Assessment of thrombin generation in plasma samples of patients under anticoagulant treatment with ST Genesia system using STG – DrugScreen

DOUXFILS Jonathan
University of Namur, Department of Pharmacy, Belgium
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University of Namur, Department of Pharmacy, Belgium
State of the art of current anticoagulant therapy and management
Pharmacodynamics of anticoagulants

**INTRINSIC PATHWAY**
- XII → XIIa
- XI → Xla
- IX* → IXa
- Vlla → Vlla
- Ca++
- PL
- Activation

**EXTRINSIC PATHWAY**
- VII* → TF → Ca++
- PL → Vlla/TF

**COMMON PATHWAY**
- X* → Xa
- Ca++
- Prothrombinase
- Prothrombin

**Direct Xa inhibitors**
- AT
- HSPGs/LMWH/UFH

**Direct thrombin inhibitors**
- Fibrinogen → Thrombin → Fibrin
- Fibrin monomer → Fibrin polymer

* VKA-dependent coagulation factors

Figure 1: Coagulation cascade depicting the intrinsic and extrinsic pathways of activation. The different classes of anticoagulants are represented in red. AT: antithrombin – HSPGs: Heparan sulfated proteoglycans – LMWH: Low molecular weight heparin – PL: Phospholipids – TF: Tissue factor – VKA: Vitamin K antagonist – UFH: Unfractionated heparin.
Pharmacodynamics of anticoagulants

Figure 2: Vitamin K acts as a cofactor of the $\gamma$-glutamyl carboxylase to form the $\gamma$-carboxyglutamic acid residues of factor II, VII, IX, X, protein C, S and Z. Vitamin K antagonists act by inhibiting the vitamin K 2,3-epoxide reductase, thus inhibiting the regeneration of vitamin K to its reduced (active) form.
### PK and PD

<table>
<thead>
<tr>
<th></th>
<th>dabigatran etexilate (PRADAXA®)</th>
<th>rivaroxaban (XARELTO®)</th>
<th>apixaban (ELIQUIS®)</th>
<th>edoxaban (LIXIANA®)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>Thrombin</td>
<td>Factor Xa</td>
<td>Factor Xa</td>
<td>Factor Xa</td>
</tr>
<tr>
<td><strong>Bioavailability (%)</strong></td>
<td>3-7% Not affected by food</td>
<td>80 to 100% for the 10 mg</td>
<td>50% Not affected by food</td>
<td>62% Not affected by food</td>
</tr>
<tr>
<td><strong>Prodrug</strong></td>
<td>Yes – activated by esterase (CES1)</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Half-life (hours)</strong></td>
<td>11-13</td>
<td>5-13</td>
<td>8-15</td>
<td>10-14</td>
</tr>
<tr>
<td><strong>T_{MAX} (hours)</strong></td>
<td>0.5-2.0</td>
<td>2.0-4.0</td>
<td>3.0-4.0</td>
<td>1.0-2.0</td>
</tr>
<tr>
<td><strong>Renal clearance</strong></td>
<td>80%</td>
<td>33%</td>
<td>25%</td>
<td>50%</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>P-gp</td>
<td>P-gp</td>
<td>P-gp</td>
<td>P-gp</td>
</tr>
</tbody>
</table>

- **P-gp**
- **CYP3A4**
- **CYP3A4/5, 1A2, 2J2**

* these dose regimens have to be taken with food.
Monitoring/point measurement

- Why to measure?
- How to measure?
WHY TO MEASURE?
VKA

There is a real need for monitoring those treatments in order to ensure their efficacy and to minimize hemorrhagic complications.
WHY TO MEASURE?
LMWH

- Unmonitored, weight-adjusted subcutaneous heparin was found to be as safe and effective as weight-adjusted LMWH in a randomized trial of patients with VTE.

- However, monitoring may be used:
  - in obese patients
  - in those with renal insufficiency or with cirrhosis
  - when therapeutic doses of LMWH are required during pregnancy and in neonates and infants.

WHY TO MEASURE?
Dabigatran concentrations and bleeding risk

The Effect of Dabigatran Plasma Concentrations and Patient Characteristics on the Frequency of Ischemic Stroke and Major Bleeding in Atrial Fibrillation Patients

The RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy)

Paul A. Reilly, PhD,* Thorsten Lehr, PhD,† Sebastian Haertter, PhD,‡ Stuart J. Connolly, MD,§ Salim Yusuf, MD, DPhil,¶ John W. Eikelboom, MB BS,§ Michael D. Ezekowitz, MD, PhD,¶ Gerhard Nehmiz, PhD,¶ Susan Wang, PhD,¶ Lars Wallentin, MD, PhD,¶ on behalf of the RE-LY Investigators

Ridgefield, Connecticut; Biberach and Saarbrücken, Germany; Hamilton, Ontario, Canada; Wynnewood, Pennsylvania; and Upplands, Sweden

Figure 2
Probability of Major Bleeding Event and Ischemic Stroke/SEE Versus Trough Plasma Concentration of Dabigatran

Calculated for 72-year-old male atrial fibrillation patient with prior stroke and diabetes. Lines and boxes at the top of the panel indicate median dabigatran concentrations in the RE-LY trial with 10th and 90th percentiles.

Conc. = concentration; DE = dabigatran etexilate; SEE = systemic embolic event(s).

WHY TO MEASURE?
Edoxaban concentrations and bleeding risk

Association between edoxaban dose, concentration, anti-Factor Xa activity, and outcomes: an analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial

Christian T Ruff, Robert P Giugliano, Eugene Braunwald, David A Morrow, Sabina A Murphy, Julia F Kuder, Naveen Deedwania, Peter Faraci, Joshua Betcher, Ningyu Shi, Karen Brown, Indravendra Puri, Michele Mercier, Elliot M Antman

Figure 2: Probability of clinical outcomes versus edoxaban concentration
Trough edoxaban plasma concentration at 1 month after randomisation versus probability of efficacy and safety outcomes (median follow-up 2.8 years). ICH=intracranial haemorrhage. SEE=systemic embolic event.

## WHY TO MEASURE?
### Drug interactions

<table>
<thead>
<tr>
<th></th>
<th>dabigatran etexilate (PRADAXA®)</th>
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<th>edoxaban (LIXIANA®)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP substrate</strong></td>
<td>×</td>
<td>✓ 3A4, 2J2 + cyp-independent mechanism</td>
<td>✓ 3A4/5, 1A2, 2J2 + cyp-independent mechanism</td>
<td>✓ 3A4/5 + cyp-independent mechanism</td>
</tr>
<tr>
<td><strong>Transport substrate</strong></td>
<td>✓ P-gp</td>
<td>✓ P-gp</td>
<td>✓ P-gp</td>
<td>✓ P-gp</td>
</tr>
<tr>
<td><strong>Anti-H₂/proton pump inhibitors</strong></td>
<td>30% reduction of the AUC No impact on efficacy in RCT</td>
<td>No impact</td>
<td>No impact</td>
<td>No impact</td>
</tr>
</tbody>
</table>

Eu-SmPC Pradaxa® Xarelto® Eliquis® Lixiana®
WHY TO MEASURE?
Renal impairment

- Renal function should be assessed in all patients by calculating the CrCl prior to initiation of treatment with DOACs

- Several dosing recommendations based on CrCl

- Algorythm for the monitoring of the renal function has been proposed*

  → CrCl divided by 10 if CrCl is below 60 mL/min

* There are several proposals for the monitoring of the kidney function, but in any case, yearly monitoring is probably not sufficient in patients with impaired renal function.

WHY TO MEASURE?
Hepatic impairment

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<th>edoxaban (LIXIANA®)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>associated with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coagulopathy</td>
<td>CONTRINDICATED</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

→ Recommendation:

Prior to initiating DOACs, liver function testing should be performed.

WHY TO MEASURE?
Extreme body weight

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</tr>
</thead>
<tbody>
<tr>
<td><strong>Low body weight</strong></td>
<td>No dose adjustment required</td>
<td>No dose adjustment</td>
<td>Dose reduction: 2.5</td>
<td>Dose reduction: 30</td>
</tr>
<tr>
<td>(&lt;50-60 kg)</td>
<td>Limited clinical data are available for patients &lt;50 kg</td>
<td>required</td>
<td>mg bid</td>
<td>mg od</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variation of less than</td>
<td>if at least 2 of the</td>
<td>C_{MAX} and AUC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25% of the exposure</td>
<td>≥80 years ≤60 kg</td>
<td>increased by 40%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>serum creat. ≥ 1.5 mg/dl or if CrCl 15-29 ml/min</td>
<td>and 13%, respectively</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 % increase of the exposure</td>
<td></td>
</tr>
<tr>
<td><strong>High body weight</strong></td>
<td>No dose adjustment required</td>
<td>No dose adjustment</td>
<td>No dose adjustment</td>
<td>No information</td>
</tr>
<tr>
<td>(&gt;120 kg)</td>
<td>Trough dabigatran concentrations were 20% lower in patients &gt;100 kg compared to the 50-100 kg group</td>
<td>required</td>
<td>required</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variation of less than</td>
<td>30% reduction of the exposure</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25% of the exposure</td>
<td></td>
<td></td>
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Eu-SmPC Pradaxa® Xarelto® Eliquis® Lixiana®
### WHY TO MEASURE?

**Other special populations**

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<th>edoxaban (LIXIANA®)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elderly</strong></td>
<td>Patients aged 80 years or above should be treated with a daily dose of 220 mg taken as one 110 mg capsule twice daily due to the increased risk of bleeding in this population.</td>
<td>Elderly patients exhibited higher plasma concentrations than younger patients, with mean AUC values being approximately 1.5 fold higher, mainly due to reduced (apparent) total and renal clearance. No dose adjustment is necessary.</td>
<td>Dose reduction: 2.5 mg bid if at least 2 of the following: ≥80 years ≤60 kg serum creat. ≥ 1.5 mg/dl or if CrCl 15-29 ml/min Elderly patients (above 65 years) exhibited higher plasma concentrations than younger patients, with mean AUC values being approximately 32% higher and no difference in C_{MAX}.</td>
<td>After taking renal function and body weight into account, age had no additional clinically significant effect on edoxaban pharmacokinetics</td>
</tr>
<tr>
<td><strong>Ethnic origin and race</strong></td>
<td>No clinically relevant ethnic differences. However, 32.8% of the white European participants of the RE-LY study presented a particular SNP that decreased trough concentrations to the rate of the 110mg BID dose</td>
<td>No clinically relevant inter-ethnic differences</td>
<td>No clinically relevant inter-ethnic differences</td>
<td>No clinically relevant inter-ethnic differences.</td>
</tr>
</tbody>
</table>
Patients or situations requiring an assessment of the response (1)

- Bleeding or recurrence of thrombosis
- Before an invasive procedure (elective or urgent surgery)
  - Laboratory vs pharmacokinetic approach
- In patients with potential drug interactions that affect the pharmacokinetics of OACs
- In patients with extreme body weight (< 50 or > 100 kg)

Patients or situations requiring an assessment of the response (2)

- In elderly patients (> 75 years of age)
- In patients with genetic mutations
- In case of accumulating interfering factors

Limitations of current measurements

- Inter-laboratory variation
- Implementation of international standards for calibration
- Turn-around time in emergent situations if not implemented in routine
- Are plasma levels the good biomarker to assess the individual response of the patient? Can we get more information from global assays such as thrombin generation test?
PERSPECTIVES
What do we need in the future?

FUTURE can be…

- Determination of plasma concentration is feasible but safe threshold have to be clearly established for all DOAC
  - depending on the population
  - proposals of cut-off for safe surgery have to be confirmed in prospective studies

- Identify those that may benefit from specific dose tailoring

- Development of (new) tests…
  1. able to provide individualized response to the treatment, not only measuring plasma levels (inter-individual variation of plasma protein levels, contribution of antiplatelet agents,...)
  2. able to guide the physician in emergent situations (POC device)
PERSPECTIVES
What do we need in the future?

FUTURE can be…

- Development of (new) **tests**…
  1. able to *provide individualized response to the treatment*, not only measuring plasma levels (inter-individual variation of plasma protein levels, contribution of antiplatelet agents,…)

![Graph showing the phases of fibrin clot formation](image)
FUTURE can be…

- Thrombin generation test was already evaluated in this way with:
  - LMWH…

<table>
<thead>
<tr>
<th>Table 2 Sensitivity to heparin treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>R.S.</td>
</tr>
<tr>
<td>UFH</td>
</tr>
<tr>
<td>MMW</td>
</tr>
<tr>
<td>Ceraptorin</td>
</tr>
<tr>
<td>Enoxaparin</td>
</tr>
</tbody>
</table>

Each figure (except N) gives the percentage of samples in which the test in the column was significantly different from the relevant normal control (see text). All, All samples except the 0 and 24-h time points; R.S. (restricted set), the previous group with the 0.5, 8 and 10-h time points omitted. Lower rows: samples after injection of the heparin indicated.

PERSPECTIVES
What do we need in the future?

• DFXa inhibitors…  ➔ Main effect on Peak, mVRI and ETP
  ➔ Non-evaluated by other coagulation tests…

PERSPECTIVES
What do we need in the future?

FUTURE can be…

• DFIllla inhibitor… → Main effect on Lag time and ETP (at high[ ])

TGA could be used to determine a risk of bleeding/thrombosis under anticoagulant therapy

Previous studies already performed using the ETP as a marker of bleeding risk (<350nM.min)

A French team studied the effect of different non-specific hemostatic agents on the reversal of apixaban

Honickel *et al.* investigated the effect of idarucizumab and different prothrombinic complex on dabigatran

- Contradictory results with **aPTT** and **PT**
- **Underlying the interest of having more sensitive assays assessing the complete coagulation process**

Problem with TGA in its current form...

... STANDARDIZATION

Many parameters influenced the TGA

- The patient’s plasma (the inner-filter effect)
- Preanalytical variables
  - tube, needle versus butterfly, fresh vs frozen samples
- Reagents:
  - PPP-Reagent Low (1pM TF + 4µM PL)
  - PPP-Reagent High (20pM TF + 4µM PL)
  - PPP-Reagent (5pM TF + 4µM PL)
  - MP-Reagent (4µM PL)
  - PRP Reagent (1pM TF)
  - + “home made” reagents
- Batch
- Method used (different depending on the manufacturer)

BUT WE HAVE A SOLUTION...
AIMS

- Validation of a new TGA system
- DrugScreen application

• **Primary objective**
  - To show that TG is significantly modified in patients with anticoagulant treatment

• **Secondary objectives**
  - To evaluate the precision performance of the device under final user setting.
  - To determine the reference interval of the method for the specific reagent tested.
THE STG-DRUGSCREEN STUDY
PHASE-1

- Familiarization period with the device

- By running calibration, quality controls and "dummy" samples by using the QC plasmas
Results – precision STG-DrugScreen

- On CAT system, typical inter-day precision is around 10 to 30% depending on the parameter analyzed.

- 41 independent runs of measurement were performed with the same batch of STG - DrugScreen on ST Genesia.

- On each run, 3 freeze-dried samples were tested.
  - 2 of these samples were internal quality control samples (hypocoagulable and normocoagulable)
  - 1 is intended to be used as reference plasma for normalizing results.
# Results – precision STG-DrugScreen

<table>
<thead>
<tr>
<th></th>
<th>Sample 1 = STG - QualiTest Norm DS</th>
<th>Sample 2 = STG - QualiTest Low DS</th>
<th>Sample 3 = STG - RefPlasma DS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>CV</td>
</tr>
<tr>
<td>n=41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time (min)</td>
<td>0,92</td>
<td>0,04</td>
<td>4,6%</td>
</tr>
<tr>
<td>Peak (nM)</td>
<td>501,92</td>
<td>16,17</td>
<td>3,2%</td>
</tr>
<tr>
<td>Time to Peak (min)</td>
<td>1,97</td>
<td>0,07</td>
<td>3,6%</td>
</tr>
<tr>
<td>ETP (nM.min)</td>
<td>1602,66</td>
<td>65,24</td>
<td>4,1%</td>
</tr>
<tr>
<td>Velocity Index (nM/min)</td>
<td>718,69</td>
<td>55,87</td>
<td>7,8%</td>
</tr>
</tbody>
</table>

[Table 1 - Performances of ST-Genesllla on reference, hypo- and normo-coagulable plasma]
THE STG-DRUGSCREEN STUDY
PHASE-2

Validation of pre-analytical standard practices for blood draw of the investigational site

The general purposes of this Phase 2 were:

- To get evidence that the pre-analytical conditions applied routinely at the investigation site are compliant with the system purpose and its intended use.
- To help proving that TG results are stable along time on samples stored at around -70°C, at least for the same duration as the maximal storage duration observed on the samples from the validation study itself.
THE STG-DRUGSCREEN STUDY
PHASE-2

SAMPLES

- 2 types of samples
  - From patients treated with anticoagulants
    - Apixaban
    - Rivaroxaban
    - Dabigatran
    - LMWH
    - VKA
  - From healthy subjects
    - Without any factors capable of interfering with coagulation
THE STG-DRUGSCREEN STUDY
PHASE-2

Testing schedule

- Samples tested fresh and at several times after freezing at -70°C

- Fresh
  - TG measurement with STG-DrugScreen
  - PT and APTT determination with Stago reagents (healthy)
  - INR, anti-Xa level, DOAC plasma concentrations determination with Stago reagents (patients)

- Freezing
  - TG measurement with STG-DrugScreen
THE STG-DRUGSCREEN STUDY
PHASE-2

6 Healthy individuals
30 patients with anticoagulant treatment (pt)

Draw 7 tubes of 3.5 mL of blood
+ discard the 1st tube for each subject

Centrifuge + Aliquot 12 vials of 600µL per subject

Day 1
Freeze 10 vials at ≤-70°C per subject
Measure TG with STG-DrugScreen with 1 vial of fresh sample for each subject
Assay PT and APTT with 1 vial of fresh sample for each healthy individual
Measure of anticoagulant activity with 1 vial of fresh sample for each patient under anticoagulant

Day 2
Thaw 1 vial at 37°C per subject

Month 1, 2, 3, 6, 9 and 10
Thaw 1 vial at 37°C per subject
Measure TG with STG-DrugScreen with 1 vial for each subject

3 back-up frozen vials
THE STG-DRUGSCREEN STUDY
PHASE-2

Results

- Impact of freezing (D1 vs D0) was assessed through paired-sample analysis (Student or Wilcoxon test) and stability through evaluation of trends overtime vs D1.

- Currently 6 healthy subjects, 7 apixaban, 3 dabigatran, 6 rivaroxaban, 5 VKA and 6 LMWH patients were included. Only results for D0, D1 and M1 are currently available. Table 1 summarizes the mean difference relative to D0 or D1 and the range of relative deviations.
## Results

<table>
<thead>
<tr>
<th></th>
<th>Lag time (min)</th>
<th>Peak Height (nM)</th>
<th>Time to Peak (min)</th>
<th>ETP (nM.min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1 vs D0</td>
<td>M1 vs D1</td>
<td>D1 vs D0</td>
<td>M1 vs D1</td>
</tr>
<tr>
<td><strong>Healthy subjects</strong></td>
<td>Mean diff. (range)</td>
<td>-1% (-15 to 11%)</td>
<td>-8% (-12 to 4%)</td>
<td>-8% (-12 to 4%)</td>
</tr>
<tr>
<td><strong>Apixaban</strong></td>
<td>Mean diff. (range)</td>
<td>0% (-10% to 12%)</td>
<td>1% (-20% to 33%)</td>
<td>15% (-4% to 28%)</td>
</tr>
<tr>
<td><strong>Dabigatran</strong></td>
<td>Mean diff. (range)</td>
<td>-10% (-14% to -4%)</td>
<td>-10% (-19% to 2%)</td>
<td>2% (-3% to 6%)</td>
</tr>
<tr>
<td><strong>Rivaroxaban</strong></td>
<td>Mean diff. (range)</td>
<td>-7% (-11% to -4%)</td>
<td>-6% (-12% to 7%)</td>
<td>13% (-5% to 25%)</td>
</tr>
<tr>
<td><strong>VKA</strong></td>
<td>Mean diff. (range)</td>
<td>-1% (-6% to 1%)</td>
<td>-3% (-13% to 2%)</td>
<td>1% (-4% to 5%)</td>
</tr>
<tr>
<td><strong>LMWH</strong></td>
<td>Mean diff. (range)</td>
<td>-1% (-9% to 6%)</td>
<td>-9% (-20% to 3%)</td>
<td>15% (-5 to 69%)</td>
</tr>
</tbody>
</table>

[Table 1 – Stability of TGA parameters after 1 day and 1 month storage at -80°C.]
Conclusions

- Automation, enhanced control of temperature throughout the assay and standardization of thrombin generation measurement help to achieve highly reproducible results, first step to introduce this assay in the clinical lab.

- Freezing slightly affects all TG parameters.

- Once plasmas are frozen, TG parameters are also slightly influenced during the first month of storage.

- As it is frequently impossible to ensure that storage duration of samples is strictly equivalent throughout a study on a plasma bank, working with fresh samples would help in avoiding the outliers observed.

- This demonstrates the utility of a fully automated TGA analyzer that could be integrated in the routine lab.
Acknowledgments

- Prof. Jean-Michel Dogné
- Prof. François Mullier
- Prof. Bernard Chatelain
- Technical staff of the CHU UCL Namur and the University of Namur
En el corazón de la hemostasia