Laboratory monitoring of oral anticoagulants

A/Prof. Lee Lai Heng
Haematology Singapore General Hospital
<table>
<thead>
<tr>
<th>Relevant Disclosures</th>
</tr>
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<tbody>
<tr>
<td>Educational and Travel Grants</td>
</tr>
<tr>
<td>Bayer, Leo, Bristol Meyer Squib</td>
</tr>
<tr>
<td>Advisory Boards</td>
</tr>
<tr>
<td>Bayer, Bristol Meyer Squib, Boehringer-Ingelheim, Pfizer, Leo, Covidien</td>
</tr>
<tr>
<td>Factor Xa and Il a Inhibitor Clinical Trials – Study Management Committee or Investigator:</td>
</tr>
<tr>
<td>VTE Prevention: apixaban (BMS), rivaroxaban (Pfizer)</td>
</tr>
<tr>
<td>VTE Therapy: apixaban (Pfizer), rivaroxaban (Bayer), edoxaban (Daiichi-Sankyo), dabigatran (Boeringher Ingelheim)</td>
</tr>
<tr>
<td>Stocks and Shares</td>
</tr>
<tr>
<td>Nil</td>
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</tbody>
</table>
Indications for Anticoagulation

Prophylaxis and Treatment of Venous Thromboembolic Disease (VTE)

Stroke Prevention Atrial Fibrillation

Prosthetic heart valves

Tissue Heart Valves

Coronary Heart Disease / Myocardial Infarction
1920s cattle in N.USA & Canada died from a haemorrhagic disease after eating mouldy silage made from sweet clover.

1940 - Karl Link & student Harold Campbell in Wisconsin discovered that the anticoagulant in sweet clover was 3,3'-methylenebis(4-hydroxyl coumarin).

1948 - Further work by Link led to the synthesis of WARFARIN.

(WARF (Wisconsin Alumni Research Foundation) and -arin From coumarin)
Vitamin K Antagonists (VKA) / Warfarin

1952 - Rodenticide in the USA
1954 - Human use

NO RCT

Warfarin - Only approved drug for 60 years
Warfarin—Mechanism of Action

- Synthesis of Dysfunctional Coagulation Factors
- Reduction of functional factors available for coagulation
- It has no effect on these factors that are already formed
- Full therapeutic action is delayed until circulating coagulation factors are removed by normal degradation

Warfarin inhibits the interconversion of vit K and its vit K 2,3 epoxide, which modulates the γ-carboxylation of glutamate residues on the N-terminal regions of the coagulation proteins.

Similar structure to vitamin K

<table>
<thead>
<tr>
<th>Protein</th>
<th>Half-Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VII</td>
<td>4–6 hours</td>
</tr>
<tr>
<td>Factor IX</td>
<td>24 hours</td>
</tr>
<tr>
<td>Factor II</td>
<td>60 hours</td>
</tr>
<tr>
<td>Factor X</td>
<td>48–72 hours</td>
</tr>
<tr>
<td>Protein C</td>
<td>8 hours</td>
</tr>
<tr>
<td>Protein S</td>
<td>30 hours</td>
</tr>
</tbody>
</table>

Ansel et al; Pharmacology and Management of the Vitamin K Antagonists: ACCP Guidelines 8th Ed; Chest 2008;133;160-198
Warfarin

- Delayed onset of anticoagulation effects
- Half-life 35 hours, anticoagulation effects persists few days after discontinuation
- Plasma concentration has poor correlation to anticoagulation effects
- Absorption influenced by drugs & diet
- Metabolism & excretion influenced by drugs & diseases
- 99% bound to albumin; Free warfarin is active

- **Monitoring of Anticoagulant Effects is ESSENTIAL**
Prothrombin Time

- First described by Quick in 1935
- One step assay - Clotting time

\[
\begin{align*}
&\text{TF- VIIa} \\
&\text{X} \quad \rightarrow \quad \text{Xa} \\
&\quad \downarrow \quad \text{Va} \\
&\text{II} \quad \rightarrow \quad \text{Thrombin} \\
&\quad \downarrow \\
&\text{Fibrinogen} \quad \rightarrow \quad \text{Fibrin Clot}
\end{align*}
\]

- Now routinely performed using an automated coagulation analyzer, commercial tissue thromboplastin reagent
  - tissue factor (extract of brain or recombinant tissue factor)
  - phospholipids
  - calcium
  - citrate-anticoagulated plasma
- Time from the mixing of the patient sample with the reagent until the formation of a clot is monitored and recorded in seconds as the clot time
- Abnormalities in VII, V, X, prothrombin and fibrinogen result in prolongation of PT

Warfarin - Monitoring

Prothrombin time ratio: PT(patient)/PT (normal control)

• using thromboplastins

• Different thromboplastins differ in their sensitivities to reduction of vitamin K dependent factors

• No standard comparisons between different labs & countries
**International Normalised Ratio (INR)**

- Developed in 1983 as a method to standardize reporting of PT ratios between laboratories to monitor patients on VKA
- Based on WHO primary international reference preparation of thromboplastin (IPR)
- ISI - Linear relationship between Log PT ratio of reference thromboplastin and that of test thromboplastin
- INR = International Normalized Ratio

\[
\text{INR} = \left( \frac{\text{Patient’s PT in seconds}}{\text{Mean normal PT in seconds}} \right) \times \text{ISI}
\]

**Graph Information:**
- PT = Prothrombin Time
- ISI = International sensitivity index
- Very sensitive thromboplastin has ISI < 1
- Less sensitive thromboplastin has ISI > 1
Vitamin K Antagonists (VKA) / Warfarin

- Oral – suitable for chronic use
- Peak anticoagulation activity delayed for 72-96 hrs
- Time to reach therapeutic range is unpredictable
- Unpredictable anticoagulation, narrow therapeutic window, and numerous interactions
  ⇒ Requires frequent monitoring and dose adjustments

Thromboembolic diseases - 2.0-2.5
Mechanical Heart Valves - 2.5-3.5
Ensuring Accurate Results

Laboratory Variables

Reagents and Controls

• Reconstitution, Stability and Storage

• Instrumentation - Temperature, Timing device, Optimal activation times, Fibrometer Probes

Accuracy of MNPC and ISI

• External QC necessary to ensure accuracy of test results
Ensuring Accurate Results

**Patient Variables - Blood Collection**

- Good venepuncture technique, fresh sample
- Blood to anticoagulant ratio not maintained at 9:1
  - incorrect blood volume, very high or low haematocrit
- Haemolysis
- Heparin contamination
Point-of-Care (POC) Testing

• POC testing devices developed for frequent monitoring VKA.

• Advantages for moving to POC devices:
  • faster turnaround time, lower required blood volumes, less invasive collection technique lower costs, and home monitoring

• Numerous reports on an acceptable correlation between various POC devices and the laboratory

• Variability and poor correlation when the INR is markedly elevated, and also a positive bias is seen with the POC device on INRs within the therapeutic range
Genetic Testing

Genes affecting response to VKA – genes encoding:
• Cytochrome P450 2C9 (CYP2C)
• Vitamin K epoxide reductase complex 1 (VKORC1)

• Early studies - pharmacogenomic testing for warfarin dosing is more accurate than other dosing schemes.
  - Improves time to a therapeutic INR
  - Requires fewer dosing adjustments.
• Patients who require higher or lower than usual doses seem to benefit the most.
• BUT cost effectiveness and prevention of bleeding or thrombosis complications required further evaluation
Genotype-guided versus standard vitamin K antagonist dosing algorithms in patients initiating anticoagulation: A systematic review and meta-analysis

- 12 studies (3,217 patients)
- 6 (2,223 patients) reported all components of the primary outcome
  - 87 events of mortality, thromboembolic events and major bleeding
- Genotype-guided compared with standard VKA dosing algorithms were not found to decrease a composite of death, thromboembolism and major bleeding, (relative risk 0.85, 95 % [CI] 0.54–1.34; p=0.35)
- (6 trials, 997 patients) - An improvement with significantly higher TTR in the genotype-guided dosing when compared with fixed VKA dosing algorithms (CI 3.50-13.31; p=0.01, )
- (4 trials, 1,770 patients) – TTR Not higher in genotype-guided dosing when compared with clinical algorithm guided dosing (95 % CI –2.48 -1.90; p=0.68)

Genetic testing is not routinely recommended.
Ideal Anticoagulant

- Oral administration
- Good Efficacy and Safety
- Metabolic Properties with No food and drug interaction
- No need for coagulation monitoring
- Reversal Agent / Antidote available
Advances in Anticoagulation -- Direct Oral Anticoagulants (DOACs)

Prevention of stroke and systemic embolism in atrial fibrillation (AF)

VTE prophylaxis in major orthopaedic surgery

Treatment of acute VTE and secondary prevention of recurrent VTE

Prevention of cardiovascular deaths after acute coronary syndrome (Rivaroxaban)
# RCTs – DOACs in Atrial Fibrillation

<table>
<thead>
<tr>
<th></th>
<th>RE-LY²</th>
<th>ROCKET-AF²</th>
<th>ARISTOTLE¹</th>
<th>ENGAGE AF-TIMI 48¹</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dabigatran 150 mg (n=5076)</td>
<td>Dabigatran 110 mg (n=5015)</td>
<td>Warfarin (n=6072)</td>
<td>Rivaroxaban (n=7131)</td>
<td>Warfarin (n=7128)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.5 (8.8)</td>
<td>71.4 (8.6)</td>
<td>71.6 (8.6)</td>
<td>72 (65-78)</td>
<td>70 (63-76)</td>
</tr>
<tr>
<td>&lt;75 years</td>
<td>40%</td>
<td>38%</td>
<td>39%</td>
<td>42%</td>
<td>31%</td>
</tr>
<tr>
<td>Women</td>
<td>37%</td>
<td>36%</td>
<td>40%</td>
<td>40%</td>
<td>36%</td>
</tr>
<tr>
<td>Atrial fibrillation type</td>
<td>67%</td>
<td>68%</td>
<td>66%</td>
<td>81%</td>
<td>85%</td>
</tr>
<tr>
<td>Persistent or permanent</td>
<td>67%</td>
<td>68%</td>
<td>66%</td>
<td>81%</td>
<td>85%</td>
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<tr>
<td>Paroxysmal</td>
<td>33%</td>
<td>32%</td>
<td>34%</td>
<td>18%</td>
<td>15%</td>
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<tr>
<td>CHA₂DS²–V栓</td>
<td>2.2 (1.2)</td>
<td>2.1 (1.1)</td>
<td>2.1 (1.1)</td>
<td>2.5 (0.94)</td>
<td>2.1 (1.1)</td>
</tr>
<tr>
<td>0-1</td>
<td>32%</td>
<td>32%</td>
<td>32%</td>
<td>33%</td>
<td>33%</td>
</tr>
<tr>
<td>2</td>
<td>32%</td>
<td>32%</td>
<td>32%</td>
<td>33%</td>
<td>33%</td>
</tr>
<tr>
<td>3-6</td>
<td>32%</td>
<td>32%</td>
<td>32%</td>
<td>33%</td>
<td>33%</td>
</tr>
<tr>
<td>Previous stroke or TIA¹</td>
<td>20%</td>
<td>20%</td>
<td>20%</td>
<td>55%</td>
<td>19%</td>
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<tr>
<td>Heart failure¹</td>
<td>32%</td>
<td>32%</td>
<td>32%</td>
<td>63%</td>
<td>33%</td>
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<tr>
<td>Diabetes</td>
<td>23%</td>
<td>23%</td>
<td>23%</td>
<td>40%</td>
<td>25%</td>
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<tr>
<td>Hypertension</td>
<td>75%</td>
<td>75%</td>
<td>75%</td>
<td>90%</td>
<td>87%</td>
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<tr>
<td>Prior myocardial infarction</td>
<td>17%</td>
<td>17%</td>
<td>17%</td>
<td>18%</td>
<td>18%</td>
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<tr>
<td>Creatinine clearance</td>
<td>&lt;50 mL/min</td>
<td>15%</td>
<td>15%</td>
<td>19%</td>
<td>21%</td>
</tr>
<tr>
<td>&gt;50 mL/min</td>
<td>45%</td>
<td>45%</td>
<td>45%</td>
<td>42%</td>
<td>42%</td>
</tr>
<tr>
<td>Previous VKA use</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
<td>62%</td>
<td>57%</td>
</tr>
<tr>
<td>Aspirin at baseline</td>
<td>35%</td>
<td>40%</td>
<td>45%</td>
<td>35%</td>
<td>35%</td>
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<tr>
<td>Median follow-up (years)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Individual median TTR</td>
<td>64 (37-71)</td>
<td>64 (37-71)</td>
<td>64 (37-71)</td>
<td>64 (37-71)</td>
<td>64 (37-71)</td>
</tr>
</tbody>
</table>

Data are mean ±SD, median (IQR), or percent, unless otherwise indicated. NOACs = new oral anticoagulants. CHA₂DS²-V栓 = stroke risk scoring system in which one point is given for history of congestive heart failure, hypertension, age ≥75 years, and diabetes, and two points are given for history of stroke or transient ischemic attack. TIA=transient ischemic attack. VKA=warfarin, AT = antithrombotic therapy. TTR= time in therapeutic range. NA=not available. ROCKET AF and ARISTOTLE included patients with systemic embolism. ROCKET AF included patients with left ventricular ejection fraction <50%; ARISTOTLE included those with left ventricular ejection fraction ≤50%. RE-LY ≤50 mL/min; SO-79 mL/min; >80 mL/min; ARISTOTLE ≤50 mL/min; >50-80 mL/min; >80 mL/min. RCTs – RE-LY, ARISTOTLE, and ENGAGE AF-TIMI 48 patients who used VKAs for ≥60 days. DOAC AF patients who used VLQs at 6 weeks of screening. TTRs not available.

Table: Baseline characteristics of the intention-to-treat populations of the included trials

42 411 NOACs vs 29 272 warfarin
### Overview of phase III clinical trials NOACs vs VKAs in VTE

<table>
<thead>
<tr>
<th>Drug</th>
<th>Trial</th>
<th>Design</th>
<th>Treatments and dosage</th>
<th>Duration</th>
<th>Patients</th>
<th>Efficacy outcome (results)</th>
<th>Safety outcome (results)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran</td>
<td>RE-COVER²⁹⁴</td>
<td>Double-blind, double-dummy</td>
<td>Enoxaparin/dabigatran (150 mg b.i.d.) vs. enoxaparin/warfarin</td>
<td>6 months</td>
<td>2539 patients with acute VTE</td>
<td>Recurrent VTE or fatal PE: 2.4% under dabigatran vs. 2.1% under warfarin</td>
<td>Major bleeding: 1.6% under dabigatran vs. 1.9% under warfarin</td>
</tr>
<tr>
<td></td>
<td>RE-COVER IP²⁹⁴</td>
<td>Double-blind, double-dummy</td>
<td>Enoxaparin/dabigatran (150 mg b.i.d.) vs. enoxaparin/warfarin</td>
<td>6 months</td>
<td>2589 patients with acute VTE</td>
<td>Recurrent VTE or fatal PE: 2.3% under dabigatran vs. 2.2% under warfarin</td>
<td>Major bleeding: 15 patients under dabigatran vs. 22 patients under warfarin</td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>EINSTEIN-DVT²⁹⁵</td>
<td>Open-label</td>
<td>Rivaroxaban (15 mg b.i.d. for 3 weeks, then 20 mg o.d.) vs. enoxaparin/warfarin</td>
<td>3.6, or 12 months</td>
<td>3449 patients with acute DVT</td>
<td>Recurrent VTE or fatal PE: 2.1% under rivaroxaban vs. 3.0% under warfarin</td>
<td>Major or CRNM bleeding: 8.1% under rivaroxaban vs. 8.1% under warfarin</td>
</tr>
<tr>
<td></td>
<td>EINSTEIN-PE²⁹⁶</td>
<td>Open-label</td>
<td>Rivaroxaban (15 mg b.i.d. for 3 weeks, then 20 mg o.d.) vs. enoxaparin/warfarin</td>
<td>3.6, or 12 months</td>
<td>4832 patients with acute PE</td>
<td>Recurrent VTE or fatal PE: 2.1% under rivaroxaban vs. 1.8% under warfarin</td>
<td>Major or CRNM bleeding: 10.3% under rivaroxaban vs. 11.4% under warfarin</td>
</tr>
<tr>
<td>Apixaban</td>
<td>AMPLIFY²⁹⁷</td>
<td>Double-blind, double-dummy</td>
<td>Apixaban (10 mg b.i.d. for 7 days, then 5 mg b.i.d.) vs. enoxaparin/warfarin</td>
<td>6 months</td>
<td>5395 patients with acute DVT and/or PE</td>
<td>Recurrent VTE or fatal PE: 2.3% under apixaban vs. 2.7% under warfarin</td>
<td>Major bleeding: 0.6% under apixaban vs. 1.8% under warfarin</td>
</tr>
<tr>
<td>Edoxaban</td>
<td>Hokusai-VTE²⁹⁸</td>
<td>Double-blind, double-dummy</td>
<td>LMWH/edoxaban (60 mg o.d., 30 mg o.d. if creatinine clearance 30–50 ml/min or body weight &lt;60 kg) vs. UFH or LMWH/warfarin</td>
<td>Variable, 3–12 months</td>
<td>8240 patients with acute DVT and/or PE</td>
<td>Recurrent VTE or fatal PE: 3.2% under edoxaban vs. 3.5% under warfarin</td>
<td>Major or CRNM bleeding: 8.5% under edoxaban vs. 10.3% under warfarin</td>
</tr>
</tbody>
</table>

27,044 patients
Robust evidence from RCTs in Atrial Fibrillation and VTE

- Unmonitored NOAC therapy is at least as effective and safe as monitored warfarin.
- Lower rates of intracranial hemorrhage and reduced mortality.
- Further research is required to determine whether routine laboratory monitoring might provide a net benefit for patients.
- Until such data are available, clinicians should continue to prescribe NOACs in fixed doses without routine monitoring.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Drug choice</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-phospholipid syndrome</td>
<td>Warfarin</td>
<td>Inadequate data for this highly thrombotic diseases</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>LMWH</td>
<td>Warfarin and NOACs cross the placenta</td>
</tr>
<tr>
<td>Active cancer</td>
<td>LMWH</td>
<td>No trials comparing NOACs with LMWH</td>
</tr>
<tr>
<td>Creatinine clearance &lt;30 mL/min</td>
<td>Warfarin</td>
<td>Such patients excluded from trials with NOACs</td>
</tr>
<tr>
<td>Creatinine clearance 30-50 mL/min (UNSTABLE)</td>
<td>Warfarin</td>
<td>Avoid overdosage in events of renal deterioration</td>
</tr>
<tr>
<td>AF with mitral stenosis, severe valve abnormalities</td>
<td>Warfarin</td>
<td>No data on efficacy</td>
</tr>
<tr>
<td>Mechanical Heart Valves</td>
<td>Warfarin</td>
<td>Clinical Trial failed</td>
</tr>
<tr>
<td>CYP3A4 and P-gp strong inducers/inhibitors</td>
<td>Warfarin/LMWH</td>
<td>Under/over exposure</td>
</tr>
</tbody>
</table>
# Pharmacological Properties of the DOACs

<table>
<thead>
<tr>
<th></th>
<th>Dabigatran</th>
<th>Rivaroxaban</th>
<th>Apixaban</th>
<th>Edoxaban</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>Factor IIa</td>
<td>Factor Xa</td>
<td>Factor Xa</td>
<td>Factor Xa</td>
</tr>
<tr>
<td><strong>Half-life (hour)</strong></td>
<td>12-17</td>
<td>5-9</td>
<td>12</td>
<td>6-10</td>
</tr>
<tr>
<td><strong>Time to peak effect (hour)</strong></td>
<td>1-3</td>
<td>2-4</td>
<td>1-3</td>
<td>1-2</td>
</tr>
<tr>
<td><strong>Dosing in non-valvular AF</strong></td>
<td>150 mg BID</td>
<td>20 mg OD</td>
<td>5 mg BID</td>
<td>60 mg OD</td>
</tr>
<tr>
<td><strong>Dosing in VTE treatment</strong></td>
<td>150 mg BID after 5-10 days of parenteral anticoagulation</td>
<td>15 mg BID for 21 days followed by 20 mg OD</td>
<td>10 mg BID for 7 days followed by 5 mg BID</td>
<td>60 mg OD after 5 days of parenteral anticoagulation</td>
</tr>
<tr>
<td><strong>Renal clearance as unchanged drug (%)</strong></td>
<td>80</td>
<td>33</td>
<td>27</td>
<td>50</td>
</tr>
<tr>
<td><strong>Drug Interactions Pathways</strong></td>
<td>P-gp</td>
<td>3A4/P-gp</td>
<td>3A4/P-gp</td>
<td>3A4/P-gp</td>
</tr>
</tbody>
</table>
**P-Glycoprotein**

- P-gp inducers reduces drug level
- P-gp inhibitors increases drug level

**CYP3A4/5 Metabolism**

- Strong inducers of CYP3A4/5 decrease exposure of drug
- CYP3A4 Inhibitors increase blood concentrations drug
DOAC Metabolism and Drug Interactions

- **Dabigatran etexilate**: Esterase-mediated hydrolysis. Bio-availability 3-7%.
  - 

- **Rivaroxaban**: Bio-availability: 66% (without food), >80% (with food).
  - 

- **Apixaban**: Bio-availability 50%.
  - 

- **Edoxaban**: Bio-availability 62%.
  - 

EHRA Practical Guideline. Europace (2013) 15, 625-651
<table>
<thead>
<tr>
<th>Interacting drug</th>
<th>Class</th>
<th>Dabigatran</th>
<th>Rivaroxaban</th>
<th>Apixaban</th>
</tr>
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<tbody>
<tr>
<td>Ketoconazole</td>
<td>Anti-fungal</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Anti-fungal</td>
<td>ND</td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
<td>Voriconazole</td>
<td>Anti-fungal</td>
<td>ND</td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
<td>Posaconazole</td>
<td>Anti-fungal</td>
<td>ND</td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
<td>Fluconazole</td>
<td>Anti-fungal</td>
<td>ND</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>Antibiotic</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
<td>Erythromycin</td>
<td>Antibiotic</td>
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<td>↑↑</td>
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<td>Verapamil</td>
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<td>Amiodarone</td>
<td>Anti-arrhythmic</td>
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<td>Diltiazem</td>
<td>Anti-arrhythmic</td>
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<td>Quinidine</td>
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<td>↑↑</td>
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<td>ND</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Anti-tuberculosis</td>
<td>↓↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Anti-convulsant</td>
<td>ND</td>
<td>↓↑</td>
<td>↓</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Anti-convulsant</td>
<td>↓↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>St John’s Wort</td>
<td>Herbal</td>
<td>↓↑</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

ND: No data; HIV: human immunodeficiency virus

↑↑: anticoagulant effect likely to be increased; ↑: anticoagulant effect may be increased; ↓: anticoagulant effect may be decreased
DOAC - Ideal Anticoagulant?

• Oral administration
• Good Efficacy and Safety
• Metabolic Properties with No FEW food and drug interaction
• Specific Reversal Agents NOT available for ALL DOACs
• No need for regular coagulation monitoring
DOACs - Coagulation Monitoring and Lab Testing

• DOACs marketed before Lab tests for drug levels anticoagulation function available
• Lab measurement for residual drug effect:
  • Before surgery or invasive procedure
  • Trauma
  • Suspected overdose - drug interactions, renal impairment
  • Recurrent Thrombosis
• The bleeding patient
  • Major Bleeds – access anticoagulation effects of drugs
  • Is bleeding due to high drug levels or other reasons?
Liquid chromatography coupled with tandem-mass spectrometry (LC-MS/MS)
- used to study plasma concentrations and PK/PD properties
- high specificity, sensitivity, selectivity, and reproducibility,
- Gold standard method for the measurement of DOACs

Not a functional assay, recommended to measure active metabolites to avoid systematical bias when assessing the performance of functional assays.
- Supplemented by specific and sensitive functional assays (i.e., dilute thrombin time [dTT] or ecarin-based assays for dabigatran and chromogenic anti-Xa assays for direct factor-Xa inhibitors)

A major limitation - absence of standardization or harmonization of mass spectrometry-based assays.
- To improve inter-laboratory reproducibility, high-quality and international reference standards are urgently needed

NOT Readily available in most labs
Clotting times and Dabigatran

Exquisite sensitivity of this test to dabigatran presence

A normal TT essentially excludes presence of drug

- APTT is more sensitive to dabigatran than the PT.
- Patients treated with 150 mg twice daily
  - PT data - Flat slope
  - PTT data - curvilinear response
- “Misprediction” of the screening test as a normal result despite therapeutic concentrations.
- The misprediction rates for PT reagents ranged from 13% to 43%
- The misprediction rates for APTT more favorable 11% to 26%

Thromb Haemost 2010;103(6):1116–1127
Seminars in Thrombosis & Hemostasis Vol. 43 No. 3/2017
Methods for Quantifying Anti-IIa Direct Oral Anticoagulant

**Diluted Thrombin Time / Hemoclot**
- Modification of TT
- Pt’s sample is diluted (e.g., 1:8) with buffer,
- Then added to an equal quantity of (NPP). Thrombin is added to the mixture and clotting times are recorded.
- Linear response to increasing concentrations of dabigatran,
- Good correlation when compared with drug measurements by mass spectrometry

**Ecarin Clotting Time**
- Ecarin is a metalloprotease from venom viper, Echis carinatus
- Converts prothrombin to meizothrombin, a potent thrombin intermediate inhibited by dabigatran but not heparin.
- Clot-based assay / but chromogenic assay
- Linear correlation to increasing drug concentration
- Quantitative measurements of dabigatran when calibrated appropriately.
- Good correlation with dabigatran levels measured by mass spectrometry.
Anti-Xa Direct Oral Anticoagulants and Clotting tests

• The PT /aPTT have varying degree of sensitivity to anti-Xa DOACs
• Edoxaban is less sensitive than rivaroxaban
• Apixaban demonstrating the least impact on these assays
• Normal PT/PTT despite presence of anticoagulation
• This inter-reagent and inter-drug variability prevents valid recommendations of cut-off points associated with a bleeding risk applicable to all reagents and all drugs
Methods for Quantifying Anti-Xa Direct Oral Anticoagulants

- Anti-Xa and dilute Russell viper venom times provide rapid and accurate drug concentrations.
- Appropriately calibrated using drug-specific standards.
- Can be adopted and performed on almost any laboratory-based coagulation analyzer.
- Should be calibrated with commercial standards in which the exact concentration is verified by a validated LC-MS/MS method.

Most anti-FXa chromogenic assays are also influenced by the presence of heparins.
**Laboratory tests for DOACs**

<table>
<thead>
<tr>
<th></th>
<th>Dabigatran</th>
<th>Rivaroxaban and Apixaban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative/semiquantitative for high levels</td>
<td>aPTT with a sensitive reagent</td>
<td>PT with a sensitive reagent</td>
</tr>
<tr>
<td>Highly sensitive screen</td>
<td>TT</td>
<td>Anti-Xa</td>
</tr>
<tr>
<td>Quantitative (using appropriate calibrators)</td>
<td>Diluted TT, factor IIa, Ecarin clotting time</td>
<td>Anti-Xa</td>
</tr>
</tbody>
</table>

Abbreviations: aPTT, activated partial thromboplastin time; DOAC, direct oral anticoagulant; PT, prothrombin time; TT, thrombin time.

- Effects of NOACs on clotting tests are variable
- Clotting times do not accurately reflect drug levels / anticoagulant effect
- Degree of prolongation is highly dependent on the reagent used for the assay.
- Detect peak or supra-therapeutic drug levels
- May be normal during low or trough drug levels.
- Calibrated assays for quantification of drug levels
- Clotting times may be abnormal from other reasons
- Does NOT provide an accurate assessment of risk of surgical bleeds

A Practical Guide to Ordering and Interpreting Coagulation Tests for Patients on Direct Oral Anticoagulants in Singapore

Wan Hui Wong, 1Esc, PhD, Christina YC Yip, 2Esc, MSc, PhD, Christina LL Sum, 2, Chuen Wen Tan, 1MBBS, MCRP, FRCPath, Lai Heng Lee, 1MBBS, MMed, FAMS, Eng Soo Yap, 4MBBS, MCRP, FCP Thom, Ponmudurai Kuperan, 5FCP, FCRCP, FAMS, Wen Chang Ting, 6MBBS, FCRCP, FAMS, Heng Joo Ng, 1MRCP, FCRCP th, FAMS

<table>
<thead>
<tr>
<th>Test</th>
<th>Activator</th>
<th>Hospital/Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time (PT)</td>
<td>Dade Innovin</td>
<td>SGH, CGH, KTPH, PLS, QL, ID, RD, SKH</td>
</tr>
<tr>
<td></td>
<td>Neoplastin C1 Plus</td>
<td>NUH, TTS, NTFH, KKH, SHP, NHGP</td>
</tr>
<tr>
<td></td>
<td>Thromborel S</td>
<td>MAH</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (aPTT)</td>
<td>Actin FSL</td>
<td>SGH, CGH, KTPH, PLS, QL, ID, RD, MAH, SKH</td>
</tr>
<tr>
<td></td>
<td>STA Cephasscreen</td>
<td>NUH, TTS, NTFH, KKH</td>
</tr>
<tr>
<td>Thrombin clotting time</td>
<td>Thromboclotin</td>
<td>SGH, CGH, KTPH, PLS, QL, ID, MAH, SKH</td>
</tr>
<tr>
<td></td>
<td>STA Thrombin</td>
<td>NUH, TTS, NTFH, KKH</td>
</tr>
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</table>

CGH: Changi General Hospital; ID: Innovative Diagnostics; KKWHC: KK Women’s and Children’s Hospital; KTPH: Khoo Teck Puat Hospital; MAH: Mount Alvernia Hospital; NHGP: National Healthcare Group Polyclinics; NTFH: Ng Teng Fong Hospital; NUH: National University Hospital; PLS: Parkway Laboratory Services; QL: Quest Laboratory; RD: Raffles Diagnostics; SGH: Singapore General Hospital; SHP: Singhealth Polyclinics; SKH: Sengkang Hospital; TTS: Tan Tock Seng Hospital
Prothrombin Time

**TEST**

**DOAC**
- Rivaroxaban
  - KKH, NTFH, NUH, TTSH, SHP, NHG P
- Apixaban
  - CGH, KTPH, SGH, PLS, QL, ID, RD, SKH, MAH

**INSTITUTION**
- All hospitals
  - Minimal effect on PT at usual therapeutic doses and drug levels. Prolonged PT may be indicative of high plasma concentrations
  - Normal PT does not exclude the presence of dabigatran. Test results are not comparable across hospitals because of differing sensitivity of test assays at high plasma concentrations. Not useful for detecting presence or absence of dabigatran

**INTERPRETATION**
- Rivaroxaban
  - Prolonged PT at therapeutic drug levels. May be prolonged at trough levels of drug
  - Normal PT does not exclude significant drug level in patients

- Apixaban
  - May be slightly prolonged at therapeutic level. Normal PT at trough level

- Dabigatran
  - Minimal effect on PT at usual therapeutic doses and drug levels. Prolonged PT may be indicative of high plasma concentrations

**ADDITIONAL COMMENTS**
- Rivaroxaban
  - Least sensitive of PT assay. Normal PT does not exclude significant drug level

- Apixaban
  - Insensitive to apixaban.
Activated partial thromboplastin time

**TEST**

**DOAC**

- **Dabigatran**
  - **INSTITUTION**: KKH, NTFH, NUH, TTSH
  - **INTERPRETATION**: Prolonged at usual doses and drug levels including at trough level
  - **ADDITIONAL COMMENTS**: Normal aPTT does not exclude presence of low concentration of drugs

- **Rivaroxaban**
  - **INSTITUTION**: CGH, KTPH, SGH, PLS, QL, ID, RD, SKH, MAH
  - **INTERPRETATION**: May be normal at trough level. aPTT prolonged at therapeutic level.
  - **ADDITIONAL COMMENTS**: Normal aPTT does not exclude presence of significant drug level.

- **Apixaban**
  - **INSTITUTION**: All hospitals
  - **INTERPRETATION**: Normal at therapeutic levels. Prolonged aPTT may be indicative of very high drug level.
  - **ADDITIONAL COMMENTS**: Normal aPTT does not exclude the presence of significant drug level. Less sensitive assay. aPTT is generally only weakly sensitive to apixaban. Test may not be prolonged until very high drug level.

## How DOACs affect the other coagulation tests

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>Lupus anticoagulant testing</th>
<th>One-stage factor assays</th>
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<tr>
<td>Dilute Russell’s viper venom time (dRVVT)</td>
<td>PTT-LA</td>
<td>Phospholipid-corrected silica clotting time (Staclot LA)</td>
<td>Antithrombin</td>
<td>Protein S</td>
<td>Protein C</td>
</tr>
<tr>
<td>• Falsely prolonged</td>
<td>• Falsely reduced</td>
<td>• Falsely reduced</td>
<td>• Functional assay: clot-based; falsely elevated even at sub-therapeutic levels.</td>
<td>• Chromogenic; not affected.</td>
<td></td>
</tr>
<tr>
<td>• dRVVT ratio cutoff exceeded even at sub-therapeutic levels.</td>
<td>• Phospholipid correction may be incomplete.</td>
<td>• Incomplete correction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• May result in false positive LA.</td>
<td>• Significant effects on factors II and V at therapeutic levels.</td>
<td>• May suggest false presence of factor inhibitor at peak levels.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Significant effects on factors VIII, IX, XI and XII at trough levels.</td>
<td>• Thrombin-based; falsely elevated AT activity in thrombin-based assays</td>
<td>• Significant effects at therapeutic levels.</td>
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<tr>
<td>• Total/free assays: antigen-based; no effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### ASSAY EFFECT

- **DOAC EFFECT**:
  - **Antithrombin**: Partially elevated at therapeutic levels; not affected at trough levels.
  - **Protein S**: Partially elevated at therapeutic levels; not affected at trough levels.
  - **Protein C**: Partially elevated at therapeutic levels; not affected at trough levels.
  - **Activated Protein C (APC) Ratio**: Partially elevated at therapeutic levels; not affected at trough levels.
  - **vWF:Ag, vWF:RCo**: Partially elevated at therapeutic levels; not affected at trough levels.
  - **Platelet function tests**: Not affected.

### RECOMMENDATIONS

- **Do not test when patient is on drug.**
  - **If testing must be done, recommended to do so at trough levels.**
  - **If testing must be done, recommended to do so at trough levels.**
  - **Do not test for clot-based functional Protein S when patient is on drug.**
  - **May test for Protein S antigen.**

- **Do not test when patient is on drug.**
  - **May test.**

- **Do not test when patient is on drug.**
  - **May test.**

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*Ann Acad Med Singapore 2016;45:98-105*
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<td>EFFECT</td>
<td>• Falsely prolonged</td>
<td>Likely not affected.</td>
<td>• Falsely reduced factors II, V, VII and X, but might still be within normal range</td>
<td>• Likely to be incompletely corrected</td>
<td>• Functional assay: clot-based; falsely elevated even at sub-therapeutic levels</td>
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<td>• dRVVT ratio cutoff exceeded even at sub-therapeutic levels.</td>
<td></td>
<td>• Significantly reduce factors VIII and IX at therapeutic levels</td>
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<td>• Total/free assays: antigen-based; no effect</td>
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<td></td>
<td></td>
<td></td>
<td>• Falsely reduced XI and XII levels</td>
<td></td>
<td>Chromogenic: not affected.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Chromogenic assays are also affected</td>
<td></td>
<td>Falsely elevated beyond peak levels.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not affected</td>
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<td>May test.</td>
<td>Do not test for clot-based functional Protein S when patient is on drug.</td>
</tr>
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</table>

---

Monitoring of patient on DOACs

• NO monitoring of INR
• Need to monitor patients
• Be mindful of the few drug interactions
• Need to monitor renal function
• Screening for presence of drug and measurement for drug levels necessary in certain circumstances
• Know how these DOACs interfere with other coagulation tests
THANK YOU