Blood Coagulation and Thrombophilia

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Disclosure Information

Cliff Takemoto MD

NONE
Objectives

- Develop a framework to understand coagulation
- Know how to approach coagulopathies
What is in a clot?

VENOUS
Slow flow
RED-(rbc’s)

ARTERIAL
Fast flow
WHITE (plts)
How we clot

Cut in the endothelium
Exposes collagen for platelet binding sites
Exposes tissue factor for activating coagulation

Platelet Activation
Platelet granule secretion
Activate fibrinogen receptor
Provides site for prothrombinase complex

Thrombin Activation
And fibrin deposition
This is how a clot is made

Falati et al., Nature Medicine 2002 with permission from Nature Publishing Group
how a clot is made in vivo

Thrombin activation INITIATED by TISSUE FACTOR
(microparticles?)

TWO pathways to PLATLET ACTIVATION:
TF (thrombin)
Collagen (vWF)
Coagulation Cascades

… the often-asked question: “Why is coagulation so complicated?” resolves itself into the question: “Why are there so many stages?”


“Waterfall” Sequence or Enzyme Cascade

XII → XIIa

XI → XIa

IX → IXa

VIII → VIIIa VIIa → VII

X → Xa

V → Va

II → IIa

I → Iαγen
Coagulation Cascades
Some concepts to remember…

• Highly regulated complexes of serine proteases and co-factors

• All paths lead to thrombin activation

• Goal is to make a fibrin clot

• They are not just a number— they have a personality too!!!
Serine Proteases

- FIX
- FX
- FVII
- FII
- PC

Procoagulant

- FVIII
- FV
- TF
- TM
- PS

Co-Factors

- A1
- A2
- B
- A3
- C1
- C2
Clot formation--physiology

CONTACT FACTORS
(prekallikrein, HMWK, XII, XI)

IXa

VIIa

Ca++, PL

extrinsic tenase

IXa

VIIa

Ca++, PL

Fibrinogen

Fibrin polymers

prothrombin

thrombin

SERINE PROTEASE

COFACTOR

“Prothrombinase”

“Prothrombinase”

Clot formation--physiology
Know the functions and mechanism of activation of factor ___ in coagulation
Know the consequences of deficiency of factor ___ on the laboratory assessment of hemostasis.
Clot formation--physiology

CONTACT FACTORS
(prekallikrein, HMWK, XII, XI)

INITIATION

AMPLIFICATION
PROPAGATION

"Prothrombinase"

fibrinogen
Fibrin clot
Clot formation--physiology

Initiation:
- IXa
- VIIIa

Propagation:
- Xa
- Va
- II
- IIa

Reactions:
- TF
- Fibrinogen → Fibrin clot
Activation of thrombin: 3 complexes

enzyme complexes: protease cofactor

Prothrombinase Xa Va
Intrinsic tenase IXa VIIIa
Extrinsic tenase VIIa TF

Also need Calcium and a phospholipid surface
A few words about contact activation...

- Regulate Inflammation
- HK, PK, FXII deficiency do not bleed
- FXI deficiency associated with bleeding

Protease
Prekallikrein (PK)
FXII
FXI

Cofactor
High Molecular-Weight Kininogen (HK)

C1 esterase inhibitor
Thrombin has both pro- and anti-coagulant functions.

Thrombin IIa

- Fibrinogen
- FV, FVIII, FXI
- FXIII
- TAFI (fibrinolysis inhibitor)
- Platelet Activation (procoagulant)

- Protein C/S bound to Thrombomodulin (anticoagulant)
Fibrinogen Structure

Know basic structure of fibrinogen and its gene control

Fibrinogen (soluble)  Fibrin (insoluble)

Fibrinogen Genes

6 polypeptide chains
3 genes: α, β, γ

Gorkun O. et al., Blood 1997; 89:4407-44. With permission from the American Society of Hematology
Fibrin Clot formation and fibrinolysis

Thrombin (IIa) → fibrinopeptides

fibrinogen → D-Dimer

FXIII → D-dimer

Plasmin → D-dimer
Plasminogen regulation

- Activators: t-PA, u-PA
- Inhibitors: PAI-1, PAI-2, $\alpha_2$ antiplasmin, $\alpha_2$ macroglobulin, TAFI
Endogenous Anticoagulants: Turning off the clot

Protein C  →  VIIIa, Va (the cofactors)
Protein S

Antithrombin  →  IIa, Xa (serine proteases)

TFPI  →  VIIa/TF
How to stop the clot

Protein C
Protein S
thrombomodulin

II

IIa

VIIa

TFPI

IIa

Xa

Va

AT III

II

IIa

IIa
Heparin—physical characteristics
It binds to Antithrombin

heparin pentasaccharide binding to antithrombin

Activation loop binds to reactive Site in serine protease
Heparin-- physical characteristics

Pentasaccharide Antithrombin binding sequence

Molecular Weight Distributions of LMW heparin and unfractionated heparin

Hirsh J, Warkentin T et al. Chest
Heparin binds and activates Antithrombin

Suicide inhibitor

Coagulation Factors

Heparin accelerates this reaction >1000fold

Pentasaccharide sequence of heparin binds antithrombin
Antithrombin Deficiency

- Prevalence 1/5000
- Risk of thrombosis $\uparrow$ 15-20X
- Acquired forms - nephrotic syndrome, liver disease
- Lab - Antithrombin activity
Protein C and Protein S

Vitamin K dependent
Protein C-serine protease
Protein S-cofactor
Activated by thrombin/TM

IIa

C

APC

Va

VIIIa

cofactors

Thrombomodulin

C4b binding protein

S

S
Protein C and S deficiency

- Protein C: 1/250-500
- Protein S: 1/1000
- Increase risk 5-10X
- Acquired causes:
  - Both: Vit. K deficiency
  - Protein S: estrogens, pregnancy
- Homozygous protein C presents with neonatal purpura fulminans
- Lab: Protein C and S activity
Thromboembolic events with other prothrombotic risk factors
Factor V Leiden

- Prevalence 5% (0-15%)
- Autosomal dominant
- Prevents inactivation of FVa
- Thrombosis risk:
  - FVL +/- = 5X
  - FVL++ = 50X
  - FVL +/- and OCPs = 30X

\[ \text{Factor Va} \downarrow \text{APC} \text{Factor Vi} \]

306 506 679
What is APC resistance?

FVL:

\[
\frac{\text{PTT (APC)}}{\text{PTT}} = 1.3
\]

normal:

\[
\frac{75\text{sec}}{30\text{sec}} = 2.5
\]

FVa

R506Q is the FV leiden mutation
## Characteristics of Clotting Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Invivo T^{1/2}</th>
<th>Synthesis</th>
<th>Function</th>
<th>vitK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (I)</td>
<td>2-4 days</td>
<td>liver</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Prothrombin (II)</td>
<td>3 days</td>
<td>liver</td>
<td>SP</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>36 hrs</td>
<td>liver/(mega)</td>
<td>CoF</td>
<td>-</td>
</tr>
<tr>
<td>VII</td>
<td>3-6 hrs</td>
<td>liver</td>
<td>SP</td>
<td>+</td>
</tr>
<tr>
<td>VIII</td>
<td>8-12 hrs</td>
<td>liver/EC</td>
<td>CoF</td>
<td>-</td>
</tr>
<tr>
<td>IX</td>
<td>22 hrs</td>
<td>liver</td>
<td>SP</td>
<td>+</td>
</tr>
<tr>
<td>X</td>
<td>40 hrs</td>
<td>liver</td>
<td>SP</td>
<td>+</td>
</tr>
<tr>
<td>XI</td>
<td>80 hrs</td>
<td>liver</td>
<td>SP</td>
<td>-</td>
</tr>
<tr>
<td>XII</td>
<td>50-70 hrs</td>
<td>liver</td>
<td>SP</td>
<td>-</td>
</tr>
<tr>
<td>XIII</td>
<td>10 days</td>
<td>liver/MΦ</td>
<td>TG</td>
<td>-</td>
</tr>
<tr>
<td>VWF</td>
<td>12 hrs</td>
<td>EC/(mega)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

mega—megakaryocyte; MΦ—macrophage; EC—endothelial cell; SP—serine protease; CoF—Cofactor; TG--transglutaminase

Coleman Hemostasis and Thrombosis 3rd Edition
## Characteristics of Clotting Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Incidence</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (I)</td>
<td>1:1 million</td>
<td>afibrinogen-recessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hypofibrinogen-dominant</td>
</tr>
<tr>
<td>Prothrombin (II)</td>
<td>1:2 million</td>
<td>recessive</td>
</tr>
<tr>
<td>V</td>
<td>1:1 million</td>
<td>recessive</td>
</tr>
<tr>
<td>VII</td>
<td>1:300,000</td>
<td>recessive</td>
</tr>
<tr>
<td>VIII</td>
<td>1:5,000</td>
<td>x-linked</td>
</tr>
<tr>
<td>IX</td>
<td>1:30,000</td>
<td>x-linked</td>
</tr>
<tr>
<td>X</td>
<td>1:1 million</td>
<td>recessive</td>
</tr>
<tr>
<td>XI</td>
<td>1:1 million</td>
<td>recessive</td>
</tr>
<tr>
<td></td>
<td>1:12 (het)</td>
<td>Ashekenazi Jewish</td>
</tr>
<tr>
<td>XII</td>
<td>1:50 (het)</td>
<td>recessive</td>
</tr>
<tr>
<td>XIII</td>
<td>1:1 million</td>
<td>recessive</td>
</tr>
<tr>
<td>VWF</td>
<td>1:100</td>
<td>Type 1 dominant</td>
</tr>
<tr>
<td></td>
<td>1:1 million</td>
<td>Type 3 recessive</td>
</tr>
</tbody>
</table>

Bolton-Maggs et. al., Haemophilia 2004
Approach to abnormal laboratory screens

Elevated aPTT

Elevated PT

Elevated aPTT and PT

Thrombin Time
Elevated aPTT

Mixing study

1:1 ratio control + patient plasma

correction

No Bleeding
Contact factors
--FXII
--PK
--HMWK

Bleeding
FVIII
FIX
FXI

no correction

Heparin
Circulating Inhibitor

Phospholipid dependent
Lupus Anticoagulant
--DRVVT
--phospholipid neutralization
--platelet neutralization

Non-specific
Elevated aPTT and PT

**Thrombin Time**

- Prolonged
  - Heparin
  - FDP (DIC)
  - Fibrinogen
- Normal
  - Factor Deficiencies
    - Common Pathway
      -- FV, FX
    - Combined Pathway
      -- Intrinsic/Extrinsic
      -- vitamin K deficiency
  - Aquired
  - Liver disease
  - DIC

**Fibrinogen activity**

- Low
  - Inherited
    - FGN ag low/absent
      -- hypofibrinogenemia
      -- afibrinogenemia
    - FGN ag normal
      -- dysfibrinogenemia

Thrombin Time--prolonged, Reptilase Time--normal

- heparin

Thrombin Time--prolonged, Reptilase Time--prolonged

- Low fibrinogen dysfibrinogenemia
Case

23 year old male with hemoptysis for 3 weeks

Seen at Eastern Shore ER and spiral CT shows large PE

Transport Team calls you—asks for management advice and recommendation for bloodwork before treatment?
Case

Antithrombin, protein C/S, APC, prothrombin 20210, Homocysteine, etc—all normal.

One test you were not able to get was the antiphospholipid antibodies. He is already on heparin. When can you do the test? Does it matter?
Case

DRVVT was tested on coumadin and prolonged. The “confirm ratio” was high, suggesting an antiphospholipid antibody.
Antiphospholipid antibodies (APA)

Lupus Anticoagulant
- prolong PTT test AND is phospholipid dependent
  (mixing study with plt neutralization, DRVVT)
  --usually benign and transient
  --can be seen in association with thrombosis
  --bleeding when directed against specific factor
    (FII, FV, FVIII)
  --can be acquired transaplacentally in neonates

Anticardiolipin Antibodies
  ELISA based detection

Anti-Beta2 Glycoprotein I Antibodies
  ELISA based detection
Dilute Russell viper venom time (DRVVT—like aPTT)

- Activate X
- Sensitive to inhibition by Antiphospholipid Ab

\[
\begin{align*}
\text{Confirm Ratio} \\
\frac{\text{dRVVT (sec)}}{\text{dRVVT + PL (sec)}} & = \frac{60\text{ sec.}}{30\text{ sec.}} = 2 \\
\text{Normal (1 - 1.4)}
\end{align*}
\]

If the confirm ratio is high
An a phospholipid ab is present
Case 1

21 year old woman with history of short gut syndrome due to small bowel atresia presents with fever, renal insufficiency and jaundice

WBC: 18K/cu mm
H/H: 9.9 g/dl/29.3%
Plt: 132K/cu mm

aPTT/PT: 62 s/ 19 s

ALT: 144 U/L
AST: 666 U/L
Bilirubin 6.2 mg/dl
Direct bilirubin 1.0 mg/dl

Fibrinogen: 150 mg/dl
D-dimer: 21 mg/L
Case 1

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>FV</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>FVII</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>FVIII</td>
<td>138%</td>
<td></td>
</tr>
</tbody>
</table>

Diagnosis?

Coagulopathy of Liver Disease
Case 2

6 week infant with presents with lethargy, bulging fontelle

On examination, extensive bruising, bleeding from the mouth
Case 2

WBC: 10.9K/cu mm
H/H: 7.8 g/dl 22.2%
Plt: 407K/cu mm

aPTT/PT: >100 s/ 65 s

Fibrinogen: 360 mg/dl
D-dimer: > 2.6 mg/L
Case 2

Diagnosis?
Late Vitamin K Deficiency Bleeding (VKDB)

Management?
FFP
IV vitamin K
Vitamin K metabolism

Vitamin K Dependent Factors
- II Protein C
- VII Protein S
- IX
- X Gla proteins
  - osteocalcin (bone)
Case 3

17 old male with presents with bruising and Petechia

WBC: 10.9K/cu mm
H/H: 7.8 g/dl 22.2%
Plt: 17K/cu mm
Blasts on peripheral smear

Fibrinogen: 112 mg/dl
D-dimer: > 54.7 mg/L

aPTT/PT: 40s/19 s
Flow

APML

normal
Case 3

Diagnosis:

Acute Promyelocytic Leukemia
With DIC
Concepts about the pathophysiology of DIC: microvascular clotting

Underlying disease

Activation of coagulation

Depression of Anticoagulant proteins impaired fibrinolysis

Consumption of Coagulation factors and platelets

Fibrin deposition Microvascular thrombosis

Severe Bleeding
Anticoagulant and Procoagulant Proteins are low in DIC

BUT:
Organ failure principally arises from microvascular clotting
Diagnosing DIC

No single test

Scoring Criteria for diagnosis and management

- Thrombocytopenia
- Elevated D-dimers/FSP (sensitive)
- Low fibrinogen (late finding)
- Elevated PT (PT more sensitive than aPTT)
Pathogenesis of DIC

Microvascular fibrin deposition
Leads to organ dysfunction

1. **Tissue Factor** initiation of coagulation from monocytes, other cells
2. Decreased **anticoagulants** leading to uncontrolled amplification of thrombin
3. **Fibrinolysis** accompanies DIC but is impaired due to upregulation of PAI with fibrin propagation
Treatment of DIC

- Treat underlying disease

- Coagulopathy? Treat bleeding/bleeding risk
  - No consensus guidelines for transfusion with platelets, FFP, cryoprecipitate

- Role for anticoagulants?
Distinguishing coagulopathies

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>DIC</th>
<th>vitamin K</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVII</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>FV</td>
<td>↓</td>
<td>↓</td>
<td>NL</td>
</tr>
<tr>
<td>FVIII</td>
<td>NL or ↑</td>
<td>↓</td>
<td>NL</td>
</tr>
</tbody>
</table>
Coagulopathy in other acquired disorders

Hemophagocytic syndromes—DIC with hypofibrinogenemia

Leukemia—DIC (APML); platelet dysfunction (M7, M5 AML)

L-Asparaginase—impaired protein synthesis with hemorrhage and thrombosis; AT deficiency

Nephrotic Syndrome—loss of coagulation factors with thrombosis; AT deficiency
Bleeding in Renal Disease

Platelet dysfunction
  --Uremia
  --Increased nitric oxide production
  --Anemia

Treatment
  --dialysis
  --RBC Txn; plt Txn
  --DDAVP; cryoprecipitate
  --conjugated estrogens
Summary

- **Function**: Serine Protease or CoFactor
  - Except FXIII transglutaminase

- **Site of synthesis**: Liver
  - FVIII liver+endothelium; vWF endothelium
  - FXIII liver+Macrophage; FV liver+megakaryocyte

- **Consequence of deficiency**: Bleeding
  - Except for most Contact Factors

- **Half life**: FVII 2hrs; FXIII 2 weeks
Summary

- Work up for PTT/PT elevation:
  - Liver coagulopathy—FVIII preserved
  - Vitamin K deficiency
  - DIC—anticoagulant and procoagulant low