Measuring concentrations of Rivaroxaban, Apixaban, Edoxaban

Methods and Challenges

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Disclosures/COI

• Speaker/advisory board/consultancy fees
  – Bayer (rivaroxaban)
  – Bristol Myers Squibb (apixaban)
  – Daiichi Sankyo (edoxaban)

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Anti Xa – Stago

Anti IIa - ecarin/chromogenic /Hyphen cals
Direct factor Xa inhibitors

Measurement of oral anti-Xa agents

Recommendation

- Anti-Xa chromogenic assays should be used to determine plasma concentration of direct FXa inhibitors.
- Product-specific calibrator should be used and results should be expressed in mass concentration.
- LMWH reference standards should not be used as calibrators for direct FXa inhibitors.
- PT and APTT should not be used to measure the plasma concentration of Xa inhibitors.
Anti Xa assay

- Xa is added to plasma sample
- Any Xa inhibitor present neutralises some of Xa
- Artificial substrate is added comprising several amino acids linked to a colourless molecule (pNA)
- Any residual Xa cleaves the bond and yellow colour develops
Anti Xa assay

- No drug, no inhibition of Xa – more colour
- More drug, more inhibition, less colour.
- Natural Xa inhibitors form test sample (AT, TFPI) usually no effect due to assay conditions
- **AT in reagents?**
- Assay calibrated by adding known concentrations of drug (commercial calibrators)

- Not specific for one drug - Heparin. LMWH inhibit via Antithrombin, any direct Xa inhibitor will be detected depending on reagents
Anti Xa assay for Rivaroxaban

![Graph and table data]

<table>
<thead>
<tr>
<th>RIVA ng ml</th>
<th>dOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.9400</td>
</tr>
<tr>
<td>37.50</td>
<td>1.6737</td>
</tr>
<tr>
<td>75.00</td>
<td>1.4310</td>
</tr>
<tr>
<td>150.00</td>
<td>1.0782</td>
</tr>
<tr>
<td>300.00</td>
<td>0.6353</td>
</tr>
<tr>
<td>420.00</td>
<td>0.4722</td>
</tr>
<tr>
<td>525.00</td>
<td>0.2639</td>
</tr>
</tbody>
</table>
Anti Xa assays for Rivaroxaban
(Mani et al 2012)

• Samples with <25 ng/ml require a low calibrator set (0, 15, 60, 100 ng/ml) for precise measurement

• Assay with added Antithrombin overestimated apparent apparent rivaroxaban by 15-30 ng/ml
Anti Xa assays are unreliable below 25-30 ng/ml (Mani et al 2012, patients on Rivaroxaban)
### Specific assays for DOAC
**UK NEQAS - May 2014. Secondary care**

<table>
<thead>
<tr>
<th></th>
<th>Apixaban</th>
<th>Dabigatran</th>
<th>Rivaroxaban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromogenic</td>
<td>43</td>
<td>13</td>
<td>123</td>
</tr>
<tr>
<td>Clotting</td>
<td>-</td>
<td>62</td>
<td>-</td>
</tr>
<tr>
<td>LC MS/MS</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>% of centres with an assay</td>
<td>7%</td>
<td>12%</td>
<td>20%</td>
</tr>
</tbody>
</table>

610 responses
Rivaroxaban assays in different centres
(Oct/Nov 2014)

55 centres
Anti Xa assays
Calibrators: Hyphen 26; Stago 10; Technoclone 6

<table>
<thead>
<tr>
<th>Sample</th>
<th>Median (ng/ml)</th>
<th>Range (ng/ml)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8*</td>
<td>0 - 102</td>
<td>186%</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>13 - 80</td>
<td>38%</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>94 - 473</td>
<td>34%</td>
</tr>
</tbody>
</table>

* Sample contained no rivaroxaban but only 7 centres recognised a lower limit of quantification by reporting as “less than”
Countries

• 19 UK
• 14 Italy
• France, Belgium, Germany, Republic of Ireland, Israel
Rivaroxaban Anti Xa assays with different calibrators  
(Oct/Nov 2014)

<table>
<thead>
<tr>
<th>Mass spec</th>
<th>Hyphen (26) median</th>
<th>Stago (10) median</th>
<th>Technolcone (5) median</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.0 ng/ml*</td>
<td>6 ng/ml*</td>
<td>15 ng/ml*</td>
<td>0 ng/ml*</td>
</tr>
<tr>
<td>37 ng/ml</td>
<td>38 ng/ml</td>
<td>38 ng/ml</td>
<td>28 ng/ml</td>
</tr>
<tr>
<td>141 ng/ml</td>
<td>143 ng/ml</td>
<td>130 ng/ml</td>
<td>145 ng/ml</td>
</tr>
</tbody>
</table>

*sample 1 contained no rivaroxaban
Apixaban assays in different centres
(Oct/Nov 2014)

24 centres (55 for rivaroxaban, 49 for dabigatran)
Anti Xa assays
Calibrators: Hyphen 6; Stago 9; Technoclone 5

<table>
<thead>
<tr>
<th>Sample</th>
<th>Median (ng/ml)</th>
<th>Range (ng/ml)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (&lt;1)</td>
<td>4.0</td>
<td>0 - 60</td>
<td>168%</td>
</tr>
<tr>
<td>2 (52)</td>
<td>45</td>
<td>21 - 69</td>
<td>22%</td>
</tr>
<tr>
<td>3 (193)</td>
<td>179</td>
<td>131 - 221</td>
<td>11%</td>
</tr>
</tbody>
</table>

(Tandem mass spec results in brackets)

Sample 1 contained no apixaban, 8 centres reported 0 and 3 centres recognised a lower limit of quantification by reporting as “less than”
## Apixaban Anti Xa assays with different calibrators
(Oct/Nov 2014)

<table>
<thead>
<tr>
<th>Mass spec</th>
<th>Hyphen (6) ng/ml</th>
<th>Stago (9) ng/ml</th>
<th>Technolcone (5) ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>0, 0, 1, 8, &lt;27, &lt;30</td>
<td>0, 5, 7, 7, 9, 12, 20, 30, &lt;20</td>
<td>0, 0, 0, 4, 10</td>
</tr>
<tr>
<td>52</td>
<td>43 (38-48)</td>
<td>46 (38-69)</td>
<td>40 (25-58)</td>
</tr>
<tr>
<td>193</td>
<td>166 (146-184)</td>
<td>182 (156-213)</td>
<td>185 (153-210)</td>
</tr>
</tbody>
</table>

Sample 1 contained no apixaban
• STA-Liquid Anti Xa
• Specific Calibrators and controls
• CV 3-7% between assay
• LLOD - 15 ng/ml
• LLOQ – 20 ng/ml
• ULOQ – 150 ng/ml or 450 with sample re-dilution

• NOT YET LAUNCHED
Development of a New Automated Assay for Measurement of the Oral Direct Factor Xa Inhibitor Edoxaban Plasma Concentration

Tristan Hervé¹, Sandra Beaufils², Ling He³, Jarrem Kochan³, François Depasse¹

¹ Diagnostica Stago, 3, allée Thérésa, 92600 Asnières sur Seine, France - ² Diagnostica Stago R&D, 125, avenue Louis Roche, 92230 Gennevilliers, France - ³ Daiichi Sankyo Inc., 399 Thomall Street, Edison, NJ 08837, USA

Anti-Xa vs LC-MS comparison for spiked plasmas:

\[ y = 0.985x + 3.5 \]

\[ R^2 = 0.99 \]
Apixaban/Eliquis SPC

- PT INR APTT are affected. Changes are small at the expected therapeutic dose and subject to a high degree of variability.

- Calibrated quantitative anti Xa may be useful in exceptional circumstances

- Detailed table of expected anti Xa activity – Max, Min, 5\textsuperscript{th}-95\textsuperscript{th} percentiles in ng/ml and IU/ml of NVAF stroke prevention and for treatment and prevention of VTE
Edoxaban/Lixiana  SPC

- Edoxaban prolongs clotting tests such as PT APTT

- Changes expected at the therapeutic dose are small, subject to a high degree of variability and not useful for monitoring

- Concomitant VKA and edoxaban – concomitant therapy can increase INR post Lixiana by up to 46%

- Pharmacodynamic effects measured by Anti Xa are predictable and correlate with dose and concentration of edoxaban

- Calibrated quantitative anti Xa may be useful in exceptional circumstances

- Detailed table of anti Xa activity by creatinine clearance
Rivaroxaban/Xarelto SPC

- PT APTT Heptest are affected by rivaroxaban

- Dose dependent effect on PT with Neoplastin. Other reagents provide different results. PT done in seconds not INR.

- Conversion between warfarin and Riva - anti Xa PiCT Heptest can be used to test for effects of Riva as these are not affected by warfarin

- Calibrated quantitative anti Xa assay may be useful in exceptional circumstances

- 20 mg dose - Geometric mean (90% prediction interval) 2-4 hr post dose 215 µg/ml (22-535) and 24 hr post dose- 32 µg/ml ( 6-239)
What is needed?

SPC

- More information/data/references to PT APTT and Anti Xa results with different reagents
- Anti Xa levels in more detail where lacking
- Statements that normal PT and/or APTT don’t exclude presence of therapeutic levels
What is needed?

Other needs

• Wider availability of anti Xa assays – more centres, 24/7

• More Proficiency testing

• POC tests for emergency depts/ thrombolysis etc?

• International Standards and International Units - each product ? Single preparation?

• Commercial available CE marked Edoxaban anti Xa assays

• Published data on stability of drugs in blood samples