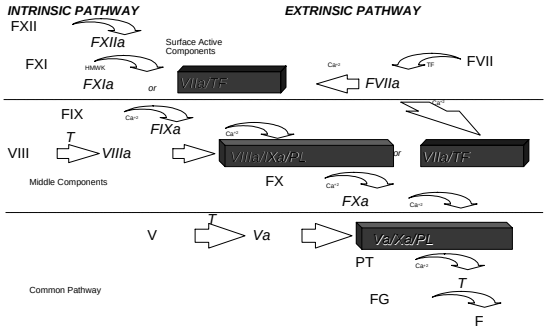
LABORATORY DIAGNOSIS OF PROTHROMBOTIC STATES

REGULATION OF COAGULATION
Introduction

- Coagulation necessary for maintenance of vascular integrity
- Enough fibrinogen to clot all vessels
- What controls clotting process?

COAGULATION CASCADE



COAGULATION INHIBITORS

- Tissue Factor Pathway Inhibitor (TFPI) Lipoprotein Associated Coagulation Inhibitor (LACI) Extrinsic Pathway Inhibitor (EPI)
  - Complexes with Factors VIIa/TF/Xa; inactivates Xa
- Antithrombin III/Heparin Cofactor II/Heparin
  - Binds and Inactivates Enzymes
- Protein C/Protein S/Thrombomodulin
  - Cleaves & Inactivates Cofactors (Va & VIIIa)
- Plasminogen - 3º hemostasis
  - Cleaves Fibrin

ANTICOAGULANT PROTEIN DEFICIENCY
Disease entities

- Heterozygous Protein Deficiency
  - Increased Venous Thrombosis
  - Occasional Increased Arterial Thrombosis
  - Warfarin Induced Skin Necrosis
- Homozygous Protein Deficiency
  - Neonatal Purpura Fulminans
  - Fibrinogenolysis
  - Chronic DIC

ANTICOAGULANT PROTEIN DEFICIENCY

- Dominant
  - Increased Venous Thrombosis
  - Young Age of Thrombosis
  - No Predisposing Factors to Thrombosis
  - Increased Thrombin Generation
  - Positive Family History
- Recessive
  - No history of thrombosis
  - No family history
  - Neonatal Purpura Fulminans
  - Increased Thrombin Generation

ACTIVATED PROTEIN C RESISTANCE

- 1st described by Dahlback, 1994
- Hallmark: Failure of activated Protein C to prolong aPTT
- First noted in screening of plasma samples of patients with increased clotting
- Functional defect described before protein defect noted

ACTIVATED PROTEIN C RESISTANCE

- Bertina et al described genetic defect
- Mutation of Arg 506 ▸ Gln
- Named Factor V Leiden
- Found in > 98% of patients with APC Resistance

ACTIVATED PROTEIN C RESISTANCE

- Extremely common (5-20% of Caucasian population with mutation)
- Increases risk of venous thromboembolism (VTE) c. 4x in heterozygous form, more in homozygous
- Can exist in combination with other defects (protein C, protein S, ATIII, plasminogen
- In combination, has synergistic effect on other anticoagulant protein deficiencies

FACTOR V LEIDEN

- Normal procoagulant activity
- Inactivated slowly by activated protein C
- Leads to increased prothrombinase complex activity due to failure to remove factor Va
- Patients also display increased factor VIIIa/tenase activity

PROTEIN C - MECHANISM OF ACTION

FACTOR Va INACTIVATION

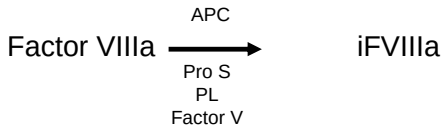
Factor Va $\xrightarrow[\text{Pro S, PL}]{\text{APC}}$ iFVa

FACTOR VIIIa INACTIVATION

- Factor V is cofactor for Factor VIIIa inactivation
- Factor V Leiden unable to act as cofactor in VIIIa inactivation
- Therefore, increased VIIIa inactivation ▸ increased tenase activity

PROTEIN C - MECHANISM OF ACTION

FACTOR VIIIa INACTIVATION



HYPERCOAGULABLE STATES

Prothrombin G20210 ▸ A

- First described by Poort et al, 11/96
- Mutation in 3' non-coding sequence of prothrombin gene
- Northern European mutation (still being studied in non-European populations)

HYPERCOAGULABLE STATES

Prothrombin G20210 ▸ A

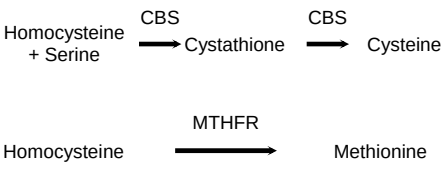
- Mechanism of increased thrombosis unknown
- Increased prothrombin synthesis seen (> 115% of normal)
- Implicated in both arterial (stroke) and venous thrombosis as well as pregnancy-related thrombosis

HYPERCOAGULABLE STATES

Hyperhomocysteinemia

- Inborn error of metabolism
- Leads to buildup of homocysteine via several pathways
- Homozygous form associated with mental retardation, microcephaly, nephrolithiasis, seizure disorder, accelerated atherosclerosis, marked increase in thromboembolic disease
- Heterozygous form assoc. with mildly increased thromboembolic disease but not other problems

HYPERCOAGULABLE STATES Hyperhomocysteinemia



HYPERCOAGULABLE STATES

Hyperhomocysteinemia - Causes

- Vitamin B_{12} deficiency
- Folic acid deficiency
- Vitamin B_6 deficiency
- Cysthathione synthase deficiency (classic form)
- Methyl tetrahydrofolate reductase deficiency (most common by far)

HYPERCOAGULABLE STATES

Hyperhomocysteinemia - Diagnosis

- Fasting homocysteine levels; considerable variability depending on assay
- Methionine loading if clinical suspicion high, but can precipitate thrombosis
- Methyl tetrahydrofolate reductase mutation (MTHFR C677 ▸ T) - Only relevant if homozygous

HYPERCOAGULABLE STATES

Acquired

- Anticardiolipin Syndrome
- Malignancy
- Immobilization
- TTP
- DIC
- Oral Contraceptive Therapy
- Prosthetic Valves
- PNH
- Myeloproliferative
- diseases
- Nephrotic Syndrome
- Inflammatory Diseases
- Atherosclerosis
- Surgery
- Diabetes mellitus

ANTICARDIOLIPIN ANTIBODY

Lupus Anticoagulant

- Not necessarily associated with lupus (< 50%)
- Not associated with bleeding except in rare circumstances
- Associated with thrombosis - arterial & venous
- Associated with false (+) RPR
- Associated with recurrent spontaneous abortions
- Mechanism of thrombotic tendency unknown

LUPUS ANTICOAGULANT

- Caused by antiphospholipid antibodies that interfere with clotting process *in vitro* but not *in vivo*
- Dilute phospholipid so level of phospholipid becomes rate-limiting
- Many add confirmatory study of either aPTT with platelets as PL source or orthogonal PL as PL source

ANTIPHOSPHOLIPID ANTIBODY

Assay

- Usually antigenic as opposed to functional assay
- True antigen is source of controversy- ? if phospholipid is true antigen or if associated protein is true antigen
- ? Pathogenicity of what is being measured
- Impossible to standardize assay even batch-to-batch of reagents

ACQUIRED HYPERCOAGULABLE STATES

Mechanisms in Acute Inflammation

- C4b Binding Protein - Acute Phase Reactant
  - Increases in inflammatory diseases
  - Binds to Protein S
  - Bound Protein S inactive as cofactor
- Inflammation Increased IL-1 & TNF
  - Both downregulate thrombomodulin
  - Thrombin becomes procoagulant instead of anticoagulant protein

PROTHROMBOTIC DISORDERS

Summary

- No screening test readily available
- Probably look at genetic tests 1st
  - Factor V Leiden
  - Prothrombin G20210A
  - MTHFR mutation
- Antiphospholipid antibody studies
- Homocysteine levels
- Protein C, Protein S, ATIII, Plasminogen
- Look for signs of inflammation
- Consider prolonged anticoagulant Rx if any of above positive
- Screen family for disease if positive