MEASUREMENT OF ANTICOAGULANT EFFECTS OF NEW ORAL ANTICOAGULANTS - PRACTICAL ASPECTS

Désirée Coen Herak
Department of Laboratory Diagnostics
University Hospital Centre Zagreb
Warfarin monopoly on oral anticoagulant therapy, as well as that of other vitamin K antagonists derived from coumarins is over.

Warfarin monopoly on oral anticoagulant therapy, as well as that of other vitamin K antagonists derived from coumarins is over.

Within a few years it is likely that NOACs (DOACs) will be the dominant chronic anticoagulation therapy.

Warfarin Replacements: Good for Patients, Challenging for Laboratories

Charles S. Eby

1109 articles

(Medline search using terms NOAC, DOAC, TSOAC, apixaban, dabigatran, rivaroxaban, edoxaban AND laboratory, monitoring, measurement or test)

Laboratory testing in the postwarfarin era - what do we need to know?
OFFICIAL COMMUNICATION OF THE SSC

Measuring oral direct inhibitors of thrombin and factor Xa: a recommendation from the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis


T. BAGLIN,* A. HILLARP,† A. TRIPODI,‡ I. ELALAMY,§ H. BULLER¶ and W. AGENO**

RECOMMENDATIONS AND GUIDELINES

Report of the Subcommittee on Control of Anticoagulation on the determination of the anticoagulant effects of apixaban: communication from the SSC of the ISTH

J. HARENBERG,* S. DU, * C. WEISS,† R. KRÄMER,‡ D. HOPPENSTEADT,§ and J. WALENGA.§, FOR THE WORKING PARTY: METHODS TO DETERMINE APIXABAN OF THE SUBCOMMITTEE ON CONTROL OF ANTICOAGULATION OF THE INTERNATIONAL SOCIETY OF THROMBOSIS AND HAEMOSTASIS

*Medical Faculty Mannheim, Department of Clinical Pharmacology, Ruprecht-Karls-University Heidelberg, Mannheim; †Medical Faculty Mannheim, Medical Statistics and Biomathematics, Ruprecht-Karls-University Heidelberg, Mannheim; ¶Inorganic Chemistry Institute, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany; and §Loyola University Chicago, Maywood, IL, USA
Recommendation from the SSC (2013) on Control of Anticoagulation for measuring NOACs

ASSAYS

SEMIQUANTITATIVE

- for the estimation of the relative intensity of anticoagulation
- indicators of supratherapeutic, therapeutic or subtherapeutic anticoagulation status

 Assays should not be used for the quantitative measurement of NOAC concentrations.

QUANTITATIVE

- for the quantitative measurement of NOAC concentrations in plasma
QUANTITATIVE ASSAYS

DIRECT

- LC tandem mass spectrometry

- **gold standard** - provide accurate measure of a wide range of NOAC concentrations in plasma samples (especially for low concentrations)

INDIRECT

- specific coagulation assays based on methods that are closely related to their anticoagulant targets and mechanism of action

- standard calibration curve has to be generated with drug specific calibrators
THROMBIN TIME (TT)

TT is very sensitive to the presence of dabigatran - suitable as a screening test for the exclusion of supratherapeutic dabigatran concentrations in plasma samples.

<table>
<thead>
<tr>
<th>Dabigatran concentration (µg/L)</th>
<th>0</th>
<th>30</th>
<th>155</th>
<th>382</th>
<th>720</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (s)</td>
<td>21.6</td>
<td>127.5</td>
<td>&gt;150</td>
<td>&gt;150</td>
<td>&gt;150</td>
</tr>
<tr>
<td>BC Thrombin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (ratio)</td>
<td>1.17</td>
<td>6.89</td>
<td>&gt;8.11</td>
<td>&gt;8.11</td>
<td>&gt;8.11</td>
</tr>
</tbody>
</table>

**TT** TT indicator of very low dabigatran concentration or no presence.
DILUTE THROMBIN TIME (dTT)

- The strong sensitivity of TT towards dabigatran led to the development of a **CALIBRATED dTT using dabigatran standards** to calculate dabigatran concentrations.
- Plasma samples are prediluted 1/8 or 1/20 in normal plasma before performing the assay.
- There is a linear relationship between dTT and dabigatran concentration in a wide range of concentrations (a little bit not sensitive for low concentrations <50 µg/L).

**QUANTITATIVE ASSAY**

- High correlation of dTT with dabigatran plasma concentrations measured by LC-MS/MS (Douxfils 2014)
DILUTE THROMBIN TIME (dTT)

Commercially available kit
HEMOCLOT ASSAY (HTI)

In-house method

Ryn JV et al, Thromb Haemost 2010; 103: 1116–1127

BC Thrombin
Anticoagulant effects of NOACs on PT and APTT

The anticoagulant effect of each NOAC on PT and APTT has been demonstrated in a number of studies.

- In general a more pronounced influence has been observed for:
  - thrombin inhibitors on APTT
  - FXa inhibitors on PT

- PT and APTT expressed in s and ratios show a dose-dependent prolongation (increase) with increasing NOAC concentrations.

However, remarkable between-reagent variability has been observed.
### PT and APTT reagent sensitivity to Dabigatran

<table>
<thead>
<tr>
<th>PT reagents</th>
<th>APTT reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromborel S</td>
<td>Actin FSL</td>
</tr>
<tr>
<td>Recombioplastine 2G</td>
<td>Actin FS</td>
</tr>
<tr>
<td>STA Neoplastin Cl plus</td>
<td>Synthasil</td>
</tr>
<tr>
<td>Innovin</td>
<td>STA Cephascreen</td>
</tr>
<tr>
<td>STA Neoplastine R</td>
<td>STA PTT-A</td>
</tr>
<tr>
<td></td>
<td>Pathromtin SL</td>
</tr>
</tbody>
</table>
**PT reagent sensitivity**

<table>
<thead>
<tr>
<th>RIVAROXABAN</th>
<th>APIXABAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innovin</td>
<td>Innovin</td>
</tr>
<tr>
<td>Thromborel S</td>
<td>Thromborel S</td>
</tr>
<tr>
<td>STA Neoplastin Cl plus</td>
<td>STA Neoplastin R</td>
</tr>
<tr>
<td>Recombioplastine 2G</td>
<td>STA Neoplastin Cl plus</td>
</tr>
<tr>
<td>STA Neoplastin R</td>
<td>Recombioplastine 2G</td>
</tr>
</tbody>
</table>

Least sensitive

Most sensitive
<table>
<thead>
<tr>
<th>RIVAROXABAN</th>
<th>APIXABAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthasil</td>
<td>Actin FS</td>
</tr>
<tr>
<td>STA Cephascreen</td>
<td>Actin FSL</td>
</tr>
<tr>
<td>Actin FSL</td>
<td>STA Cephascreen</td>
</tr>
<tr>
<td>APTT-SP</td>
<td>Synthasil</td>
</tr>
<tr>
<td>Actin FS</td>
<td>APTT-SP</td>
</tr>
<tr>
<td>Pathromtin SL</td>
<td>Pathromtin SL</td>
</tr>
</tbody>
</table>

**Least sensitive**

**Most sensitive**
Determination of local PT and APTT reagents sensitivities to NOAC using NOAC specific calibrators (commercially available from several suppliers) IN-VITRO using plasma samples from patients receiving a specific NOAC (NOAC concentrations determined with the appropriate quantitative assay) EX-VIVO

In-vitro determination of local PT and APTT sensitivity to NOACs

1. PT and APTT performed in pooled normal plasma samples spiked with increasing NOAC concentrations corresponding to through, peak and supratherapeutic levels:
   - **Dabigatran concentrations** 0, 30, 150, 382 and 720 μg/L
     (Neqas supplementary exercise, April 2012)
   - **Rivaroxaban concentrations** 0, 29, 121, 346 and 734 μg/L
   - **Apixaban concentrations** 0, 33, 164, 398 and 737 μg/L
     (Neqas supplementary exercise, May 2014)

2. PT and APTT performed using commercial **Rivaroxaban calibrators** (STA-Rivaroxaban Calibrator, Stago, France) with concentrations 0, 100, 254 and 494 μg/L
Local PT and APTT sensitivity to NOACs

Neqas supplementary exercise, April 2012

Neqas supplementary exercise, May 2014
Local PT and APTT sensitivity to Rivaroxaban determined using Rivaroxaban calibrators
Ex-vivo effect of rivaroxaban

**PATIENTS:**

21 patients with nonvalvular atrial fibrillation (creatinine clearance >50 mL/min) receiving 20 mg of Rivaroxaban once daily.

**SAMPLES:**

For each patient blood samples were taken:
- before the next dose (through concentration)
- 3h after drug administration (peak concentration)

**METHODS:**

PT (Innovin), APTT (Actin FS) and anti-Xa assay (Berichrom Heparin calibrated with STA-Rivaroxaban Calibrators)
Real-world variability in rivaroxaban levels in patients with atrial fibrillation
Real-world variability in rivaroxaban levels in patients with atrial fibrillation
Attempts to adapt the INR system for specific NOAC

The International Normalized Ratio calibrated for rivaroxaban has the potential to normalize prothrombin time results for rivaroxaban-treated patients: results of an in vitro study

A. Tripodi, V. Chantarangkul, C. Guinet, and M. M. Samama


\[ \text{INR}_{\text{rivaroxaban}} = \left( \frac{\text{PT}_{\text{patient}}}{\text{PT}_{\text{normal}}} \right) \times \text{ISI}_{\text{rivaroxaban}} \]

Table 1: General effects of thrombin and factor Xa inhibition by drugs such as dabigatran and rivaroxaban (and expected with other FXa inhibitors)

<table>
<thead>
<tr>
<th>Test</th>
<th>Thrombin inhibition</th>
<th>Clinical utility</th>
<th>FXa inhibition</th>
<th>Clinical utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>++</td>
<td>No</td>
<td>++</td>
<td>Qualitative</td>
</tr>
<tr>
<td>INR</td>
<td>Not applicable</td>
<td>No</td>
<td>Not applicable</td>
<td>No</td>
</tr>
<tr>
<td>POC PT</td>
<td>Not applicable</td>
<td>No</td>
<td>++</td>
<td>Uncertain</td>
</tr>
<tr>
<td>APPT</td>
<td>+++</td>
<td>Qualitative</td>
<td>+</td>
<td>Uncertain</td>
</tr>
<tr>
<td>ACT</td>
<td>++</td>
<td>No</td>
<td>+</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 1: International sensitivity index (ISI) calibration

<table>
<thead>
<tr>
<th>Thromboplastin</th>
<th>ISIvka</th>
<th>Recombiplastin as arbitrary standard</th>
<th>Neoplastin-Plus as arbitrary standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombiplastin</td>
<td>1.00</td>
<td>1.000</td>
<td>0.987 (1.0)</td>
</tr>
<tr>
<td>Thrombord S</td>
<td>1.07</td>
<td>1.382 (1.8)</td>
<td>1.361 (1.8)</td>
</tr>
<tr>
<td>Neoplastin-Plus</td>
<td>1.17</td>
<td>1.014 (1.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Neoplastin</td>
<td>1.75</td>
<td>1.196 (1.0)</td>
<td>1.177 (0.8)</td>
</tr>
<tr>
<td>Trinidrot</td>
<td>2.22</td>
<td>1.224 (1.6)</td>
<td>1.208 (1.6)</td>
</tr>
<tr>
<td>Innovin</td>
<td>0.93</td>
<td>1.712 (1.4)</td>
<td>1.691 (1.6)</td>
</tr>
</tbody>
</table>

CV, coefficient of variation of the slope. ISIvka and ISI_{rivaroxaban} refer to the ISIs valid for vitamin K antagonists and rivaroxaban, respectively.
Measurement of NOACs in other media

The NOACs can be assessed in alternate (nonplasma) samples.

- **urine** - measurement of dabigatran and rivaroxaban (qualitative assessment)
  

- **serum** - measurement of rivaroxaban and apixaban
  
Perspectives (the future)

- Indirect quantitative assays seem to be unreliable for the measurement of low plasma concentrations - there is a need to develop specific coagulation assays that are accurate for these low concentrations.
  - separate calibration curves in the low range?
- There is a need for the development of a global coagulation test which is sensitive to all NOACs and for which a cut-off associated with bleeding or thrombotic can be provided.
  - dRVVT seems to be promising
- POCT including alternate samples such as urine and serum is under development - providing additional opportunities when plasma samples are unavailable.
Conclusions

- Up to now no biological assay can be recommended for the assessment of all NOACs.

- Each coagulation laboratory should be familiar with the sensitivity of its own PT and APTT reagents to each NOAC.

- A normal thrombin time suggests very low (not clinically relevant) level of dabigatran.

- For apixaban both PT and APTT are insensitive and patients may have normal coagulation times despite therapeutic concentrations, and anti-Xa assays should be used to determine plasma concentrations.

- As there are no therapeutic intervals set, threshold values of NOAC concentrations that might still be assumed as safe must be defined.
Postwarfarin era and laboratory responsibilities

Coagulation laboratory experts have a major role in ensuring patients receive appropriate testing and accurate interpretation of results, and must protect patients from inaccurate test results due to NOAC interference by educating clinicians and providing interpretive comments warning of possible confounding of results due to the presence of NOACs.


The laboratory needs to know the drug being used in order to perform appropriate testing and apply the appropriate calibration curve.