Direct-Acting Anticoagulants: Practical Laboratory Aspects That Practicing Physicians Need to Know About

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Disclosures

• Relevant Financial Relationship(s)
  • None

• Off Label Usage
  • None
Learning Objectives:

• To know the effects of novel anticoagulants on commonly ordered coagulation assays
• To know the circumstances in which monitoring may be useful
• Understand limitations of currently available assays
• Review availability of assays to monitor novel anticoagulants
Oral anticoagulant use by quarter

Office visits (thousands)

- Warfarin
- All DOACs
- Rivaroxaban
- Apixaban
- Dabigatran

Case history

- 89 year old male
  - New onset atrial fibrillation and recent thrombotic stroke: s/p thrombolytic therapy
  - Slow recovery, needed PEG tube placement

<table>
<thead>
<tr>
<th>Date</th>
<th>Day 15</th>
<th>Day 18</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prothrombin time, sec (INR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 (1.6) (Vitamin K replacement)</td>
<td>12 (1.2)</td>
<td>15 (1.5)</td>
</tr>
</tbody>
</table>

Apixaban (Eliquis) initiated
Newer Anticoagulant Drugs: Effect on laboratory tests

- **Routine assays:**
  - Prothrombin Time
  - Activated partial thromboplastin time
  - Fibrinogen
  - Thrombin Time

- **Specialized tests**
  - AT, PC, PS, Lupus anticoagulant assays
  - Anti-Xa assays
  - APCR-V
  - Coagulation factor assays
  - Others

- **Should you measure levels?**
**Activated Partial thromboplastin Time (APTT)**

**Intrinsic**

- XII
- XI
- IX
- VIII

**Extrinsic**

- VII

**Prothrombin Time (PT)**

**Dilute Russel Viper Venom Time (DRVVT)**

**Fibrinogen**

- **Fibrin clot**

**dabigatran**

**rivaroxaban/apixaban/edoxaban**

**TT: thrombin time; RT: reptilase time**
Dabigatran effect on PT (INR): multiple reagents

Innovin
Simple simon
Owren’s
Nycotest
Owren’s

Potential reasons for such discrepancy

\[ \text{INR} = \left( \frac{\text{PT (patient)}}{\text{PT (Geo mean)}} \right)^\text{ISI} \]

- PT reagent (thromboplastin)
  - Recombinant vs tissue derived
- PT geometric mean
  - Changes between reagents and between lots of reagents
- ISI: varies with reagent and regent/instrument combination

ISI: international sensitivity index
Dabigatran effect on multiple APTT reagents

Potential reasons for such discrepancy

- Sensitivity of aPTT reagent to anticoagulant
  - Heparin sensitivity varies
- Effect of instrument
  - Mechanical endpoint vs optical endpoint
Implications

• PT and/or aPTT in patients on therapeutic dabigatran doses:
  • PT: 30% of results were normal
  • aPTT: 18% of results were normal

Hawes EM et al JTH 2013; 11: 1493
Effect on thrombin time

Multiple dose
R2 = 0.8568

Van Rynn Thromb Haemst 2010;103:1116
Dabigatran effect on thrombin time: (in house Mayo data, spiked pooled plasma sample)

Dabigatran level (mcg/mL)

seconds

0  40  80  120  160  200  240  280  320

0.02  0.04  0.06  0.08  0.1  0.12  0.14  0.16  0.18
Dabigatran effect on PT and APTT: Take home message

- Prolongs respective clotting times
- Degree of prolongation will vary with reagent instrument combinations
- Not accurately reflective of drug levels
- Although theoretically possible, calibration of individual labs PT and APTT reagents is possible but not advisable
  - Would need to be revalidated with each change in lot
- No known ‘safe’ PT/APTT level for invasive procedures (in patients on dabigatran)
- Prolongs APTT (Mixing study inhibited)
- May be mistaken for presence of inhibitor
  - FVIII Inhibitor or lupus anticoagulant
Activated Partial thromboplastin Time (APTT)

*Intrinsic*

- XII
- XI
- IX
- VIII

*aPTT*

dabigatran

*Extrinsic*

- VII
- PT

*Prothrombin Time (PT)*

- V
- X
- II

Fibrinogen

- Fibrin clot

*Dr. Russell Viper Venom Time (DRVVT)*

- Dilute Russel Viper Venom Time (DRVVT)

- rivaroxaban/
apixaban/
edoxaban

*TT: thrombin time; RT: reptilase time*
Varying effect of rivaroxaban on different prothrombin time reagents

- Recombiplastin (ISI: 1.0)
- Triniclot (ISI 1.22)
- Neoplastin Plus ISI 1.17
- Neoplastin (ISI 1.75)
- Thromborel (ISI 1.07)
- Innovin (ISI 0.93)
Plasma concentration of rivaroxaban & APTT

Samama MM et al. T&H 103;2010:815-825
Implications

- PT and/or aPTT in patients on therapeutic rivaroxaban doses:
  - PT: 32% of results were normal
  - aPTT: 80% of results were normal (trough levels)
    - 13% results normal (peak levels)

Francart et al T&H 2014; 111: 1133
Effect of direct anti-Xa inhibitors

- Variable prolongation of PT/aPTT
  - Reagent/instrument dependent
- Cannot establish a cut off to detect or exclude presence of drug
- No ‘safe’ level of PT/aPTT
### Newer Anticoagulant Drugs: Effect on laboratory tests

<table>
<thead>
<tr>
<th></th>
<th>DTI</th>
<th>Anti-Xa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin Time</td>
<td>Prolongs*</td>
<td>Prolongs*</td>
</tr>
<tr>
<td>Activated partial</td>
<td>Prolongs*</td>
<td>Prolongs*</td>
</tr>
<tr>
<td>thromboplastin time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (Clauss)</td>
<td>Artifactually low at high</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td>concentrations</td>
<td></td>
</tr>
<tr>
<td>Thrombin Time</td>
<td>Prolongs</td>
<td>No effect</td>
</tr>
</tbody>
</table>

*inhibited on mixing study with normal pooled plasma
Newer Anticoagulant Drugs: Effect on laboratory tests

- Routine assays:
  - Prothrombin Time
  - Activated partial thromboplastin time
  - Fibrinogen:
  - Thrombin Time

- Specialized tests
  - AT, PC, PS, Lupus anticoagulant assays
  - Anti-Xa assays
  - APCR-V
  - Coagulation factor assays
  - Others

- Should you measure levels?
60 year old male with deep vein thrombosis

• PT 11.2
• APTT 44
• 1:1 mix 36
• DRVVT 2.6
• 1:1 mix 1.7
• Confirm 1.4

Data are consistent with lupus anticoagulant.

How long would you continue anticoagulation?

• A) Long term
• B) Three months
• C) 6 months
• D) one year

• TT >240

Staclot APTT 77
Staclot Δ 8
60 year old male with deep vein thrombosis

<table>
<thead>
<tr>
<th></th>
<th>Jan 6th</th>
<th>Jan 13th (off dabigatran)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PT</strong></td>
<td>11.2</td>
<td>10.6 (8.3-10.8)</td>
</tr>
<tr>
<td><strong>APTT</strong></td>
<td>44</td>
<td>40 (21-33)</td>
</tr>
<tr>
<td><strong>1:1 mix</strong></td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td><strong>DRVVT</strong></td>
<td>2.6</td>
<td>1.2 (&lt;1.2)</td>
</tr>
<tr>
<td><strong>1:1 mix</strong></td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Confirm</strong></td>
<td>1.4</td>
<td>ND</td>
</tr>
<tr>
<td><strong>TT</strong></td>
<td>&gt;240</td>
<td>36 (16-25)</td>
</tr>
<tr>
<td><strong>Staclot APTT</strong></td>
<td>77</td>
<td>54</td>
</tr>
<tr>
<td><strong>Staclot Δ</strong></td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

*ND: not done*
Dabigatran effect on specialized assays

- Prolongs and inhibits screening assays
  - False impression of either lupus anticoagulant of specific factor inhibitors
- Results in artifactually reduced factor levels
  - False positive Bethesda assays
- No effect or falsely lowers Clauss fibrinogen
- False positive dRVVT
  - Lupus anticoagulant
## Effect on specialty assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>DTI</th>
<th>Anti-Xa</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT (FXa based)</td>
<td>No effect</td>
<td>artifactual overestimation</td>
</tr>
<tr>
<td>AT (FIIa based)</td>
<td>artifactual overestimation</td>
<td>No effect</td>
</tr>
<tr>
<td>PC activity (clot based)</td>
<td>artifactual overestimation</td>
<td>artifactual overestimation</td>
</tr>
<tr>
<td>PC activity (chromogenic)</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>PS activity (clot based)</td>
<td>artifactual overestimation</td>
<td>artifactual overestimation</td>
</tr>
<tr>
<td>Free PS Ag</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>APC-R (2nd generation)</td>
<td>Falsely normal</td>
<td>Falsely normal</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>False positive</td>
<td>False positive</td>
</tr>
</tbody>
</table>
Direct acting anticoagulants and the laboratory: Conclusions

• Oral direct acting anticoagulants have variable effects on routine and specialty coagulation assays

• Measurement of presence or absence of drug may be useful in selected circumstances
  • Assess compliance
  • Hemorrhagic or thrombotic complications
  • Pre-operative for urgent surgery
  • Cases of overdosing
Direct acting anticoagulants and the laboratory: Conclusions

• Currently no FDA approved kits/calibrators
• Selected reference laboratories offer measurement of drug levels
  • Mass spectrometry
  • Laboratory developed anti-Xa assays
Thank you for your attention

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Questions & Discussion