Direct Oral Anticoagulants and the Coagulation Laboratory
Outline

Direct Oral Anticoagulants and the Coagulation Laboratory

• Effect on routine coagulation assays
• Assays to detect drug presence
• Laboratory methods to measure drug concentration
  • Drug calibrated assays
• Effect on specialty coagulation assays
Direct Oral Anticoagulant Agents

Direct Thrombin Inhibitors

- Dabigatran \textit{Pradaxa}®
- Ximelagatran

Direct Xa Inhibitors

- Rivaroxaban \textit{Xarelto}®
- Apixaban \textit{Eliquis}®
- Edoxaban \textit{Savaysa (US), Lixiana (EU)}®

Direct Oral Anticoagulants (DOACs)

Fast acting anticoagulant agents

- Predictable pharmacokinetic responses with little dietary effect or drug interactions
- Administered in fixed dose
- Do not require therapeutic monitoring
  - Dose is not adjusted based on laboratory measures
  - Pharmaceuticals do not provide therapeutic ranges
  - Wide “on therapy” ranges
  - No published studies correlating plasma drug levels with thrombotic or hemorrhagic complications
• Trough levels should be drawn just before the next dose

• Peak levels should be drawn 2 to 4 hours after administration of the last dose

• All DOACS are cleared, at least partially, by the kidneys
  • In all patients on DOACS, renal function should be monitored carefully and at least annually
Coagulation Cascade: *In vitro* model

- APTT
- FXII
- FXI
- FIX
- FVIII
- FX
- FXa
- FV
- FII
- Thrombin
- Fibrinogen
- Fibrin
- Ecarin
- dRVVT
- PT
Coagulation Cascade: *In vitro* model
DOAC Treated Patient Samples in Unmodified APTT, PT and Thrombin Time Assays
Dabigatran (DTI) Effect on PT and APTT

On therapy range

Upper limit of normal range

Dabigatran – TT Response

3 reagent manufacturers for 11 results at 10 sites#

25ng/mL

Dabigatran level, ng/ml


*Hawes E, ...Adcock D, Gosselin R. J Thromb Haemost. 2013;11:1493-1502
Amount of rivaroxaban needed to double the PT varies from 66ng/mL to 700ng/mL depending on the reagent, with no correlation to ISI.

Edoxaban and Apixaban Effect on PT and APTT

APTT and PT:

- Responsiveness of different reagents varies significantly and also depends on the particular DOAC
  - Apixaban has little effect on APTT and PT
- Relationship of [DOAC] to clotting time is not predictable
- Non-linear dose response with direct thrombin inhibitors

Thrombin Time:

- Too sensitive to DTI for quantitation

Unmodified APTT, PT and Thrombin time assays are not suitable for DOAC quantitation and are not a reliable measure of anticoagulant effect
Assays to Determine DOAC Presence

In some clinical situations it may be important to determine if DOAC is present, such as an unconscious patient in the emergency room

- Normal APTT and PT are not reliable to R/O the presence of either a DTI or DXa*#
- Normal TT rules out significant DTI present*
- Chromogenic anti-Xa assay calibrated with either UFH or LMWH can R/O significant DXa presence§

Chromogenic Anti-Xa Assay

plasma [Dxa]
Test Sample

+ Excess FXa

DXa-FXa

+ residual FXa

Chromogenic substrate

Peptide cleavage

yellow color
The chromogenic anti-Xa assay can be used to detect any factor Xa inhibitor anticoagulant and this includes:

- Heparin
- Low Molecular Weight Heparin
- Fondaparinux
- Rivaroxaban
- Apixaban
- Edoxaban

Calibrating the assay with a specific drug as calibrator (i.e. heparin) merely measures the drug level (i.e. apixaban) compared to the calibrator drug and it does not make the assay specific for the drug (i.e. apixaban).
# Chromogenic Anti-Xa Assay

<table>
<thead>
<tr>
<th>Manufacturer Reagent</th>
<th>Calibrator source</th>
<th>LLQ Anti-Xa U/mL</th>
<th>Lowest Rivaroxaban level with LLQ</th>
<th>ULQ Anti-Xa U/mL</th>
<th>Highest Rivaroxaban level with ULQ</th>
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<tbody>
<tr>
<td>Chromogenix COAMATIC</td>
<td>UFH</td>
<td>0.03</td>
<td>~15ng/mL</td>
<td>1.0</td>
<td>&gt;200ng/mL</td>
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<tr>
<td>Siemens Berichrom®</td>
<td>UFH</td>
<td>0.03</td>
<td>~10ng/mL</td>
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<tr>
<td>Stago STA® Liquid Heparin</td>
<td>UFH</td>
<td>0.00</td>
<td>&lt;5ng/mL</td>
<td>0.60</td>
<td>~24ng/mL</td>
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<tr>
<td>Chromogenix COAMATIC</td>
<td>LMWH</td>
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<tr>
<td>Stago STA® Liquid Heparin</td>
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<td>&lt;5ng/mL</td>
<td>1.86</td>
<td>~80ng/mL</td>
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</table>

Case #1

47 year old male with a history of atrial fibrillation is brought to the ED unconscious and bleeding internally following a car accident. Which of the following tests could be beneficial to determine if the patient is anticoagulated?

1. APTT
2. PT
3. Thrombin time
4. Heparin chromogenic anti-Xa assay
Laboratory Results

- APTT 31.5s  Normal 27 – 32s
- PT 12.8s  Normal 11 – 13s
- TT 18s  Normal 13 – 20s

- Heparin Anti Xa  4.0 IU/mL  High
  Therapeutic Range LMWH 1.0 – 2.0 IU/mL

The fact that a patient can be fully anticoagulated with a normal APTT and PT is a major patient safety issue
Accurate assays are available over a broad drug concentration range using a variety of methodologies

- No assay is FDA approved
- None of the DOACs has a validated expected therapeutic range
- There are no published data on plasma drug concentration associated with an increased hemorrhagic or thrombotic risk
- While these methods measure drug concentration (ng/mL), they do not provide a direct measurement of the degree of anticoagulation
  - A paradigm shift compared to VKA monitoring
  - Results are reported in ng/mL of drug
Assays to Quantitate DOACs

- Use appropriate drug-specific calibrators
- Assays can be based on various methodologies
  - **LC-MS/MS** – only assay specific to each DOAC
  - **Chromogenic**: inhibition of FIIa (DTI) or FXa (DXa)
  - **Clot-based**
    - Modified TT (dTT) and ecarin based (DTIs)
    - Modified RVV based (DTIs and DXa)
    - PT, dPT, modified INR (DXa)
Total, Unconjugated Dabigatran by LC-MS/MS

The assay is calibrated with the specific DTI

<table>
<thead>
<tr>
<th>Replicate</th>
<th>LLOQ</th>
<th>QC1</th>
<th>QC2</th>
<th>QC3</th>
<th>ULOQ</th>
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<tbody>
<tr>
<td>1</td>
<td>4.806</td>
<td>13.567</td>
<td>118.026</td>
<td>363.762</td>
<td>482.598</td>
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<td>2</td>
<td>5.196</td>
<td>14.252</td>
<td>116.981</td>
<td>357.539</td>
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<td>4</td>
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<td>14.065</td>
<td>119.239</td>
<td>358.943</td>
<td>486.911</td>
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<td>5</td>
<td>5.121</td>
<td>14.050</td>
<td>120.728</td>
<td>359.518</td>
<td>494.197</td>
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<td>6</td>
<td>4.694</td>
<td>14.145</td>
<td>119.761</td>
<td>359.396</td>
<td>482.350</td>
</tr>
</tbody>
</table>

Mean: 4.829, 14.134, 118.986, 358.395, 489.607
Std Dev: 0.376, 0.373, 1.318, 4.091, 6.742
Imprecision (%): 7.78, 2.64, 1.11, 1.14, 1.38
Inaccuracy (%): -3.42, NA, NA, NA, -2.08
LC-MS/MS Considerations

How is the assay standard (calibrator) prepared and qualified:

• DOAC used for calibration may be obtained from a chemical manufacturer, it may be a synthesized compound. Composition and purity must be qualified.
  • Should this be fingerprinted (i.e. product ion scan) to the compound manufactured by the pharmaceutical company?
• There are no universal DOAC standards nor methods for making DOAC calibration material
  • This potentially impacts the accuracy of all calibrated assays
Quantitative measure of DTIs
A modified thrombin time method

Diluted test plasma is mixed with normal pooled plasma; clotting initiated by addition of a constant amount of alpha thrombin and clotting time is directly proportional to [DTI]

The assay is calibrated with the specific DTI; calibration range 50-500ng/mL Lacks low-end sensitivity

Insensitive to:
- Levels of coagulation factors
- VKA
- Lupus anticoagulant

*RUO Assay

Ecarin Chromogenic Assay* Diagnostica Stago, Inc

Quantitative measure of DTIs

Ecarin

Prothrombin (excess) → Meizothrombin → DTI → Chromogenic Substrate

OD of pNA is inversely proportional to [DTI] in plasma

The assay is calibrated with the specific DTI

Insensitive to:

• Levels of coagulation factors except fibrinogen & FII
• VKA
• Lupus anticoagulant


*RUO Assay
Anti-Xa Assays for DXa Inhibitors

Anti-Xa Assay Principal

Patient sample (DXa) + FXa (in excess)

Residual Xa = [1/DXa]

Measured with a chromogenic substrate

The assay is calibrated with the specific DXa (i.e. rivaroxaban), results are reported in ng/mL

• All calibrators for the DOACS are RUO
• An Anti-IIa assay is available for the direct thrombin inhibitors

Apixaban Treated Patients

- Apixaban was administered at 5 or 10 mg bid or 20 mg qd in patients with acute VTE
- Strong, linear correlation between plasma [drug] and anti-Xa activity
- Use of drug-specific calibrator does not make the assay specific for that DXa drug

* Barrett YC. Thromb Haemost 2010;104:1263-1271

Calibrated with apixaban
Calibrated with LMWH
Assays to Quantitate DOACs

• **LC-MS/MS** – only assay specific to each DOAC
  • Considered gold standard assay
  • Specific, sensitive, precise, accurate, robust, broad range

• **Chromogenic**: inhibition of FIIa (DTI) or FXa (DXa)
  • Will not differentiate between DOACs within a class
  • Sensitive, precise, accurate, robust, broad range

• **Clot-based**
  • Non-specific, may not distinguish DTI from DXa
  • Potential for interference by underlying coagulopathies, LA
  • Potential for limited sensitivity, greater imprecision and lot-to-lot variability
DOACS can have a significant effect on clot-based and/or chromogenic assays, not ELISA

Not always obvious, based on screening assays, that patients are on a DOAC

- Especially with apixaban that has little impact on some APTT and PT reagents

Unlike heparin, currently cannot be neutralized in the laboratory
Dabigatran and Rivaroxaban Effect on Coagulation Assays

- **Drugs act as inhibitors:**
  - Incomplete correction with 1:1 plasma mix
  - Prolongation with incubation
  - Can cause a false positive Bethesda assay

- **False positive Lupus Anticoagulant Assays**
  - Dabigatran can elevate dRVVT and hexagonal PL neutralization
  - Anti-Xa tend not to cause false + hexagonal PL neutralization

- **DXa agents falsely decrease chromogenic FVIII activity**

- **No effect on:** free protein S antigen, chromogenic protein C activity, reptilase time, D-dimer, VWF assays

Adcock D, Gosselin R. *Thromb Res.* 2015
Dabigatran and Rivaroxaban Effect on Coagulation Assays

AT assays either IIa or Xa based
PC and PS assays: clot-based

<table>
<thead>
<tr>
<th>Assay</th>
<th>NOAC Anti-IIa</th>
<th>NOAC Anti-Xa</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCT</td>
<td>Markedly ↑</td>
<td>No effect</td>
</tr>
<tr>
<td>Clauss Fibrinogen</td>
<td>No effect or falsely ↓</td>
<td>No effect</td>
</tr>
<tr>
<td>APTT Mixing Study</td>
<td>Incomplete correction</td>
<td>Incomplete correction</td>
</tr>
<tr>
<td>PT Mixing Study</td>
<td>Incomplete correction</td>
<td>Incomplete correction</td>
</tr>
<tr>
<td>Bethesda assay</td>
<td>Falsely present</td>
<td>Not tested</td>
</tr>
<tr>
<td>APTT- based factor assays, one stage</td>
<td>Possibly Falsely ↓</td>
<td>Possibly Falsely ↓</td>
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<tr>
<td>PT- based factor assays, one stage</td>
<td>Possibly Falsely ↓</td>
<td>Possibly Falsely ↓</td>
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<tr>
<td>Chromogenic FVIII activity</td>
<td>No effect</td>
<td>Possibly Falsely ↓</td>
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<tr>
<td>AT Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. FXa based</td>
<td>a. No effect</td>
<td>a. Falsely ↑</td>
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<tr>
<td>b. FIIa based</td>
<td>b. Falsely ↑</td>
<td>b. No effect</td>
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<tr>
<td>PC Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Clot based</td>
<td>a. Falsely ↑</td>
<td>a. Falsely ↑</td>
</tr>
<tr>
<td>b. Chromogenic</td>
<td>b. No effect</td>
<td>b. No effect</td>
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<tr>
<td>PS Activity</td>
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<tr>
<td>a. Clot based</td>
<td>a. Markedly ↑</td>
<td>a. Falsely ↑</td>
</tr>
<tr>
<td>b. ELISA or LIA based</td>
<td>b. No effect</td>
<td>b. No effect</td>
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<td>LA Tests</td>
<td>Possible to misclassify as LA</td>
<td>Possible to misclassify as LA</td>
</tr>
<tr>
<td>APCR</td>
<td>Falsely ↑ ratio</td>
<td>Falsely ↑ ratio</td>
</tr>
</tbody>
</table>
Thank you

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720 568 4328