Learning Objectives

DOACs:

- Discuss current methods for monitoring traditional anticoagulants
- Demonstrate the impact of DOACs on routine and specialty coagulation assays.
- Discuss a case study highlighting the role of the laboratory in this testing regime.
Heparin Anticoagulation Overview and Monitoring Methods
Anticoagulant drugs used to:
avoid abnormal clot formation
to stop the extension of an abnormal clot into the blood vessels.

- **Stroke**: 15 million people per year
- **Myocardial infarction**: 15 million people per year
- **VTE**: 10 million people per year
- **AF (risk of stroke)**: 30 million people
- **Orthopedic & Cardiac surgeries (risk of DVT)**

AF: Atrial Fibrillation  
VTE: Venous Thromboembolism  
DVT: Deep Vein Thrombosis  
PE: Pulmonary embolism
Heparin Comparison

- **Unfractionated (UFH)**
  - Primarily obtained from porcine intestine
  - Varied molecular weight
  - Binds to plasma proteins, PF4, macrophages & endothelial cells
    - Limits bioavailability & accounts for varied response
  - **MONITORING NECESSARY**

- **Low Molecular Weight (LMWH)**
  - Derived from UFH by depolymerization
    - Smaller molecules
  - Lower affinity for binding proteins other than AT
    - More predictable response

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UFH vs. LWMH Comparison – FXa vs. Thrombin

Potential UFH and LMWH Therapy Complications

Bleeding complications
• Difficult to define risk because dependent on numerous parameters: indication, dosage, method, duration of therapy, patient characteristics, co-medication

Non-bleeding complications
• Caused by UFH and LMWH binding to proteins other than AT and cells
• HIT is the most severe complication; mostly associated with UFH

UFH Monitoring Assays

- aPTT
- Anti-Xa
- ACT
Reasons for a Prolonged aPTT

Blood Sampling (Pre-analytical)
- Tube fill
- Tube type

UFH
- LMWH: no relation to APTT/drug dosage

Congenital Deficiencies
- Mainly hemophilia (VIII, IX)
- VWF/VIII (von Willebrand’s disease)
- II, V, X
- XI, XII
- Fibrinogen (hypo <0.8 g/L)
- Dysfibrinogenemia

Acquired Deficiencies
- Liver disease
- DIC
- Vit K deficiency
- Warfarin

Auto-Antibodies
- Specific (factor)
- Nonspecific (LA)
How did the APTT Become the Standard?

- 1972 landmark study concluded that a range APTT of 1.5 - 2.5 x the baseline value is required\(^1\)
  - This range was determined in animal studies and had not been tested clinically in humans
  - There was a question of whether you needed to monitor UFH or just use a standard dose
  - They were prospectively looking for a relationship between the APTT and recurrent venous thromboembolism (VTE) or bleeding

- 5 patients being treated for VTE developed a new thrombosis and all 5 had lower APTT than patients without new thrombus development\(^1\)

- Later, it was determined that the 1.5 - 2.5 x the baseline corresponded to 0.2 - 0.4 U/mL UFH by protamine sulfate titration\(^2,3\)

CAP 1998 Guidelines for UFH Monitoring

- Adjusted dose & therapeutic UFH requires monitoring using method with a defined range, testing at 6 hour intervals until a stable response achieved, then daily
- aPTT reagents for UFH therapy should be calibrated against an anti-Xa reagent corresponding to 0.3 - 0.7 IU/mL
- Anti-Xa method is the preferred alternative method to the aPTT for monitoring UFH

ACCP 2001 Guidelines for UFH Monitoring

- UFH monitoring with aPTT can be done but the therapeutic range should be calibrated against an anti-Xa range of 0.3 – 0.7 IU/mL
- Doses for intravenous (IV) and subcutaneous UFH therapy are described along with weight-based dosing strategies but the aPTT-based nomograms have the shortcoming of being reagent-dependent
- UFH monitoring with anti-Xa may be useful for patients at high risk of bleeding

Since 1998, CAP (and ACCP in 2001) has recommended calibrating aPTT reagents used for UFH therapy against an anti-Xa range of 0.3 – 0.7 IU/mL
### Heparin Therapy for Different Populations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bolus</th>
<th>Infusion rate</th>
<th>Target (APTT)</th>
<th>Target (anti-FXa) (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult (VTE and ACS)</td>
<td>80 U/kg (maximum 5,000 U)</td>
<td>18 U/kg/h (maximum 1,200 U/h and maximum 1,800 U/h for obese)</td>
<td>HTR</td>
<td>0.3–0.7</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 12 y</td>
<td>80 U/kg (maximum 5,000 U)</td>
<td>18 U/kg/h (maximum 1,200 U/h and maximum 1,800 U/h for obese)</td>
<td>HTR</td>
<td>0.3–0.7</td>
</tr>
<tr>
<td>&gt; 1 y</td>
<td>75 U/kg (maximum 5,000 U)</td>
<td>20 U/kg/h (maximum 1,200 U/h)</td>
<td>Do not use</td>
<td>0.3–0.7</td>
</tr>
<tr>
<td>0–12 mo</td>
<td>75 U/kg (maximum 5,000 U)</td>
<td>28 U/kg/h (maximum 1,200 U/h)</td>
<td>Do not use</td>
<td>0.3–0.7</td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>5,000 USQ every 8–12 h</td>
<td></td>
<td>APTT 1–5 above reference interval</td>
<td></td>
</tr>
</tbody>
</table>

The 0.3-0.7 target anti-Xa range is suitable for most patient therapeutic needs and is the only choice for therapy in patients < 12 yrs.

aPTT vs. anti-Xa Discordance
Assay Discordance

Discordance between the aPTT and anti-Xa assays is a well documented problem

- Preanalytic variables
- Biologic factors
- aPTT reagent/instrument combination variability
- Differences in testing targets
UFH Therapeutic Range Graph

Discordant values skew the best fit line

y = 63.712x + 53.099
R^2 = 0.1068

n=80
Final UFH Therapeutic Range Graph

Patients are dosed based on the graph constructed from these samples

\[ y = 87.76x + 42.542 \]

Concordant therapeutic results

\[ R^2 = 0.2397 \]

\[ n = 60 \]
• Case #1:
  - APTT: 75.4 seconds
  - Anti-Xa: 0.12 IU/mL

APTT is prolonged in relation to anti-Xa level.

\[ y = 87.76x + 42.542 \]

\[ R^2 = 0.2397 \]
• Case #2:
  - APTT: 113.3 seconds
  - Anti-Xa: 0.38 IU/mL

APTT is prolonged in relation to anti-Xa level

APTT=Super therapeutic
Anti-Xa=Therapeutic

\[ y = 87.76x + 42.542 \]
\[ R^2 = 0.2397 \]
anti-Xa Methodology and Determination of aPTT based Heparin Therapeutic Range
Anti-Xa Assay Overview

- **Quantitative determination of the plasma levels of both UFH and LWMH**
  - Direct sensitivity to UFH through measurement of anti-Xa activity on antithrombin

- **Few interferences**
  - Patient variables: not susceptible to interference from elevated factor VIII or fibrinogen and not influenced by factor deficiencies
  - Pre-analytical variables: not affected by under-filled collection tubes
  - Analytical variables: reagents are calibrated to international standards for UFH and LMWH

Anti-Xa Methodology

Sample plasma (with anti-Xa drug + Free AT) + Chromogenic Substrate → Colored product

Inverse relationship between chromogenic readout and drug concentrations

- UFH concentration
- Inhibition of FXa
- Chromogenic substrate cleavage
- Color development
Need for an aPTT Heparin Therapeutic Range

Due to different sensitivities of different aPTT reagents and between lots of the same reagent, if aPTT is used for monitoring, it must be calibrated to 0.3 – 0.7 anti-Xa

CAP Lot Change Recommendations

Compare the original PTT reagent lot to the new PTT lot to determine clinically equivalent response on samples of patients receiving UFH.

- The mean difference must not exceed 7 seconds.
- Each subsequent reagent lot is compared against the preceding lot, so laboratories must monitor the sum of differences from the reagent lot used in the original validation to ensure that the cumulative mean PTT difference does not exceed 7 seconds.

Establish a new therapeutic range


Cumulative Summation of Reagent Mean Differences - UF Heparin Samples

<table>
<thead>
<tr>
<th>Lot Conversion Sequence</th>
<th>Patient Data Set Date</th>
<th>Current Lot Mean Seconds</th>
<th>New Lot Mean Seconds</th>
<th>Difference in Seconds (New lot - Current Lot)</th>
<th>% Diff* from Current Reagent</th>
<th>Cumulative Summation Difference in Seconds</th>
<th>Significant?</th>
<th>Therapeutic Range Change</th>
<th>Date</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Mar-05 003221</td>
<td>56.4</td>
<td>56.0</td>
<td>-2.00</td>
<td>-4.43</td>
<td>-2.63</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>Mar-06 041951</td>
<td>66.7</td>
<td>65.5</td>
<td>-1.23</td>
<td>-1.84</td>
<td>-3.86</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>Mar-07 050849</td>
<td>70.1</td>
<td>70.0</td>
<td>-0.15</td>
<td>-0.21</td>
<td>-0.41</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td>Mar-08 061952</td>
<td>65.1</td>
<td>65.3</td>
<td>0.21</td>
<td>0.32</td>
<td>-3.80</td>
<td>no</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5th</td>
<td>Mar-09 101122</td>
<td>70.7</td>
<td>72.3</td>
<td>2.59</td>
<td>3.67</td>
<td>-1.21</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6th</td>
<td>Feb-10 102997</td>
<td>66.6</td>
<td>67.2</td>
<td>0.63</td>
<td>0.95</td>
<td>-0.57</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th</td>
<td>Feb-11 105351</td>
<td>63.7</td>
<td>62.6</td>
<td>-1.07</td>
<td>-1.68</td>
<td>-1.64</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8th</td>
<td>Feb-11 105261</td>
<td>63.7</td>
<td>62.6</td>
<td>-1.10</td>
<td>-1.73</td>
<td>-2.74</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9th</td>
<td>Jan-12 106272</td>
<td>66.6</td>
<td>64.7</td>
<td>-1.90</td>
<td>-2.85</td>
<td>-4.64</td>
<td>no</td>
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<td></td>
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<tr>
<td>10th</td>
<td>Jan-13 107812</td>
<td>66.7</td>
<td>67.6</td>
<td>0.90</td>
<td>1.35</td>
<td>-3.74</td>
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<td></td>
</tr>
<tr>
<td>11th</td>
<td>Feb-14 109355</td>
<td>76.7</td>
<td>79.3</td>
<td>2.60</td>
<td>0.76</td>
<td>-3.14</td>
<td>no</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reagents Change

Significance: (Arrows ref for HTR change: < 3 seconds is insignificant; >5 is cause for concern; >7 necessitates action [probable change of range])

Reviewed by: 
Date: 
Comments: 

Cumulative Sum
The cumsum method only examines the mean value of the HTR and does not take into account potential shifts in the upper and lower limits of the HTR. Thus, the cumsum method is not recommended.

Laboratory Options for Heparin Monitoring

For small and medium sized hospitals who may have trouble validating the aPTT HTR due to sample availability and technical difficulties, anti-Xa is preferred for UFH monitoring.

<table>
<thead>
<tr>
<th>APTT-based methods</th>
<th>Non-APTT-based method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex vivo comparison of heparin level with APTT</td>
<td>Direct assay of anti-FXa activity</td>
</tr>
<tr>
<td>Use fresh samples from larger hospital for ex vivo method</td>
<td></td>
</tr>
<tr>
<td>Freeze samples for APTT and heparin levels (platelet count in plasma is critical) and should be &lt; 10,000/μL</td>
<td></td>
</tr>
<tr>
<td>At multihospital networks, perform at &gt; 2 facilities and if agree within 10%, accept for all hospitals in network</td>
<td></td>
</tr>
</tbody>
</table>

**Reagent Formulation Differences: Antithrombin**

**Containing Exogenous AT**
- Standard AT concentration in the reaction
- Measuring a standardized heparin inhibitory activity in the sample
- Possibility for overestimation of heparin levels
  - Drug unbound to AT in vitro (inactive) to bind exogenous AT and have an anticoagulant effect in vitro

**Without Exogenous AT**
- AT-Heparin complex is already formed in the sample
- Heparin activity depends on the sample concentration of AT
- Reaction of the AT-Heparin complex inhibiting FXa happens all in one step
  - Measures the true heparin inhibitory activity in the sample
  - *In vitro* effect closely emulates *in vivo* effect

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The bleeding incidence was four times higher in the APTT-monitored group who were given an average of 4000 units/day more UFH than those monitored by the anti-Xa assay.

**APTT vs. Anti-Xa – Time to Therapeutic Range**

![Graph showing comparison between aPTT and Anti-Xa](image)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>aPTT</th>
<th>Anti-Xa</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTR (hr)</td>
<td>48 (26)</td>
<td>28 (16)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% tests within TR</td>
<td>42 (20)</td>
<td>66 (18)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tests performed per 24 hr period</td>
<td>2.8 (0.6)</td>
<td>2.5 (0.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dosage changes per 24 hr period</td>
<td>1.6 (0.7)</td>
<td>0.8 (0.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Major hemorrhage (n (%))</td>
<td>6 (12)</td>
<td>5 (10)</td>
<td>1</td>
</tr>
<tr>
<td>Mean LOS (days)</td>
<td>25 (34)</td>
<td>17 (15)</td>
<td>0.13</td>
</tr>
<tr>
<td>VTE/bleeding mortality (n (%))</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

UFH monitoring using anti-Xa enables a faster time to therapeutic anticoagulation, higher numbers of tests within the TR, fewer tests and dosage changes, and potential LOS improvement.

In a study from Stanford of 2,321 paired aPTT and anti-Xa values from 539 patients, a majority of aPTT values were discordant with anti-Xa.

Assay Discordance: Mortality Outcomes

Baseline PT and aPTT of patients who died within 30 days in group with consistently high aPTT values relative to anti-Xa values

### Exempla St. Joseph’s Institutional Impact

<table>
<thead>
<tr>
<th></th>
<th>Anti-Xa</th>
<th>APTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number tests /patient / day</td>
<td>2.08</td>
<td>2.73</td>
</tr>
<tr>
<td>Mean number UFH dosage adjustments /patient / day</td>
<td>0.62</td>
<td>1.47</td>
</tr>
<tr>
<td>Reagent cost</td>
<td>$2.55 / test</td>
<td>$0.65 / test</td>
</tr>
<tr>
<td></td>
<td>$5.30 / patient / day</td>
<td>$1.77 / patient / day</td>
</tr>
<tr>
<td>Lab tech and phlebotomy labor</td>
<td>$3.08 / test</td>
<td>$3.08 / test</td>
</tr>
<tr>
<td></td>
<td>$6.40 / patient / day</td>
<td>$8.41 / patient / day</td>
</tr>
<tr>
<td>Nurse labor</td>
<td>$2.58 / dosage adjustment</td>
<td>$2.58 / dosage adjustment</td>
</tr>
<tr>
<td></td>
<td>$1.60 / patient / day</td>
<td>$2.58 / patient / day</td>
</tr>
<tr>
<td>Total cost / patient / day</td>
<td>$13.30</td>
<td>$13.97</td>
</tr>
</tbody>
</table>

Anticoagulation Overview
Indications for Anticoagulation

• Venous thrombosis
  - Treatment
  - Prophylaxis

• Atrial fibrillation

• Mechanical heart valve

• Arterial thrombosis
  - Acute heart attack
  - Cardiac surgery
DVT/VTE Prevention

• > 2 million people acquire VTE per year; 50% acquire it as inpatients or 30 days post hospitalization
  ♦ PE is the most common cause of preventable death

• 23 million people have surgery per year
  ♦ up to 20% of high risk surgical patients acquire DVT, and only 5% are at low risk of VTE
  ♦ Beginning in 2015, the perioperative DVT/PE rate is one of the measures used for Medicare’s Hospital Value-Based Purchasing that links quality to payment

• In one year, a single 400 bed hospital will have 200 hospital acquired VTEs with 50% of those having been preventable (!!!)

• Anticoagulant drug prophylaxis reduces all DVT/PE incidence by 50-65%

Traditional anticoagulants and DOACs play large roles in effective prevention and treatment of DVT, VTE, and PE, with a need for more clinical guidelines

US Department of Health and Human Services Agency for Healthcare Research and Quality
Atrial fibrillation is a serious cardiac disease that can cause stroke; it is the most common type of irregular heartbeat. When the upper chambers of the heart don’t beat the way they should, blood pools in the left atrium where a clot can form.

Projections for Atrial Fibrillation Incidence

AFib currently affects more than 2 million people in the US. The incidence of atrial fibrillation will continue to increase as the population ages.

HAS-BLED Clinical Scoring

<table>
<thead>
<tr>
<th>Letter</th>
<th>Clinical Characteristic</th>
<th>Points Awarded</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Hypertension</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>Abnormal renal and liver function (1 point each)</td>
<td>1 or 2</td>
</tr>
<tr>
<td>S</td>
<td>Stroke</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>Bleeding</td>
<td>1</td>
</tr>
<tr>
<td>L</td>
<td>Labile INRs</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>Elderly</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>Drugs or alcohol (1 point each)</td>
<td>1 or 2</td>
</tr>
</tbody>
</table>

Use of the CHADS$_2$ and HAS-BLED scores enables clinicians to decide which patients should get anticoagulation and are at risk of 1 year major bleeding.

CHADS$_2$ Clinical Scoring for AF

<table>
<thead>
<tr>
<th>Letter</th>
<th>Risk Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Congestive heart failure</td>
<td>1 point</td>
</tr>
<tr>
<td>H</td>
<td>Hypertension</td>
<td>1 point</td>
</tr>
<tr>
<td>A</td>
<td>Age &gt; 75 years</td>
<td>1 point</td>
</tr>
<tr>
<td>D</td>
<td>Diabetes Mellitus</td>
<td>1 point</td>
</tr>
<tr>
<td>S</td>
<td>Stroke/transient ischemic attack</td>
<td>2 points</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CHADS$_2$ Score</th>
<th>No. of Patients (n = 1733)</th>
<th>No. of Strokes (n = 94)</th>
<th>NRAF Crude Stroke Rate per 100 Patient-Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>120</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td>1</td>
<td>463</td>
<td>17</td>
<td>2.8</td>
</tr>
<tr>
<td>2</td>
<td>523</td>
<td>23</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>337</td>
<td>25</td>
<td>6.4</td>
</tr>
<tr>
<td>4</td>
<td>220</td>
<td>19</td>
<td>8.0</td>
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<tr>
<td>5</td>
<td>65</td>
<td>6</td>
<td>7.7</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>2</td>
<td>44.0</td>
</tr>
</tbody>
</table>

Traditional and Direct Oral Anticoagulants (DOACs)
Anticoagulant Targets in the Coag Cascade

DOACs Target a Single Coagulation Factor

- TF/VIIa
- X
- IX
- VIIIa
- IXa
- Va
- Xa
- II
- IIa
- Fibrinogen
- Fibrin
- Dabigatran (PRADAXA®)
- Rivaroxaban (XARELTO®)
- Apixaban (ELIQUIST®)
- Edoxaban (SAVAYSA®)
DOACs (Direct Oral Anticoagulants)

- Rivaroxaban (XARELTO®)
- Apixaban (ELIQUIS®)
- Edoxaban (SAVAYSA®)
- Dabigatran (PRADAXA®)

Diagram:
- Xa
- TF/VIIa
- IX
- VIIIa
- IXa
- Va
- II
- Fibrinogen
- Fibrin

DOAC
Direct Oral Anticoagulant
## Warfarin vs. DOACs

<table>
<thead>
<tr>
<th>Drug Feature</th>
<th>Warfarin&lt;sup&gt;10&lt;/sup&gt;</th>
<th>Dabigatran&lt;sup&gt;20&lt;/sup&gt;</th>
<th>Rivaroxaban&lt;sup&gt;11&lt;/sup&gt;</th>
<th>Apixaban&lt;sup&gt;22&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Vitamin K</td>
<td>Thrombin</td>
<td>Factor Xa</td>
<td>Factor Xa</td>
</tr>
<tr>
<td>Dose frequency</td>
<td>Daily</td>
<td>Once or twice daily</td>
<td>Once or twice daily</td>
<td>Twice daily</td>
</tr>
<tr>
<td>Onset</td>
<td>Slow</td>
<td>Rapid</td>
<td>Rapid</td>
<td>Rapid</td>
</tr>
<tr>
<td>Peak effect</td>
<td>4 to 5 days</td>
<td>1 to 2 hours</td>
<td>2 to 3 hours</td>
<td>1 to 2 hours</td>
</tr>
<tr>
<td>Offset</td>
<td>Long</td>
<td>Short</td>
<td>Short</td>
<td>Short</td>
</tr>
<tr>
<td>Half-life</td>
<td>40 hours</td>
<td>12 to 17 hours</td>
<td>7 to 11 hours</td>
<td>12 hours</td>
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<tr>
<td>Renal clearance</td>
<td>None</td>
<td>80%</td>
<td>33%</td>
<td>25%</td>
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<tr>
<td>Interactions</td>
<td>Many</td>
<td>P-gp</td>
<td>CYP3A4; P-gp</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Monitoring</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dialyzable</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Antidote</td>
<td>Vitamin K</td>
<td></td>
<td></td>
<td>YES</td>
</tr>
</tbody>
</table>

b.i.d. indicates twice daily; CYP3A4, cytochrome P<sub>450</sub> 3A4 enzyme; P-gp, P-glycoprotein; q.d., once daily.

Half lives of DOACs are much shorter vs. warfarin but DOACs are cleared through the renal system, and no antidotes are available.

DOACs – Commonalities

- Predictable Pharmacokinetics and pharmacodynamics in most patient populations
- Promise of little to no routine laboratory monitoring

Gosselin RC, Douxfils J, Adcock A. Clinical Pearls: Laboratory assessment of direct oral anticoagulants (DOACS); Sem Throm Haemostasis, July 2017
### DOAC Approvals – US/Canada

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hip/Knee surgery</th>
<th>Atrial Fibrillation (AF)</th>
<th>DVT/PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran etexilate (Pradaxa®)</td>
<td>✓*</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Rivaroxaban (Xarelto®)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Apixaban (Eliquis®)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Edoxaban (Savaysa®)</td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Betrixaban (Bevyxxa®)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* prophylaxis of venous thromboembolism (VTE) in adults hospitalized for an acute medical illness who are at risk for thromboembolic complications due to moderate or severe restricted mobility and other risk factors for VTE.

*Canada only*
## Warfarin vs. DOACs

<table>
<thead>
<tr>
<th>Drug Feature</th>
<th>Warfarin&lt;sup&gt;19&lt;/sup&gt;</th>
<th>Dabigatran&lt;sup&gt;20&lt;/sup&gt;</th>
<th>Rivaroxaban&lt;sup&gt;21&lt;/sup&gt;</th>
<th>Apixaban&lt;sup&gt;22&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Vitamin K</td>
<td>Thrombin</td>
<td>Factor Xa</td>
<td>Factor Xa</td>
</tr>
<tr>
<td>Dose frequency</td>
<td>Daily</td>
<td>Once or twice daily</td>
<td>Once or twice daily</td>
<td>Twice daily</td>
</tr>
<tr>
<td>Onset</td>
<td>Slow</td>
<td>Rapid</td>
<td>Rapid</td>
<td>Rapid</td>
</tr>
<tr>
<td>Peak effect</td>
<td>4 to 5 days</td>
<td>1 to 2 hours</td>
<td>2 to 3 hours</td>
<td>1 to 2 hours</td>
</tr>
<tr>
<td>Offset</td>
<td>Long</td>
<td>Short</td>
<td>Short</td>
<td>Short</td>
</tr>
<tr>
<td>Half-life</td>
<td>40 hours</td>
<td>12 to 17 hours</td>
<td>7 to 11 hours</td>
<td>12 hours</td>
</tr>
<tr>
<td>Renal clearance</td>
<td>None</td>
<td>80%</td>
<td>33%</td>
<td>25%</td>
</tr>
<tr>
<td>Interactions</td>
<td>Many</td>
<td>P-gp</td>
<td>CYP3A4; P-gp</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Monitoring</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dialyzable</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Antidote</td>
<td>Vitamin K</td>
<td>YES</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b.i.d. indicates twice daily; CYP3A4, cytochrome P<sub>450</sub> 3A4 enzyme; P-gp, P-glycoprotein; q.d., once daily.

Half lives of DOACs are much shorter vs. warfarin but DOACs are cleared through the renal system, and no antidotes are available.

## DOAC Bridging to Other Anticoagulants

<table>
<thead>
<tr>
<th>Bridging therapy</th>
<th>Moderate-high bleeding or thrombotic risk. CrCl&lt;50 ml/min</th>
<th>DOAC OR LMWH (WHEN HAEMOSTATIC COMPETENCE IS REACHED)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAST DOSE DOAC</td>
<td>FIRST DOSE LMWH*</td>
<td>LMWH</td>
</tr>
<tr>
<td>-5</td>
<td>-4</td>
<td>-3</td>
</tr>
<tr>
<td>TIME (DAYS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAST DOSE OF DOAC</td>
<td>NO DOAC</td>
<td>SURGERY</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### NO bridging therapy
Low bleeding or thrombotic risk. CrCl≥50 ml/min

---

Anticoagulant Market Trends
Anticoagulant Market Trends – Aetna Cohort

The Laboratory and DOACs
Potential DOAC Laboratory Testing Situations

- **Before surgery or invasive procedure**
  - If the patient has taken the drug
    - 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)**

"On Therapy" vs Therapeutic Range

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Trough plasma level (ng/mL)</th>
<th>Peak plasma level (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median 5&lt;sup&gt;th&lt;/sup&gt;-95&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>Median 5&lt;sup&gt;th&lt;/sup&gt;-95&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td></td>
</tr>
<tr>
<td>Dabigatran</td>
<td>150 mg BID</td>
<td>90 (31-225)</td>
<td>184 (64-443)</td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>20 mg daily</td>
<td>26 (6-87)</td>
<td>270 (189-419)</td>
</tr>
<tr>
<td>Apixaban</td>
<td>5 mg BID</td>
<td>103 (41-230)</td>
<td>171 (91-321)</td>
</tr>
<tr>
<td>Edoxaban</td>
<td>60 mg daily</td>
<td>22 (10-40&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>170 (120-250&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Interquartile range

Data from Adam Cuker presentation at AC Forum April 2015
<table>
<thead>
<tr>
<th>Coagulation Test</th>
<th>Below on-therapy range</th>
<th>On-therapy range</th>
<th>Above on-therapy range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dabigatran</strong></td>
<td></td>
<td>TT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dilute TT, ECT, ECA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>APTT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT</td>
<td></td>
</tr>
<tr>
<td><strong>Rivaroxaban</strong></td>
<td>Anti-Xa Activity</td>
<td>PT</td>
<td>APTT</td>
</tr>
<tr>
<td><strong>Apixaban</strong></td>
<td>Anti-Xa Activity</td>
<td>PT</td>
<td>APTT</td>
</tr>
</tbody>
</table>

The Laboratory and DOACs; Screening Assays
DOACs and PT

- Though rivaroxaban prolongs the PT, the degree of prolongation depends on the reagent
  

- Apixaban does not significantly prolong the PT at therapeutic does, though the degree of prolongation depends on the reagent
  
DOACs and APTT

**APTT prolongation with DOAC is moderate, depending on the reagent**


**APTT response is curvilinear**

Limitations of aPTT for Dabigatran

aPTTs are sensitive to various factors, the response of the test is curvilinear, and a normal aPTT cannot exclude the presence of dabigatran.

PT & APTT – Pros/Cons

**PROS**
- Widely available
- Cost effective

**CONS**
- Non specific
- Poor sensitivity
- Platform and batch dependent
- Inter and intra-individual variability

➤ A more specific tool is required
The Laboratory and DOACs; Measurement Assays
DOAC Recommendations - ISTH

- **Dabigatran**
  - APTT is acceptable in emergency situations; reagent response varies; normal TT indicates low or undetectable dabigatran

- **Rivaroxaban**
  - PT is acceptable in emergency situations; reagent response varies
  - Anti-Xa in combination with rivaroxaban cals/QC used for measurement

- **Apixaban**
  - PT reagents less sensitive to apixaban compared to rivaroxaban; reagent response varies; use of clotting assays limited to acute situations
  - Anti-Xa variability minimized when assay adapted to specific platform


Different PT and anti-Xa tests have different sensitivities for rivaroxaban, but the agreement between assays is superior for anti-Xa vs. PT.

PT tests are not sensitive to apixaban, making anti-Xa the preferred test for apixaban.

Relationship Between Anti-Xa & Rivaroxaban

Anti-Xa and Rivaroxaban Result Interpretation

- Anti-Xa assays calibrated with either UFH or LMWH demonstrate a linear, concentration-dependent relationship.

- Correlation only occurs over the reportable range for the assay, which is dependent on the calibration material, number of calibrators and highest calibrator used (determines the ULQ).

- STA Liquid Anti-Xa demonstrated the highest sensitivity.

Relationship Between Anti-Xa & Apixaban

STA Liquid Anti-Xa Hybrid – Spiked Samples

\[
y = 0.0151x - 0.0369 \\
R^2 = 0.99916
\]

Relationship Between Anti-Xa & Apixaban

STA Liquid Anti-Xa Hybrid – Patient Samples

Dabigatran and TT

The TT response to dabigatran level is very steep.


The TT response to dabigatran is dependent on the thrombin concentration in the reagent.

Ecarin Clotting Time (ECT) and Dabigatran

<table>
<thead>
<tr>
<th>ECT</th>
<th>ECA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting test</td>
<td>Chromogenic assay</td>
</tr>
<tr>
<td>Sensitive to prothrombin and fibrinogen levels</td>
<td>insensitive to factor levels</td>
</tr>
<tr>
<td>Not standardized</td>
<td>Standardized, fully automated</td>
</tr>
<tr>
<td>No commercially available kits</td>
<td>RUO</td>
</tr>
<tr>
<td>Carry over artifacts</td>
<td>No carry over artifacts</td>
</tr>
</tbody>
</table>
Ecarin Chromogenic Assay (ECA)

ECA is a chromogenic, concentration based DTI assay derived from the ecarin clotting time and is not affected by coagulation factors or inhibitors.
Ecarin Chromogenic Assay (ECA) and Dabigatran

ECA shows a linear relationship to dabigatran levels, whereas the aPTT shows curvilinear behavior

ECA vs. dilute TT – Low Dabigatran Range

ECA shows a better correlation to LC-MS at the low range of dabigatran concentrations compared to dilute TT

The ECA assay showed better performance compared to the HTI assay with the ECA showing the tightest results around the mean. In addition, the ECA could go even lower than LC-MS in concentration.

## Recommended Tests for DOACs

<table>
<thead>
<tr>
<th></th>
<th>Rivaroxaban</th>
<th>Apixaban</th>
<th>Edoxaban</th>
<th>Dabigatran</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>Screening only*</td>
<td>No</td>
<td>Screening only*</td>
<td>No</td>
</tr>
<tr>
<td>aPTT</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Screening only*</td>
</tr>
<tr>
<td>TT</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Screening only</td>
</tr>
<tr>
<td>Dilute TT</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Screening/ Measurement</td>
</tr>
<tr>
<td>ECA</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Measurement</td>
</tr>
<tr>
<td>Anti-Xa</td>
<td>Measurement</td>
<td>Measurement</td>
<td>Measurement</td>
<td>No</td>
</tr>
</tbody>
</table>

*Due to intersubject variability of factor levels and presence of other potential interferences, along with different assay/platform sensitivities, PT and aPTT assays cannot reliably be used to rule out DOAC presence.
Correct Laboratory Interpretation under DOAC treatment

Knowledge of influencing parameters

Test method

Drug

Test reagent

Time of latest drug intake

Conclusions

- UFH is widely used and continues to be an anticoagulant of choice due to economy, fast onset, and ease of reversal.

- Discordance between aPTT and anti-Xa is a well documented issue; best practices dictate use of aPTT for baseline testing and anti-Xa for UFH monitoring.

- Full time use of anti-Xa for UFH monitoring has positive institutional impact to save money, improve outcomes, and potentially reduce length of stay.

- DOACs are becoming a mainstay of anticoagulant therapy and if measured require dedicated laboratory assays.