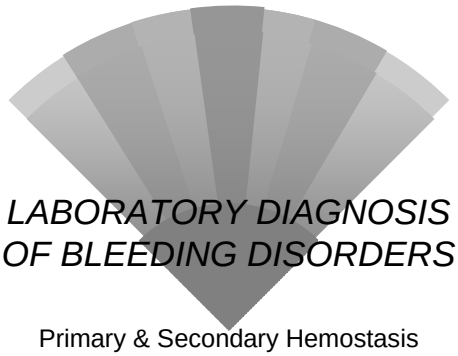


LABORATORY DIAGNOSIS OF BLEEDING DISORDERS

Primary & 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

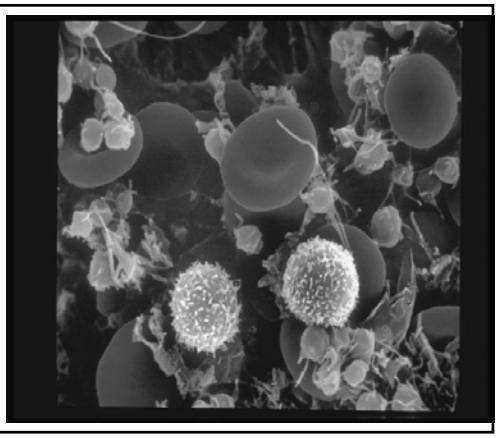
COAGULATION TESTING

Basic Testing

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

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 DYSFUNCTION

Clinical Features

- Mucosal bleeding common
- Often see diffuse oozing
- Often suspected as a diagnosis of exclusion - *ie* clotting studies normal, but patient has clinical bleeding disorder
- #1 cause of bleeding disorder post-bypass surgery

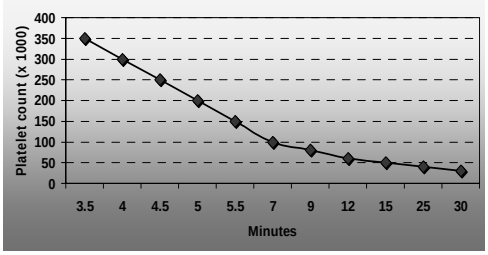
PLATELET FUNCTION STUDIES

- Bleeding Time
- Platelet Count
- Platelet aggregation studies

BLEEDING TIME

- Bioassay
- Difficult to standardize
- Most reproducible measure of platelet function

BLEEDING TIME vs. PLATELET COUNT



PLATELET FUNCTION DEFECTS

Prolonged Bleeding Time

- Congenital
- Drugs
- Alcohol
- Uremia
- Hyperglobulinemias
- Fibrin/fibrinogen split products
- Thrombocythemia
- Cardiac Surgery

PLATELET AGGREGATION STUDIES

- Multiple agonists used (ADP, epinephrine, collagen, ristocetin)
- Add agonists to platelet rich plasma, then measure increase in light transmission as platelets aggregate
- Difficult to standardize
- Useful for determining cause of platelet dysfunction

PLATELET FUNCTION DEFECTS

Congenital

- Bernard-Soulier disease (Decreased platelet adhesion
- Glanzmann's thrombasthenia (Decreased platelet aggregation)
- γ or δ-storage pool disease (Defective platelet release)
- Gray platelet syndrome (Defective platelet release)
- Von Willebrand Disease

PLATELET FUNCTION DEFECTS

Treatment

- Attention to drugs
- Platelet transfusion - for bleeding or pre-procedure, esp with congenital defects
- Desmopressin (DDAVP) - Shortens bleeding time; ? if decreases bleeding.
- Causes release of vWF from endothelial cells
- Cryoprecipitate-Same as DDAVP
- Dialysis
- ? RBC transfusion

THROMBOCYTOPENIA

Causes-Miscellaneous

- Factitious
  - Macroplatelets
  - Platelet aggregation
  - Platelet satellitism
- Splenic sequestration
- Hemodilution

THROMBOCYTOPENIA

Decreased production

- Decreased megakaryocytes
  - Normal platelet life span
  - Good response to platelet transfusion
- Neoplastic Causes
  - Leukemias
  - Aplastic Anemia
  - Metastatic Carcinoma
  - Drugs
  - Radiotherapy
- Primary Marrow Disorders
  - Megaloblastic Anemias
  - Myelodysplastic syndromes
  - Myeloproliferative diseases
  - Some congenital syndromes

THROMBOCYTOPENIA

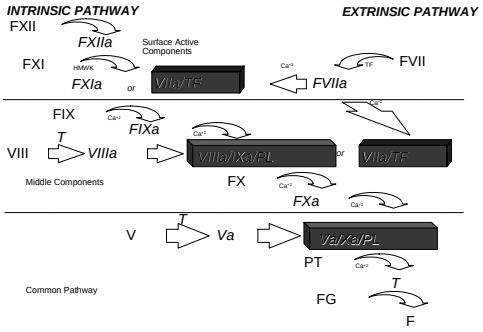
Increased Destruction - Causes

- Increased megakaryocytes
  - Shortened platelet life span
  - Macroplatelets
  - Poor response to platelet transfusion
- Causes
  - Immune
    - ITP
    - Lymphoma
    - Lupus/rheumatic diseases
    - Drugs
  - Consumption
    - Disseminated intravascular coagulation
    - Thrombotic thrombocytopenic purpura
    - Hemolytic/uremic syndrome
  - Septicemia

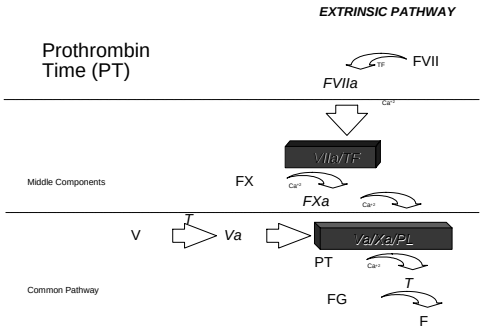
COAGULATION CASCADE
General Features

- 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

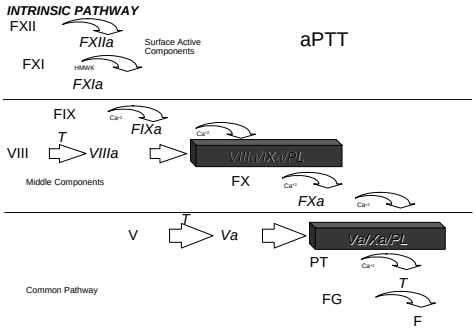
COAGULATION CASCADE



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
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

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 > 800
  - Factor VIII > 1000

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 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

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

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

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 vs. VON WILLEBRAND DISEASE

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

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

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*

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)

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

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