HEMOSTASIS/THROMBOSIS III

Regulation of Coagulation/
Disseminated Intravascular Coagulation

REGULATION OF COAGULATION

Introduction

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

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
COAGULATION CASCADE

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ANTITHROMBIN III – Mechanism of Action

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PROTEIN C/PROTEIN S – Mechanism of Action

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- Plasminogen - 3rd hemostasis
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ANTICOAGULANT PROTEIN DEFICIENCY

**Disease entities**

- **Heterozygous Protein Deficiency**
  - Increased Venous Thrombosis
  - Occasional Increased Arterial Thrombosis
- **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 in offspring
  - 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

PROTEIN C - MECHANISM OF ACTION

FACTOR Va INACTIVATION

\[ \text{Factor Va} \xrightarrow{\text{APC}} \text{iFVa} \]
PROTEIN C - MECHANISM OF ACTION

FACTOR VIIIa INACTIVATION

APC

Factor VIIIa → iFVIIIa

Pro S
PL
Factor V

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
- Found in 1-3% of persons of Northern European descent
HYPERCOAGULABLE STATES

Prothrombin G20210 ▶ A

- Increased prothrombin synthesis seen (> 115% of normal)
- Primary risk is in pregnancy-associated thrombosis & venous thromboembolic disease
- ??? Increased risk of stroke
- Mechanism of increased thrombosis unknown

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

Homocysteine + Serine \(\xrightarrow{CBS}\) Cystathione \(\xrightarrow{CBS}\) Cysteine

Homocysteine \(\xrightarrow{MTHFR}\) Methionine

HYPERCOAGULABLE STATES
Hyperhomocysteinemia - Causes

- Vitamin B\(_{12}\) deficiency
- Folic acid deficiency
- Vitamin B\(_6\) 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 C677 ▶ T) - Only relevant if homozygous

HYPERCOAGULABLE STATES

Acquired

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

Mechanisms

- 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

NEPHROTIC SYNDROME

- Loss of glomerular filtration & reabsorption capability
- Leads to excretion of large amounts of protein in the urine, including
  - Antithrombin III (MW 65,000)
  - Protein S (MW 70,000)
  - Protein C (MW 56,000)
NEPHROTIC SYNDROME (2)

- C4b Binding Protein has MW c. 250,000, & is markedly elevated in nephrotic syndrome
- Therefore, any protein S left in the circulation is bound to C4b Binding Protein & is inactive as an anticoagulant

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
DIC

- Acute
  - Shock
  - Sepsis
  - Allergic reactions
  - Mismatched transfusion
  - Obstetrical problems
  - Trauma
  - Burns
  - Extracorporeal circulation
  - Acidosis
  - Purpura fulminans

- Subacute/Chronic
  - Acute leukemia
  - Carcinomas
  - Hemangiomata
  - Aortic aneurysms
  - ??? liver disease

ACUTE DIC

- Almost always secondary
- Consumptive coagulopathy
- Decreases in both coagulants & anticoagulants
- Severity may relate to levels of anticoagulants
DIC

Plasminogen Activation

F XII

F XIIa

Prekallikrein

Kallikrein

Urokinase

TPA

Plasminogen

Plasmin

Fibrinogen SPLIT PRODUCTS

Fibrinogen, 340,000

Fragment X, 250,000

Fragment D, 90,000

Fragment Y, 150,000

Fragment E, 50,000

Fragment D, 90,000
DEFIBRINATION

**Mechanisms**

- Release of Tissue Procoagulants
  - Tumor
  - Fetal/Placental/Amniotic
  - Prostatic
  - Pancreatic
  - WBC
  - RBC
  - Shock

- Damage to Vascular Tree
  - Septicemia
  - Aortic aneurysm
  - Hemangioma
  - Tumor emboli
  - ? Shock

- Decreased Clearance
  - Liver disease
  - ? Shock

DIC

**Testing (Acute)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin Time</td>
<td>Slightly to grossly prolonged</td>
</tr>
<tr>
<td>aPTT</td>
<td>Variable</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Usually low</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>Usually prolonged</td>
</tr>
<tr>
<td>Factor levels</td>
<td>Variable</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>Usually low</td>
</tr>
<tr>
<td>RBC fragmentation</td>
<td>Sometimes present</td>
</tr>
<tr>
<td>Fibrin split products</td>
<td>Usually present</td>
</tr>
</tbody>
</table>
DIC

Therapy

- Depends on primary manifestation
  - Thrombosis - Anticoagulant therapy
  - Bleeding - Replacement therapy
- Primary treatment
  - TREAT UNDERLYING DISEASE
- Replacement
  - Cryoprecipitate - Fibrinogen
  - Fresh frozen plasma - Other factors
  - Platelets
- Heparin
  - Rarely indicated

LIVER DISEASE

- Factor deficiencies - 2° Decreased synthesis
- Abnormal fibrinogen
  - Excess sialic acid
  - Prolonged thrombin time
- Low Grade DIC - Difficult Dx to make
- Increased fibrinolysis
  - Plasminogen activator inhibitor
  - α-2 antiplasmin
  - Tissue plasminogen activator