

Hypercoagulable States

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Faculty Disclosure

- Dr. Rizal has no actual or potential conflict of interest associated with this presentation

Learning Objectives

At the conclusion of this activity, pharmacists will be able to:

- Describe inherited hypercoagulable states
- Describe acquired hypercoagulable states
- Apply management strategies for various hypercoagulable states including Anithrombin III Deficiency, Protein C or S Deficiency, Factor V Leiden, Prothrombin gene mutation, Hyperhomocysteinemia and Antiphospholipid Antibody Syndrome

Normal Physiology: Coagulation Cascade

The diagram illustrates the coagulation cascade. It starts with two main pathways: the Contact activation (intrinsic) pathway and the Tissue factor (extrinsic) pathway. The intrinsic pathway begins at a 'Damaged surface' which activates Factor XII to XIIa, which then activates XI to XIa, IX to IXa, and VIII to VIIIa. IXa and VIIIa then activate X to Xa. The extrinsic pathway starts with 'Trauma' activating VII to VIIa, which then activates IX to IXa. IXa and VIIIa also activate X to Xa. Xa, along with Va, activates II (Prothrombin) to IIa (Thrombin). Thrombin (IIa) activates V to Va, VIII to VIIIa, and XIII to XIIIa. Thrombin (IIa) also activates XI to XIa. Finally, Xa and Va activate I (Fibrinogen) to Ia (Fibrin), which is then cross-linked by XIIIa to form a 'Cross-linked Fibrin clot'. Regulatory factors like Active Protein C, Protein S, and Protein C + thrombomodulin are shown inhibiting Va. Tissue Factor Pathway Inhibitor (TFPI) is shown inhibiting VIIa. Antithrombin is shown inhibiting Xa and IIa.

Hereditary Thombophilia

- Genetic tendency to develop venous thromboembolism
- Total incidence of inherited thrombophilia: 24-37% in pt's w/ DVT vs. 10%
- Most Common >50% of cases
 - Factor V Leiden mutation
 - Prothrombin G20210A mutation
- Remainder
 - Deficiencies of antithrombin III, protein C or protein S
 - Hyperhomocysteinemia

Acquired Hypercoagulable States

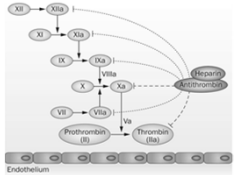
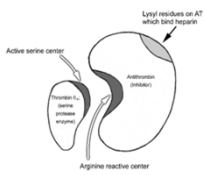
- Numerous Include:
 - Antiphospholipid antibody syndrome
 - Cancer
 - Some medications used to treat cancer, such as tamoxifen, bevacizumab, thalidomide and lenalidomide
 - Recent trauma or surgery
 - Central venous catheter placement
 - Obesity
 - Prolonged bed rest or immobility
 - Extended travel
 - Hyperviscosity
 - Smoking
 - Pregnancy
 - Estrogen use eg. Oral contraceptives
 - Hormone replacement therapy
 - Glucocorticoids
 - Heparin-induced thrombocytopenia
 - Thrombotic thrombocytopenic purpura
 - Congestive failure
 - Diabetes Mellitus
 - Hyperlipidemia
 - Previous history of deep vein thrombosis or pulmonary embolism
 - Myeloproliferative disorders such as polycythemia vera or essential thrombocytosis
 - Inflammatory bowel syndrome
 - Nephrotic syndrome (too much protein in the urine)

VIRCHOW'S TRIAD

The diagram shows three overlapping circles representing the components of Virchow's Triad: Hypercoagulability, Venous Stasis, and Endothelial Injury. The central area where all three overlap is labeled 'Thrombosis'.

Antithrombin III Deficiency

- Physiology
 - Serine protease inhibitor produced in the liver
 - Half life 2.8-4.8 days
 - Inactivates several clotting factors including Factor Xa, Factor IXa, Factor XIa, Factor XIIa, Factor VIIa and Factor IIa
 - Heparin enhances binding of Antithrombin to Factor II and Factor X

Antithrombin III Deficiency

- 2 Forms:
 - Inherited
 - Inherited deficiencies are due to AT gene mutations
 - Type I deficiency-reduced AT level
 - Type II deficiency-functionally defective AT
 - Acquired
 - Impaired production of functional AT
 - eg. due to liver disease, warfarin therapy, asparaginase therapy
 - Increased excretion
 - eg. in nephrotic syndrome, heparin
 - Accelerated consumption
 - as occurs in acute thrombosis or disseminated intravascular coagulation

Acquired Antithrombin III Deficiency

- Reduced plasma AT levels
 - Extracorporeal membrane oxygenation (ECMO)
 - Hemodialysis
 - Major surgery
 - Estrogen therapy
 - Pregnancy
 - AT level reduced during pregnancy induced hypertension, preeclampsia, eclampsia

Antithrombin III Deficiency

- Incidence
 - Prevalence in general population: 0.2-0.02% (1 in 500 to 1 in 5,000 individuals)
- Autosomal dominant
- Homozygous antithrombin deficiency incompatible with life
- Heterozygous antithrombin deficiency
 - 4% of families with inherited thrombophilia
 - 1% of consecutive patients with first episode DVT
- Age at presentation
 - Varies widely, some individuals never have a thromboembolic event in their lifetime

Antithrombin III Deficiency

- Consequences:
 - Increased risk of VTE
 - Risk varies among populations
 - 40-60% of normal AT allows thrombin generation and fibrin deposition to veins
 - Common Sites: DVT of leg, iliofemoral and mesenteric veins
 - Less common sites: Vena cava, renal, retinal, cerebral, or hepatic veins
 - Arterial thrombosis has been reported but is not characteristic
 - High risk of thrombosis during pregnancy
 - Heparin insensitivity
 - Heparin and LMWH require AT to inactivate factor Xa

Antithrombin III Deficiency

- Diagnosis
 - Differential diagnosis of AT deficiency includes other causes of thromboembolism and other causes of heparin resistance
 - Perform laboratory testing only on:
 - Patients with suspected inherited thrombophilia based on family history or atypical presentation
 - Suspected heparin resistance
 - Asparaginase therapy or extracorporeal membrane oxygenation
 - Normal antithrombin level sufficient to exclude the disorder
 - Low levels should be confirmed at a later date

Antithrombin III Deficiency

- Choice of assay
 - AT-heparin cofactor assay
 - A functional assay for plasma AT activity
 - Measures ability of heparin to inhibit coagulation factor IIa or Xa which requires AT activity
 - Factor Xa inhibition generally preferred due to improved specificity
 - Normal AT activity range: 80-120% at most laboratories
 - Laboratory-specific values should be used to take into account instrument and assay variability
 - AT-Protein level assay
 - Enzyme-linked immunosorbent assay (ELISA)
 - Unable to identify individuals with Type II AT deficiency
 - Can be used to distinguish between type I and type II defects in individuals with deficient AT activity
- Timing of testing
 - Do not perform test:
 - During acute thrombosis or comorbid illness (transient low levels)
 - Pt's on heparin therapy (increased clearance of AT thus false low reading)
 - Warfarin therapy (plasma antithrombin levels elevated to normal range)
 - Wait >2 weeks after discontinuation



Antithrombin III Deficiency

- Management
 - Anticoagulation
 - Thrombin inhibitors eg. Argatroban
 - Prophylaxis for high-risk patients with AT deficiency eg: pregnancy, surgery
 - Lifelong anticoagulation for patients with unprovoked VTE
 - AT replacement
 - Recombinant human antithrombin produced from the milk of transgenic goats (rhAT, ATryn)
 - Antithrombin concentrate derived from pooled human plasma (Thrombate III)
 - Dose and schedule depend on the product used
 - once daily dosing for plasma-derived AT
 - continuous infusion for recombinant AT
 - AT is dosed in units of activity
 - » one unit is defined as the amount of AT in one milliliter of pooled normal human plasma
 - Patient's baseline AT activity level and body weight (used for initial dose calculation), and the clinical setting (dosing of the concentrate differs in pregnancy versus surgery)



Case Presentation

60 y.o. male presents to ER with leg pain. On examination, MD notes left lower extremity swelling and tenderness. Ultrasound is positive for LLE DVT

Patient's medication list as follows: MVI, Calcium with Vitamin D, Fish oil, Thalidomide and Dexamethasone

Further history reveals that pt. just returned home after 24 hour flight from Nepal and has history of multiple myeloma

Question: How would you manage this patient's VTE?



Case Presentation

Answer:

- Initiate Heparin infusion x 5 days followed by warfarin therapy for 3-6months.
- Testing for hypercoagulable state is unnecessary in the pt. because he has known risk factors for developing clot including long flight, thalidomide, dexamethasone and cancer



Case Presentation Continued

Heparin 80units/kg bolus and 18units/kg/hr infusion started and nomogram provided to achieve aPTT levels 60-80. However despite significant heparin dose increases aPTT levels not being achieved.

Question: What is likely cause? How should we manage this patient?



Case Presentation Continued

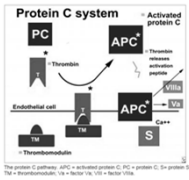
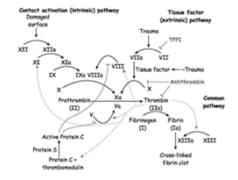
Answer:

- Suspect antithrombin III deficiency.
- Stop heparin drip and initiate pt. on argatroban drip to treat VTE.
- Perform laboratory testing to confirm antithrombin III diagnosis



Protein C Deficiency

- **Physiology**
 - Vitamin K-dependent protein synthesized in the liver
 - Activated by thrombin (bound to endothelial thrombomodulin) to the serine protease activated protein C (aPC)
 - aPC inactivates factors Va and VIIIa
 - The inhibitory effect of aPC is markedly enhanced by protein S

Protein C Deficiency

- **2 Forms:**
 - **Inherited**
 - Due to protein C gene mutations
 - Type I deficiency-Quantitative deficiency
 - » Heterozygous patients have plasma protein C concentration which is 50% of normal in antigen and activity levels
 - » More common
 - » Marked phenotypic variability
 - Type II deficiency-Functional deficiency
 - » normal plasma protein C antigen levels with decreased functional activity due to point mutations
 - **Acquired**
 - Liver disease
 - Severe infection
 - Septic shock
 - Disseminated intravascular coagulation
 - Acute respiratory distress syndrome
 - Postoperative state
 - Breast cancer patients receiving cyclophosphamide, methotrexate, and 5-fluorouracil
 - L-asparaginase therapy
 - Acute viral or bacterial infections, including meningococemia

Protein C Deficiency

- **Incidence**
 - **Prevalence:**
 - 1 in 200 - 1 in 500 individuals in healthy population
 - Congenital deficiency present in 2-5% of pt's w/ thromboembolism
- **Inheritance is autosomal dominant**
- **Age at presentation**
 - 20-50yrs old
 - **Median age at onset:**
 - 45yrs in unselected patients
 - 30yrs in members of thrombophilia families

Protein C Deficiency


- **Clinical Presentation**
 - **Venous thromboembolism**
 - 7 fold increased risk of developing VTE
 - Risk of developing VTE varies among families
 - Presence of 2nd thrombotic defect eg. factor V leiden increases risk of VTE
 - 60% of patients develop recurrent venous thrombosis
 - 40% of patients have signs of PE
 - Risk of VTE increases with age
 - **Initial episode**
 - 70% of cases-Spontaneous
 - 30% of cases-Risk factors (eg. pregnancy, oral contraceptives, surgery, or trauma)
 - DVT of legs, iliofemoral and mesenteric veins most common
 - Cerebral venous thrombosis
 - Arterial thrombosis reported but not common

Protein C Deficiency

- **Clinical Presentation**
 - **Neonatal purpura fulminans**
 - Rare, life-threatening condition that occurs in newborns with homozygous or compound heterozygous protein C deficiency
 - Presents within several hours to days of life
 - Disseminated intravascular coagulation and hemorrhagic skin necrosis
 - Extremely low levels of protein C antigen (<1% of normal)
 - **Fetal loss**
 - Increased odds ratio for fetal loss

Protein C Deficiency

- **Clinical Presentation**
 - **Warfarin-induced skin necrosis**
 - Associated with large loading doses of warfarin
 - Occurs within 1st several days of warfarin therapy due to transient hypercoagulable state
 - Warfarin initiation decreases protein C activity to approximately 50% of normal within 1 day
 - Increased thrombin generation at warfarin initiation
 - Diffuse microthrombi within dermal and subcutaneous capillaries, venules, and deep veins, with endothelial cell damage, resulting in ischemic skin necrosis and marked red blood cell extravasation
 - The skin lesions occur on the extremities, breasts, trunk, and penis (in males)
 - Rapid reversal important to avoid necrosis



Protein C Deficiency

- **Diagnosis**
 - Differential diagnosis of protein C deficiency includes other causes of thromboembolism
 - Mean protein C concentrations increase by approximately 4% per decade
 - Average concentration of protein C in human plasma= 4mcg/ml
 - Protein C antigen levels in adults range from 70-140% of normal
 - Protein C levels <55% of normal: Genetic abnormality
 - Protein C levels 55-65% of normal: Indeterminate and require re-testing
 - Full term infants: protein C levels 20-40% of normal adult levels, preterm infants have even lower levels
 - Use age-based norms for the specific laboratory performing the test in neonates
 - Methodologies for measurement of protein C differ among laboratories



Protein C Deficiency

- **Assays**
 - **Functional Assay**
 - Preferred for screening
 - Detect both type I and II defects
 - Activate protein C using either thrombin, thrombin-thrombomodulin complex or venom of southern copperhead snake (Agkistrodon contortrix)
 - Assess enzyme activity using either a chromogenic substrate or by measuring activated protein C anticoagulant activity in a factor Xa one-stage clotting assay or partial thromboplastin time
 - **Immunologic Assay**
 - Useful in characterizing patients as having a type I or II defect
 - Types
 - Electroimmunoassay
 - Enzyme-linked immunosorbent assay
 - Radioimmunoassay
- **Timing of testing:**
 - Wait 2 weeks after discontinuing oral vitamin K antagonist
 - Warfarin therapy reduces functional and immunologic measurement of protein C
 - Heparin therapy does not alter plasma protein C levels
 - Testing should not be performed during acute thrombosis or comorbid illness
 - Normal plasma protein C levels at presentation exclude deficiency
 - Low plasma protein C levels at presentation must be confirmed by repeat testing after anticoagulation is discontinued



Protein C Deficiency

- **Management**
 - **Thromboprophylaxis**
 - Strong family history of thrombosis
 - Pregnancy and the postpartum state
 - Surgery
 - Trauma
 - **VTE**
 - Lifelong anticoagulation following a spontaneous thromboembolic event
 - Ensure therapeutic aPTT or Xa levels before starting warfarin
 - Start low dose warfarin to avoid warfarin induced skin necrosis
 - **Warfarin-induced skin necrosis**
 - Immediate discontinuation of warfarin
 - Vitamin K administration
 - Heparin therapy
 - Exogenous protein C administration for patients with protein C deficiency
 - FFP
 - Purified protein C concentrate (Ceprotin)
 - **Neonatal purpura fulminans**
 - Immediate exogenous protein C administration for patients with protein C deficiency
 - Lifelong anticoagulation



Case Presentation

- 25 y.o. male presents to ER with spontaneous Doppler confirmed iliofemoral VTE. History is unremarkable for acquired hypercoagulable states but pt. remarks that his sister and mom both have had frequent fetal losses and were found “to have some sort of clotting disorder”. How would you manage this patient's VTE?



Case Presentation

- Suspect inherited hypercoagulable state and perform tests
- Initiate pt. on heparin drip



Case Presentation Continued

- Laboratory testing reveals that pt. has protein C deficiency
- How will your management of this patient change?



Case Presentation Continued

Answer:

- Repeat testing after acute event to confirm diagnosis
- Start low dose warfarin and titrate dose slowly to avoid warfarin induced skin necrosis
- Lifelong anticoagulation

Protein S Deficiency

- Vitamin K-dependent glycoprotein
- Enables activated protein C to inactivate factor Va and VIIIa at an increased rate
- Serves as a cofactor for protein C enhancement of fibrinolysis
- Directly inhibits prothrombin activation via interactions with other coagulation factors
- Synthesized by hepatocytes, endothelial cells, and megakaryocytes
- Circulates in two forms:
 - Free Form (40-50%)
 - Has activated protein C cofactor activity
 - Bound to the complement component, C4b-binding protein
- Average plasma concentration of total PS antigen in normal adults: 23 mcg/ml
- Total protein S levels increase with age
- Levels significantly lower and more variable in females than males

Protein S Deficiency

- Incidence
 - Occurs in 10% of families with inherited thrombophilia
 - Prevalence 1% among consecutive patients with 1st episode DVT
 - Prevalence 0.03 - 0.13% general population
- Homozygous protein S deficiency incompatible with life
- Inheritance of protein S is autosomal dominant
- Heterozygous individuals in these families frequently had recurrent thromboembolism
- Age at presentation
 - First thrombotic event occurs between 15-68 years (Mean age-28yrs)
 - 56% spontaneous
 - Remainder precipitated by an identifiable factor eg. age, surgery, immobility, pregnancy

Protein S Deficiency

- 2 Forms
 - Inherited
 - Caused by mutations in the PROS1 gene
 - 3 phenotypes of protein S deficiency
 - Type I-Quantitative defect
 - 50% of normal total S antigen level
 - Marked reductions in free protein S antigen and protein S functional activity
 - Type II-Qualitative defect
 - Normal total and free protein S levels
 - Diminished protein S functional activity
 - Type III
 - Normal total protein S antigen measurements
 - Selectively reduced levels of free protein S and protein S functional activity to <40% of normal
 - Acquired
 - Pregnancy
 - Oral Contraceptive use
 - Disseminated intravascular coagulation
 - Acute thromboembolic disease
 - L-asparaginase chemotherapy
 - Liver disease
 - Total and free protein S levels moderately decreased
 - Nephrotic syndrome
 - Increased total protein S antigen but reduced protein S functional assays
 - Loss of free protein S in the urine and elevation in plasma C4b-binding protein concentrations
 - HIV infection
 - Significantly reduced total and free protein S levels

Protein S Deficiency

- Clinical Presentation
 - Similar to patients with antithrombin and protein C deficiency
 - VTE
 - 8.5x higher lifetime probability of developing thrombosis
 - Presence of 2nd thrombotic defect increases risk of VTE
 - Risk of VTE is not increased in the absence of a family history of VTE
 - Axillary, mesenteric, and cerebral vein thrombosis
 - Arterial thrombosis reported but not common
 - Warfarin-induced skin necrosis
 - Thrombophlebitis
 - PE
 - Fetal loss

Protein S Deficiency

- Diagnosis
 - Difficult to document with certainty
 - Free protein S is the best screening test
 - Genetic testing not readily available
 - Testing restricted to VTE patients with strong family history
 - Total protein S antigen <60 - 65 international units/dL are considered to be in the deficient range
 - Free protein S levels <33 units/dL clinically significant for asymptomatic individuals and those with 1st VTE without a positive family history
 - Functional protein S assay
 - Larger coefficient of variation
 - Occasional false positive when factor V Leiden mutation present
 - Repeat testing necessary

Protein S Deficiency

- Assays:
 - Protein S antigen
 - Both free and total protein S are measured by ELISA methods in the laboratory
 - Total protein S antigen is measured by various types of immunoassay techniques
 - Dilution of plasma samples favors dissociation of the PS-C4b-binding protein complexes
 - Free protein S is measured using monoclonal antibody-based assay and ligand sorbent assays
 - Functional protein S assays
 - Based upon the ability of protein S to serve as a cofactor for the anticoagulant effect of activated protein C
 - Lack specificity for protein S thus can lead to erroneous diagnosis
- Timing of testing:
 - Wait 2 weeks after discontinuing oral vitamin K antagonist
 - Warfarin therapy substantially reduces antigenic and functional levels of protein S
 - Heparin therapy does not alter plasma protein S levels
 - Normal plasma protein S levels at presentation exclude deficiency
 - Low plasma protein S levels at presentation must be confirmed by repeat testing after anticoagulation is discontinued
 - Testing should not be performed during acute thrombosis or comorbid illness



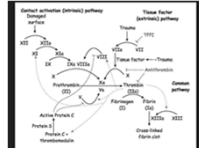
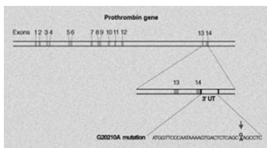
Protein S Deficiency

- Management of VTE
 - Heparin
 - IV heparin or SC LMWH for 1st 5 days
 - Warfarin
 - Initiate after 2 days and overlap with heparin for 5 days
 - Decision for lifelong anticoagulation made based on patient specific factors
- Prophylaxis
 - Administer heparin to asymptomatic carriers:
 - Pregnant
 - Undergoing surgery
 - Orthopedic surgery
 - Avoid medications that can cause clots eg. oral contraceptives



Prothrombin Gene Mutation

- Physiology
 - Prothrombin (factor II) is the precursor of thrombin, the end-product of the coagulation cascade
 - Vitamin k-dependent protein which is synthesized in the liver and circulates with a half-life of approximately 3-5 days
 - Prothrombin G20210A - human prothrombin gene
 - Guanine to adenine substitution at nucleotide 20210 of the prothrombin gene
 - Heterozygous carriers have 30 percent higher plasma prothrombin levels than normal



Prothrombin Gene Mutation

- Incidence
 - 2nd most common hereditary defect
 - Overall prevalence 2% in general Caucasian population
 - 0.7-6.5% of Caucasians heterozygous for the allele
 - Infrequent among individuals of Asian or African descent
 - Variability in geographic distribution
 - Spain (highest)
 - Europe (Southern higher than northern)



Prothrombin Gene Mutation

- Consequences
 - VTE
 - Increased risk for deep vein and cerebral vein thrombosis
 - Increased risk of cerebral vein thrombosis in patients using oral contraceptives
 - 2.8 fold increased risk for 1st episode DVT in both sexes and all age groups
 - Presence 2nd thrombotic defect (eg. factor V Leiden) increases the thrombotic risk 3-5 times over a single defect
 - Unclear increased risk of recurrent VTE
 - Increased risk of VTE in pregnancy
 - Arterial thrombosis
 - Not a risk factor for cerebrovascular ischemic disease in older patients
 - Possible increased risk in younger patients



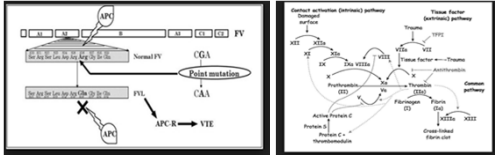
Prothrombin Gene Mutation

- Diagnosis
 - Polymerase chain reaction
 - Used to detect mutation
 - Plasma prothrombin activity & antigen levels
 - Cannot be used to screen patients due to significant overlap with the normal population



Factor V Leiden

- Physiology
 - Factor V circulates in plasma in inactive form
 - Thrombin activates factor V by limited proteolysis
 - Factor Va serves as a cofactor in the prothrombinase complex, which cleaves prothrombin to generate more thrombin, in a positive feedback loop
 - Single point mutation in factor V of arginine at position 506 to glutamine
 - Abolishes a cleavage site (Arg 506) of activated protein C
 - Renders factor V resistant to activated protein C inactivation
 - Accounts for >95% of cases of protein C resistance



Factor V Leiden

- Acquired causes of activated protein C resistance
 - Elevated factor VIII levels
 - Levels increased in inflammatory disorders and pregnancy
 - Increased estrogen
 - Mechanism
 - Reduced protein S
 - Increased prothrombin
 - Increased factors VIII, IX, X and others
 - Oral contraceptives
 - 2nd generation oc associated with acquired protein C resistance and increased thrombotic risk
 - Risk of thrombosis 1 in 345 (10x that of oc use in pt's w/o factor v leiden mutation)
 - Hormone Replacement Therapy
 - Hazard ratio of 2 for acquired protein C resistance
 - Pregnancy
 - Cancer
 - Antiphospholipid antibodies
 - Protein S deficiency
 - Can cause appearance of activated protein C resistance in some functional assays
 - Not an independent cause of activated protein C resistance
 - Other factors
 - Proteinuria, elevated body mass index, and smoking

Factor V Leiden

- Prevalence
 - Most common hereditary defect in Caucasians
 - Found in 1-8% of Caucasians (European, Jewish, Israeli, Arab, Canadian and Indian)
 - Accounts for 40-50% of cases
 - Not found in African Americans, Chinese, Japanese
 - Occurs in 5% of general population
 - Occurs in 20% of patients with 1st DVT
- 3.5 fold increased risk of VTE
- Risk of thrombosis increases dramatically in the homozygous, pseudohomozygote state or in the presence of a 2nd hereditary defect
- Homozygotes account for approximately 1% of patients with factor V leiden mutation

Factor V Leiden

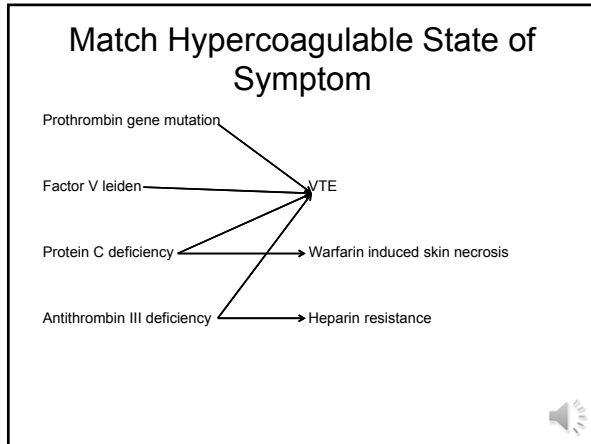
- Clinical Significance
 - Approximately 5% of factor V Leiden heterozygotes will experience venous thromboembolism during their lifetime
 - VTE
 - 10-26% of pt's with VTE have factor V Leiden mutation
 - Risk of VTE increases with advancing age
 - Major manifestation DVT±, PE (large proximal iliofemoral vein)
 - Isolated PE
 - Risk factor for cerebral, mesenteric, portal vein thrombosis and superficial vein thrombosis
 - Risk of recurrent DVT
 - Though conflicting data exists, presence of factor V Leiden mutation is likely not associated with increased risk of recurrent DVT
 - Does not increase overall mortality
 - Arterial thrombosis-weak or no association
 - Unexplained recurrent late pregnancy loss
 - Possibly due to thrombosis of placental vessels

Factor V Leiden

- Diagnosis
 - Genetic Testing
 - Detect directly by analyzing genomic DNA from peripheral blood mononuclear cells
 - Genetic testing which assays DNA sequence unaffected by anticoagulants and other drugs
 - Can determine if patient is homozygous or heterozygous for the mutation
 - Use in patients suspected of having inherited factor V leiden mutation based on family history
 - Functional activated protein C resistance assays
 - 1st Generation
 - Measure aPTT level using unaltered plasma
 - Not sensitive or specific for factor V leiden mutation
 - 2nd Generation-Preferred
 - Measure aPTT level after pt's plasma diluted with factor V deficient plasma
 - Limits influence of other coagulation factor levels on clotting time determinations
 - Correlate very well with the presence of factor V leiden mutation
 - Cost less than genetic tests
 - False normal results possible with:
 - » Presence of lupus anticoagulant
 - » Therapy with direct thrombin inhibitors eg. Argatroban, Dabigatran
 - » Therapy with factor Xa inhibitor eg. Rivaroxaban
 - Abnormal results should be confirmed by genotyping for the factor V leiden mutation

Match Hypercoagulable State of Symptom

| | |
|-----------------------------|--------------------------------|
| Prothrombin gene mutation | |
| Factor V leiden | VTE |
| Protein C deficiency | Warfarin induced skin necrosis |
| Antithrombin III deficiency | Heparin resistance |



Hyperhomocysteinemia

- Physiology
 - Homocysteine is a sulfur-containing amino acid involved in metabolic pathways leading to the formation of other amino acids
 - Deficiency in the cystathionine B-synthase (CBS) enzyme, defective methylcobalamin synthesis, or abnormality in methylene tetrahydrofolate reductase (MTHFR)
- Prevalence
 - Occurs in 5-7% of population

Hyperhomocysteinemia

- Causes
 - Genetic defects in the enzymes involved in homocysteine metabolism
 - Thermolabile variant of methylene tetrahydrofolate reductase (MTHFR)
 - Occurs in 5-14% of population
 - Nutritional deficiencies in vitamin cofactors
 - Folate
 - Vitamin B12
 - Vitamin B6
 - Cigarette smoking
 - Chronic kidney failure
 - Decreased renal removal and impaired metabolism
 - Medications
 - Fibrates and nicotinic acid, can raise homocysteine levels by approximately 30%
 - Metformin
 - Methotrexate
 - Cholestyramine

Hyperhomocysteinemia

- Vascular Injury
 - Intimal thickening
 - Elastic lamina disruption
 - Smooth muscle hypertrophy
 - Increases smooth muscle cell proliferation and enhances collagen production
 - Marked platelet accumulation
 - Impairment in endothelium-mediated platelet inhibition
 - Direct proaggregatory effects of homocysteine
 - Formation of platelet-rich occlusive thrombi
 - Atherosclerotic plaques
 - Thiolactone metabolite of homocysteine combines with LDL-cholesterol to produce aggregates that are taken up by vascular macrophages in the arterial intima
 - These foam cells may then release the lipid into atherosclerotic plaques
 - Direct injury to endothelial cells
 - Oxidative stress by free radicals formed during oxidation of reduced homocysteine

Hyperhomocysteinemia

- Prothrombotic effects
 - Attenuation of endothelial cell tissue plasminogen activator binding sites
 - Activation of factor VIIa and V
 - Inhibition of protein C and heparin sulfate
 - Increased fibrinopeptide A and prothrombin fragments 1 and 2
 - Increased blood viscosity
 - Decreased endothelial antithrombotic activity due to changes in thrombomodulin function

Hyperhomocysteinemia

- Clinical Significance
 - Increased risk of cardiovascular and cerebrovascular disease
 - Myocardial infarction, other acute coronary syndromes, and recurrent coronary events
 - Premature coronary heart disease
 - Atherosclerosis
 - Heart failure
 - Cardiovascular and total mortality
 - Adverse outcomes after angioplasty
 - Carotid artery stenosis
 - Stroke, recurrent stroke, and silent brain infarct
 - Venous thromboembolism
 - PE/DVT
 - Moderate hyperhomocysteinemia may be risk factor for recurrent VTE
 - Others
 - Obstetric complications
 - Birth defects
 - Osteoporosis
 - Dementia

Hyperhomocysteinemia

- **Diagnosis**
 - Laboratory assays measure total plasma homocysteine concentrations
 - Levels:
 - Normal
 - 5-15 µmol/L
 - Hyperhomocysteinemia:
 - Moderate: 15-30 µmol/L
 - Intermediate: 30-100 µmol/L
 - Severe: >100 µmol/L
- **Treatment**
 - Direct vitamin supplementation
 - Do not treat pt's w/ cardiovascular disease/VTE with vitamin supplementation aimed at decreasing homocysteine levels

Antiphospholipid Antibody Syndrome

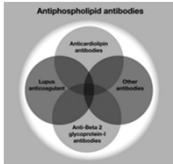
- **Physiology**
 - Coagulation reactions must take place on a phospholipid surface
 - Tissue injury damages cell membranes which allows negatively charged phospholipids (normally present on the underside of cell membranes) to come into contact with the blood
 - Phospholipids also appear on activated platelet surfaces
 - Several clotting factors bind directly to negatively charged phospholipids

Antiphospholipid Antibody Syndrome

- **Pathophysiology**
 - Immune system produce antibodies which attacks the phospholipids or proteins bound to phospholipids leading to increased clot formation
 - Occurs in susceptible patients following incidental exposure to infectious agents or in patients with rheumatic disease eg. SLE
 - Once antiphospholipid antibodies are present, a 2nd cause is required for the development of antiphospholipid antibody syndrome eg. Smoking, prolonged immobility, pregnancy, postpartum period, oral contraceptive use, hormone replacement therapy, malignancy, nephrotic syndrome etc..
 - Antibodies have procoagulant effect on:
 - Protein C
 - Annexin V
 - Platelets
 - Serum proteases
 - Toll-like receptors
 - Tissue factor
 - Impaired fibrinolysis
 - Antiphospholipid antibodies increase vascular tone
 - Atherosclerosis
 - Fetal loss
 - Neurological damage

Antiphospholipid Antibody Syndrome

- **Antiphospholipid antibodies**
 - Anti-Beta 2 glycoprotein 1 antibody
 - B2 glycoprotein
 - Phospholipid binding protein
 - Apolipoprotein H
 - Lupus anticoagulant
 - Anticardiolipin antibodies



Antiphospholipid Antibody Syndrome

- **Prevalence**
 - Occurs in 1-5% of population
 - Incidence: 5 cases per 100,000 persons per year
 - Twice as common in women than in men
 - Antiphospholipid antibody syndrome is the cause:
 - 14% of all strokes
 - 11% of MI
 - 10% of DVT
 - 6% of pregnancy morbidity
 - 9% of pregnancy losses
- **Recurrent events likely**
 - High rate of recurrence if anticoagulation is d/c'd 3months after initiation of anticoagulation
 - Lifelong anticoagulation recommended

Antiphospholipid Antibody Syndrome

- **Clinical Significance**
 - 30 fold increased risk for thrombosis
 - Venous thrombosis
 - DVT
 - Calf
 - Renal veins
 - Hepatic
 - Axillary
 - Subclavian
 - Retinal
 - Cerebral sinuses
 - Venae cavae
 - PE
 - Superficial thrombophlebitis
 - Arterial thrombosis
 - Stroke
 - Heart attack
 - Clots in the arteries of the arm, leg, eye, kidney, or abdomen
 - Pregnancy complications
 - High risk of pregnancy loss ≥ 10 weeks of pregnancy
 - Eclampsia
 - Preeclampsia
 - Placental insufficiency
 - Recurrent miscarriage

