Laboratory Diagnosis of Prothrombotic Disorders

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 - 3rd hemostasis
  - Cleaves Fibrin

Coagulation Cascade

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

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

**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

**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 Va INACTIVATION**
- Factor Va
- APC
- Pro S
- PL
- iFVa

**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
PROTEIN C - MECHANISM OF ACTION

FACTOR VIIIa INACTIVATION

\[ \text{APC} \]

\[ \text{Factor VIIIa} \rightarrow \text{iFVIIIa} \]

PROTEIN C - MECHANISM OF ACTION

HYPERCOAGULABLE STATES

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

Prothrombin G20210 ▶ A

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

Prothrombin G20210 ▶ A

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HYPERCOAGULABLE STATES

Prothrombin G20210 ▶ A

Hyperhomocysteinemia - Causes

Vitamin B12 deficiency

Folic acid deficiency

Vitamin B6 deficiency

Cystathionine 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

ANTSICARDIOLIPIN 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 (PROBABLY NOT)
- 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

**Heparin-Induced Thrombocytopenia (HIT): Terminology**

<table>
<thead>
<tr>
<th>HIT Type I</th>
<th>HIT Type II</th>
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<tbody>
<tr>
<td>Transient, mild, non-immune mediated</td>
<td>Not transient, severe, immune mediated</td>
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<tr>
<td>Early onset (&lt;4 d of heparin treatment)</td>
<td>Typically 4 to 14 d after start of heparin</td>
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<tr>
<td>Reversible, asymptomatic</td>
<td>Can occur within 12 h with recent exposure</td>
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<tr>
<td>Associated with thromboembolic complications (HIT with TECs) also known as HITTS</td>
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**Heparin-Induced Thrombocytopenia (HIT): Paradoxes**

- Anticoagulant-induced thrombosis
- Clotting disorder, not bleeding disorder
- Platelet transfusions can increase thrombosis risk
- Simply stopping heparin may not prevent thrombosis
- Warfarin contraindicated as acute monotherapy

**Heparin-Induced Thrombocytopenia (HIT): Pathophysiology**

*Places patient at greater risk from primary thrombotic problem.*


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**Heparin-Induced Thrombocytopenia (HIT): An Overview**

More than 1 trillion units of heparin are used each year in the United States.

Prevalence: up to 1% to 3% of heparin-treated patients.

Consequences: ~50% of untreated HIT patients are at risk for developing life- or limb-threatening thromboembolic complications (TECs).

Management: immediate cessation of heparin; strongly consider use of alternative anticoagulant.
Laboratory Diagnosis of Prothrombotic Disorders

Heparin-Induced Thrombocytopenia (HIT): The Nature of Heparin Exposure

- HIT can occur with any exposure to heparin
  - Type of heparin: UFH > LMWH
  - Dose and duration: high dose > low dose
  - Dose and duration of current exposure: long-term > short-term
  - Route of administration: IV > SC, flushes, catheters, heparin-coated devices
  - Clinical setting: especially cardiac, orthopedic, or intensive care


Laboratory Testing for HIT

<table>
<thead>
<tr>
<th>Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>SRA</td>
<td>Sensitivity: high</td>
<td>Technically demanding (radioisotopes)</td>
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<tr>
<td></td>
<td>Specificity: high</td>
<td>Not readily available</td>
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<tr>
<td></td>
<td>(false positives rare)</td>
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</tr>
<tr>
<td>Platelet aggregation</td>
<td>Specificity: high</td>
<td>Sensitivity: low</td>
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<tr>
<td></td>
<td>Rapid turnaround time</td>
<td>Technique-dependent</td>
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<tr>
<td>ELISA</td>
<td>Sensitivity: high</td>
<td>Specificity: low (false positives common for some populations)</td>
</tr>
<tr>
<td></td>
<td>Technically easy</td>
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</tr>
</tbody>
</table>

HIT Requires a Clinical Diagnosis

SRA=serotonin-release assay; ELISA=enzyme-linked immunosorbent assay.