# LABORATORY DIAGNOSIS OF PROTHROMBOTIC STATES

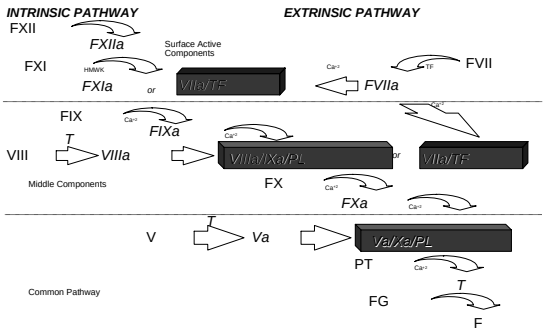
## REGULATION OF COAGULATION
*Introduction*

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

## 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




## 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%

From: Heijboer, H. et al; NEJM 323: 1512 (1990)

## Prothrombotic States - Screening

- Before discovery of Factor V Leiden & Prothrombin G20210A mutations
- Together c. 6-7% of northern European populations are positive for one of these mutations
- Factor V Leiden increases VTE risk c. 4x over lifetime; Prothrombin G20210A c. 2-3x over lifetime

## 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

Deitcher. *Formulary*. 2001;36:26-41; Kelton. *Semin Hematol*. 1999;36(suppl 1):17-21; Matthai. *Semin Thromb Hemost*. 1999;25(suppl 1):57-60; Warkentin et al. *N Engl J Med*. 1995;332:1330-1335; Warkentin. *Thromb Haemost*. 1999;82:439-447.

## 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]

1. Fahey VA. *J Vasc Nurs*. 2. Warkentin TE et al. *N Engl J Med*. 3. Warkentin TE, Kelton JG. *Am J Med*. 4. Warkentin TE, Barkin KL. *Pharmacotherapy*. 5. Chong BH. *Brit J Haematol*.

## Heparin-Induced Thrombocytopenia (HIT): Terminology

| HIT Type I[1,2] | HIT Type II[1-4] |
|---|---|
| Transient, mild, non–immune mediated | Not transient, severe, immune mediated |
| Early onset (<4 d of heparin treatment) | Typically 4 to 14 d after start of heparin<br>– Can occur within 12 h with recent exposure |
| Reversible, asymptomatic | Associated with thromboembolic complications (HIT with TECs)<br>also known as HITTS |

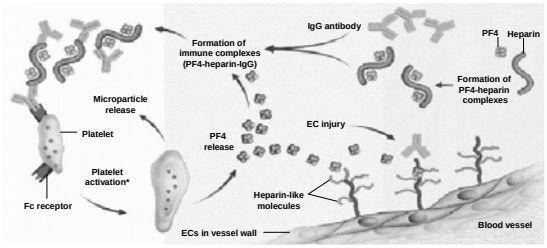
1. Warkentin TE, Greinacher A, eds. *Heparin-Induced Thrombocytopenia*. 2. Chong BH. *Br J Haematol*. 3. Lewis BE et al. *Circulation*. 4. Greinacher A et al. *Circulation*.

## Heparin-Induced Thrombocytopenia (HIT): Paradoxes[1]

- 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

1. Warkentin TE. *Thromb Haemost*.

## Heparin-Induced Thrombocytopenia (HIT): Pathophysiology[1]




*Places patient at greater risk from primary thrombotic problem.

1. Adapted from Aster RH. *N Engl J Med*. 1995;332(20):1374-1376.

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

- HIT can occur with any exposure to heparin
  - Type of heparin: UFH > LMWH[1]
  - Dose and duration: high dose > low dose
  - Dose and duration of current exposure: long-term > short-term[1]
  - Route of administration: IV > SC, flushes, catheters, heparin-coated devices[2-4]
  - Clinical setting: especially cardiac, orthopedic, or intensive care[1,2]

1. Warkentin TE. *Drug Saf*. 2. Warkentin TE, Greinacher A, eds. *Heparin-Induced Thrombocytopenia*. 3. Nand S et al. *Am J Hematol*. 4. Kadidal VV et al. *J Intern Med*.

## Laboratory Testing for HIT

| Test | Advantages | Disadvantages |
|---|---|---|
| **SRA** | Sensitivity: high<br>Specificity: high<br>(false positives rare) | Technically demanding<br>(radioisotopes)<br>Not readily available |
| **Platelet aggregation** | Specificity: high<br>Rapid turnaround time | Sensitivity: low<br>Technique-dependent |
| **ELISA** | Sensitivity: high<br>Technically easy | Specificity: low (false positives common for some populations) |

**HIT Requires a Clinical Diagnosis**

SRA=serotonin-release assay; ELISA=enzyme-linked immunosorbent assay.
Fabris et al. *Arch Pathol Lab Med.* 2000;124:1657-1666; Kelton. *Semin Hematol.* 1999;36(suppl 1):17-21.