LABORATORY DIAGNOSIS OF BLEEDING DISORDERS

Secondary Hemostasis Disorders

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

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

HEMOSTASIS

Primary vs. Secondary vs. Tertiary

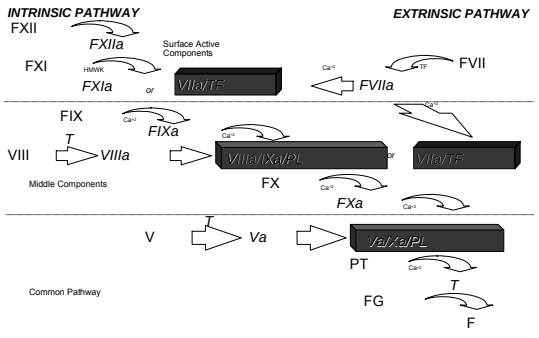
- Primary Hemostasis
  - Platelet Plug Formation
  - Dependent on normal platelet number & function
- Secondary Hemostasis
  - Activation of Clotting Cascade → Deposition & Stabilization of Fibrin
- Tertiary Hemostasis
  - Dissolution of Fibrin Clot
  - Dependent on Plasminogen Activation

COAGULATION CASCADE

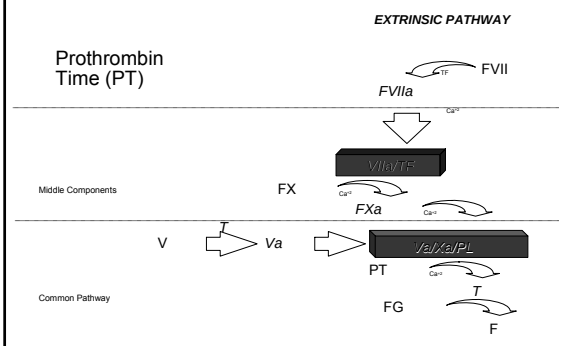
General Features

- 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

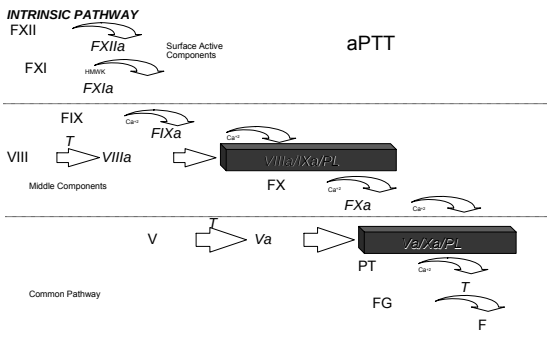
COAGULATION CASCADE



COAGULATION CASCADE



COAGULATION CASCADE



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

Factor Assays

- Assays based on fact that 50% of normal levels of any clotting protein → normalization of clotting time
- Reaction: 0.1 ml patient plasma, 0.1 ml factor-deficient plasma, 0.1 ml phospholipid/activator agent, 0.1 ml calcium; measure time to clot formation

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

CLOTTING FACTOR DEFICIENCY

Factor Assays

- EG – If patient is Factor VIII deficient:
  - Assay using factor IX-deficient plasma will have 50% factor VIII from the factor deficient plasma and 50% factor IX from the patient's plasma, & so aPTT for factor IX will be normal

CLOTTING FACTOR DEFICIENCY
Factor Assays

- If patient is factor VIII deficient:
  - Assay using factor VIII deficient plasma will have no factor VIII from the patient and no factor VIII from the factor deficient plasma, yielding a prolonged aPTT using factor VIII deficient plasma
- 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

- 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

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

HEMOPHILIA – General Rules RE: Rx

- Treat first; ask questions later
- Bleeding into closed spaces stops!!
- AVOID EMERGENT PROCEDURES IF POSSIBLE
- No procedures without replacement Rx
- Avoid weekend/night procedures
- No procedures without Hematology & Lab backup

Initial Therapy of Hemophilia A

Indication	Hemophilia A Factor VIII:C (u/kg)	Factor VIII Desired Level (%)
Mild Hemorrhage	15	30
Major Hemorrhage	25	50
Life-Threatening Lesion	40-50	80-100

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

Indication	Hemophilia B Factor IX:C (U/kg)	Factor IX Desired Level (%)
Mild Hemorrhage	30	30
Major Hemorrhage	50	50
Life-Threatening Hemorrhage	80	80

Modified from Levine, PH. "Clin. Manis. of Hem. A & B", in *Hemost. & Thromb., Basic Principles & Practices*

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

HEMOPHILIA Rx Subsequent Treatment

- Dependent on:
  - Procedure being done
  - ½-life of factor VIII or factor IX IN THAT PATIENT! (Monitored by factor assay)
    - Should be determined in each case
  - Generally, ½ life 8-12 hours for VIII, 24 hours for IX
- ε-amino caproic acid (Amicar®) – a plasminogen inhibitor sometimes useful to limit bleeding

Factor Concentrates

ALL FACTOR CONCENTRATES REQUIRE HEMATOLOGY APPROVAL!!

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

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

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

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