LABORATORY DIAGNOSIS OF BLEEDING DISORDERS

Secondary Hemostasis Disorders

HEMOSTASIS
Primary vs. Secondary vs. Tertiary

- Primary Hemostasis
  - Platelet Plug Formation
  - Dependent on normal platelet number & function
- Secondary Hemostasis
  - Activation of Clotting Cascade
  - Deposition & Stabilization of Fibrin
- Tertiary Hemostasis
  - Dissolution of Fibrin Clot
  - Dependent on Plasminogen Activation

CIRCULATORY SYSTEM

- Low volume, high pressure system
- Efficient for nutrient delivery to tissues
- Prone to leakage due to endothelial surface damage
- Small volume loss results in large decrease in nutrient delivery
- Minimal extravasation in critical areas may lead to irreparable damage/death of organism

HEMOSTATIC DISORDERS

- History critical to assessment of presence of disorder
  - History of bleeding problems in the family
  - History of spontaneous bleeding
  - History of heavy menses
  - History of easy bruising
  - History of prior blood transfusion
  - History of prior tooth extractions
  - History of prior surgery/pregnancy
- Physical exam rarely useful except for petechiae or severe hemophiliac arthropathy
- Laboratory essential for determining specific defect & monitoring effects of therapy

COAGULATION TESTING
Basic Testing

- Prothrombin Time
- Activated partial thromboplastin time (aPTT)
- Thrombin Time (Thrombin added to plasma, & time to clot measured)
- Fibrinogen
- Platelet Count
- Bleeding Time

COAGULATION CASCADE
General Features

- Zymogens converted to enzymes by limited proteolysis
- Complex formation requiring calcium, phospholipid surface, cofactors
- Thrombin converts fibrinogen to fibrin monomer
- Fibrin monomer crosslinked to fibrin
- Forms "glue" for platelet plug
**Laboratory Diagnosis of Bleeding Disorders**

October 13, 2003 10:00 am

---

**COAGULATION CASCADE**

**INTRINSIC PATHWAY**

- FXII
- FXIIa
- Surface Active Components

**EXTRINSIC PATHWAY**

- FX
- FXa
- FXIIa/VIIa/T

**Common Pathway**

- FX
- FXa
- Ca+2

**Middle Components**

- FXIIa
- FXIa
- FXIIa/T

**Surface Active Components**

- V
- Va
- FXa
- FXIIa/VIIa/T

---

**CLOTTING FACTOR DEFICIENCY**

* Determination of missing factor

- Done only if one of screening tests is abnormal
- Run panel of assays corresponding to the abnormal screening test, using factor deficient plasmas
  - PT abnormal - Factors II, V, VII, X
  - aPTT abnormal - Factors XII, XI, IX, VIII

---

**CLOTTING FACTOR DEFICIENCY**

* Determination of missing factor

- For all but the deficient factor, there will be 50% of normal level of all factors, & clotting assay will be normal
- For missing factor, clotting time will be prolonged
- If more than one factor level abnormal, implies inhibitor to clotting testing

---

**CLOTTING FACTOR DEFICIENCY**

* Circulating Inhibitor to Clotting Protein

- Mixing studies will be abnormal
- Need to ensure no heparin is in the specimen
- Important to distinguish lupus anticoagulant from circulating anticoagulant to a clotting factor
  - Former associated with thrombosis
  - Latter with major hemorrhage
- Factor to which inhibitor is directed needs to be determined, along with titer of inhibitor
### HEMOPHILIA

**Clinical Severity - Correlates with Factor Level**
- **Mild** – > 5% factor level – Bleeding only with significant trauma or surgery; only occasional hemarthroses, with trauma
- **Moderate** – 1–5% factor level – Bleeding with mild trauma; hemarthroses with trauma; occasionally spontaneous hemarthroses
- **Severe** – < 1% factor level – Spontaneous hemarthroses and soft tissue bleeding
- Within each kindred, similar severity of disease
- Multiple genetic defects
  - Factor IX > 1000
  - Factor VIII > 1000

### VON WILLEBRAND FACTOR

- Large Adhesive Glycoprotein
- Polypeptide chain: 220,000 MW
- Base structure: Dimer; Can have as many as 20 linked dimers
- Multimers linked by disulfide bridges
- Synthesized in endothelial cells & megakaryocytes
- Constitutive & stimulated secretion
- Large multimers stored in Weibel-Palade bodies
- Functions:
  1) Stabilizes Factor VIII
  2) Essential for platelet adhesion

### HEMOPHILIA

- Sex–linked recessive disease
- Disease dates at least to days of Talmud
- Incidence: 20/100,000 males
- 85% Hemophilia A; 15% Hemophilia B
- Clinically indistinguishable except by factor analysis
- Genetic lethal without replacement therapy

### VON WILLEBRAND DISEASE

- Autosomal Dominant Inheritance
- Variable Penetrance
- 1953 - Patients lack factor VIII
- 1957 - Plasma from hemophiliac increase in factor VIII
- 1976 - Von Willebrand Antigen discovered
- Prevalence: 0.8–1.6% (probable underestimate)
- Generally mild bleeding disorder
- Variable test results

### Factor XI Deficiency

- 4th most common bleeding disorder
- Mostly found in Ashkenazi Jews
- Mild bleeding disorder; bleeding mostly seen with procedures/accidents
- Levels don’t correlate with bleeding tendency
- Most common cause of lawsuits vs. coagulationists

### VON WILLEBRAND DISEASE

**Diagnostic Studies**
- aPTT - Prolonged
- vWF Activity Level (Ristocetin Cofactor Activity) - Decreased
- vWF Antigen Level (“Factor VIII Antigen”) - Decreased
- Factor VIII Activity - Decreased
- Bleeding Time - Increased
- Ristocetin-Induced Platelet Aggregation - Decreased
- Multimer Structure - Variable
VON WILLEBRAND DISEASE

Classification
- Type I – Quantitative Defect
- Type II – Qualitative Defect
  - Type IIa – No multimer formation
  - Type IIb – Decreased multimers, decreased platelets
  - Type IIc – Other Protein Defects
  - Type IIIn – Defect in Factor VIII Binding
- Type III – Severe Quantitative Defect

VON WILLEBRAND DISEASE

Treatment
- DDAVP – Releases vWF from stores
  - 70% respond; must test prior to use in critical situation
- Humate-P – Factor VIII concentrate rich in vWF; approved for Rx of vWD
  - Dosage: 60-80 units/kg initial dose
- Cryoprecipitate – Gold standard; 40 units/kg for 0-100% of normal; ½ life 12-24 hours

HEMOPHILIA vs. VON WILLEBRAND DISEASE

<table>
<thead>
<tr>
<th>Test</th>
<th>Hemophilia A</th>
<th>Von Willebrand Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding time</td>
<td>Normal</td>
<td>Prolonged</td>
</tr>
<tr>
<td>aPTT</td>
<td>Prolonged</td>
<td>Prolonged</td>
</tr>
</tbody>
</table>

FACTOR VIII vs. VWF

<table>
<thead>
<tr>
<th>Von Willebrand Factor VIII</th>
<th>Platelet adhesion, Factor VIII stability</th>
<th>Fibrin Clot Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of synthesis</td>
<td>Endothelial cells, Megakaryocytes</td>
<td>Hepatocytes</td>
</tr>
<tr>
<td>Genetic control</td>
<td>Autosomal dominant</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>Normal</td>
<td>Low</td>
</tr>
<tr>
<td>Von Willebrand Disease</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

Hemophilia A - Treatment

- Plasma-derived Factor VIII
  - Now virally inactivated; safest blood products derived from humans
    - Intermediate purity – Cheapest, but does result in immune deficiency
    - Monoclonal purified – 1.5-2X the cost of intermediate purity; most common product used
- Recombinant Factor VIII
  - No more effective than plasma-derived factor VIII
  - 2x cost of monoclonal purified factor VIII
**Initial Therapy of Hemophilia B**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Hemophilia B Factor IX:C (U/kg)</th>
<th>Factor IX Desired Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Hemorrhage</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Major Hemorrhage</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Life-Threatening Hemorrhage</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>


**Hemophilia B - Treatment**

- Monoclonal purified product – Most effective; virally inactivated
- Recombinant Factor IX
  - Slightly less effective for equivalent units
  - Priced: Same as monoclonal purified factor IX
  - Used almost exclusively at present

**CLOTTING FACTOR DEFICIENCY**

**Treatment**

- For Factor XII & above, no treatment needed
- FFP for Factor XI deficiency, factor XIII deficiency
- Cryoprecipitate for low fibrinogen, factor XIII deficiency
- Factor IX concentrate for deficiency of Vitamin K-dependent clotting factors (important to make sure the one you are using has the factor that you need)

**CLOTTING DISORDERS**

**Acquired**

- Vitamin K deficiency
- Liver disease
- Coumadin therapy
- Heparin therapy
- Disseminated Intravascular Coagulation

**VON WILLEBRAND DISEASE**

**Therapy**

- Goal: Correct bleeding time and Factor VIII level
- Ideal test for monitoring efficacy of therapy never documented
- Treatment usually needed only for surgery or major trauma
- DDAVP (Desmopressin - 0.3 μg/kg by infusion
  - Often effective for Type I; tachyphylaxis develops
  - Ineffective in Type IIa; relatively contraindicated in Type IIb
  - MUST TEST FOR EFFICACY PRIOR TO USE
- Cryoprecipitate - 1000-1200 units every 12 hours for Types I & II vWD; 2000-2400 units every 12 hours for Type III vWD
- Factor VIII concentrate - Do not use, except:
  - Humate-P (only one containing significant vWF)

**VITAMIN K DEFICIENCY**

- Almost always hospitalized patients
- Require both malnutrition & decrease in gut flora
- PT goes up 1st, 2º to factor VII's short half-life
- Treatment: Replacement Vitamin K
- Response within 24-48 hours
CLOTTING DISORDERS

Acquired

- Vitamin K deficiency
- Liver disease
- Coumadin therapy
- Heparin therapy
- Disseminated Intravascular Coagulation

LIVER DISEASE

- Decreased synthesis, vitamin K dependent proteins
- Decreased clearance, activated clotting factors
- Increased fibrinolysis 2º to decreased antiplasmin
- Dysfibrinogenemia 2º to synthesis of abnormal fibrinogen
- Increased fibrin split products
- Increased PT, aPTT, TT
- Decreased platelets (hypersplenism)
- Treatment: Replacement therapy
  - Reserved for bleeding/procedure