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

October 14, 2003 9:00 am

COAGULATION CASCADE

INTRINSIC PATHWAY
FXII
FXI
FIX
VIII

Surface Active Components
VIIIa/VIIIa/IXa
IXa/PL/PL or
VIIa/TF/VIIa/TF

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

![Diagram showing the process of Factor Va inactivation involving APC, Pro S, and PL.]

**Factor V** is a cofactor for the inactivation of **Factor VIIIa**.

- **Factor V Leiden** is unable to act as a cofactor in VIIIa inactivation.
- Therefore, there is increased VIIIa inactivation, leading to increased tenase activity.

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**FACTOR VIIIa INACTIVATION**

- Factor V is a cofactor for Factor VIIIa inactivation.
- Factor V Leiden is unable to act as a cofactor in VIIIa inactivation.
- Therefore, there is increased VIIIa inactivation, with increased tenase activity.
PROTEIN C - MECHANISM OF ACTION

FACTOR VIIIa INACTIVATION

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

Protein C - Mechanism of Action

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

Hyperhomocysteinemia - Causes

- Vitamin B₁₂ deficiency
- Folic acid deficiency
- Vitamin B₆ deficiency
- Cystathione 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 C677T) - 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
Laboratory Diagnosis of Prothrombotic Disorders

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)
- Immunoglobulin-mediated allergic reaction to heparin/platelet factor 4 complex
- Thrombocytopenia
  - Platelet count <150,000 thrombocytes/µL or a 30% to 50% drop from baseline during heparin exposure
  - Onset 5 to 14 days after initiating heparin
- With or without thrombotic complications
- Any type of heparin or route of administration can lead to HIT

Heparin-induced Thrombocytopenia (HIT): An Overview

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

Prevalence: up to 1% to 3% of heparin-treated patients.\(^2\)

Consequences: ~50% of untreated HIT patients are at risk for developing life- or limb-threatening thromboembolic complications (TECs).\(^3\)

Management: immediate cessation of heparin; strongly consider use of alternative anticoagulant.\(^4,5\)

Heparin-Induced Thrombocytopenia (HIT): Terminology

**HIT Type I\(^1,2\)**
- Transient, mild, non-immune mediated
- Early onset (<4 d of heparin treatment)
- Reversible, asymptomatic

**HIT Type II\(^1\)**
- Not transient, severe, immune mediated
- Typically 4 to 14 d after start of heparin
  - Can occur within 12 h with recent exposure
- Associated with thromboembolic complications (HIT with TECs)
  - also known as HITTS

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


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Heparin-Induced Thrombocytopenia (HIT): Pathophysiology

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

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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</thead>
<tbody>
<tr>
<td>SRA</td>
<td>Sensitivity: high</td>
<td>Technically demanding (radioisotopes)</td>
</tr>
<tr>
<td></td>
<td>Specificity: high</td>
<td>Not readily available</td>
</tr>
<tr>
<td></td>
<td>(false positives rare)</td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>Specificity: high</td>
<td>Sensitivity: low</td>
</tr>
<tr>
<td>aggregation</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</td>
</tr>
<tr>
<td></td>
<td>Technically easy</td>
<td>some populations)</td>
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HIT Requires a Clinical Diagnosis

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