Hemostasis/Thrombosis I

Normal Hemostasis/Thrombosis;
Assessment of Clotting System

HEMOSTASIS & THROMBOSIS

- Platelets
- Coagulation Cascade
- Regulation of Coagulation
CIRCULATORY SYSTEM

- Low volume, high pressure system
- Efficient for nutrient delivery to tissues
- Prone to leakage 2º to endothelial surface damage
- Small volume loss ➔ large decrease in nutrient delivery
- Minimal extravasation in critical areas ➔ irreparable damage/death of organism

HEMOSTASIS

Primary vs. Secondary vs. Tertiary

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

**Suspicions**

- Spontaneous bleeding
- Prolonged or excessive bleeding after procedures or trauma
- Simultaneous bleeding from multiple sites

<table>
<thead>
<tr>
<th>Bleeding Problem</th>
<th>Platelet Disorder</th>
<th>Coagulation Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petechiae</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bleeding Sites</td>
<td>Mucous Membrane</td>
<td>Deep Tissue</td>
</tr>
<tr>
<td>Time of onset of bleeding</td>
<td>Immediate</td>
<td>Delayed</td>
</tr>
<tr>
<td>Ecchymoses /Hematomas</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>
HEMOSTATIC ABNORMALITIES
"Screening Tests" (History)

- Dental Extractions
- Prior Surgery/Biopsy
- Easy bruising
- Heavy menses
- Deliveries
- Blood transfusion

PLATELETS

- Anucleate cellular fragments; multiple granules, multiple organelles
- Synthesis controlled by IL-6, IL-3, IL-11, & thrombopoietin
- Circulate as inactive, non-binding concave discs
- On stimulation, undergo major shape change
- Develop receptors for clotting factors
- Develop ability to bind to each other & subendothelium
PLATELET ACTIVATION

Resting Platelets

Platelet Adhesion

Initial Thrombin Formation

IIa

II → IIa

X → Xa

VIIa

TF
PLATELET ACTIVATION

Platelet Activation

VIIa - TF

Platelet Release

TxA2, ADP,
Serotonin,
Fibrinogen,
Thrombospondin

II → IIa
X → Xa
VIIa - TF
PLATELET PLUG FORMATION

Platelets/Introduction to Hemostasis Tuesday, February 24, 2004 – 10:00 am
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
COAGULATION CASCADE

THROMBIN

- Serine protease
- Cleaves fibrinogen to fibrin
- Activates V to Va, VIII to VIIIa
- Activates platelets-cleaves thrombin receptor
- Activates XIII to XIIIa
- In presence of thrombomodulin activates Protein C to APC
COAGULATION CASCADE

Platelet Release

TxA2, ADP, Serotonin, Fibrinogen, Thrombospondin

VIII → VIIIa
V → Va
II → IIα

IX → IXa

VIIa-TF

X → Xα

VIIa-TF
Tenase/Prothrombinase complex assembly

\[ \text{X} \rightarrow \text{Xa} \]

\[ \text{VIIa/IXa} \]

\[ \text{VIIa R} \]

\[ \text{VIIIa/IXa} \]

\[ \text{Va/Xa} \]

\[ \text{Va R} \]

\[ \text{II} \rightarrow \text{IIa} \]

FIBRIN FORMATION

\[ \begin{array}{c}
\alpha \\
\beta \\
\gamma \\
T \\
F \text{XIIIa}
\end{array} \]

\[ \begin{array}{c}
\alpha \\
\beta \\
\gamma \\
\alpha \\
\beta \\
\gamma
\end{array} \]
VITAMIN K DEPENDENT CARBOXYLASE

- Post-translational modification
- Factors II, VII, IX, X; proteins C & S
- Converts 1st 7-12 glutamic acids to γ-carboxyglutamic acid
- Confers calcium binding and lipid binding on these proteins
- Without vitamin K, secrete des-γ-carboxyglutamic acid containing proteins (inactive in coagulation)

γ-CARBOXYGLUTAMIC ACID

\[ \text{Glu} \rightarrow \gamma\text{-carboxy Glu} \]

\[ \text{Vit K Carboxylase} \]

\[ \text{O}_2, \text{CO}_2 \]
PLATELET FUNCTION STUDIES

- Bleeding Time
- Platelet Count
- Platelet Aggregation Studies

BLEEDING TIME vs. PLATELET COUNT

Minutes

Platelet count (x 1000)
Clotting Tests

- Recalcification times
- Blood collected in sodium citrate, a weak calcium chelator
- Amount designed to drop calcium concentration to < 1 mM
- At that level, blood won’t clot spontaneously
- RBC’s & platelets centrifuged off, leaving plasma (unclotted liquid portion of blood)
Prothrombin Time

- Mixture of:
  - 50% patient’s platelet poor plasma
  - 25% Mixture of Tissue Factor & phospholipid species
  - 25% Calcium chloride (to bring final calcium concentration to c. 3-5 mM)
  - Time to clot formation measured
aPTT (activated partial thromboplastin time)

- 50% patient’s platelet-poor plasma
- 25% mixture of phospholipid & surface active agent (Celite, Kaolin)
- 25% Calcium Chloride to bring calcium concentration to 3-5 mM
- Time to clot formation measured
COAGULATION CASCADE

Contact Phase

Prekallikrein → Kallikrein

F XII → HMWK → F XIIa

F XI → F Xa

Intermediate Phase

F VII/TF → Ca²⁺ → F VIIa/TF

IXa/VIIIa/PL

F IX → Ca²⁺ → F IXa

VIII + F IXa → IXa/VIIIa/PL

F X → Ca²⁺ → F Xa

Tenase Complex
COAGULATION CASCADE

Common Pathway

IXa/VIIa/PL  VIIa/TF/PL

F X

Ca²⁺

Ca²⁺

V → Va + F Xa → Xa/Va/PL

Prothrombinase Complex

Fibrinogen

PT

t

Fibrin monomer

CLOTTING ASSAYS

Specific factors/Inhibitors

- Deficiency states of every clotting protein described
- 50% of any clotting factor will yield normal PT and/or aPTT
- Mix 1/2 patient plasma with 1/2 factor deficient plasma; then perform PT and/or aPTT
- Factor deficient plasma with missing factor will prolonged clotting time; all others WNL
- If more than one clotting assay prolonged, implies inhibitor to clotting protein