using a **mixing study** as described in the first lecture.
- Deficiencies (or less commonly, inhibitors) of these coagulation factors are seen in several clinically recognized situations…



↑ PT; Normal PTT
Clinical Conditions (Differential Diagnosis)

- Differential Diagnosis:
  - **Mixing Study Corrects** (suggesting a ***deficiency***)
    - Inherited Factor VII deficiency
      - Very rare; autosomal recessive
    - Inherited mild deficiency of II, V, X, I
      - All very rare
    - Liver disease
      - Since the coagulation factors are produced in the liver, mild to moderate liver disease causes a prolonged PT due to decreased synthesis of coagulation factors; more severe liver disease may cause both the PT and PTT to be prolonged
    - Vitamin K deficiency
      - Vitamin K is a required cofactor in the synthesis of factors II, VII, IX, and X. A deficiency of vitamin K due to poor dietary intake or malabsorption can result in a prolonged PT (and in more severe cases , a prolonged PT and PTT
    - Warfarin
      - Warfarin is a vitamin K antagonist. Usually, warfarin causes a prolonged PT but in more severe cases of Warfarin overdose can cause a prolonged PT and PTT
  - **Mixing Study does NOT Correct** (suggesting an ***inhibitor***)
    - Inhibitors of Factor VII (or II, V, X) are extremely rare

Case Study 1

- A 55 year-old man with a history of alcohol abuse and malnutrition presented with rectal bleeding
  - Laboratory values:
    - Hgb: 11.0 g/dL 14-18
    - PT: 18 sec 11-13
    - PTT: 30 sec 27-33
    - Mix: Corrects ?
  - Differential Diagnosis: ?

- Diagnosis:

?

Case Study 1 (*Continued*)

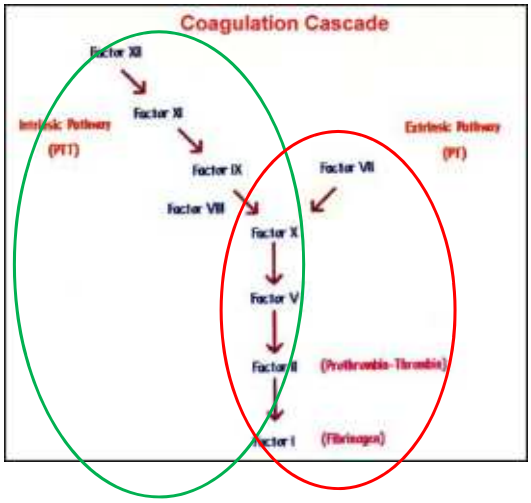
- A 55 year-old man with a history of alcohol abuse and malnutrition presented with rectal bleeding
  - Laboratory values:
    - Hgb: 11.0 g/dL 14-18
    - PT: 18 sec 11-13
    - PTT: 30 sec 27-33
    - Mix: Corrects ?
  - Differential Diagnosis:
    - Liver disease
    - Vitamin K deficiency
    - Warfarin

- Answer:
  - Due to his malnutrition, the patient was given 4 mg of Vitamin K subcutaneously
  - 4 hours later the PT was repeated and the PT was found to be within the normal range

- Vitamin K deficiency

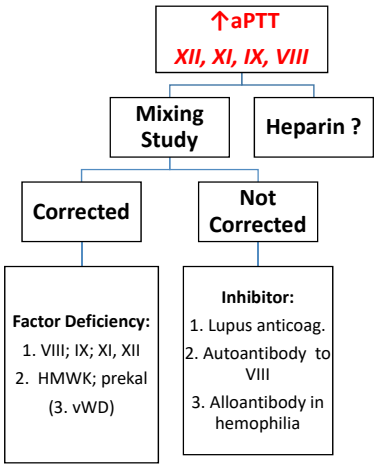
PT Normal; aPTT ↑

- Abnormal aPTT:
  - XII, XI, IX, VIII, X, V, II, Fibrinogen
- Normal PT ***excludes:***
  - VII, X, V, II, Fibrinogen (I)
- Leaving only 4 coagulation factors that could be abnormal in this case:
  - **XII, XI, IX, VIII**
- **But, are we dealing with an inhibitor or a deficiency of one of these factors???**
- Mixing Study will help distinguish—but before we do a mixing study we should ask one more question:
  - **IS THIS PATIENT ON HEPARIN?????**



PT Normal; aPTT↑

- Heparin causes an isolated prolongation of the aPTT
  - Since so many hospitalized patients are on heparin (IV lines and other sites of venous access often contain heparin) this source of error must be considered whenever there is an isolated ↑aPTT)
- If the patient is NOT on heparin we can proceed to the mixing study:
  - Corrected: **DEFICIENCY** of VIII, IX, XI, XII (*HMWK, Prekallekrein*), or vWD (*remember why????*)
  - Not Corrected: **INHIBITOR** most commonly lupus inhibitor, autoantibody to VIII, or alloantibody to VIII or IX in hemophilia patients being treated
  - *Prekallekrein and HMWK can affect the PTT, but deficiencies are NOT thought to cause a risk of bleeding*



PT: Normal; aPTT ↑

- **Factor deficiencies** as a cause of a prolonged PTT (but normal PT):
  - Three things to consider:
    1. XII, Prekallekrein, and HMWK deficiencies do ***NOT*** cause bleeding even though the aPTT may be extremely prolonged
    2. VIII and IX are relatively common deficiencies ***IN MALES*** (1:10,000 to 1:25,000 males)
       (we will talk about this in more detail in the next few slides)
    3. XI deficiencies (or inhibitors) are extremely rare (~1:1,000,000 general population)
- **Factor inhibitors as a cause of a prolonged PTT (but normal PT):**
  - Three inhibitors most frequently seen in patients with prolonged PTT:
    - Lupus inhibitor
    - Autoantibody to Factor VIII
    - Alloantibody in patients treated for hemophilia A or B

PT Normal; ↑PTT
Clinical Conditions (Differential Diagnosis)

- Deficiencies:
  - Hemophilia A (Factor VIII deficiency)
  - Hemophilia B (Factor IX deficiency)
  - Hemophilia C (Factor XI deficiency)
  - (Von Willebrand Disease)
  - Heparin
- Inhibitors:
  - Lupus inhibitor
  - Alloantibody to Factor VIII or IX
  - Autoantibody to Factor VIII

Hemophilia A

- **Hemophilia A** is caused by an inherited deficiency of Factor VIII
  - Typical Symptoms (severe disease):
    - Easy bruisability
    - Hemarthroses (*bleeding into joint spaces*)
    - Soft tissue hematomas
    - GI / GU bleeding
    - Poor wound healing
    - Intracranial bleeding (*esp. post-traumatic*)
    - (*Petechiae-usually **absent**!*)

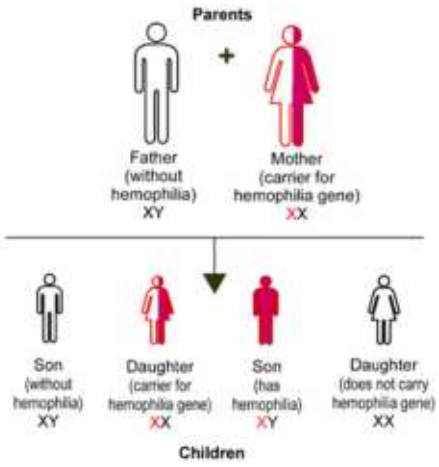
Hemophilia A

- The severity of bleeding is directly related to the level of Factor VIII in the patient as noted in the adjacent table
- Female carriers normally have Factor VIII levels approximately 50% of normal and generally do not need treatment

Severity	Factor VIII (%)	Spontaneous Hemorrhage	Traumatic Hemorrhage	aPTT
Very mild	>20	Never	Rare	Slight ↑ or Normal
Mild	5-20	Rare	Common	↑
Moderate	2-5	Unusual	Always	↑↑
Severe	<2	Common	Always	↑↑↑

Hemophilia A

- Hemophilia A
  - Factor VIII deficiency
  - Inherited as a sex-linked recessive trait (affecting boys and men)
  - Spontaneous mutations in up to 30% of cases
  - The disease occurs at a rate of 1:10,000 males



Hemophilia A

- Treatment
  - Mild to moderate disease may respond to DDAVP (stimulates release of Factor VIII and vWF)
  - Factor VIII concentrates
    - Manufactured using recombinant technologies
    - Monitor therapy with Factor VIII assay
  - Female carriers typically have Factor VIII levels of ~50% and rarely need treatment

Hemophilia B and Hemophilia C

- **Hemophilia B**
  - Deficiency of Factor IX
  - "Christmas disease"
  - Pattern of inheritance (sex-linked recessive), symptoms of severe disease are *the same as Hemophilia A*
  - Hemophilia B will have a prolonged aPTT
  - Frequency:
    - 1:25,000 males
  - Treatment:
    - Factor IX concentrates
      - Manufactured by recombinant technology
- **Hemophilia C**
  - Deficiency of Factor XI
  - Autosomal recessive inheritance
  - Bleeding symptoms are variable; may be milder than expected based on factor levels but occasionally more severe than expected
  - Frequency:
    - Rare; 1:1,000,000 (high prevalence in Ashkenazi Jews; ~13%)
  - Treatment:
    - There is no commercial concentrate of factor XI
    - Fresh frozen plasma is treatment of choice

Acquired Inhibitors of Coagulation

- Coagulation Factor inhibitors have been mentioned previously and we perform mixing studies to distinguish between factor deficiencies and the presence of factor inhibitors.
- What are coagulation factor inhibitors?
  - Two categories:
    - Inhibitors to single coagulation factors
      - Usually IgG antibodies (IgG4)
      - **Autoantibodies** for normal Factor VIII and von Willebrand Factor (vWF) are the most common and give rise to acquired hemophilia A or acquired von Willebrand Disease
      - **Alloantibodies** to Factor VIII or Factor IX can develop in patients with hemophilia A or hemophilia B being treated with commercial concentrates of Factor VIII or Factor IX
    - Antiphospholipid antibodies
      - This group of **autoantibodies** include anti-cardiolipin antibodies and lupus anticoagulant cause a prolonged aPTT, but are only rarely associated with bleeding but can be associated with thromboembolic events and miscarriage

Inhibitors to Single Coagulation Factors:
Antibodies to Factor VIII (or other factors)

- **Alloantibody** (*patients who have Hemophilia A or B and who are being treated with commercial factor VIII or factor IX may develop antibodies to the infused factor concentrate!!*)
  - Among patients with severe hemophilia A or B who are treated with infused coagulation factor concentrates (VIII or IX), 10-15% will develop alloantibodies to the infused coagulation factors
- **Autoantibody** (***patients who DO NOT have inherited hemophilia A or B***)
  - Very rarely (~0.5 per 1,000,000 people) spontaneously develop autoantibodies to specific coagulation factors with autoantibodies to factor VIII being the most common but *autoantibodies to vWF also occur*):
    - 50% of these have no recognizable underlying illness
    - 10-15% are associated with pregnancy (most often, first pregnancy and within 2-3 months post-partum)
    - Remainder are associated with underlying autoimmune disease, malignancy, or allergy (often to medication

Alloantibodies to Factors VIII or IX

- Clinical scenario:
  - A patient is found to have hemophilia A or B
  - The mixing study corrects indicating a factor deficiency
  - Patient is treated with the appropriate Factor concentrate

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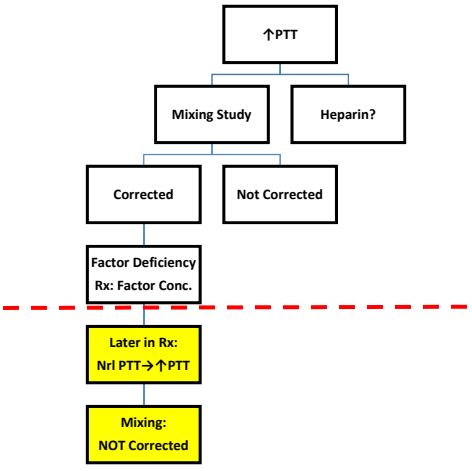
  - At some time after the initiation of therapy, the patient becomes resistant to the factor concentrate (↑aPTT)
  - A Mixing study done at this time will **NOT correct** suggesting the presence of an inhibitor (an alloantibody) to the factor concentrate
  - RX: Different manufactured concentrate; Factor VIIa; immunosuppression is ineffective

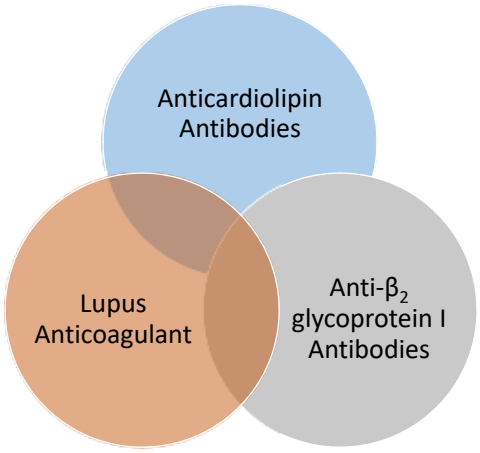


Autoantibody to Factor VIII

- Often referred to as "acquired hemophilia"
- Autoantibodies to most factors have been identified, but autoantibodies to Factor VIII is ***by far*** the most common
  - Autoantibodies to factor VIII are rare but occur more commonly in the elderly. 50% of cases have no apparent predisposing while 30-40% occur in patients with other autoimmune diseases
  - Autoantibodies also occur in occasional patients who are pregnant usually within 2-3 months postpartum
- Risk of hemorrhage can be severe
- Treatment:
  - Patients can be treated with Factor VIII concentrates
  - But:
    - May respond to immunosuppression
    - Of the cases associated with pregnancy, ¾ will resolve spontaneously and may require only transient corticosteroid treatment

Lupus Anticoagulant (LA)

- Lupus Anticoagulants are confusing:
  - While LAs can be seen in patients with SLE, they are seen in many other situations
  - LAs are NOT associated with increased risk of bleeding (in spite of ↑aPTT); they are associated with venous thrombosis and pregnancy complications
- Most LAs are antiphospholipid antibodies and the thrombotic complications are associated with the antiphospholipid antibody syndrome (APLA)
  - There are several "categories" of APLAs, but our focus will be the Lupus Anticoagulant



Lupus Anticoagulants (LA)

- **What do you really need to know about LAs?**
  1. LAs are acquired IgG (usually) or IgM autoantibodies
  2. These antibodies cause an ↑aPTT, but there is not an increased risk of bleeding
     - Testing for LAs can be difficult since excess phospholipids in patient serum can neutralize testing reagents even in the presence of *clinically significant* LAs. Therefore, multiple tests might be necessary when a patient is suspected of having an LA (e.g. recurrent fetal loss; thrombosis in atypical patients)
     - Additional tests might include: ***dilute Russell's viper venom test*** or ***Textarin/Ecarin time***.
  3. LAs are associated with venous or arterial thromboses ***and*** increased risk of miscarriage before 10 weeks or unexplained fetal death after 10 weeks, etc.
  4. While LAs are common in SLE and other autoimmune conditions, most patients with LAs do ***NOT*** have an underlying autoimmune condition

Case Study 2

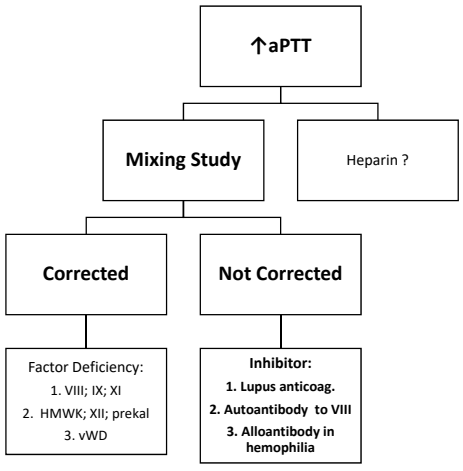
- Medical History:
  - A 7 year old boy is brought to the emergency room. He has a grossly enlarged and painful knee joint. On history, it is learned that several males in his family including his brother have had similar complaints. His sister is unaffected.
- Laboratory Testing:
  - PT: 13.5 sec *(12.2-14.2)*
  - aPTT: 47 sec. *(23-36)*
- What coagulation factors might be involved?
- Should a mixing study be done?

Case Study 2 (continued)

- What coagulation factors might be involved?
  - *XII, XI, IX, VIII*
- Should a mixing study be done?
  - *Yes*
  - *Mixing study "corrects" implying that the prolonged PTT is due to a deficiency*
- Since Factor VIII and IX deficiencies (and inhibitors) are relatively common in males (and Factor XII does not cause bleeding)----
  - Factor VIII Deficiency (Hemophilia A): 1:10,000 males
  - Factor IX Deficiency (Hemophilia B): 1:25,000 males
- Specific Factor Assays for VIII or IX will probably give us an answer:
- Current patient results:
  - Factor VIII = 5% (probably mild-moderate disease)
  - Factor IX = 93%
- Probable Diagnosis:
  - **HEMOPHILIA A**

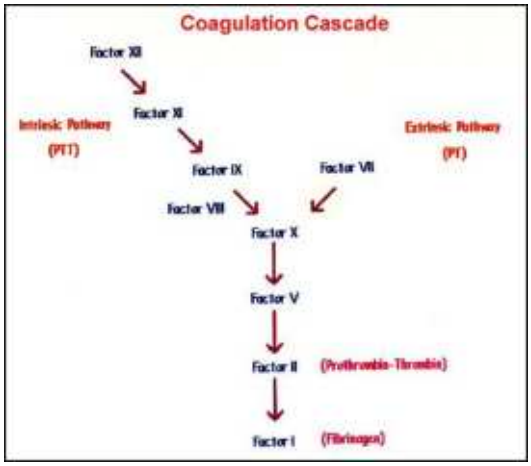
Case Study 3

- Patient History:
  - 23 year old woman with Hashimoto's thyroiditis is admitted to the hospital
  - Laboratory coagulation Studies:
    - PT: 12.5 sec. (12.2-14.2)
    - aPTT: 53.2 sec. (23-36)
    - Fibrinogen: 273 (150-400)
  - A mixing study was done which did not correct the aPTT:
    - aPTT (immediate): 47 sec.
    - aPTT (incubated): 45 sec.
  - Probable Diagnosis:
    - Prolonged aPTT probably due to the presence of an inhibitor (*Lupus anticoagulant or Autoantibody to Factor VIII*)



↑PT and ↑PTT

- What abnormality might cause a ↑PT ***and*** ↑aPTT?
  - **Factor Deficiencies:**
    - Deficiency of a **Common Pathway** factor: Factor I (fibrinogen), II, V, or X
    - Multiple factor deficiencies (Liver Disease; Vitamin K deficiency)
    - Dilutional Coagulopathy
      - Massive transfusion without plasma
  - **Inhibitors:**
    - II, V, X (*all very rare*)
    - Direct thrombin inhibitors (DTI)
      - *Argatroban; bivalirudin*
  - **Disseminated Intravascular Coagulation (DIC)**
    - In patients with a prolonged Thrombin Time (TT) or decreased fibrinogen
    - Positive D-Dimer Test
    - "Next lecture"



Normal PT; Normal PTT

Rarely, patients may present with a strong clinical history of bleeding yet have a normal PT and PTT:

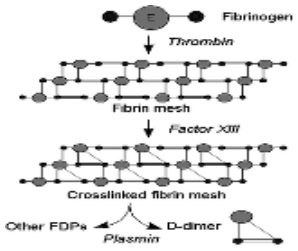
Previously we noted that patients with vWD (particularly Type 1) will have evidence of bleeding but a normal PT and PTT. However, platelet studies and specific tests for vWD will generally identify these patients

A second possibility for this pattern of testing would be patients who have a deficiency of Factor XIII which is not assessed by the PT or PTT

Factor XIII is activated by thrombin and in its active form crosslinks fibrin molecules to improve clot stability

Factor XIII deficiency is rare (~1:2,000,000) but severe if <3% of normal levels which can be associated with:

Umbilical site bleeding
Delayed bleeding from trauma
Intracranial bleeds
Pregnancy loss due to bleeding



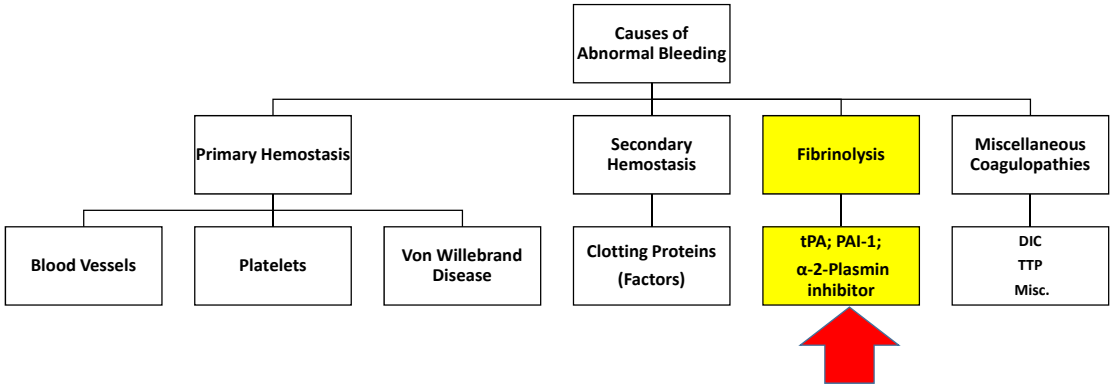
Fibrinogen Abnormalities

- **Hypofibrinogenemia**
  - Fibrinogen levels <150 mg/dL
  - Causes:
    - Severe liver disease—impaired fibrinogen production
    - DIC—increased consumption of fibrinogen by thrombin or plasmin
    - High volume fluid replacement—volume replacement without plasma or cryoprecipitate supplementation
  - Testing:
    - The most sensitive and specific test for hypofibrinogenemia is the Thrombin Time (TT)

Fibrinogen Abnormalities

- **Dysfibrinogenemia**
  - This is a blood clotting disorder caused by a structurally abnormal fibrinogen
  - Inherited as a mutation of the coding region of fibrinogen
    - Inherited dysfibrinogenemia is **very rare** (200-300 families have been reported)
  - There is also an acquired form of dysfibrinogenemia usually associated with severe liver or biliary tract disease which is also very rare
  - Clinical presentation:
    - Asymptomatic (55%)
    - Major bleeding (25%)
    - Thrombosis (20%)
  - The primary screening test for dysfibrinogenemia is the Thrombin Time (TT)
    - In addition, the patient generally has low fibrinogen activity compared to normal fibrinogen antigen level. There is also a prolonged “reptilase time”

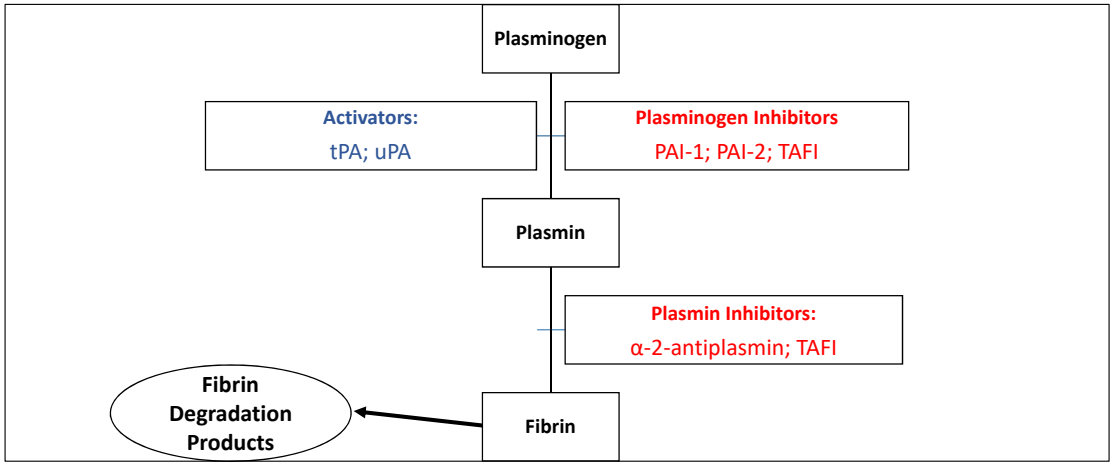
Bleeding Disorders



Fibrinolysis

- Fibrin plays a critical role in coagulation by stabilizing the initial platelet-based clot (primary hemostasis)
- The fibrinolytic system controls the extent of the clot or thrombus—keeping it confined to the area of vascular injury--and also is active in removing the thrombus once the blood vessel has "healed"
- The "primary" fibrinolysin is **plasmin** which is controlled by a number of activators and inhibitors as outlined in the next slide……

Regulation of Fibrinolysis



Fibrinolytic Bleeding Disorders

- Bleeding due to abnormalities in the fibrinolytic system are usually manifest as:
  - Mild-severe ***delayed bleeding*** after surgery, trauma, or childbirth
    - (*Why **DELAYED** bleeding???????*)
  - Excessive wound hematomas
  - Easy bruising
  - Occasional bleeding into joints and intracranial bleeding following trauma
- Fibrinolytic bleeding disorders can be either inherited or acquired

Fibrinolytic Bleeding Disorders

Inherited		Acquired	
PAI-1 deficiency	Rare	Cardiopulmonary bypass	CPB is a strong stimulator of tPA release Treated with aprotinin
A-2-antiplasmin deficiency	Rare	Cirrhosis	Reduced hepatic clearance of tPA
		Nephrotic syndrome	Urinary loss of α-2-antiplasmin
		Trauma	Release endothelial tPA and inhibition of PAI-1
		DIC	Multiple defects; *to be discussed in next lecture*

Fibrinolytic Bleeding Disorders

- While very rare, the diagnosis of inherited fibrinolytic bleeding disorders can be considered in patients who have characteristic delayed onset bleeding after surgery or trauma and who have a normal PT, PTT, and platelet count.
- The tests available include:

Test					
PAI-1 activity	Normal	Undetectable	Undetectable	Reduced	-----
PAI-1 antigen	-----	Undectectable	Low or normal	-----	-----
tPA antigen	-----	Reduced	Reduced	Normal or elevated	-----
Antiplasmin activity	-----	Normal	Normal	Normal	Reduced
Interpretation	No evidence of PAI-1 deficiency	Complete PAI-1 deficiency	Deficiency of active PAI-1	Increased tPA release	Antiplasmin deficiency

- Summary of this lecture:
  - It is important to be able to determine the possible coagulation factor deficiency based on the PT and PTT results
  - Further, based on the interpretation of the PT and PTT, be able to construct a differential diagnosis
  - Some of the most important coagulopathies include:
    - Hemophilia A, B, C
    - While less common, the various inhibitors (autoantibody and alloantibody to factor VIII and Lupus inhibitor, are also important)
  - Fibrinolysis is an important condition that may result in severe bleeding abnormalities