Laboratory Diagnosis of Prothrombotic Disorders

REGULATION OF COAGULATION
Introduction

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

Prothrombotic States

- No screening test available
- ? – How do we make the diagnosis of a prothrombotic condition?
- Does diagnosis of a prothrombotic state lead to a change in treatment?

COAGULATION CASCADE

Common Pathway

Intrinsic Pathway

Extrinsic Pathway

COAGULATION INHIBITORS

- Tissue Factor Pathway Inhibitor (TFPI)
  - 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

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HYPERCOAGULABLE STATES
Acquired
- Antiphospholipid Antibody Syndrome
- Malignancy
- Immobilization
- TTP
- DIC
- Oral Contraceptive Therapy
- Prosthetic Valves
- HIT
- Paroxysmal Nocturnal Hemoglobinuria
- Myeloproliferative diseases
- Nephrotic Syndrome
- Inflammatory Diseases
- Atherosclerosis
- Surgery
- Diabetes mellitus

Prothrombotic State - Diagnosis
- Prevalence of all inherited prothrombotic conditions - ? 1/15 people
- Mostly autosomal dominant conditions, with incomplete penetrance
- Therapy for prothrombotic conditions problematic – risk of bleeding from anticoagulants often outweighs risks of thrombosis

Venous Thromboembolism
- Prevalence in general population: c. 0.1% risk of developing VTE over a lifetime
- Risk of warfarin therapy: c. 9%/patient-year of significant bleed
- Therefore, prophylactic therapy not warranted

Prothrombotic States
- Should not screen general population
- ? Utility of screening at-risk populations
  - Patients with history of VTE
  - Patients with family history of VTE
  - Immobilized patients
  - Paraplegic/hemiplegic patients

Prothrombotic States
- Screening
  - Screening for:
    - Protein C, Protein S, Antithrombin III, Plasminogen deficiency
    - In Patients with
      - VTE, Age < 40
      - Recurrent VTE
      - Family Hx VTE
      - Incidence of one of above disorders 30%
    - For all other populations, incidence < 10%

Prothrombotic States
- These are synergistic with other defects, such that
- Multiple defects lead to multiplication of risk
- In general, if acute risk of thrombosis is greater than 3-5%, probably worthwhile using prophylactic therapy, & therefore probably worthwhile screening

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
  - 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.
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.

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)</td>
<td>Also known as HITTS</td>
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Heparin-Induced Thrombocytopenia (HIT): Pathophysiology

1. 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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<tbody>
<tr>
<td>SRA</td>
<td>Sensitivity: high</td>
<td>Technically demanding (radioisotopes)</td>
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<tr>
<td></td>
<td>Specificity: high (false positives rare)</td>
<td>Not readily available</td>
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<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>
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<tr>
<td></td>
<td>Technically easy</td>
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**HIT Requires a Clinical Diagnosis**