Case studies in hemostasis and unprovoked thrombosis: questions and conundrums in coagulation

Christine Daniele MT(ASCP)
Claudia Escobar MT(ASCP)SH
Learning Objectives

• Describe the process of coagulation in the context of overall hemostasis
• Identify the role of different routine and specialized coagulation assays in diagnosis of hemostatic problems, especially unprovoked thrombosis
• Present current methods for screening and confirmation of problems related to hypo- and hypercoagulability
• Correlate coagulation testing to specific clinical cases
What is Hemostasis?
Blood Circulation

- Blood flow through blood vessels
- The heart pumps the blood
  - Arteries carry oxygenated blood away from the heart under high pressure
  - Veins carry de-oxygenated blood back to the heart under low pressure
Hemostasis

• The mechanism that maintains blood fluidity

• Keeps a balance between bleeding and clotting

• 2 major roles
  - To stop bleeding by repairing breaks in the blood vessels
  - To clean and maintain the inside of blood vessels
    o Removes temporary clot that stopped bleeding
    o Remove old clot fragments that may cause blood flow blockages
Two Major Diseases Linked to Hemostatic Abnormalities

• Bleeding = Hemorrhage

• Blood clot = Thrombosis
Physiology of Hemostasis
Wound Sealing

break in vessel

PRIMARY HEMOSTASIS

strong clot

wound sealing $\rightarrow$ blood flow $\pm$ stopped

FIBRINOLYSIS

clot destruction

PLASMATIC COAGULATION
The 3 Steps of Hemostasis

• Primary Hemostasis
  - The interaction between the injured vessel wall, platelets and adhesive proteins ➔ platelet clot

• Coagulation Factors
  - The consolidation of the platelet clot ➔ insoluble fibrin net
    o Coagulation factors and inhibitors

• Fibrinolysis
  - Clot lysis ➔ clot is digested
    o Fibrinolytic activators and inhibitors
Vessel Wall

- Intact endothelium ➔ non thrombogenic
  - Synthesis of vasodilators (prostacyclin)
  - No activation of platelets or factors
  - Limitation of thrombin generation
  - Regulation of fibrinolysis
When a vessel wall is damaged
- Exposure of the subendothelium
- Platelet adhesion
- Initiation of the mechanisms of coagulation and fibrinolysis
Primary Hemostasis

Aim is to clog the damaged vessel
($\approx$ bricks without cement)
Primary Hemostasis

- Vasoconstriction occurs first

- Platelets then aggregate on the break in the vessel wall
Assays for Primary Hemostasis

• Bleeding time / PFA
• Von Willebrand Factor
  - Antigen determination
  - Activity (ristocetin cofactor)
  - Factor VIII
• Platelet count
• Platelet aggregation
• Activation markers (β-TG, PF4, GPV)
• Specialized tests for platelet function
Primary Hemostasis Case Study

• 50 year old male with persistent left knee swelling/pain, increased over 4 months secondary to minor trauma. Received cryoprecipitate infusions, moderate improvement in swelling/pain.
• Arthroscopy was performed due to diffuse hemosiderin deposition. Pain worsened, leading to arrival at ER.
• History of hemophilia A: diagnosis at age 18 months after prolonged bleeding time observed.
• Recurrent spontaneous and trauma-induced hemarthroses in ankles/knees, episodes of GI and soft tissue bleeds, along with bleeding during dental procedures.
• Bleeding was treated with cyroprecipitates and FVIII concentrate during a 2 year period. No family history of bleeding.

### Primary Hemostasis Case Study – Lab Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>1.04</td>
<td>0.9 – 1.1</td>
</tr>
<tr>
<td>aPTT</td>
<td>61.2 seconds</td>
<td>24 – 30 seconds</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>2%</td>
<td>55 – 180%</td>
</tr>
<tr>
<td>Bethesda Assay</td>
<td>0 Bethesda units</td>
<td>0 Bethesda units</td>
</tr>
</tbody>
</table>

# Primary Hemostasis Case Study – Lab Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>1.04</td>
<td>0.9 – 1.1</td>
</tr>
<tr>
<td>aPTT</td>
<td>61.2 seconds</td>
<td>24 – 30 seconds</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>2%</td>
<td>55 – 180%</td>
</tr>
<tr>
<td>Bethesda Assay</td>
<td>0 Bethesda units</td>
<td>0 Bethesda units</td>
</tr>
<tr>
<td>% vWF antigen</td>
<td>16%</td>
<td>50 – 200%</td>
</tr>
<tr>
<td>Ristocetin cofactor</td>
<td>&lt; 12%</td>
<td>55 – 200%</td>
</tr>
<tr>
<td>vWF multimer test</td>
<td>Barely detectable, but</td>
<td>Normal size and distribution</td>
</tr>
<tr>
<td></td>
<td>present at normal range of sizes</td>
<td></td>
</tr>
</tbody>
</table>

Case Study – Diagnosis and Therapy

• When consideration of clinical findings and lab results are taken together, Hemophilia A was a misdiagnosis.
• Most likely diagnosis is type 3 von Willebrand Disease (VWD). This is the rarest subtype of VWD, which is a deficiency of vWF.
• Treatment
  - FVIII concentrates are not enough.
  - vWF replacements must also be given to correct bleeding episodes.
  - Desmopressin (DDAVP) cannot be given to stimulate secretion of vWF from endothelial cells, since is not indicated in type 3 VWD.

Coagulation Factors

Aim is to strengthen the platelet plug
Coagulation is a balance between pro- & anti-coagulant mechanisms → bleed & clot, → hemorrhage & thrombosis

**PROCOAGULANT FACTORS**

- Triggering agents
- pro-enzyme → enzyme (serine-protease: IIa, VIIa, IXa, Xa)
- Cofactors (V & VIII)

**ANTICOAGULANT FACTORS**

- Serine-protease inhibitor: AT (ATIII)
- Cofactors inhibitors: PC / PS
- (TFPI)

THROMBIN

Fibrinogen → Fibrin
## Coagulation factors

<table>
<thead>
<tr>
<th>Usual name</th>
<th>Factor</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>I</td>
<td>Substrate</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>II</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Proaccelerin</td>
<td>V</td>
<td>Cofactor</td>
</tr>
<tr>
<td>Proconvertin</td>
<td>VII</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Anti-hemophilic factor A</td>
<td>VIII</td>
<td>Cofactor</td>
</tr>
<tr>
<td>Anti-hemophilic factor B</td>
<td>IX</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Stuart factor</td>
<td>X</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Rosenthal factor</td>
<td>XI</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Hageman factor</td>
<td>XII</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Fibrin Stabilizing Factor</td>
<td>XIII</td>
<td>Pro-enzyme</td>
</tr>
</tbody>
</table>

Pro-enzyme = Zymogen ➔ activation ➔ Active Enzyme
Coagulation Cascade

- FXII
- FXI
- FIX
- FVII
- FV
- FX
- Prothrombin
- Thrombin
- Fibrinogen
- Fibrin

Extrinsic pathway:
- PT
- Tissue Factor Activation
- FXII
- FXI
- FIX
- FVII
- FV
- FX
- Prothrombin
- Thrombin
- Fibrinogen
- Fibrin

Extrinsic - PT

We ❤️ Coag
Prothrombin Time (PT)

- Developed by Dr. Quick 1935
- Citrated plasma + Thromboplastin + Ca\textsuperscript{++} = Time to clot in seconds
- Thromboplastin = Tissue factor + PL
- Especially sensitive to abnormalities of the “extrinsic system” including the common pathway; FVII, FX, FV, FII
- Useful for testing levels of extrinsic factors
- Not sensitive to abnormalities of fibrinogen unless fibrinogen < 100 mg/dL
Coagulation Cascade

Intrinsic – aPTT

Contact Activation

FXII
FXI
FIX
FVIII
FVII

FV
FX
Prothrombin
Thrombin
Fibrinogen
Fibrin

aPTT
Intrinsic pathway
Activated Partial Thromboplastin Time (aPTT)

- Screening test for > 50 years
- Citrated plasma + contact activator (kaolin, ellagic acid, celite) + PL + Ca\(^{++}\) = Time to clot in seconds
- Especially sensitive to abnormalities of the “intrinsic factors”; FXII, FXI, FIX, FVIII
- Useful for testing intrinsic factor levels as well as acquired factor inhibitors (Bethesda assay)
- Not sensitive to abnormalities of fibrinogen unless fibrinogen < 100 mg/dL, not sensitive to FII, little sensitivity for FV, FX
Abnormal PT and/or aPTT

- PT abnormal – aPTT normal
  - FVII
- PT normal – aPTT abnormal
  - FVIII, FIX, FXI, FXII
- PT abnormal – aPTT abnormal
  - FV, FX, FII, Fibrinogen
Responsiveness of aPTT and PT to Factor Activity Levels

- Reagent and even lot dependent
- Typical normal range 50 - 150%
  - Level where bleeding occurs depends on the factor and the situation but is generally lower
- May not detect deficiency of some factors until levels are below 30%
  - May not detect clinically significant factor deficiencies
- Factor sensitivity should be determined by each laboratory for each reagent or provided by manufacturer
Responsiveness of aPTT and PT to Factor Deficiency

- Normal aPTT and mild ↓ FVIII, FIX, FXI
- Evaluate these factors when patient has a history of a mild bleeding disorder even with a normal aPTT
- Most PT reagents are sensitive to ↓ FVII, II, V, X
- Abnormalities of these factors are uncommon
- Neither aPTT or PT is prolonged unless fibrinogen < 100mg/dL
Factor Testing Case Study

• 5 month old male presented for evaluation of markedly elevated aPTT, first seen immediately following birth. Factor IX was unquantifiable due to possible inhibitor presence.
• Test was repeated and found to be markedly decreased.
• Inhibitor screen was negative.
• Born without complications vaginally, unremarkable pregnancy. No bleeding since birth.
• Family history included mother who was a symptomatic hemophilia B carrier with FIX of 16%, father had FIX of 2%.

We ❤️ Coag

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (initial)</td>
<td>13.0 seconds</td>
<td>9.2 – 12.5 seconds</td>
</tr>
<tr>
<td>PT (repeated)</td>
<td>11.1 seconds</td>
<td>9.2 – 12.5 seconds</td>
</tr>
<tr>
<td>aPTT (initial)</td>
<td>104.2 seconds</td>
<td>23.5 – 33.5 seconds</td>
</tr>
<tr>
<td>aPTT (repeated)</td>
<td>84.7 seconds</td>
<td>23.5 – 33.5 seconds</td>
</tr>
<tr>
<td>Inhibitor screen</td>
<td>Not done</td>
<td>Negative</td>
</tr>
<tr>
<td>(initial)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibitor screen</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>(repeated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor IX (initial)</td>
<td>Unable to quantify due to potential</td>
<td>82 – 155%</td>
</tr>
<tr>
<td>Presence of inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor IX (repeated)</td>
<td>&lt; 1%</td>
<td>82 – 155%</td>
</tr>
</tbody>
</table>
Case Study – Diagnosis and Therapy

• Most likely diagnosis is hemophilia B, since not on heparin therapy, family history was positive for hemophilia B, and lab tests indicated markedly decreased FIX.

• If mild to moderate bleeding occurs, provide FIX infusions with goal to achieve FIX level of at least 30%.

• If more severe bleeding occurs, need to achieve FIX levels of 100 – 150% aggressively then maintain at 80 – 100% over next 5 – 7 days, followed by vigorous maintenance of these levels.

• Monitor for presence of factor inhibitors which commonly occur in patients receiving factor infusions.

Fibrinolysis

(Digestion of Fibrin)
Fibrin Formation (Clot)

Formation of Fibrin

- Fibrinogen
- Thrombin
- Fibrin Monomer + fibrinopeptides A & B
- Soluble Fibrin Polymer
- XIII $\rightarrow$ XIIIa
- Stabilized Fibrin clot (not soluble)

Thrombin crosslinking
Fibrinolysis

• Destroys fibrin fibers

• Destroys the scab (*dried wound*) that stopped bleeding

• Maintains vessel integrity
Fibrinolysis

- Plasmin acts on fibrin
Fibrinolysis Releases D-dimers

D-dimer presence: fibrin has been formed and digested in patient's body

Normal D-dimer level: no thrombosis occurred in the patient
Fibrinolysis

Extrinsic pathway
(endothelial cells)

1st Step

- t-PA
- PAI-1

Intrinsic pathway
(plasma)

Pro Urokinase

Proteolysis

PK → Kallikrein

Urokinase

2nd Step

Fibrin clot

- TAFIa

Plasminogen → Plasmin

- α2AP
- a2MG

Antiplasmin

AP

Fibrin degradation products

- FDP

D-dimer

Fibrin clot

PK: Prekallikrein

FDP: Fibrinogen degradation products

AP: Antiplasmin = a2AP: alpha 2 Antiplasmin / a2MG: alpha 2 Macroglobulin

t-PA : tissue-type plasminogen activator / PAI-1 : Plasminogen activator inhibitor 1 / PK : Prekallikrein

We ❤️ Coag
Main Assays for Fibrinolysis

- Fibrin/Fibrinogen Degradation Products
- D-dimer
- Plasminogen
- Antiplasmin
- Plasminogen Activator
- Plasminogen Activator Inhibitor
Fibrinolysis Testing Case Study

- 18 year old male presented to the ED after 3 weeks of nosebleeds and increasing levels of severe fatigue. No medical history, born at term, all developmental milestones achieved. No family history of bleeding or thrombosis. No medications, denies recreational drugs/alcohol. Physical exam finds blood clots in both nostrils and petechial hemorrhages in mouth and lower extremities. Patients bleeding subsided but lab results were monitored closely during hospitalization while blood products were administered.

## Fibrinolysis Testing Case Study – Lab Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count</td>
<td>7.7 K/μL</td>
<td>4.23 – 9.07 x K/μL</td>
</tr>
<tr>
<td>RBC count</td>
<td>1.7 M/μL</td>
<td>13.7 – 17.5 x M/μL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>6.7 g/dL</td>
<td>13.7 – 17.5 g/dL</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>19.5%</td>
<td>40.1 – 51.0%</td>
</tr>
<tr>
<td>MCV</td>
<td>95 fL</td>
<td>79.0 – 92.2 fL</td>
</tr>
<tr>
<td>MPV</td>
<td>12 fL</td>
<td>9.4 – 12.4 fL</td>
</tr>
<tr>
<td>Platelet count</td>
<td>9 K/μL</td>
<td>161 – 347 K/μL</td>
</tr>
<tr>
<td>Metamyelocytes, promyelocytes, myelocytes, myeloblasts</td>
<td>all elevated above normal level of 0</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes, monocytes, eosinophils, basophils</td>
<td>all below normal range</td>
<td></td>
</tr>
<tr>
<td>PT</td>
<td>47 seconds (corrected on mixing study)</td>
<td>11.6 – 15.2 seconds</td>
</tr>
<tr>
<td>APTT</td>
<td>75 seconds (corrected on mixing study)</td>
<td>25.3 – 37.3 seconds</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>&lt; 76 mg/dL</td>
<td>177 – 466 mg/dL</td>
</tr>
<tr>
<td>D-dimer</td>
<td>9.00 μg/mL FEU</td>
<td>0 – 0.50 μg/mL FEU</td>
</tr>
</tbody>
</table>

Case Study – Microscopy

Arrow shows giant platelets in this peripheral smear

Promyelocytes apparent in this peripheral smear

Case Study – Diagnosis and Therapy

• Profound anemia, significant reticulocytosis, and increased mean corpuscular volume (MCV), decreased platelets with increased mean platelet volume (MPV), numerous promyelocytes, High D-dimer, with PT/APTT correcting on mixing study, along with low fibrinogen indicate Disseminated intravascular coagulation (DIC) secondary to acute myelogenous leukemia (AML); most likely acute promyelocytic leukemia (APL). DIC is due to release of tissue factor (TF) by APL blasts.

• Molecular studies of PML-retinoic acid receptor-alpha (RARA) gene fusion was positive, which occurs in >95% of APL cases.

• Transfusions to replace factors, along with platelets and RBCs are needed during APL treatment.

The Regulation of Coagulation
Coagulation Inhibitors

• **AT** = Antithrombin
  - Old name: ATIII = Antithrombin III

• **PC** = Protein C
  - APC: Activated Protein C

• **PS** = Protein S
  - C4bBP = C4b Binding Protein

• (TFPI = Tissue Factor Pathway Inhibitor)
AT, PC, PS

- Inhibition systems of the coagulation cascade
  - Stops the \textit{in vivo} clot formation

- Synthesized in liver

- PC & PS = vitamin K dependent

- Normal values very close to abnormal values: approx. 70%
Main Assays for Thrombophilia

- Global screening tests: PT, PTT, TT, Fibrinogen
- APCR to screen for Factor V Leiden (FVL) and prothrombin G20210A mutation
- Factor V Leiden (FVL) mutation
- Prothrombin G20210A mutation
- AT activity
- PC activity
- PS activity
- Factor VIII activity
- Antiphospholipid antibodies
- Homocysteine level
Thrombophilia Case Study

- 42 year old female presents with right-side chest pain followed by dyspnea, hemoptysis, and a low-grade fever. Also reported calf pain 3 days earlier.
- History of craniotomy for left ophthalmic artery aneurysm repair 6 days prior, hypertension, gastroesophageal reflux, spontaneous pregnancy loss, and thyroidectomy.
- Only medication is lisinopril, no oral contraceptive use.
- Chest radiograph showed enlarged cardiac silhouette, bibasilar lung atelectasis, CT scan showed bilateral lower lobe pulmonary embolism (PE) with possible right posterior basal segment infarct

## Thrombophilia Testing Case Study – Lab Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count</td>
<td>6.9 x 10³/μL</td>
<td>4.0 – 11.0 x 10³/μL</td>
</tr>
<tr>
<td>RBC count</td>
<td>4.55 x 10³/μL</td>
<td>3.8 – 5.2 x 10³/μL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>13.3 x 10³/μL</td>
<td>11.3 – 15.2 g/dL</td>
</tr>
<tr>
<td>Platelet count</td>
<td>324 x 10³/μL</td>
<td>150 – 400 x 10³/μL</td>
</tr>
<tr>
<td>PT</td>
<td>13.5 seconds</td>
<td>12.6 – 14.6 seconds</td>
</tr>
<tr>
<td>INR</td>
<td>1.04</td>
<td>0.9 – 1.1</td>
</tr>
<tr>
<td>aPTT</td>
<td>24 seconds</td>
<td>25 – 35 seconds</td>
</tr>
<tr>
<td>Activated protein C resistance</td>
<td>Abnormal</td>
<td>Normal</td>
</tr>
<tr>
<td>Factor V Leiden (FVL) mutation</td>
<td>Heterozygous</td>
<td>Wild type</td>
</tr>
<tr>
<td>Prothrombin G20210A mutation</td>
<td>Heterozygous</td>
<td>Wild type</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>3.5 μmol/L</td>
<td>5.0 – 12.0 μmol/L</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>IgG anticardiolipin Ab</td>
<td>&lt; 9</td>
<td>&lt; 15 GPL/mL</td>
</tr>
<tr>
<td>IgM anticardiolipin Ab</td>
<td>&lt; 9</td>
<td>&lt; 12 MPL/mL</td>
</tr>
</tbody>
</table>

Case Study – Diagnosis and Therapy

• PE caused by history of untreated deep vein thrombosis (DVT). Abnormal APCR test along with heterozygosity for FVL and prothrombin G20210A mutation show elevated inherited risk for thrombophilia. Homocysteine was normal, but if elevated could be corrected by vitamins.

• PC/PS tests are not appropriate because acute thrombosis will reduce levels.

• Prolonged anticoagulation treatment for venous thromboembolism (VTE) is required.

Coagulation Gone Crazy
Venous Thromboembolism (VTE)

VTE
Occurs 1:1000

DVT
Deep Vein Thrombosis
70% occurrences
30 day mortality 6%

PE
Pulmonary Embolism
30% occurrences
30 day mortality 12%
VTE by the Numbers

An estimated 300,000-600,000 people are affected by VTE annually.

60,000-100,000 Americans are estimated to die each year from VTE.
Deep Vein Thrombosis (DVT)

A blood clot (thrombus) that forms in a deep vein of the leg or pelvis either partially or totally blocking the flow of blood

Pulmonary Embolism (PE)

1. A Deep Vein Thrombosis (blood clot), or part of it, breaks off from the vein.
2. The breakaway clot travels through the bloodstream, to the heart and migrates towards the lung.
3. The clot blocks a vessel in the lung, interrupting blood supply.

VTE Risk Factors - Provoked

- Prolonged immobility
- Major surgery/trauma
- Increasing age
- Cardiac failure
- Pregnancy

Unprovoked VTE

• 50% of VTE occurrences
• Anticoagulation cessation – recurrence rate
  - 10% within 1 year
  - 30% within 5 years
• Recurrent DVT causes Post Thrombotic syndrome
  - 20 – 50% patients
  - Leg swelling and ulcers
• Recurrent PE causes Chronic Thromboembolic Pulmonary Hypertension
  - 2 – 4%
  - Life threatening

Anticoagulation Treatment for VTE Stages

• Initial therapy
  - UFH/LMWH – 5 days
  - Warfarin – therapeutic INR

• Long term treatment
  - Warfarin – 30 days therapeutic INR

• Extended anticoagulation
  - Warfarin – life long
Historical Anticoagulants

- UFH Heparin – immediate anticoagulation
  - Administered IV or subcutaneous
  - UFH – unpredictable dose response
    - Bio-availability varies widely among patients
    - Requires AT for action
    - Heparin Induced Thrombocytopenia (HIT)
    - Overdose → bleeding
    - Short term use
    - Requires monitoring
    - Discrepancies with APTT and Heparin level
Historical Anticoagulants

- **LMWH**: quick acting, good bioavailability
  - Comparable raw material to UFH: shorter chain
    - Smaller molecule makes it’s action more predictable
  - Monitoring not recommended except:
    - Obesity
    - Elderly & very young
    - Renal failure
  - HIT antibody can react with LMWH
  - Cannot use the APTT to monitor
  - LMWH $\rightarrow$ F. Xa
Historical Anticoagulants

- Coumadin® – delayed anticoagulation
  - Oral anticoagulant
  - Narrow therapeutic range
  - Large inter and intra individual dose response
  - Slow onset
  - Extensive food and drug interaction
  - Effectiveness & dosing may be determined by genetics
  - Need to monitor
The “Ideal” Anticoagulant

- Oral fixed dose, preferably once per day
- Rapid onset
- No need for renal or hepatic adjustments
- Predictable pharmacokinetics/dynamics
- No need to ever switch therapies
- Wide therapeutic window
- No need for routine anticoagulation effect monitoring
- Low propensity for food/drug interactions
- Available antidote
- Reasonable cost
NOACs Target a Single Coagulation Factor

- rivaroxaban (XARELTO®)
- apixaban (ELIQUIS®)
- edoxaban (SAVAYSA®)
- dabigatran (PRADAXA®)
### NOAC Approvals – US and Canada

<table>
<thead>
<tr>
<th></th>
<th>Hip/Knee surgery</th>
<th>AF</th>
<th>Long term anticoagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dabigatran etexilate (Pradaxa®)</strong></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>Rivaroxaban (Xarelto®)</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Apixaban (Eliquis®)</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Edoxaban (Savaysa®)</strong></td>
<td>Filed with FDA Jan 2014</td>
<td>Filed with FDA Jan 2014</td>
<td></td>
</tr>
</tbody>
</table>
Comparison of the Pharmacologic Properties

<table>
<thead>
<tr>
<th></th>
<th>Warfarin</th>
<th>Dabigatran</th>
<th>Rivaroxaban</th>
<th>Apixaban</th>
<th>Edoxaban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>VKORC1</td>
<td>Thrombin</td>
<td>FXa</td>
<td>FXa</td>
<td>FXa</td>
</tr>
<tr>
<td>Prodrug</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>100</td>
<td>7</td>
<td>80</td>
<td>60</td>
<td>62</td>
</tr>
<tr>
<td>Dosing</td>
<td>OD</td>
<td>BID</td>
<td>OD(BID)</td>
<td>BID</td>
<td>OD</td>
</tr>
<tr>
<td>Time-to-peak</td>
<td>4-5 d</td>
<td>1-3 h</td>
<td>2-4 h</td>
<td>1-2 h</td>
<td>1-2 h</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>40</td>
<td>14-17</td>
<td>7-11</td>
<td>8-14</td>
<td>5-11</td>
</tr>
<tr>
<td>Renal</td>
<td>None</td>
<td>80</td>
<td>33</td>
<td>27</td>
<td>50</td>
</tr>
<tr>
<td>Monitoring</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Interactions</td>
<td>Multiple</td>
<td>P-gp</td>
<td>3A4/P-gp</td>
<td>3A4/P-gp</td>
<td>P-gp</td>
</tr>
</tbody>
</table>

Potential NOAC Laboratory Testing Situations

• Before **surgery** or invasive procedure
  - If the patient has taken the drug
    o in previous 24 hrs (or longer if creatinine clearance is > 50 mL / min)

• Identification of **sub- and supratherapeutic levels**
  - taking other drugs known to significantly affect pharmacokinetic
  - at extremes of body weight
  - with deteriorating renal function

• **Reversal** of anticoagulation

• Suspicion of **overdose**

• Assessment of **compliance** (If thrombosis or bleeding occurs during therapy)

FXa Inhibitors

• FXa inhibitors will affect common coagulation assays
  - PT & PTT assays will be prolonged and are dose dependent (to a certain degree)
  - Fibrinogen: Clauss methodology – not affected
  - Factor assays: falsely decreased
  - Thrombin Time is not affected.
  - Clot based Protein S – may be falsely elevated
  - APCR PTT assays: will be affected
    o DRVV based APCR assays are not affected

Reference: Lindahl et al., Effects of dabigatran on coagulation assays; Thrombosis and Haemostasis, 105/2, 2011 pp. 371 - 378
Direct Thrombin Inhibitors

- DTIs will affect common coagulation assays
  - PT assays much less affected – but may be prolonged
  - PTT is prolonged and is dose dependent (to a certain degree)
  - Fibrinogen: reagent dependent. Some reagents give correct results, other are falsely decreased.
  - Thrombin Time is elevated
  - ATIII assays: those based on thrombin inhibition give falsely elevated results.
  - Clot based Protein C & S – may be falsely elevated
  - APCR assays: strongly affected. May make heterozygous patients for FV Leiden appear normal

Reference: Lindahl et al., *Effects of dabigatran on coagulation assays; Thrombosis and Haemostasis, 105/2, 2011 pp. 371 - 378*
Influence on routine and special coagulation assays

<table>
<thead>
<tr>
<th></th>
<th>Dabigatran</th>
<th>Rivaroxaban</th>
<th>Apixababan</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (INR/Sec)</td>
<td>↑</td>
<td>↑↑</td>
<td>(↑)</td>
</tr>
<tr>
<td>aPTTT</td>
<td>↑↑</td>
<td>↑</td>
<td>(↑)</td>
</tr>
<tr>
<td>TT</td>
<td>↑↑↑</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fib (Clauss)</td>
<td>↓ (↓)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fib (derived)</td>
<td>-</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>AT Fxa</td>
<td>-</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>F IIa</td>
<td>↑</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>aPTTT Factors</td>
<td>↓↓</td>
<td>↓</td>
<td>(↓)</td>
</tr>
<tr>
<td>PT Factors</td>
<td>↓</td>
<td>↓↓</td>
<td>(↓)</td>
</tr>
<tr>
<td>FXIII Chrom</td>
<td>↓</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Influence depends on drug concentrations, reagents, assays
## Influence on thrombophilia assays

<table>
<thead>
<tr>
<th></th>
<th>Dabigatran</th>
<th>Rivaroxaban</th>
<th>Apixaban</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein S clotting</strong></td>
<td>↑↑↑</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
</tr>
<tr>
<td><strong>Free Protein S</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>PC clotting</strong></td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td><strong>PC Chrom</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Lupus - DRVV</strong></td>
<td>↑↑↑</td>
<td>↑↑↑</td>
<td>↑ (↑)</td>
</tr>
<tr>
<td><strong>APC - aPTT</strong></td>
<td>↑↑↑</td>
<td>↑</td>
<td>(↑)</td>
</tr>
</tbody>
</table>

Influence depends on drug concentrations, reagents, assays.
Case Study - NOACs

• Presentation:
  - 75-yr old patient, 65 kg
  - CrCl: 28 mL/min (NR 88 – 128 mL/min)
  - Atrial fibrillation for 3 years

• Treatment:
  - Initially treated with warfarin after diagnosis, was stably anticoagulated, but patient complained about limitations of warfarin therapy (no leafy greens, too many trips to clinic to monitor)
  - Put on dabigatran after 2 years on warfarin
  - Treatment for 1 year with dabigatran, 75 mg/day

• Acute event:
  - Fell in home, cranial hemorrhage resulted, transported to hospital
## Case Study NOAC Laboratory Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>2.8</td>
<td>0.9 – 1.1</td>
</tr>
<tr>
<td>APTT</td>
<td>55 sec</td>
<td>24 – 30 sec</td>
</tr>
<tr>
<td>TT</td>
<td>90 sec</td>
<td>&lt; 18 sec</td>
</tr>
<tr>
<td>Fib (derived)</td>
<td>138 mg/dL</td>
<td>195 – 450 mg/dL</td>
</tr>
<tr>
<td>Fib (Clauss)</td>
<td>295 mg/dL</td>
<td>177 – 466 mg/dL</td>
</tr>
<tr>
<td>Anti-Xa</td>
<td>0.11 IU/mL</td>
<td>UFH: 0.3 – 0.7 IU/mL</td>
</tr>
<tr>
<td>ECA</td>
<td>270 ng/mL</td>
<td>60 – 160 ng/mL</td>
</tr>
</tbody>
</table>
## Case Study NOAC Laboratory Results – Dabigatran Influence

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>INR</td>
<td>2.8</td>
<td>0.9 – 1.1</td>
</tr>
<tr>
<td>APTT</td>
<td>55 sec</td>
<td>24 – 30 sec</td>
</tr>
<tr>
<td>TT</td>
<td>90 sec</td>
<td>&lt; 18 sec</td>
</tr>
<tr>
<td>Fib (derived)</td>
<td>138 mg/dL</td>
<td>195 – 450 mg/dL</td>
</tr>
<tr>
<td>Fib (Clauss)</td>
<td>295 mg/dL</td>
<td>177 – 466 mg/dL</td>
</tr>
<tr>
<td>Anti-Xa</td>
<td>0.11 IU/mL</td>
<td>UFH: 0.3 – 0.7 IU/mL</td>
</tr>
<tr>
<td>ECA</td>
<td>270 ng/mL</td>
<td>60 – 160 ng/mL</td>
</tr>
</tbody>
</table>
Case Study NOAC – Treatment (From Guidelines)

• Half life of dabigatran for patient with this CrCl is up to 28 hrs
• Estimate normalization of hemostasis in ≥ 48 hrs
• If non-life threatening
  - RBC/platelet infusion/fresh frozen plasma (if necessary)
  - Tranexamic acid/desmopression/dialysis could be considered
• If life threatening
  - Prothrombin complex concentrate (PCC) 25 U/kg
  - Activated PCC 50 IE/kg, max 200 IE/kg/day
  - Activated rFVIIa, 90 μg/kg
  - All of the above

Conclusions

• NOAC use can improve overall clinical outcomes

• NOACs should be measured in certain circumstances

• NOAC measurement solutions exist

• Reversal of NOACs currently requires haemodialysis or PCCs/rFVIIa

• **NOACs do interfere with many coagulation assays**
Conclusions

• Hemostasis is a constant balancing act
• Bleeding and clotting are always ongoing
• Many components involved in hemostasis
  - Imbalance in any part of the process can cause disease
• Laboratories investigate the cause of coagulation imbalances