Acquired Disorders of Coagulation

Margaret E. Rick, M.D.
Adjunct Professor, Medicine
Uniformed Services University of the Health Sciences
DISCLOSURES

Off-Label Usage
• None

Interests
• None
ACQUIRED ANTICOAGULANTS
Acquired Anticoagulants

- Circulating proteins that interfere with coagulation reactions
  - Usually IgG Ab to coagulation factor; some IgM, IgA
  - Antibodies may be directed against a structurally related protein, but cross react with a clotting factor
  - Classically, Ab inhibits active site on protein

- Other mechanisms
  - Proteins or cells may complex with clotting factor and cause its clearance from circulation
  - Disease/Rx may increase proteolytic enzyme action, destroying activity of the coagulation protein
Acquired Anticoagulants

- May cause life-threatening bleeding
- May be asymptomatic
- May cause thrombosis – APS and Lupus AC (Ab inhibits coagulation reaction in vitro, not in vivo); most common cause for prolonged aPTT
ANTIPHOSPHOLIPID SYNDROME (APS)
Antiphospholipid Syndrome (APS)

**Definition** (Dx demands both clinical and lab):
- Clinical syndrome
  - Venous or arterial thrombosis +/or pregnancy morbidity
- Laboratory-defined antibodies directed against proteins that binds phospholipid (PL)
  - Anti-beta$_2$glycoprotein I
  - Anti-cardiolipin
  - Lupus anticoagulants (many are anti-$\beta$2-GPI and anti-prothrombin); measured in coagulation tests
- These ACs probably represent the “tip of the iceberg” of antibody production in these patients
Antiphospholipid Syndrome

Classification

- **Primary** - No Underlying Disease
- **Secondary** - Immune diseases (eg, SLE), medications, infections, malignancies

**Clinical Presentations**
- Thrombosis*
- Pregnancy loss*
- Thrombocytopenia
- Autoimmune hemolytic anemia
- CNS syndromes (stroke)
- Cardiac valve disease, renal disease
Antiphospholipid Syndrome Diagnosis

Clinical Criteria

- Arterial or venous thrombosis (cerebral, lower limb)
- Pregnancy morbidity: a) 1st trimester - 3 recurrent miscarriages; b) >10 weeks – 1 fetal loss; c) severe pre-eclampsia requiring delivery before 34 weeks

Laboratory Criteria – (1) High titer Abs, (2) IgG or M, (3) Persist for 12 weeks

- Anti-cardiolipin (aCL) >99th percentile or >40GPL or MPL
- $\beta_2$ glycoprotein I antibody ($\beta_2$GPI) - >99th percentile
- Lupus anticoagulant (clotting assay, one or more positive tests)

Must persist for 12 weeks, due to the frequency of transient Ab’s in the normal population, 1-5%
Antiphospholipid Syndrome
Laboratory Diagnosis

- ANTICARDIOLIPIN and β2-GPI ANTIBODIES
  - Cardiolipin Ab (ELISA) - wells coated with cardiolipin (usually contains a mixture including β2-GPI)
  - β2-GPI Ab (ELISA) - wells coated with β2-GPI

- LUPUS ANTICOAGULANTS
  - Measured in PL-dependent clotting assays. Antibodies cause a prolonged clotting time that corrects with the addition of extra phospholipid (2 steps in these assays).
  - Some LAs are also directed against β2-GPI; some are anti-prothrombin Ab’s
Antiphospholipid Syndrome
Lupus AC

EXPERIMENTAL FRAMEWORK

XII

XI

IX

VIII

X

VIIa

TISSUE FACTOR

aPTT

PT

V, PL, Ca++

“PROTHROMBINASE”

II ➔ Thrombin

Fibrin Clot
Antiphospholipid Syndrome Coagulation Tests

- **DRVVT** - Venom activates FX directly (final common pathway); prolonged by LAC’s and corrects with increased PL

- **APTT** - Usually prolonged; usually does not correct in 1:1 mix. Corrects with addition of increased PL. Basis for Staclot™

- **Prothrombin Time** - Seldom very prolonged unless PL is very limited (“Dilute Thromboplastin Inhibition Test”)

- **Multiple positive LAC assays are more likely to lead to correct diagnosis** (and predict higher risk of thrombosis)
Antiphospholipid Syndrome
Lupus anticoagulant

- Collection and preparation of plasma for tests is very important
- Must eliminate platelets and platelet fragments which may bind the Ab, making the test falsely normal
- Use double centrifugation for preparation of samples; avoid freeze-thaw cycles
APS Tests that predict greater risk of thrombosis

- **Lupus anticoagulants** are most predictive for thrombosis risk.
  - Risk of VTE >3 times greater with +LAC vs (-)LAC
- **Anti-β2-GPI** associated with increased thrombosis risk, especially higher titers. (Domain I Abs are a selected class of Anti-β2-GPI with ↑risk)
- **IgG** (vs IgM) antibodies predict greater risk of clinical thrombosis
- **Multiple positive laboratory assays** (2-3) predict increased risk for clinical thrombosis
Antiphospholipid Syndrome
Mechanisms of thrombosis

- Two-Hit Theory

- Disruption of endothelium, activating it
  - Disturb reduction-oxidation balance, other mechanisms
  - Anti-GPI ab’s to bind to EC proteins and
    - Activate/express coagulation factors (TF, XIa),
      leukocytes, other cells, leading to thrombus formation

- Second hit – potentiate thrombus formation
  - Increase tissue factor; activate monocytes, platelets
  - Disruption of annexin 5 shield which protects EC
  - Activate complement
Antibodies to Prothrombin in APS (Bleeding vs Thrombosis)

- Ab’s to prothrombin are common, but most anti-prothrombin Ab’s are low affinity or low concentration; they do not inhibit prothrombin action or cause clearance of the factor (or cause thrombosis)

- **Bleeding**: Higher affinity or ↑ concentration of Ab: May cause hypoprothrombinemia by forming complexes with prothrombin that are cleared from the circulation
  - In patients with LACs, check the level of prothrombin if the PT is prolonged >2-3 sec
Antiphospholipid Syndrome
Treatment of Thrombosis

- **Acute Venous and Arterial Thrombosis**
  - Treat as other acute thrombosis - heparin followed by coumadin
  - Coumadin - 6 months - longer if bleeding risk in patient is not too high; INR 2-3
  - Low-dose ASA may be added

- **Duration of treatment and risk of recurrence**
  - Generally 6 months or longer; some indefinite
  - Recurrence risk **20-45% after stopping AC** – new and better studies needed to determine risk.
  - Some risk of recurrence even while on ACs
  - **?** Role of d-dimer in predicting risk of recurrence
Antiphospholipid Syndrome Treatment and Pregnancy

**During Pregnancy**
- Patients with recurrent losses should receive heparin (± ASA, 75-100 mg/day) during Pg
- Fetal survival increases 50-80%

**Experimental treatments**
- Hydroxychloroquine — prevents interaction
  - Antibody and β2-GPI
  - Anti-β2-GPI complex to cells
- C5 inhibitor eculizumab (for catastrophic APS)
- Statins may protect against thrombosis
ACQUIRED FACTOR VIII INHIBITORS
Acquired Factor VIII Inhibitors
Occurrence & Associations

- Spontaneous inhibitors
  - Uncommon - Estimated at 1.3 in $10^6$
  - Spontaneous - 50% without underlying disease
- Associations
  - Autoimmune (SLE, RA), lymphoproliferative diseases
  - Peripartum – especially first pregnancy
  - With allergic reactions (e.g., penicillin, sulfa, fludarabine)
  - Malignancy

- Alloantibodies in hemophilia A
  - Occur in >25% in severe hemophilia A
Acquired Factor VIII Inhibitors

Clinical Presentation

- Ecchymoses, soft tissue bleeding (intramuscular) – majority of patients
- Sites of Invasion - IV catheters, IM injection sites, surgical sites
- Epistaxis
- Intracranial
- Retroperitoneal - Associated with fatal bleeding
- Gastrointestinal
- Gross hematuria
- Hemarthroses - less common
Acquired Factor VIII Inhibitors Diagnosis

- **Prolonged aPTT**: Does not correct with 1:1 mix or added phospholipid; may need 1-2 hour incubation to demonstrate (Ag-Ab kinetics)

- **Factor VIII Activity Assay**: Decreased activity

- **Factor VIII Inhibitor Assay (Bethesda assay with buffering)**: Calculate Bethesda titer
Acquired Factor VIII Inhibitors Diagnosis

- **BETHESDA ASSAY** - Mix dilutions of patient plasma 1:1 with normal plasma; incubate at 37°C, 2 hr; perform factor VIII assay

- **1 BETHESDA UNIT** = amount of antibody that inhibits 50% of factor VIII in normal plasma after incubation (1 unit = reciprocal of dilution of patient plasma that inhibits 50% FVIII in 1:1 mix)

- **SPONTANEOUS INHIBITORS** - Often have non-linear kinetics
Acquired Factor VIII Inhibitors
Treatment of Acute Bleeding

- **Careful Nursing Care**
  - No IM injections; special care to prevent falls.

- **Bypassing agents** - recommended as first-line Rx
  - *Recombinant activated factor VII*
  - *Factor VIII by-passing Concentrates* (FEIBA).

  Consider adding tranexamic acid

  Consider risk of thrombosis, esp in older patients

- **If titer <5 BU** - **Factor VIII** high dose; rVIII or intermediate purity; DDAVP

- **Plasma exchange** or **immunoadsorption column**
  - temporarily remove Ab; combine with FVIII infusion
Acquired Factor VIII Inhibitors
Immunosuppressive Treatment

- **Immunosuppression** - CR in ~ 5 weeks
  - *Prednisone plus cyclophosphamide - CR 70%
  - Rituximab – CR 59%
  - Single agent - prednisone or cyclophosphamide or azathioprine
  - Cyclosporine A, tacrolimus
  - IV IgG – probably not useful

- European registry – 331 patients (Blood May, 2012)

- Regimens Inducing Tolerance - Daily Factor VIII ± immunosuppression - usually reserved for hemophiliacs with inhibitors; expensive but effective in Acq FVIII Inh
ACQUIRED VON WILLEBRAND DISEASE
Acquired Von Willebrand Disease

Pathophysiology

- Antibody formation against VWF
  - Interfere with binding to platelets or collagen
  - Cause increased clearance of VWF-Ab complex
- Shear and proteolysis of VWF – ADAMTS13
- Adsorption by cells and clearance
- Decreased synthesis - hypothyroidism
- Drug-associated - hydroxyethyl starch, valproic acid, ciprofloxacin, griseofulvin
Acquired Von Willebrand Disease

Disease Associations

- Lymphoproliferative and autoimmune diseases (antibody interferes with activity or causes clearance)
- Diseases with increased proteolysis
  - Noncyanotic congenital heart disease, aortic stenosis (vascular shear leading to ↑ lysis by ADAMTS13)
  - DIC, Thrombolytic therapy (plasmin)
- Myeloproliferative diseases (↑ platelet binding, proteolysis)
- Wilm’s tumor, other solid tumors (clearance)
- Hypothyroidism (decreased synthesis)
Acquired Von Willebrand Disease Diagnosis

- **Presentation** - mucocutaneous bleeding and no past bleeding history; *negative* family history

- **Laboratory** - VWF Ag, ristocetin cofactor, and collagen binding usually decreased

- **Antibodies** often difficult to demonstrate on 1:1 mixing studies

- **Multimeric structure of VWF** shows decreased high molecular weight multimers in 2/3 of patients

- **VWF propeptide levels** - normal (ratio of pro-peptide:VWF↑); helpful assay to confirm diagnosis – but not specific
Acquired Von Willebrand Disease

Treatment (D/C ASA)

- Control bleeding and treat underlying disease
- DDAVP - assess clinical bleeding and ristocetin cofactor to follow patient
- **VWF concentrates** - Factor VIII concentrates of “Intermediate purity” contain VWF. (Humate-P, Alphanate, and Wilfactin are factor VIII concentrates labeled with VWF ristocetin cofactor units)
- **IV IgG** - especially if demonstrate antibody
- **Combination** of IV IgG and concentrates
- **Immunosuppressive agents** - if Ab causal; start at the same time as treatment for bleeding
OTHER FACTOR INHIBITORS
Other Factor Inhibitors: general principles of treatment

Control bleeding and treat any related underlying disease

Bleeding control may include

- Factor replacement – often fresh frozen plasma (FFP) since a specific factor concentrate may not be available. Occasionally platelets in addition.
- Plasma exchange if life-threatening bleeding despite FFP; immunoadsorption; IVIg
- Recombinant activated factor VII or FEIBA
- DDAVP (acquired FVIII, VWF)

Immunosuppression - begin early to eliminate the inhibitor
Inhibitors of Human Factor V

- **Mechanism - Antibodies to human factor V**
  - May be associated with systemic infections, aminoglycoside antibiotics, cephalosporins, immune disease, or be “spontaneous”
  - Ab to C2 domain of FV commonly cause bleeding

- **Diagnosis - Prolonged aPTT and PT, not correcting with 1:1 mix or PL; factor V ↓**

- **Treatment - Plasma; platelets** (often effective as platelet factor V “protected” from antibody).
  - Immunosuppressive Rx to decrease Ab
Inhibitors to Factor V (Bovine cross reactivity with human factor V)

- **Mechanism** - Exposure to bovine (‘topical’) thrombin preparations (contain bovine factor V)
  - Elicits antibody that cross reacts with human factor V
  - May occur in 38% of exposures

- **Diagnosis**
  - *History of exposure to bovine thrombin - chart review
  - Prolonged aPTT and PT, not correcting with 1:1 mix or PL; factor V ↓

- **Treatment** - Plasma; platelets often effective as platelet factor V “protected” from antibody
## Antibodies to Prothrombin and Thrombin

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mechanism</th>
<th>Diagnosis</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>Prothrombin</td>
<td>“Clearance” Ab in pts with APS → bleed; suspect when PT is also ↑ &gt;2-3 sec</td>
<td>↑PT+↓immunologic &amp; functional prothromb. PT will correct in 1:1; clearance vs act site</td>
<td>FFP to reach prothrombin level of ~40%</td>
</tr>
<tr>
<td>Bovine Thrombin (topical)</td>
<td>Ab to bovine thrombin after topical thrombin use; rare cross-rx w human IIa</td>
<td>↑TT (thrombin time) using bovine thrombin; NI TT w human thrombin; Hx</td>
<td>Rarely necessary (Usually not cross react with human thrombin)</td>
</tr>
<tr>
<td>Human Thrombin</td>
<td>Autoantibodies in pts w SLE, RA, Imm Dis. If block IIa act site → bleed; If block AT III binding → thrombosis</td>
<td>↑TT (thrombin time) using human thrombin; no corr in 1:1 mix</td>
<td><strong>Bleeding</strong>: FFP, Plasma exchange; immmsuppress <strong>Thrombosis</strong>: AC</td>
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Antibodies to Factor VII

- Rare; few reported cases
  - Associations: penicillin, cephalosporins, ATG, IL-2, pancreatitis, malignancy

- Dx
  - Prolonged PT and normal aPTT; no correction on 1:1 mix or with added PL
  - Specific Factor VII assay – ↓ level

- Rx
  - Bypass agents; plasma exchange severe bleeding
  - Immunosuppression and steroids
INHIBITORS OF FACTOR IX

- Rare in patients who do not have Hemophilia B

**Associations** - SLE and post-partum state

**Dx** - prolonged aPTT with no correction on 1:1 mix or with added PL. ↓ Factor IX level

**Therapy** - Factor IX conc, FEIBA or rVIIa. Limited experience with immunosuppression; may spontaneously disappear over 1-7 months
INHIBITORS OF FACTOR X

- **Antibody-mediated**
  - Some associated with respiratory infection, malignancy
  - Dx - prolonged PT and aPTT; no corr in mix or PL.
  - ↓ Factor X level
  - Treatment - FFP, FEIBA, plasma exchge, steroids,

- **Associated with Amyloidosis**
  - Mechanism - Factor X binds to amyloid protein which is deposited extracellularly
  - Diagnosis - Prolonged aPTT and PT, corrects with 1:1 mix (clearance vs active site inhibition); factor X ↓
  - Treatment – Plasma, FX conc, and/or splenectomy (removes large deposit of amyloid)
# Inhibitors to Factor XI and Factor XII

<table>
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<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XI</td>
<td>Ab to -domain important for activation -to active site -to sites for binding to subendothelium</td>
<td>↑ aPTT not corr 1:1 mix or PL; ↓ Factor XI</td>
<td>May not require Rx; bleeding varies. FFP or XI concs (Europe); rVIIa</td>
</tr>
<tr>
<td>Factor XII</td>
<td>Ab to factor XII active site</td>
<td>↑ aPTT not corr 1:1 mix or PL; ↓ Factor XII</td>
<td>None required; no bleeding associated</td>
</tr>
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INHIBITORS OF FACTOR XIII

- **Mechanism**
  - Antibodies, some associated with isoniazid therapy, SLE
  - Cause delayed bleeding after invasion due to unstable clot. May cause serious bleeding (CNS)

- **Diagnosis** - Normal PT and aPTT
  - Specific assay for F XIII
  - Rapid clot dissolution in 1% monochloroacetic acid; 1:1 mix may also cause rapid dissolution of normal plasma clot

- **Treatment** – FXIII conc, plasma, immunosuppression
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<th>Diagnosis</th>
<th>Treatment</th>
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<tr>
<td>Dysfibrinogenemias</td>
<td>Abnormal glycosylation</td>
<td>↑ TT and reptilase time; immunologic fibrinogen &gt;&gt; activity of fib</td>
<td>Seldom required; if bleeding, plasma or cryoppt</td>
</tr>
<tr>
<td>DYSFIBRINOGENEMIA</td>
<td>(Qualitative defect; mutations)</td>
<td></td>
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<tr>
<td>Antibodies to fibrinogen</td>
<td>Interfere with</td>
<td>↑ TT and reptilase time; no corr with 1:1 mix</td>
<td>Can cause serious bleeding. Fibrinogen concs or cryoppt. Immunosupp</td>
</tr>
<tr>
<td></td>
<td>Cleavage of fibrinopeptides</td>
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<td>Monomer polymerization</td>
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<td>Cross linking</td>
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Heparin-like Anticoagulants

**Mechanism**
- Increased levels of heparan sulfate (HS) released from cell membranes
- HS binds to antithrombin and inhibits activated coagulation factors (eg, thrombin, Xa)

**Diagnosis** - Prolonged aPTT and thrombin time, not correcting with 1:1 mix or PL

**Treatment** - FFP, Protamine sulfate (?)
Summary - Laboratory Evaluation for Inhibitors

- ↑ PT, aPTT or both; some ↑ TT (thrombin time)
- No correction on 1:1 mixing study with normal plasma (usual)
- For Lupus Anticoagulant - Ask if tests correct with added PL

Yes = LAC

No = Factor Inhibitor
Factor VIII assay - most common factor inhibitor
Acquired Anticoagulants

- Circulating proteins that interfere with coagulation reactions
  - Usually IgG antibodies; some IgM; rarely IgA
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