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 2º to endothelial surface damage
- Small volume loss ➔ large decrease in nutrient delivery
- Minimal extravasation in critical areas ➔ irreparable damage/death of organism

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

HEMOSTATIC DISORDERS

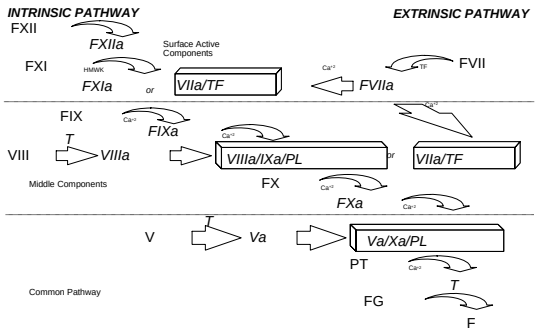
- History critical to assessment of presence of disorder
  - History of bleeding problems in the family
  - History of spontaneous bleeding
  - History of heavy menses
  - History of easy bruising
  - History of prior blood transfusion
  - History of prior tooth extractions
  - History of prior surgery/pregnancy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

COAGULATION 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

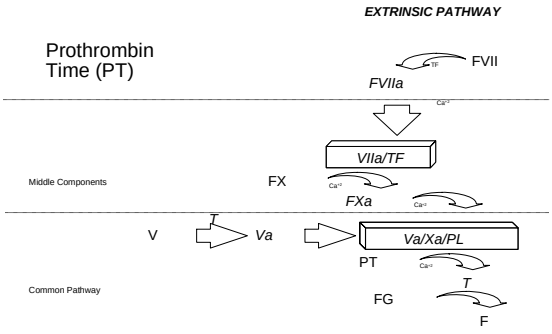


CLOTTING FACTOR DEFICIENCY

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

COAGULATION CASCADE

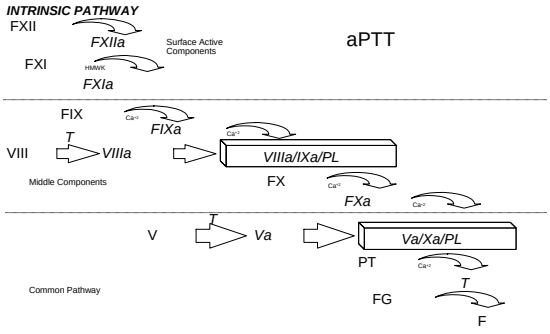


CLOTTING FACTOR DEFICIENCY

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

COAGULATION CASCADE



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

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

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 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 IIn – Defect in Factor VIII Binding
- Type III – Severe Quantitative Defect

HEMOPHILIA vs. VON WILLEBRAND DISEASE

Test	Hemophilia A	Von Willebrand Disease
Bleeding time	Normal	Prolonged
aPTT	Prolonged	Prolonged

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

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

FACTOR VIII *vs.* VWF

	Von Willebrand Factor	Factor VIII
Function	Platelet adhesion, Factor VIII stability	Fibrin Clot Formation
Site of synthesis	Endothelial cells, Megakaryocytes	Hepatocytes
Genetic control	Autosomal dominant	X-linked recessive
Hemophilia	Normal	Low
Von Willebrand Disease	Low	Low

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*

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)

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 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