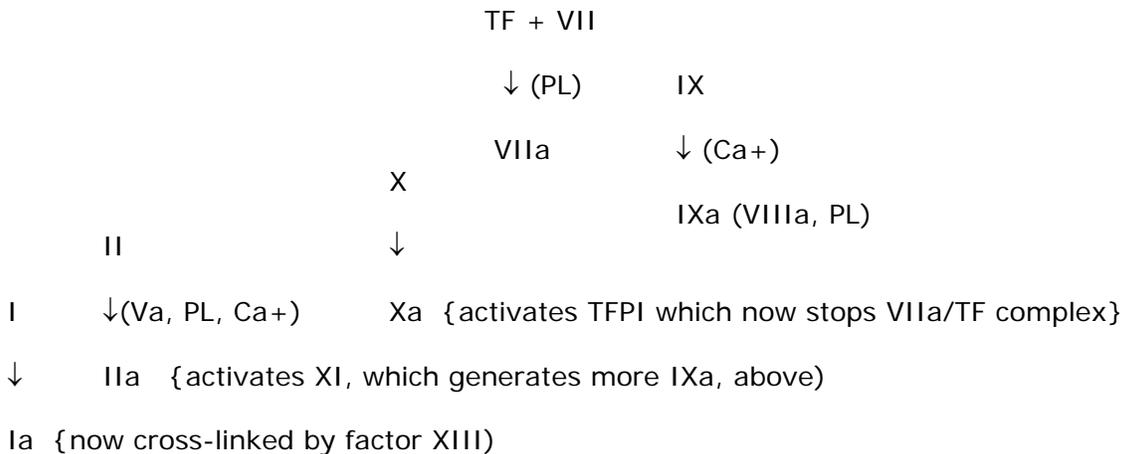


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Coagulation Lecture Outline
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- I. Brief overview of normal hemostasis
- A. primary hemostasis (vascular and platelet phase)
1. Vasoconstriction, exposure of collagen
 2. Adhesion (vWF is bridge between gpIb and collagen)
 3. Secretion (release of ADP, calcium, fibrinogen, factor V, vWF)
 4. Aggregation (gpIIb/IIIa bridges platelets with fibrinogen)
- B. secondary hemostasis
1. exposure of tissue factor
 2. activates factor VII ("extrinsic" pathway)
 3. this activates factor X (with factor VIII and calcium as cofactors)
 4. also activates factor IX ("intrinsic" pathway)
 5. activated factor X activates prothrombin (with factor V and phospholipid surface as cofactor), as well as tissue factor pathway inhibitor (TFPI), which binds up and shuts down activated factor VII
 6. thrombin cleaves fibrinogen to fibrin
 7. thrombin also activates factor XI, which then activates more factor IX
 8. activated factor IX (generated by both TF-VIIa and thrombin-activated XI) keeps the cycle going by complexing with factor VIIIa to activate more factor X.
 9. the fibrin monomers cross link and are stabilized by factor XIII

"My interpretation of what happens"



"Traditional coagulation cascade"

Intrinsic

(HMWK, PK)
XII → XIIa

XI → XIa

IX → IXa
(Ca⁺)

Common

X → Xa
(VIIIa, PL, Ca⁺)

II → IIa (thrombin)
(Va, PL, Ca⁺)

I → Ia (fibrin)

Extrinsic

VIIa ← VII + TF
(Ca⁺, PL)

X ← X
(VIIIa, PL, Ca⁺)

C. fibrinolysis

1. Plasmin is main player

a. Plasminogen is activated by t-PA (in endothelial cells and plasma) or u-PA (kidney, endothelium, malignant cells, plasma) to plasmin

b. degrades **both** fibrin clot and fibrinogen (only fibrin cleavage yields D-dimers; both yield FSP's)

c. inactivates factors **Va** and **VIIIa**

2. contact factors (XII, HMWK, PK) and IXa may activate plasminogen

3. Plasmin inhibited by alpha-2-antiplasmin (located in platelet alpha granules, hepatocytes)

4. Plasminogen activator inhibitor-1 (PAI-1) inhibits t-PA and u-PA

5. Antithrombin III inhibits thrombin (IIa), Xa, IXa, and probably VIIa

D. anticoagulation

1. protein C

a. activated by thrombin in conjunction with thrombomodulin

b. activated protein C inactivates VIIIa and Va

2. protein S – potentiates the effect of APC; other effects not figured out yet

3. thrombomodulin –

a. on surface of vascular endothelium

b. is receptor for thrombin; then activated protein C

II. Brief review of lab coagulation tests

A. "Screening labs"

1. CBC – platelet count; morphology helpful if abnormal

a. bleeding time controversial, but on an exam, a prolonged bleeding time = platelet dysfunction.

2. PT – "extrinsic pathway" (VII, X, V, II, I)

a. uses calcium and tissue thromboplastin to activate VII

b. INR = (pt's PT/mean PT of normal range)^{ISI}

3. APTT – "intrinsic pathway" (XII, XI, IX, VIII, X, V, II, I)

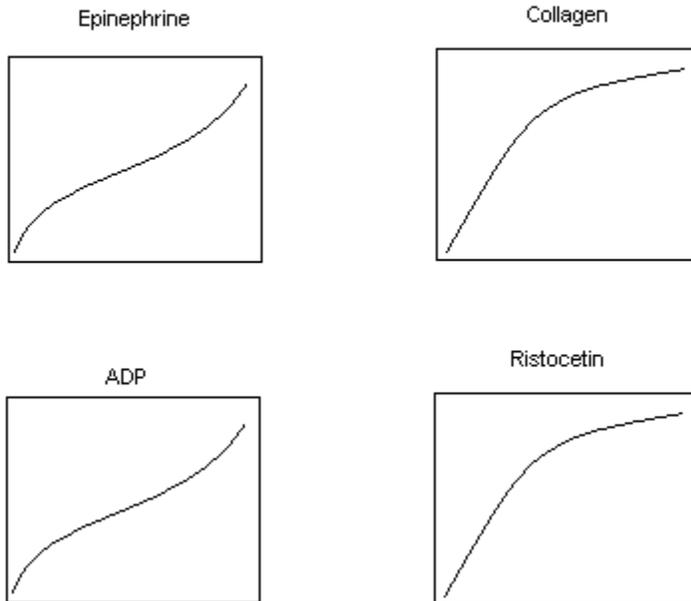
a. add calcium, partial thromboplastin (platelet substitute), and activator

b. affected by deficiencies in all but XIII and VII

B. Helpful additional tests

1. thrombin time – measures functional fibrinogen
 - a. prolonged with levels below 75 mg/dl
 - b. prolonged by fibrinogen split products, heparin, dysfunctional fibrinogen, thrombolytic agents
2. reptilase time – converts fibrinogen to fibrin
 - a. unaffected by heparin
 - b. from Bothrops atrox snake
3. mixing studies
 - a. correction suggests deficiency
 - b. no correction suggests lupus anticoagulant
 - c. initial correction and prolongation after incubation suggests inhibitor (although they may not correct at all)
4. antiphospholipid antibody tests
 - a. anticardiolipin antibody levels (>20 GPL, persistent, and IgA or IgG to be significant; IgM usually transient and insignificant)
 - b. DRVVT – Russell's viper venom, calcium, and a limited amount of phospholipid are added to patient's plasma; normally, these will activate factor X and cause clot formation. A lupus anticoagulant will bind up the limited phospholipid and prolong the test.
 - c. DRVVT confirm – basically a mixing study of the above test. Mix 1:1 patient to control plasma, do DRVVT, and calculate ratio of DRVVT to DRVVT with mix (> or = 1.20 is positive)
 - d. Platelet neutralization procedure -phospholipid overwhelms the antibody and normalizes the aPTT, confirming the presence of the lupus anticoagulant.
5. factor assays
 - a. PT (for II, V, VII, X) is performed on dilutions of patient's plasma mixed with factor-deficient substrates and compared with reference curve
 - b. aPTT (for VIII, IX, XI, XII) is performed in similar fashion for other factors
6. Bethesda unit measurement of inhibitors – serial dilutions of patient mixed with "normal" pool plasma, incubated, and then factor activity is measured. One Bethesda unit leaves 50% of the factor activity. Two Bethesda units leave 25%. Three Bethesda units leave 12.5% of the activity, and so on.
7. vonWillebrand tests
 - a. Factor VIIIc – really factor VIII activity
 - b. von Willebrand factor antigen (vWF:Ag) – variable measurement methods (ELISA, RIA, EIA)
 - c. Ristocetin cofactor activity (patient plasma mixed with ristocetin and formalin-fixed platelets) measures platelet aggregation
 - d. von Willebrand multimer analysis – for evaluation of types 2A, 2B, and 3
8. 5 M (molar) urea test
 - a. also called factor XIII screening test
 - b. plasma is clotted, then urea is added
 - c. in factor XIII deficiency, clot dissolves within 24 hours

9. platelet aggregation studies



- add different reagents to platelet rich plasma
- measure light transmittance (y axis) versus time (x axis)
- ADP causes two waves; abnormal in ASA, uremia, storage pool disease (SPD), Glanzman's
- Epinephrine usually causes two waves; abnormal in ASA, uremia, SPD, Glanzman's.
- Collagen usually causes one wave; abnormal in ASA, uremia, SPD, Glanzman's.
- Ristocetin usually occurs rapidly (looks like single wave); abnormal in von-Willebrands (except IIb, which has exaggerated response) and Bernard-Soulier

10. hypercoag workup

- Good H&P (R/O post-op, pregnant, trauma, OCP's, obesity, immobility, HIT, chronic DIC, malignancy, nephrotic syndrome, PNH, SLE, myeloproliferative disorders, and hyperhomocysteinemia).
- APC resistance – do PTT in presence of activated protein C; this normally prolongs the PTT. Compare ratio of PTT with APC to baseline PTT. Normal is ≥ 2.0 .
- Factor V Leiden – PCR amplification of mutation site followed by digestion with Mnl I. Factor V Leiden lacks digestion site.
- Prothrombin 20210 mutation - PCR
- Protein C – can measure both qualitative and quantitative (decreased in liver disease, DIC, ARDS, vitamin K deficiency, L-asparaginase therapy)
- Protein S – (decreased with oral contraceptives, liver disease, vitamin K deficiency, pregnancy, DIC, and acute inflammatory states {increased C4b binding protein binds up free protein S})
- Also certain elevated factors are associated with thrombosis – VII, VIII, I, and II (due to 20210 mutation above)

III. Work-up of the bleeding patient

A. Laboma

1. thrombocytopenia
 - a. spurious (platelet cold agglutinins, paraproteinemias, giant platelets, satellitism, lipemia, EDTA-effect)
 - b. real (ITP, TTP, DIC, lymphoproliferative disorders, infection, drugs, sequestration)
2. increased aPTT (heparin contamination, lupus anticoagulant, inadequate collection)
3. increased PT (inadequate collection)

B. Real bleeding categorization (in general)

<u>Purpuric (vessels/platelets)</u>	vs.	<u>coagulation disorder</u>
petechiae		deep dissecting hematoma
small, solitary bruises		large, solitary ecchymoses
bleeding from superficial cuts, scratches		hemarthroses
female		male
no family history (except vWD)		+ family history

C. Etiology (in general)

1. Inherited
 - a. onset in infancy/childhood
 - b. + family history
 - c. single lab abnormality, usually one factor deficiency
2. Acquired
 - a. usually less severe
 - b. clinical picture dominated by underlying disorder, not just bleeding (for example, baby with DIC will have sepsis, hypoxia, acidosis, etc.)
 - c. multiple hemostatic defects
 - d. frequently history of drug therapy

D. Sequence of testing (assuming clinical bleeding and normal platelet count)

1. Good H&P (see above)
2. CBC, PT, aPTT
 - a. elevated PT only
 - i. check drug history, liver function
 - ii. do mixing studies – if no correction, then probable inhibitor
 - iii. check factor levels (VII, V, X, II)
 - b. elevated aPTT only
 - i. check drug history
 - ii. check mixing studies (correction suggests deficiency; no correction suggests lupus anticoagulant, heparin; initial correction only suggests inhibitor)
 - iii. check thrombin time and reptilase time
 - iv. check factor levels (XII, XI, IX, VIII, vWF)
 - v. pursue lupus anticoagulant work-up if suspected
 - c. elevated PT and aPTT
 - i. check drug history
 - ii. check mixing studies
 - iii. check thrombin time, FSP's, D-dimers
 - iv. check factor levels (X, V, II, I)
 - d. normal PT and aPTT

- i. check von Willebrand screen
- ii. check 5M urea
- iii. check fibrinolytic pathway (alpha-2 anti-plasmin levels, PAI-1 levels, etc.)
- iv. check platelet aggregation studies

IV. Disorders

- A. Most common non-patient causes
 - 1. Lack of surgical hemostasis
 - 2. Drug effect (heparin, coumadin)
- B. Vascular disorders
 - 1. Infections – meningococemia, etc., due to vasculitis/DIC
 - 2. Drug reactions – hypersensitivity (immune complex deposition in vessel wall)
 - 3. Impaired collagen formation – Scurvy, Ehlers-Danlos, hypercortisolism (including steroid therapy)
 - 4. Henoch-Schonlein purpura – immune complex deposition in vasculature
 - 5. Hereditary hemorrhagic telangiectasia – dilated, tortuous vessels prone to bleeding
 - 6. Amyloidosis – amyloid deposition may cause vessel fragility
 - 7. Surgery – open vessels bleed . . .
- C. Platelet disorders
 - 1. Too few (thrombocytopenia) –
 - a. decreased production – marrow infiltration, marrow failure, etc.
 - b. decreased survival – auto- or allo-immune antiplatelet antibodies
 - mechanical – mechanical valve, TTP, HUS, etc.
 - c. sequestration – splenomegaly
 - d. dilutional – s/p massive transfusion
 - 2. Don't work
 - a. adhesion defect – Bernard Soulier (GP Ib-IX deficiency)
 - b. activation defect – various storage pool defects with deficient release/production of various mediators.
 - c. aggregation defect - Glanzman's thrombasthenia (GP Iib-IIIa deficiency)
 - d. aspirin – inhibits cyclo-oxygenase leading to defective activation and aggregation.
 - e. uremia – poorly understood, but platelets won't work
 - 3. Most common platelet disorders
 - a. Drug effect (aspirin, etc.), uremia, thrombocytopenia (ITP, TTP, DIC)
 - 4. Most testable platelet disorders
 - b. Bernard-Soulier, Glanzman's thrombasthenia, storage pool defects
- D. Most common factor deficiencies
 - 1. VIII, IX, vWD, XII
- E. Most testable factor deficiencies
 - 1. XIII
- F. Most common hypercoagulable states
 - 1. Factor V Leiden (5% of general population), prothrombin mutation (2.3%), protein S deficiency (0.7%), protein C deficiency (0.14-0.5%), antithrombin deficiency (0.17%).

- V. Differentials of screening panel patterns (in a patient with bleeding)
- A. ↑PT, nl aPTT, nl platelet count
 - 1. common – acquired VII deficiency (malnourished, pancreatobiliary dysfunction, prolonged antibiotic therapy), coumadin, liver disease
 - 2. rare – VII deficiency, VII inhibitor, dysfibrinogenemia
 - B. nl PT, ↑aPTT, nl platelet count
 - 1. common – VIII, IX, XI deficiency, vWD, heparin effect
 - 2. rare – lupus with platelet defect, inhibitor
 - C. ↑PT, ↑aPTT, nl platelet count
 - 1. common – vitamin K deficiency, liver disease, coumadin, heparin
 - 2. rare – DIC, dysfibrinogenemia, primary fibrinolysis, or deficiency or inhibitor of X, V, II, I.
 - D. ↑PT, ↑aPTT, ↓ platelet count
 - 1. common – DIC, liver disease
 - 2. rare – heparin with thrombocytopenia
 - E. nl PT, nl aPTT, ↓platelet count
 - 1. common – destruction (DIC, TTP, ITP, drug effect, etc.), sequestration (splenomegaly DDX), ↓production (infiltrated bone marrow-lymphoproliferative D/O, met, infection)
 - 2. rare – Bernard-Soulier
 - F. nl PT, nl aPTT, ↑platelet count
 - 1. myeloproliferative disorders (ET, PV, CML, CIMF)
 - G. nl PT, nl aPTT, nl platelet count
 - 1. common – mild vWD, uremia, surgery
 - 2. rare – inherited qualitative platelet D/O, vascular D/O, fibrinolytic D/O, factor XIII deficiency, dysfibrinogenemia, mild factor deficiency (VIII, IX, XI)

V. Case study notes:

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