Thrombophilia and Treatments

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Today's Discussion

Intro to Thrombophilia

Acquired Thrombophilia Disorders

Inherited Thrombophilia Disorders

Anticoagulants used for Therapy of Thrombophilia

Intro to Thrombophilia

Thrombosis and Thrombophilia

Thrombosis

- Inappropriate formation of platelet or fibrin clot obstructing blood vessels
- Multifaceted disorder resulting from abnormalities in blood flow (stasis), the coagulation system, platelet function leukocyte activation molecules and the blood vessel wall
  - Ischemia= loss of blood supply
  - Necrosis= tissue death

Thrombophilia (a.k.a hypercoagulability)

- Defined as the predisposition to thrombosis secondary to a congenital or acquired disorder
  - Physical, chemical, or biologic events such as chronic or acute inflammation releasing prothrombotic mediators from damaged vessels
  - Inappropriate and uncontrolled platelet activation
  - Uncontrolled blood coagulation system activation
  - Uncontrolled fibrinolysis suppression

Thrombosis- VTD and Arterial Thrombosis

Venous Thromboembolic Disease

- Annual incidence of VTE (Venous Thromboembolic event) in the US is 1 in 1000
- Most prevalent VTE is deep vein thrombosis (DVT)
  - Caused by clots forming in the iliac, popliteal and femoral veins of calves and upper legs
- Emboli- fragments of proximal ends of venous thrombi
  - Can dislodge and cause ischemia and necrosis in the lungs= pulmonary embolism
  - 95% of pulmonary embolism occur from deep vein thrombi
    - 10-15% of these patients die within 3 months

Arterial Thrombosis

- 80% of acute myocardial infarctions (heart attacks) and 85% of strokes occur due to thrombi blocking coronary arteries and carotid end arteries of vertebrobasilar system, respectively
- Transient ischemic attacks and peripheral arterial occlusions are most frequent than strokes and coronary artery disease
- Unstable atherosclerotic plaques= activated platelets, monocytes, and macrophages embedded the fatty plaque within the endothelial lining
  - Suppress release of antithrombotic molecules
  - Exposed prothrombotic substances such as tissue factor

Non-disease Factors Contribute to Thrombotic Events

Age

Immobilization

Diet

Lipid metabolism imbalance

Oral contraceptive use

Pregnancy

Hormone replacement therapy

Femoral or tibial fracture

Hip, knee, gynecologic, prostate surgeries

Smoking

Inflammation

Central venous catheter

Thrombotic Disorders

Acquired

- Antiphospholipid Syndrome *
  - Chronic antiphospholipid antibody often secondary to autoimmune disorder
- Myeloproliferative Neoplasms
  - Increased risk due to plasma viscosity, platelet activation
- Hepatic Disease
  - Irregular coagulation pathways due to lack of control proteins, excess thrombin formation
- Cancer: Adenocarcinoma
  - 20 x increased risk of thrombosis, chronic DIC
- Leukemia
  - Increased risk for chronic DIC
- PNH (Paroxysmal Nocturnal Hemogobinuria)
  - Increased risk for DVT, PE and DIC; platelet-related thrombosis
- Chronic Inflammation
- Diabetes Mellitus
- TTP/HUS

Congenital/Inherited

- Factor V_{leiden}
- PC Deficiency
- PS Deficiency
- AT Deficiency
- Dysfibrinogenemia
- tPA deficiency
- ↑ prothrombin
- ↑ homocysteine
- ↑ Factor VIII

Evaluation of Thrombophilia

Initial Studies

- APC resistance
- Protein C
- Protein S
- Antithrombin
- Prothrombin mutation
- Antiphospholipid antibodies

Acquired Thrombophilia Disorders

Acquired Thrombophilia

Myeloproliferative neoplasms- essential thrombocythemia and polycythemia vera
- Trigger thrombosis through platelet hyperactivity

Acute promyelocytic leukemia
- DIC is secondary to release of procoagulant granule content from malignant promyelocytes
  - May intensity during therapy at the time of vigorous cell lysis

Chronic inflammatory diseases
- Cause thrombosis through a variety of mechanisms
  - Elevation of fibrinogen and FVIII
  - Suppressed fibrinolysis
  - Promotion of atherosclerotic plaque formation
  - Reduced free protein S activity secondary to raised C4b-binding protein (C4bBP)
- Example:
  - Diabetes mellitus- raised risk of cardiovascular disease six fold
  - Conditions associated with venous stasis (congestive heart failure)
  - Untreated atrial fibrillation
  - Nephrotic syndrome

Antiphospholipid Antibodies

Comprise a family of immunoglobulins that bind protein-phospholipid complexes

Includes

- Lupus anticoagulant (LAC)
- Anticardiolipin antibody (ACA)
- Anti-β_2-GPI antibody

Disorders associated with

- Recurrent/unexplained venous and arterial thrombosis, strokes, coronary and peripheral artery disease, pregnancy loss, thrombocytopenia

Arise as immunoglobin M (IgM) or IgG isotypes

- Nonspecific inhibitors
- Bind to proteins that assemble on anionic phospholipid surfaces

Lupus Anticoagulant

Most common acquired predisposing causes of thrombosis

LA is an antiphospholipid antibody

- Interferes with coagulation testing by binding to phospholipid-protein complexes- prolonging clotting times

Commonly found in asymptomatic elderly individuals and patients with autoimmune disorders

Associated with anticardiolipin antibodies and β_2-GPI antibodies

Lupus Anticoagulant Test Profile

Results in prolongation of phospholipid-dependent assays

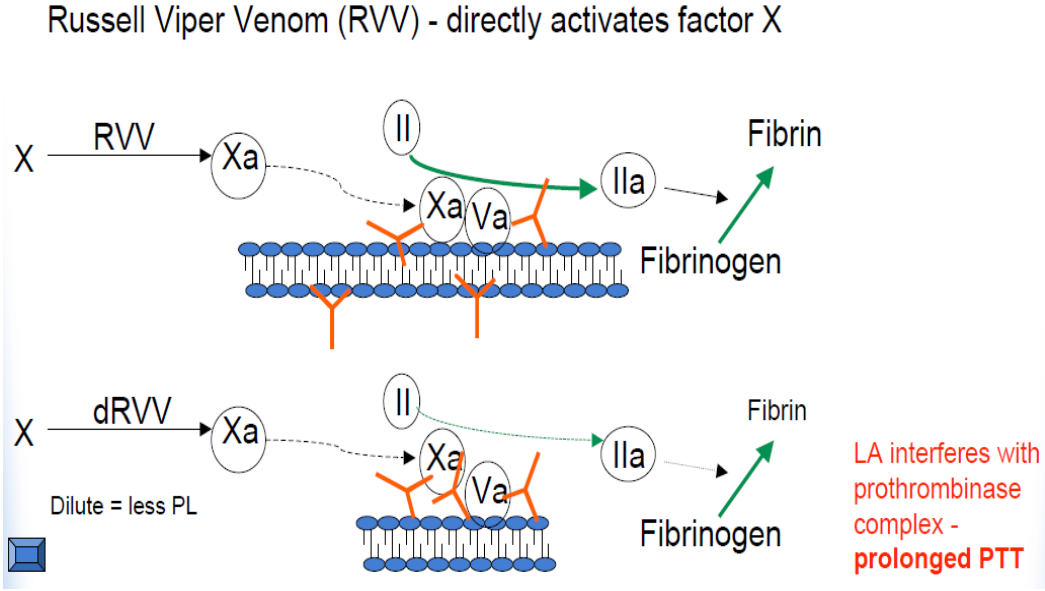
4 features of a Lupus Anticoagulant Diagnosis

1. Prolongation of at least 1 phospholipid-dependent clotting test (test with low phospholipid content)
   - Low-phospholipid PTT and DRVVT screen
   - Note that certain drugs can cause a falsely elevated PTT during initial testing: Heparin, Dabigatran, Anti-IIa drugs, Anti-Xa drugs
2. Failure to correct the prolonged clot time when mixing with normal platelet-poor plasma
   - Mixing study
3. Evidence that the inhibitory activity depends on phospholipid
   - STACLT LA, DRVVT confirm, and PNP
4. Exclusion of other coagulopathies
   - Specific inhibitors (FVIIIC, DTIs, heparin)

Lupus Anticoagulant Test Profile

Dilute Russell Viper Venom Test (DRVVT)

- Based on the activation of FX by Russell viper venom (RVV) and varying amounts of phospholipids
- When the LA antibody is present, it binds to the phospholipid and inhibits thrombin generation
  - Prolonging the clotting time
- Performed in 2 parts
  - Screen: Uses a reagent with a low concentration of phospholipid which, in the presence of LA, will cause a prolonged result
  - Confirm: Uses a reagent of high concentration of phospholipid, which will mask the LA, causing a shortened result
- Ratio of the Screen to Confirm is calculated
  - >1.2 = positive for LA
- DRVVT Mix
  - Performed as part of the assay
  - Performed as a dRVV screen with the patient sample being incubated with normal plasma. A prolonged result for this test would indicate possible LA in the plasma

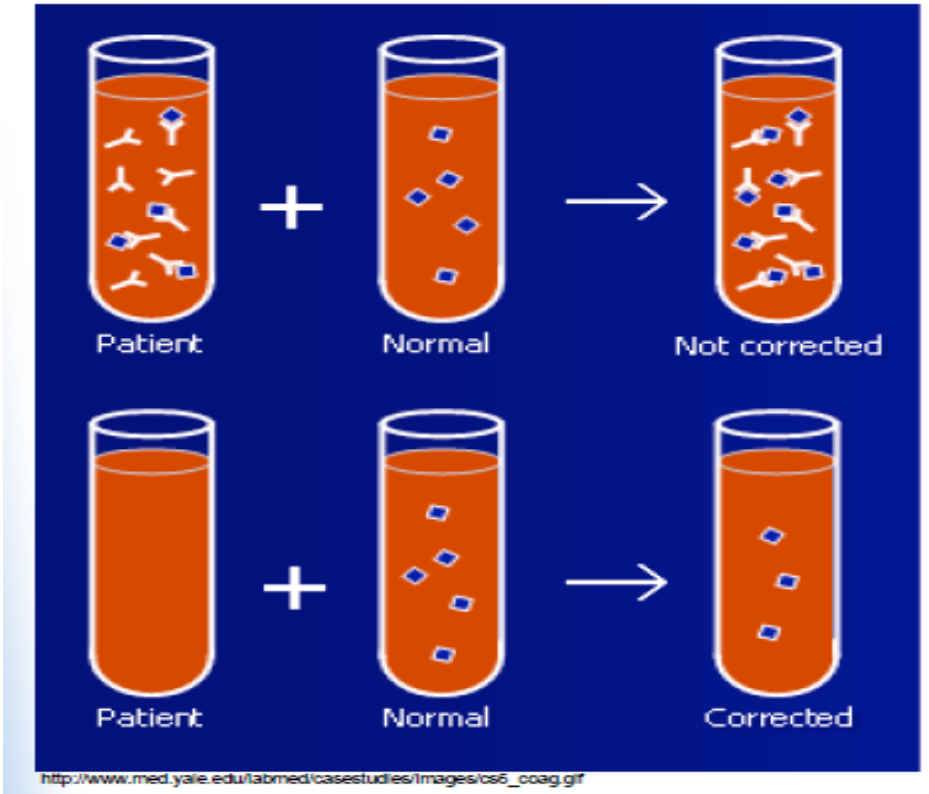


* A prolongation of the DRVVT test may be obtained on plasma from patients who have factor II, V and X deficiencies as well as those on warfarin therapy. A DRVVT Mix adds back the factors that are lacking originally. If still prolonged after mix, then anticoagulant therapy is indicative.

Lupus Anticoagulant Test Profile

Mixing study

- PTT is performed on patient plasma combined 1:1 with pooled normal plasma (NP)
- The patient:NP sample incubates at 37°C for 1 hour and the PTT is measured again
  - Most specific inhibitors such as anti-factor VIII and 15% of LACs require incubation to enhance their avidity
- Results
  - Initial 1:1 PTT uncorrected, 1 hr incubation 1:1 PTT uncorrected
    - LAC
  - Initial 1:1 PTT corrected, 1 hr incubation 1:1 PTT uncorrected
    - Assay for specific factor inhibitor
      - Time dependent
  - Initial 1:1 PTT corrected, 1 hr incubation 1:1 PTT corrected
    - Assay for factor deficiency



Platelet Neutralization Procedure (PNP) and Staclot LA

PNP

- PNP is based on the ability of platelets to significantly correct in vitro coagulation abnormalities
- Disrupted platelet membranes present in the freeze-thawed platelet suspension neutralized phospholipid antibodies present in patient's plasma with LA
- When patient plasma is mixed with the freeze-thawed platelet suspension, the pTT will be shortned in comparison to the baseline PTT
- A correction of the baseline PTT of a defined amount of time (3 to 5 seconds or more) by the platelet suspension as compared with the QC is indicative of the presence of LA

Staclot LA

- Sensitive APTT
- Performed with and without addition of hexagonal phase phospholipids
- Measures delta seconds (APTT-APTT with PL)
- Positive: delta >6.2 seconds

Antiphospholipid Antibodies Testing

Tested using immunoassays

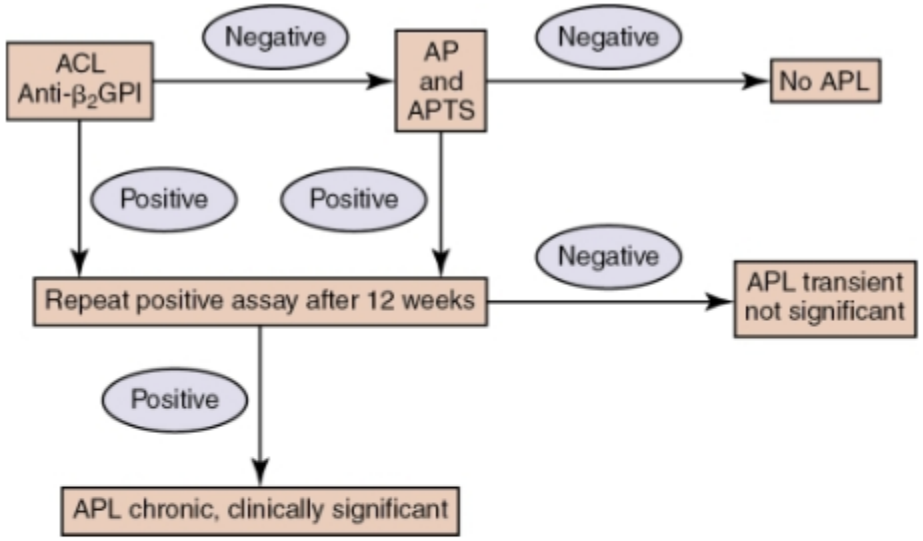


Figure 39.4**Anti-phospholipid Antibody *(APL)* Immunoassay Algorithm.** If APL is suspected, perform an anti-cardiolipin *(ACL)* or anti-β_2-glycoprotein I (anti-β_2-GPI) immunoassay. If either is positive, confirm chronicity using a new specimen collected at least 12 weeks later. If negative, perform immunoassay to detect an anti-prothrombin *(AP)* or anti-phosphatidylserine *(APTS)* antibody. If positive, repeat after 12 weeks.

DIC and HIT

- Both of these thrombotic disorders can also be characterized as hemorrhagic disorders

DIC

- a.k.a *defibrination syndrome* or *consumptive coagulopathy*
- Thrombi that form are small and ineffective, so hemorrhage is often first clinical sign of DIC

- Lab Profile:
  - Platelet Count: <150,000/uL
  - PT: >14 seconds
  - PTT: >35 seconds
  - D-dimer: >240ng/mL
    - Often 10,000 to 20,000 ng/mL
  - Fibrinogen: <220 mg/dL
    - often higher because fibrinogen is an acute phase reactant

HIT

- Between 1-5% of patients receiving unfractionated heparin (UFH) for more than 5 days develop IgG antibody specific for heparin – platelet factor 4 complexes
  - LMWH also can cause HIT
- Risk factors for HIT
  - Patients who receive surgery
  - Female sex
- PF4 antibody immunoassay
- HIPA (heparin induced platelet aggregation)
  - Reflex test
- Serotonin release assay
  - Send out

Inherited Thrombophilia Disorders

Most Common Congenital Disorders

Factor V Leiden

Mutations Prothrombin

Protein C deficiency

Protein S deficiency

Antithrombin deficiency

Factor V Leiden

Resistance to Activated Protein C (APC)

Most common congenital disorder associated with thrombophilia

Due to a mutation (Arg → Gln) in factor V (factor V Leiden)

Mutation alters an APC cleavage site in factor Va

Altered factor Va cannot be inactivated by APC

Resistant factor Va remains active (a gain of function)

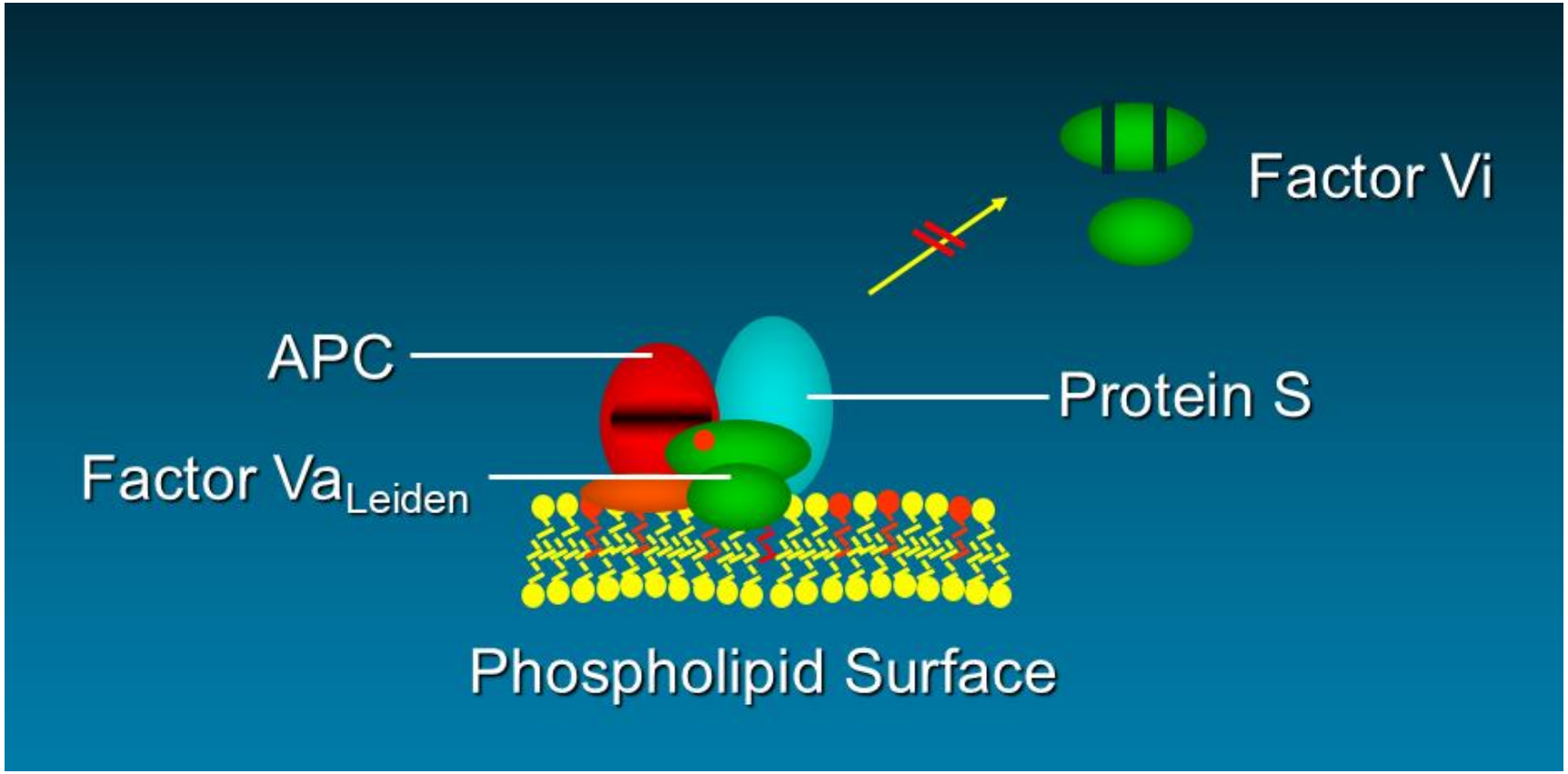
- Raises the production of thrombin, leading to thrombosis

Named Leiden for the city in the Netherlands for which it was discovered

- Between 3% and 8% of northern European Caucasians possess the FVL mutation
- Thresfold higher thrombosis risk

Tested through the APC resistance panel and molecular FVL mutation test

APC Resistance Clot-Based Assay



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Mutations in Prothrombin

Increased prothrombin

Mutation in the 3’ untranslated region of the prothrombin (FII) gene has been associated with mildly elevated plasma prothrombin levels

- Averaging 130%

Increased risk of thrombosis (x3 higher risk)

Relative risk of thrombosis 5%-18% with familial thrombosis and 0.3-2.4% worldwide

Diagnosed through molecular genetic testing

Protein C Deficiency

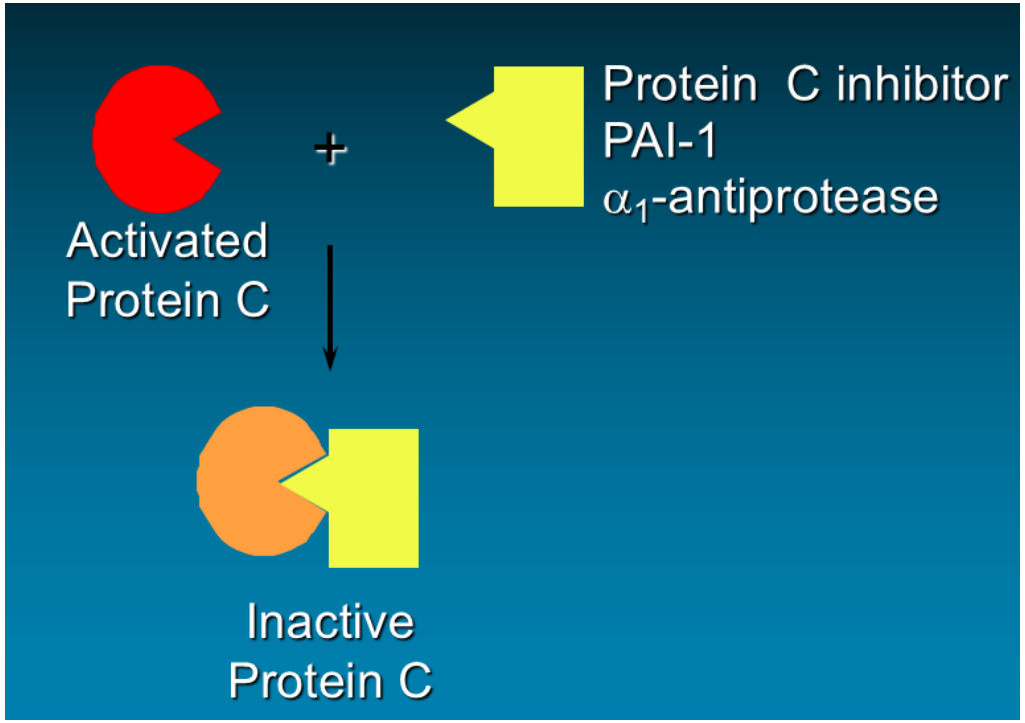
Protein C

- Synthesized in the liver, vitamin K-dependent factor
- Activated by thrombin-thrombomodulin on endothelial cell surface
- Requires protein S, which acts as a cofactor to stabilize activated PC (aPC)
- Naturally occurring inhibitor of the coagulation cascade
  - Will degrade FVa and FVIIIa

<u>PC deficiency</u>

- Autosomal dominant disorder with 30-60% protein C activity
- Leads to a 1.6 fold-11.5-fold increased risk of recurring DVTs and PEs
  - Can also see superficial thrombophlebitis, cerebrovascular events, and myocardial events
- Onset of symptoms during or after adolescence
- Associated with to warfarin-induced skin necrosis
  - Patients who receive warfarin without heparin, develop geographic areas of skin necrosis

Neutralization of Protein C



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Protein S Deficiency

Protein S

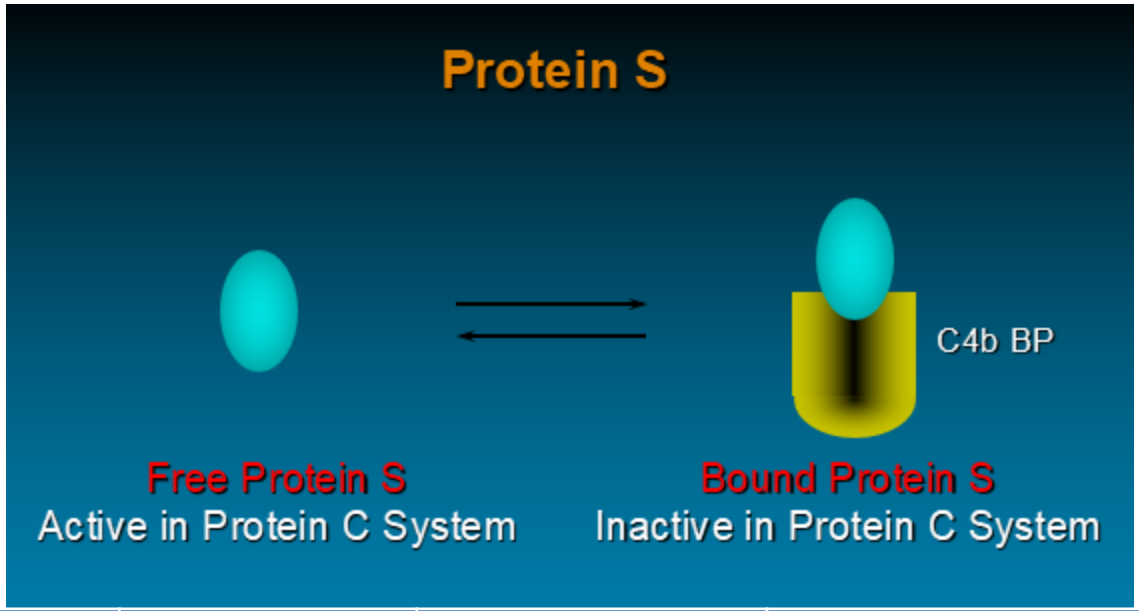
- Cofactor for PC-mediated inhibitor of activated FV and FVIII
- Reversible complex with C4bBP
  - 40% travel as Protein S Free, 60% circulates bound to C4bBP
- Vitamin K dependent factor→ decreased under oral anticoagulation

Protein S Deficiency can be hereditary or acquired (pregnancy)

Protein S Deficiency

- Autosomal dominant disorder with <60% protein S activity
- Usually associated with a decrease in free protein S
- Onset of symptoms during or after adolescence
- Affected individuals may be at risk for warfarin-induced skin necrosis
  - Patients who receive warfarin without heparin, develop geographic areas of skin necrosis

Protein S Deficiency



C4b binding protein will bind and inactivate protein S

- Complement will be elevated in acute phase response

Type of PS Deficiency	PS Activity	PS Free Antigen	PS Total Antigen	C4bBP
I Quantitative	<65%	<65%	<65%	Normal
II Qualitative	<65%	>65%	>65%	Normal
III Inflammation	<65%	<65%	>65%	Elevated

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Protein C and S Deficiency

Acquired PC/PS deficiency
- DIC
- Liver disease
- Sepsis
- Oral anticoagulant therapy (warfarin)
  - PC has a ½ life of 6 hours, decreases rapidly
- Pregnancy
- Oral contraceptive use

Treatment
- No treatments except for surgery
- Heparin when administering warfarin
- PC concentrates

Diagnosis
- Molecular testing

Antithrombin Deficiency

Antithrombin

- Major regulatory of coagulation that is a natural inhibitor of activated serin proteinases
  - IIa, IXa, Xa, XIa, XIIa, and to a limited extent VIIa
- Serves as a cofactor for heparin and other heparinoids

AT deficiency

- Autosomal dominant trait with AT activity of 40-60%
- Most severe of the inherited conditions, relatively uncommon
- Venous thrombosis predominates, but arterial thrombosis may occur
- Deficiency diminishes therapeutic response to heparin therapy
  - Because antithrombin is the binding site for heparin

Symptoms appear in adolescence or later, but may appear early in life

Antithrombin Deficiency

Types:

- Congenital
- Acquired
  - Liver failure
  - Nephrotic syndrome
  - Tumors
  - DIC
  - Pregnancy
  - Acute blood clots
  - Severe trauma
  - Severe burns

Require anticoagulant therapy for life

Anticoagulants used in Therapy

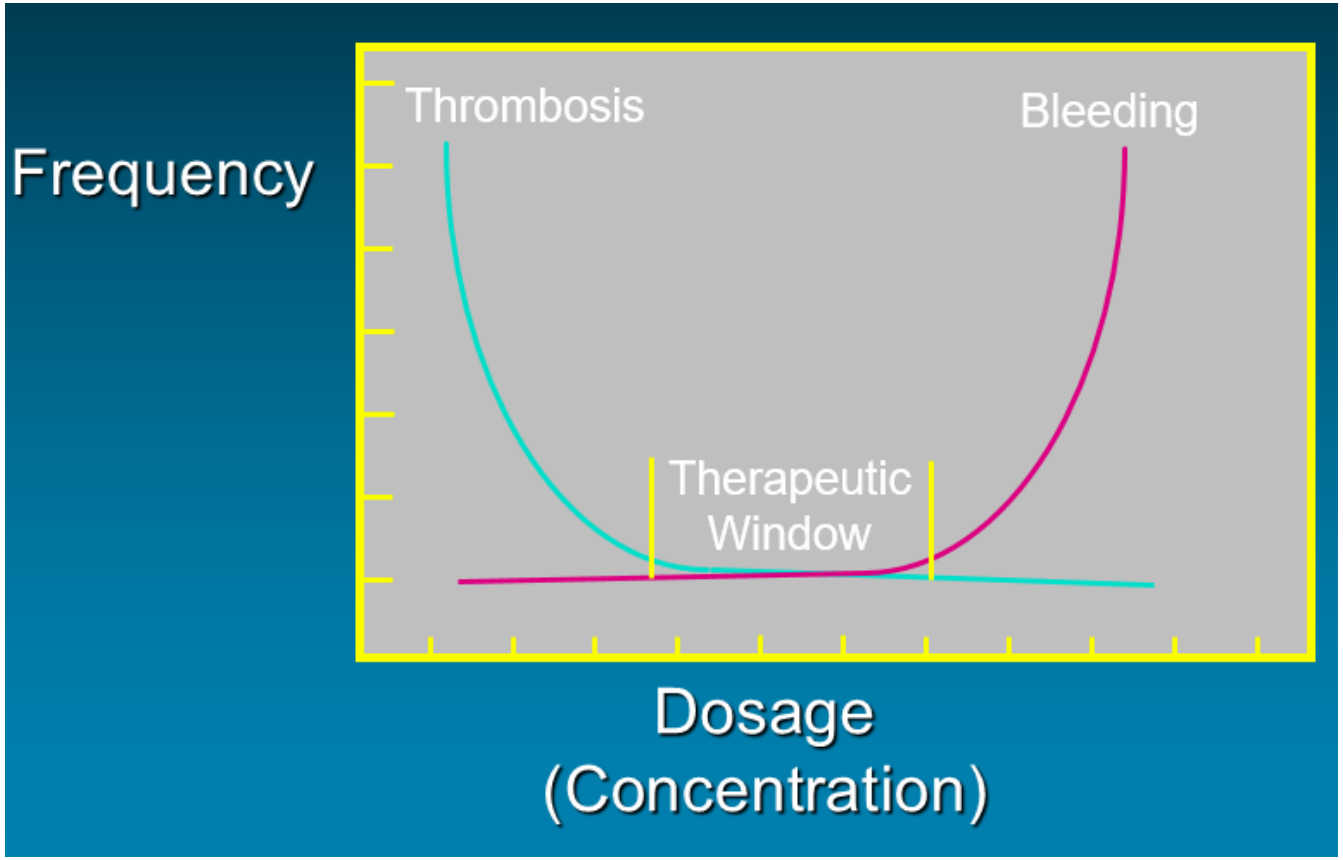
Thrombosis and Anticoagulation

Antithrombotic drugs have been employed to prevent and treat thrombosis

Anticoagulants- suppress coagulation and reduce thrombin formation

Antiplatelet drugs- suppress platelet activation

Fibrinolytics- disperse or reduce existing clots clogging veins and arteries



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Antithrombotic Drugs

Categorized into 3 groups:

- Original drugs
  - Heparin
  - Warfarin/ Coumadin
  - Aspirin
- Drugs that entered clinical use in the 1990s
  - Heparin derivatives
    - Low molecular weight heparin
    - Fondaparinux
  - Antiplatelet drugs
- Newest drugs
  - Direct-acting oral and intravenous anticoagulants (DOACs)
    - Direct IIa inhibitors
    - Direct Xa inhibitors

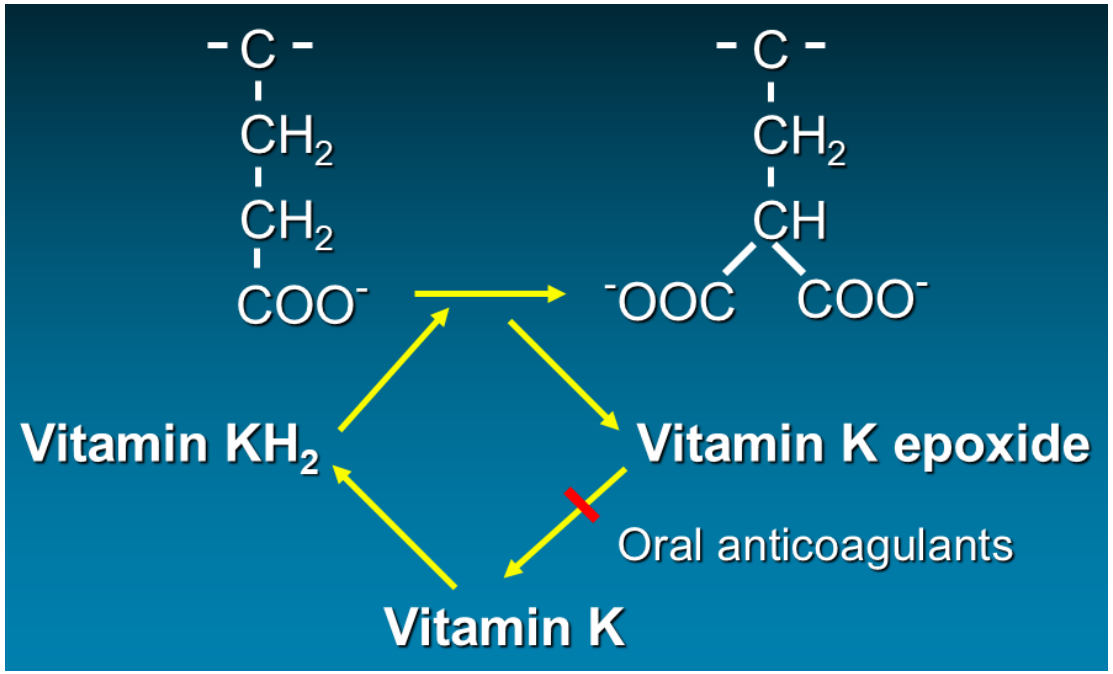
Warfarin/Coumadin

Interferes with vitamin K dependent proteins (II, VII, IX, X, PC, PS, and PZ)

Goal is to reduce but not eradicate thrombin generation

How it works:

- Absence of Vit. K results in production of nonfunctional des-γ carboxyl forms of the vitamin K dependent factors and control proteins
  - Proteins induced by vitamin K antagonists (PIVKA)
  - Bind few calcium ions but are not able assemble on the phospholipid surfaces
    - Do not participate in coagulation



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Warfarin/ Coumadin

Narrow therapeutic range

- Overdose can cause hemorrhage

Warfarin Monitoring

- Monitored by the PT and reported out as INR
- INR must fall between 2-3
- Requires frequent blood draws

Antidote for high levels of warfarin

- Vitamin K administration

Antithrombotic Drugs

Heparin-like drugs

- Unfractionated heparin (UFH)
- Low molecular weight heparin (LMWH)
- Fondaparinux

Unfractionated Heparin

Naturally occurring anticoagulant and can also be administered as a drug

Polymer composed of glycosaminoglycan (sulfated disaccharide repeat unit) –minimum of 18

A naturally occurring anticoagulant

- Produced by basophils and mast cells

Activates antithrombin (1000x) to cause inactivation of thrombin (IIa) and Xa through a “bridging mechanism”

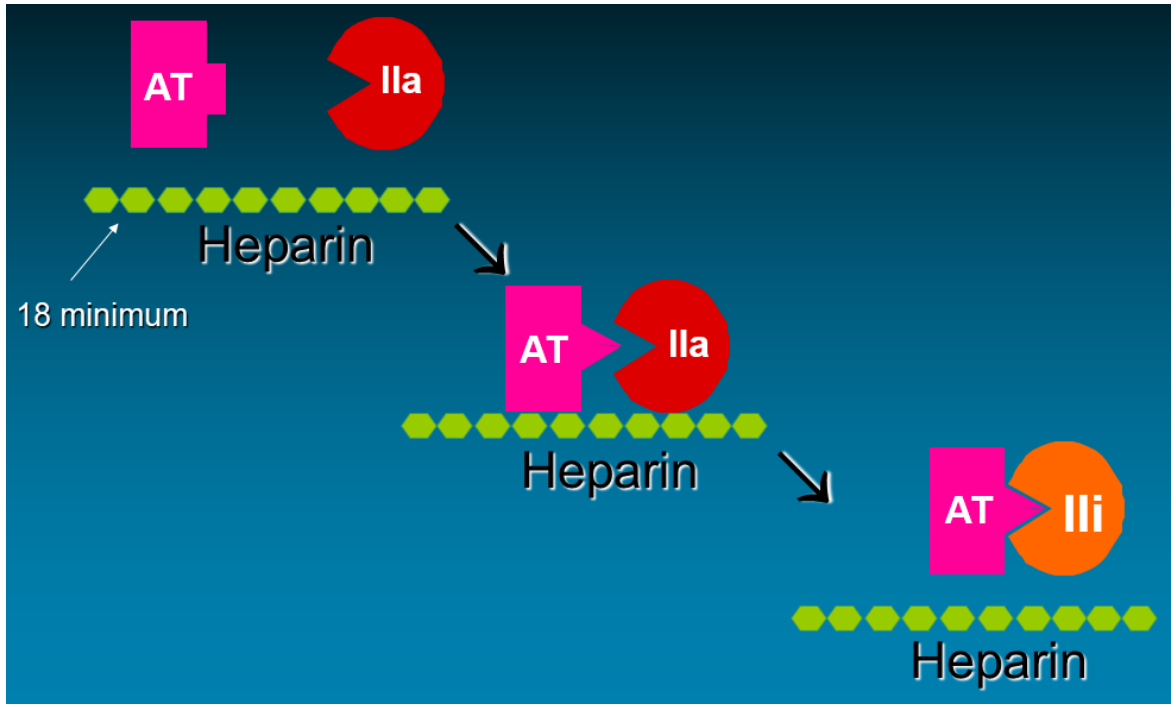
- Thrombin (IIa) will assemble on the UFH molecule near the activated antithrombin and become inactivated
- Xa becomes inactivated by binding to the modified antithrombin molecule (does not bind to UFH)

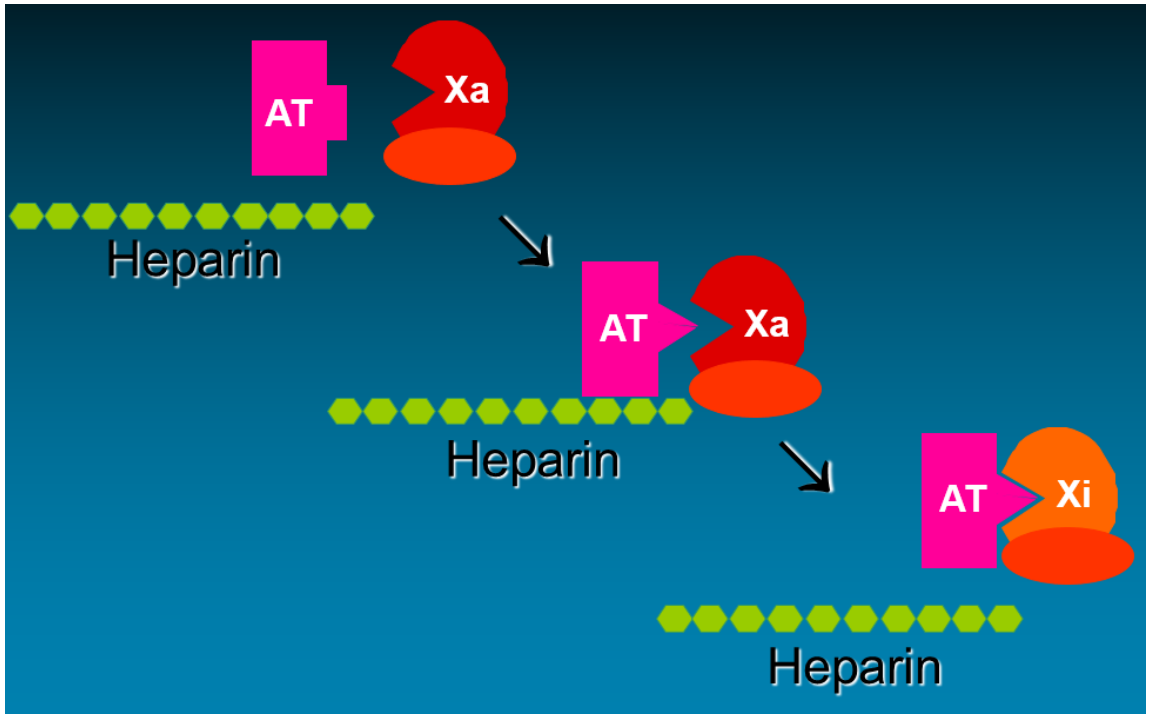
Monitored through

- Anti-Xa testing
- PTT
- ACT

Must monitor platelets for possibility of developing HIT*

Unfractionated Heparin





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Low-Molecular Weight Heparin (LMWH)

Prepared by cleaving the long polysaccharide chains of UFH to yield a product 1/3 the mass of UFH

- Less than 18 repeat units

Possess the same antithrombin-binding except the shorter polysaccharide chain does not support the entire thrombin-antithrombin bridging

- Unable to inactivate IIa

Factor Xa neutralization response is unchanged

- Does not rely on factor Xa binding to heparin's polysaccharide chain

Selective for Xa

Fondaparinux

Synthetic heparin

Inhibits only factor Xa through antithrombin

- Raises antithrombin activity 400-fold

Comparable to LMWH except:

- Fondaparinux has a reduced major bleeding effect
- Fondaparinux has a longer half life
- PTT is not sensitive to Fondaparinux

New Direct Oral Anticoagulants (DOAC)

Goal is to offer more specific targeting with more affordable and predictable results

Does not require periodic laboratory monitoring

Replacing Coumadin

Two different types:

- Direct thrombin (IIa) inhibitors
  - Dabigatran
- Direct Xa inhibitors
  - Rivaroxaban
  - Apixaban
  - Edoxaban

Direct thrombin Inhibitor

Dabigatran

- DTI that binds both free thrombin and clot-bound thrombin
  - Direct acting inhibitor
    - Does not require antithrombin as a cofactor
- aPTT and TT elevation gives a qualitative measure of effect
- TT is highly sensitive to presence of dabigatran and will be markedly prolonged
- Only requires measurement with a clinical condition that requires anticoagulant measurement
- Effect can be reversed with idarucizumab

Direct Xa inhibitors

Apixaban, rivaroxaban, edoxoban, and betrixaban are direct FXa inhibitors which can be taken orally

Inhibit FX whether it is free, clot-bound, or bound to coagulation factor IXa

Do not require antithrombin to express their anticoagulant activity

Cleared by the liver

PT and aPTT are usually prolonged when there is a theraupetic effect

Only requires measurement with a clinical condition that requires anticoagulant measurement

Reversal agent is andexanet alfa

References

Rodak's Hematology, 6th Edition

Dr. Theil, MD Staff Pathologist Hematology and Coagulation