NEW ORAL ANTICOAGULANTS: A VIEW FROM THE LABORATORY

Jing Jin

Clinical laboratory Scientist (MLS, ASCP) - Coagulation/Hematology
Stanford University Hospital and Clinics
OBJECTIVES

1. Describe the mechanisms of action of the new anticoagulants and their effects on the clotting cascade.

2. Discuss the available laboratory tests used to monitor these new anticoagulants.
In the early 1920s, there was an outbreak of cattle disease in the northern United States and Canada. Cattle were haemorrhaging after minor procedures, such as dehorning and castration. Some bled to death.

In 1921, Frank Schofield, a Canadian veterinary pathologist, determined that the spoiled hay from sweet clover eaten by those cattle functioned as a potent anticoagulant and caused bleeding.

In 1940, Dicoumarol (a product of the plant molecule Coumarin) was isolated from spoiled sweet clover.

Warfarin was invented in 1948. The name “Warfarin" stems from the acronym WARF, for Wisconsin Alumni Research Foundation + the ending -arin indicating its link with coumarin.

It was initially introduced in 1948 as a rats poison and is still used for this purpose.

In the early 1950s, Warfarin was found to be effective and relatively safe for preventing thrombosis and embolism and has remained popular ever since.

Warfarin (brand name Coumadin) is the most widely prescribed oral anticoagulant drug in North America.
Mechanism of Warfarin

Vitamin K-dependent factors need vitamin K to become functional. Warfarin inhibits vitamin K epoxide reductase (VKOR), which leads to vitamin K depletion. Therefore, the levels of functional vitamin K-dependent factors are decreased.

Warfarin can prevent clotting by decreasing functional vitamin K-dependent clotting factors (II, VII, IX, X).
WHAT’S WRONG WITH WARFARIN?

- Narrow therapeutic range
- Slow onset of action
- Slow offset of action (long duration of action, long elimination half life)
- Multiple drug and dietary interactions
- Monitoring required to maintain in therapeutic range

Thromb Haemost 2010;103:34-39
MULTIPLE DRUG AND DIETARY INTERACTIONS

- A total of 727 drugs (4773 brand and generic names) are known to interact with Coumadin (warfarin).
  - **194 major** drug interactions (Aspirin, Ibuprofen)
  - **341 moderate** drug interactions
  - **192 minor** drug interactions
- Certain foods and beverages can increase or decrease the effect of warfarin
  - **May decrease the effectiveness warfarin therapy**
    Kale, Spinach, Broccoli, Turnip greens, Cauliflower, Chick peas, Brussels sprouts (*Rich in vitamin K*)
  - **may increase warfarin activity**
    Cooked onions
WHAT ARE THE ATTRIBUTES OF THE IDEAL ANTICOAGULANTS

1. Oral administration
2. Rapid onset of action/rapid offset of action
3. Wide therapeutic range
4. Predictable therapeutic effect with fixed or weight-based dosing.
5. No food or drug-drug interactions
6. No monitoring required (but the ability to monitor if desired)
7. Well defined pharmacokinetics in presence of renal or hepatic disease
8. Easily reversible.
9. Cost effective
# Advantages of New Oral Anticoagulants Over Warfarin

<table>
<thead>
<tr>
<th>Feature</th>
<th>Warfarin</th>
<th>New Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>Slow</td>
<td>Rapid</td>
</tr>
<tr>
<td>Dosing</td>
<td>Variable</td>
<td>Fixed</td>
</tr>
<tr>
<td>Food effect</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Interactions</td>
<td>Many</td>
<td>Few</td>
</tr>
<tr>
<td>Monitoring</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Offset</td>
<td>Long</td>
<td>Short</td>
</tr>
</tbody>
</table>
NEW ANTICOAGULANTS

The new oral anticoagulants (such as Dabigatran, Rivaroxaban and Apixaban) are rapidly replacing Warfarin.

They directly inhibit either Factor IIa and Factor Xa, while Warfarin lowers the functional levels of all of the vitamin K-dependent clotting factors (II, VII, IX, X, PC and PS).

New oral anticoagulants have pharmacological, biochemical, and clinical features distinct from those of Warfarin.
Fibrinogen → Fibrin →硬 clot

Extrinsic Pathway
TF → XIIa → Xa → Thrombin

Intrinsic pathway
XIIa → Xla → IXa → Va

Common pathway
VIIIa → Xa → VIIa

New anticoagulants target at Va

Soft clot → Fibrin

Hard clot → Fibrin → XIIIa
Factor Xa and IIa Inhibitors

- Two classes of **Factor IIa** and **Xa** inhibitors
  - **Indirect**, *Anti-thrombin-III (ATIII) dependent*
    - Catalytic
    - Irreversible ATIII-mediated inhibition of factors IIa and Xa
    - Inhibits only free factor
  
- **Direct** (*Non-ATIII-dependent*)
  - Stoichiometric
  - Specific and reversible inhibition of a single factor
  - Inhibit both free factor and bound factor

*Traditional anticoagulants*

*New anticoagulants*
Factor Xa Inhibitors

Indirect ATIII-dependent inhibitors
- Unfractionated Heparin
- LMWHs
  - Enoxaparin
  - Dalteparin
  - Tinzaparin
- Pentasaccharides
  - Fondaparinux

Direct Xa Inhibitors
- Apixaban (Eliquis®)
- Rivaroxaban (Xarelto®)
- DU176b Edoxaban (Savaysa®)

Vitamin K Antagonists (VKAs)
- Warfarin

Make functional factor X depleted. Not a Factor Xa inhibitor.
Factor IIa Inhibitors

**Indirect ATIII-dependent inhibitors**
- Unfractionated Heparin
- LMWHs
  - Enoxaprin
  - Dalteparin
  - Tinzaparin

**Direct IIa Inhibitors**
- Lepirudin
- Bivalirudin
- Argatroban
- Dabigatran (Pradaxa®)
- Ximelagatran

**Vitamin K Antagonists (VKAs)**
- Warfarin

Make functional factor II depleted. Not a Factor IIa inhibitor.
New Oral Anticoagulants on Market

- **Direct** thrombin (IIa) inhibitor
  - Dabigatran (Pradaxa®)

- **Direct** Factor Xa inhibitors
  - Rivaroxaban (Xarelto®)
  - Apixaban (ELIQUIS®)
  - Edoxaban (Savaysa®)

- Dabigatran, Rivaroxaban, Apixaban and Edoxaban are both on the market. They are rapidly replacing Warfarin.
# Oral Anticoagulants

<table>
<thead>
<tr>
<th>Drug</th>
<th>Warfarin</th>
<th>Rivaroxaban</th>
<th>Apixaban</th>
<th>Dabigatran</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>Vitamin K epoxide reductase</td>
<td>Factor Xa</td>
<td>Factor Xa</td>
<td>Thrombin</td>
</tr>
<tr>
<td><strong>Half-life (hours)</strong></td>
<td>40</td>
<td>3.2-9.1</td>
<td>8-15</td>
<td>7.1-17</td>
</tr>
<tr>
<td><strong>Monitoring</strong></td>
<td>INR-adjusted</td>
<td>Not needed</td>
<td>Not needed</td>
<td>Not needed</td>
</tr>
<tr>
<td><strong>Administration</strong></td>
<td>Once daily</td>
<td>Once daily</td>
<td>Once-twice daily</td>
<td>Once-twice daily</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>CYP450</td>
<td>66% fecal; 33% renal</td>
<td>75% fecal; 25% renal</td>
<td>20% fecal; 80% renal</td>
</tr>
<tr>
<td><strong>Antidote or treatment of bleeding</strong></td>
<td>Vit K + FFP, APCC, or recombinant FVIIa</td>
<td>Recombinant Factor Xa derivative, APCC, recombinant FVIIa</td>
<td>Recombinant Factor Xa derivative</td>
<td>No antidote anymore</td>
</tr>
<tr>
<td><strong>Assay</strong></td>
<td>PT/INR</td>
<td>Antifactor Xa, PiCT, HepTest</td>
<td>Antifactor Xa</td>
<td>Ecarin Clotting time</td>
</tr>
<tr>
<td><strong>Drug Interactions</strong></td>
<td>CYP 2C9, 1A2, 3A4</td>
<td>CYP 3A4 Inhibitor</td>
<td>CYP 3A4 Inhibitor</td>
<td>PPI decrease absorption</td>
</tr>
</tbody>
</table>

# Pharmacology of the DOACs

<table>
<thead>
<tr>
<th></th>
<th>Rivaroxaban</th>
<th>Apixaban</th>
<th>Edoxaban</th>
<th>Dabigatran etexilate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target</strong></td>
<td>FXa</td>
<td>Fxa</td>
<td>FXa</td>
<td>Thrombin</td>
</tr>
<tr>
<td><strong>Peak activity</strong></td>
<td>1-3 hr</td>
<td>1-3 hr</td>
<td>0.5-2 hr</td>
<td>1-3 hr</td>
</tr>
<tr>
<td><strong>Half-life</strong></td>
<td>7-11 hr</td>
<td>12 hr</td>
<td>8-10 hr</td>
<td>14-17 hr</td>
</tr>
<tr>
<td><strong>Protein binding</strong></td>
<td>92-95%</td>
<td>84%</td>
<td>40-59%</td>
<td>35%</td>
</tr>
<tr>
<td><strong>Renal clearance</strong></td>
<td>66%</td>
<td>25%</td>
<td>35%</td>
<td>80%</td>
</tr>
<tr>
<td><strong>Assay</strong></td>
<td>Anti-Xa</td>
<td>Anti-Xa</td>
<td>Anti-Xa</td>
<td>Ecarin Clotting time</td>
</tr>
<tr>
<td><strong>FDA-approved indications</strong></td>
<td>NVAF, VTE, TKA/THA</td>
<td>NVAF, VTE, TKA/THA</td>
<td>NVAF, VTE</td>
<td>NVAF, VTE</td>
</tr>
</tbody>
</table>

* NVAF: Nonvalvular atrial fibrillation; VTE: Venous thromboembolism; VTE prophylaxis for THA (total hip arthroplasty) and TKA (total knee arthroplasty).

Eikelboom JW et al., Circulation 2010;121:1523; Samama MM et al., Clin Lab Med 2014;34:503
### Anticoagulants usage in Stanford Hospital

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Brand name</th>
<th>count</th>
<th>percentage</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td></td>
<td>123660</td>
<td>55.7%</td>
<td>Unfractionated Heparin</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Coumadin®</td>
<td>38807</td>
<td>17.5%</td>
<td>Vitamin K Antagonist</td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>Lovenox</td>
<td>34646</td>
<td>15.6%</td>
<td>LMWH</td>
</tr>
<tr>
<td>Alteplase</td>
<td>Activase®</td>
<td>9825</td>
<td>4.4%</td>
<td>Tissue plasmin activator</td>
</tr>
<tr>
<td>Argatroban</td>
<td></td>
<td>9504</td>
<td>4.3%</td>
<td>Thrombin inhibitor</td>
</tr>
<tr>
<td>Dabigatran</td>
<td>Pradaxa®</td>
<td>1952</td>
<td>0.9%</td>
<td>Thrombin inhibitor</td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>XARELTO®</td>
<td>1520</td>
<td>0.7%</td>
<td>Xa inhibitor</td>
</tr>
<tr>
<td>Apixaban</td>
<td>ELIQUIS®</td>
<td>1210</td>
<td>0.5%</td>
<td>Xa inhibitor</td>
</tr>
<tr>
<td>Fondaparinux</td>
<td>Arixtra</td>
<td>747</td>
<td>0.3%</td>
<td>Xa inhibitor</td>
</tr>
<tr>
<td>Bivalirudin</td>
<td>Angiomax</td>
<td>128</td>
<td>0.1%</td>
<td>Thrombin inhibitor</td>
</tr>
<tr>
<td>Dalteparin</td>
<td>Fragmin</td>
<td>1</td>
<td>0.0%</td>
<td>LMWH</td>
</tr>
</tbody>
</table>

### Patient Demographic Overview

<table>
<thead>
<tr>
<th>Gender</th>
<th>n, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>4,522 (58.9)</td>
</tr>
<tr>
<td>Female</td>
<td>3,224 (41.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race</th>
<th>n, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian</td>
<td>804 (10.1)</td>
</tr>
<tr>
<td>Black</td>
<td>416 (7.2)</td>
</tr>
<tr>
<td>White</td>
<td>4,876 (60.5)</td>
</tr>
<tr>
<td>Other</td>
<td>1,650 (22.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>n, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Hispanic</td>
<td>6,472 (82.5)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>928 (13.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>346 (4.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Death</th>
<th>n, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recorded</td>
<td>2,159 (27.9)</td>
</tr>
<tr>
<td>Within 30 days of testing</td>
<td>865 (11.1)</td>
</tr>
<tr>
<td>Within 3 days of testing</td>
<td>360 (4.6)</td>
</tr>
</tbody>
</table>

Total 7746

Total 222000

* Unpublished data from Saurabh Gombar MD, PhD; Anandi Krishnan PhD; James Zehnder MD Department of Pathology, Stanford University.
OUTCOMES COMPARED WITH WARFARIN

- Dabigatran, Rivaroxaban, and Apixaban have all been shown to be non-inferior to warfare. (Non-inferior clinical trial: A clinical trial that shows that a new treatment is equivalent to standard treatment.)

- All three of the new oral anticoagulants were associated with less intracranial bleeding and the rates of major bleeding were similar or lower than those with Warfarin. (Bleeding is the primary complication of anticoagulant therapy.)

- Approximately 10% reduction in mortality with the new oral anticoagulants when compared with Warfarin.

- No evidence of hepatic toxicity with any of the new agents, which was an important safety consideration after the experience with Ximelagatran (a direct thrombin inhibitor, withdrawn in 2006 due to hepatotoxicity).
# Class and Differentiating Effects of the New Oral Anticoagulants

<table>
<thead>
<tr>
<th>Class effects (compared with Warfarin)</th>
<th>Differentiating effect (compared with Warfarin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As effective as Warfarin</td>
<td>Small increase in risk of myocardial infarction with Dabigatran</td>
</tr>
<tr>
<td>Less intracranial bleeding</td>
<td>More gastrointestinal bleeding with Dabigatran and Rivaroxaban</td>
</tr>
<tr>
<td>Reduced mortality</td>
<td>Dabigatran (150 mg bid) lowers risk of ischemic stroke</td>
</tr>
<tr>
<td>No hepatic toxicity</td>
<td>Apixaban associated with lower risk of stroke and major bleeding compared with Warfarin</td>
</tr>
</tbody>
</table>
**Why is there more GI bleeding with the new oral anticoagulants?**

- All of the new oral anticoagulants are partially excreted in the feces as active drug.

- Even though Dabigatran etexilate is a prodrug, gut esterases transform unabsorbed drug into Dabigatran during its passage through the GI tract.

- May exacerbate bleeding from surface lesions. Such lesions are more common in the elderly.
Why is there Less Intracranial Hemorrhage (ICH) with New Oral Anticoagulants?

- More predictable anticoagulant effect
- No reduction in the level of FVII
- Less impairment of post-clot thrombin generation
WHY THROMBIN GENERATION IS SO IMPORTANT FOR HEMOSTASIS

- Over 95% of thrombin generated after clot formation
- Post-clotting thrombin generation essential for stabilizing the thrombus and enhancing its barrier properties
THE MANY ROLES OF THROMBIN
Thrombin Generation Assay

CALIBRATED AUTOMATED THROMBOGRAM

- Time to peak
- Peak
- ETP
- Tail
- Clot time
- Lag time

MEASUREMENT PRINCIPLE

- Lag Phase: clotting time
- Slope: 
  \[ \text{Velocity index} = \frac{\text{peak thrombin}}{\text{peak time} - \text{lag phase}} \]
- Peak thrombin: Max \([\text{FIIa}]\) formed
- Inactivation Phase
- AUC = Thrombin Potential (ETP)

With conventional coagulation tests only the initial phase of thrombin generation is covered.
Comparison of Effects of Rivaroxaban, Dabigatran, and Warfarin on Thrombin Generation

LAbORATORY MONITORING

- Food does not influence their metabolism.
- Drug–drug interactions are uncommon.
- Predictable anticoagulant effect.

Therefore, new anticoagulants can be given in fixed doses without the need for routine coagulation laboratory monitoring although monitoring tests are available.

CONCERN:

Lack of specific reversal agents and monitoring parameters is a concern in the milieu of hemorrhagic emergency
Liquid Chromatography/tandem mass spectrometry
### Plasma drug levels

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Trough plasma level (ng/mL)</th>
<th>Peak plasma level (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>5 th-95th percentile</td>
</tr>
<tr>
<td>Dabigatran</td>
<td>150 mg BID</td>
<td>90</td>
<td>31-225</td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>20 mg daily</td>
<td>26</td>
<td>6 - 87</td>
</tr>
<tr>
<td>Apixaban</td>
<td>5 mg BID</td>
<td>103</td>
<td>41-230</td>
</tr>
<tr>
<td>Edoxaban</td>
<td>60 mg daily</td>
<td>22</td>
<td>10-40*</td>
</tr>
</tbody>
</table>

* Interquartile range

---

Variability in trough levels

US adult male height:
5th percentile: 5’ 4”
95th percentile: 6’ 3”

If variation in height was equivalent to variation in rivaroxaban trough levels

U.S. Census Bureau, Statistical Abstract of the United States: 2012
Therapeutic On-therapy range

- Below on-therapy range
- On-therapy range
- Above on-therapy range

DOAC level

- 5th percentile trough level
- 95th percentile peak level
Why measure?

- Treatment failure
- Preoperative state
- Non-compliance
- Obesity
- Renal hyperfunction
- GI malabsorption
- Drug interaction

Trauma
Emergent procedure
Reversal agent

Bleeding
Overdose
- Renal dysfunction
- Low body weight
- Advanced age
- Drug interaction

Below on-therapy range
On-therapy range
Above on-therapy range

Drug level
OVERDOSE PROBLEM

- Dabigatran’s anticoagulant effect is through direct inhibition of clotting factors and not through clotting factor depletion. 

  **Therefore the administration of clotting factors (FFP,PCC,etc) is NOT very effective in reversing the effects of Dabigatran**

Supportive care and control of bleeding site are cornerstones of therapy in patients who have life threatening Dabigatran-related bleeding.

In the event of an acute (<1-2 hous) overdose, the administration of activated charcoal may be helpful in absorbing Dabigatran.

- Dabigatran is mostly cleared by kidneys. Renal failure will lead to a half-life time in excess of 24 hours.

  *The primary means of reversing the effects is through natural renal elimination. Hemodialysis may be effect in removing about 60% of Dabigatran.*
AVAILABLE

PRAXBIND — Immediate PRADAXA Reversal

ONLY FDA-approved specific reversal agent for a NOAC

LEARN MORE

When reversal of the anticoagulant effects of Pradaxa® (dabigatran etexilate) is needed:

▸ For emergency surgery/urgent procedures
▸ In life-threatening or uncontrolled bleeding

This indication is approved under accelerated approval based on a reduction in unbound dabigatran and normalization of coagulation parameters in healthy volunteers. Continued approval for this indication may be contingent upon the results of an ongoing cohort case series study.

NOAC=novel oral anticoagulant.
*Accurate as of 07/01/2016.
MONITORING DIRECT THROMBIN INHIBITORS (DTIs)

- DTIs include Argatroban (Argatroban), bivalirudin (Angiomax®), Dabigatran (Pradaxa®) and Recombinant hirudin.

- We use Ecarin chromogenic / Clotting assay (ECA) to monitor DTIs.
Hirudin is naturally produced in the salivary glands of medicinal leeches. It has a blood anticoagulant property; it keeps the blood flowing after the initial bite by the worm on the host’s skin.

Hirudin is the most potent natural inhibitor of thrombin.

It is difficult to extract large amounts of hirudin from natural sources. Recombinant biotechnology has been developed to produce and purify this protein. Recombinant hirudin can be as an intravenous anticoagulant, particularly useful for patients who are allergic to or cannot tolerate heparin.
Plasma Dabigatran level measurement

Dabigatran can inhibit the cleavage of a chromogenic substrate by Meizothrombin. Thus, the generation of pNA or the color development is inversely proportional to the concentration of the Dabigatran in the plasma.

It is a User-defined test run on STAGO instruments.
Ecarin is extracted from snake venom of the saw-scaled viper *Echis carinatus* and converts prothrombin to meizothrombin, which promotes clot formation.
PNA released in the reaction (color development) is inversely proportional to the concentration of Rivaroxaban in patient’s plasma.

- Run on STAGO instruments. **Reagents:** STA®-Liquid Anti-Xa.
- FDA approved.
CONCLUSIONS

- Fixed-dose, unmonitored and orally taken new anticoagulants represent a giant step forward to replace warfarin.

- They have the potential to be more effective than Warfarin.

- They have the potential to be safer than Warfarin such as less major bleeding, especially intracranial bleeding, the most feared complication of anticoagulant therapy.

- No laboratory monitoring needed for most cases.

- December of 2011, the FDA announced that it was investigating reports of serious bleeding in patients taking dabigatran. The bleeding risks strongly suggest that drug monitoring may be recommended.
Common snake venoms used in the Diagnostic Hamostasis Laboratory
<table>
<thead>
<tr>
<th>Common Name</th>
<th>Taxonomic Name</th>
<th>Location</th>
<th>Clotting Compound</th>
<th>Action</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saw Scaled Viper</td>
<td><em>Echis carinatus</em></td>
<td>Middle east and Central Asia</td>
<td>Ecarin A</td>
<td>Prothrombin activator (generates meizothrombin)</td>
<td>Textarin:Ecarin ratio for screening for lupus anticoagulants Ecarin time to monitor hirudin activity and other direct thrombin inhibitors.</td>
</tr>
<tr>
<td>Eastern Brown Snake</td>
<td><em>Pseudonaja textilis</em></td>
<td>Australia (kills more humans than any other Australian snake)</td>
<td>Pseutarin C</td>
<td>Prothrombin activator</td>
<td>Textarin:Ecarin time (test for lupus anticoagulant)</td>
</tr>
<tr>
<td>Costal Taipan</td>
<td><em>Oxyuranus scutellatus</em></td>
<td>Australia (4th most potent venom by LD50 of any snake in the world)</td>
<td>Oscutarin C</td>
<td>Prothrombin activator</td>
<td>Taipan snake venom test (test for lupus anticoagulant)</td>
</tr>
<tr>
<td>Common Lancehead</td>
<td><em>Bothrops atrox</em></td>
<td>South America (kills more humans than any other American snake)</td>
<td>Reptilase</td>
<td>Fibrinogen activator, (uninhibited by antithrombin so unaffected by Heparin)</td>
<td>Reptilase time (commonly used to screen for heparin contamination of a plasma sample)</td>
</tr>
<tr>
<td>Copperhead</td>
<td><em>Agkistrodon contortrix</em></td>
<td>North America (kills more humans than any other snake)</td>
<td>Protac</td>
<td>Protein C activator</td>
<td>The basis for functional Protein C and S assays and for activated protein C resistance</td>
</tr>
<tr>
<td>Russell's Viper</td>
<td><em>Daboia russelii</em></td>
<td>Asia (kills more humans than any other snake)</td>
<td>Dilute Russell's Viper Venom [dRVVT - test for lupus]</td>
<td>Activates factors V and X</td>
<td>dRVVT - test for lupus anticoagulant</td>
</tr>
</tbody>
</table>
Briefly outline the roles for the following snake venoms:

<table>
<thead>
<tr>
<th>Venom</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botrocetin</td>
<td>Botrocetin is a snake venom isolated from <em>Bothrops jararaca</em> which alters the conformation of VWF and increases its affinity for GpIb. The Botrocetin assay of VWF is similar to that of the ristocetin cofactor assay but some patients have been reported with 100% botrocetin cofactor activity and 0% ristocetin cofactor activity.</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>Ristocetin is an antibiotic obtained from <em>Amycolatopsis lurida</em> but was found to cause thrombocytopenia and so removed from the market. It causes thrombocytopenia by inducing platelet agglutination but only in the presence of VWF.</td>
</tr>
<tr>
<td>Russell Viper Venom</td>
<td>Isolated from <em>Daboia russellii</em> and is a specific activator of factors V and X. It is used the dRVVT as a screening test for a Lupus anticoagulant.</td>
</tr>
<tr>
<td>Textarin</td>
<td>Textarin, a protein fraction of <em>Pseudonaja textilis</em> venom (Australian Eastern brown snake), activates prothrombin in the presence of PL, factor V and calcium ions.</td>
</tr>
<tr>
<td>Ecarin</td>
<td>Isolated from <em>Echis carinatus</em> and is a Prothrombin activator [generates meizothrombin]. Ecarin will activate prothrombin in the absence of any cofactors. It forms the basis for the assay of direct thrombin inhibitors and in addition forms the basis for a screening test for a lupus anticoagulant.</td>
</tr>
<tr>
<td>Botox</td>
<td>Botulinum toxin is a protein produced by the bacterium <em>Clostridium botulinum</em>, and is extremely neurotoxic. It is used medically to treat a number of disorders but its major role appears to be for cosmetic purposes. It does not as far as we are aware have any role in the haemostasis lab.</td>
</tr>
<tr>
<td>Ancrod</td>
<td>A defibrinogenating agent derived from the venom of the Malayan pit viper [<em>Aglkistrodon rhodostoma</em> also called <em>Calloselasma rhodostoma</em>]. It also has an action on platelet function.</td>
</tr>
<tr>
<td>Protac</td>
<td>Isolated from <em>Aglkistrodon contortrix</em> and is a specific activator of Protein C to Activated Protein C [APC]. It forms the basis for the chromogenic PC assay and is also used in a functional PS assay.</td>
</tr>
<tr>
<td>Reptilase</td>
<td>Isolated from <em>Bothrops atrox</em> and is an activator of fibrinogen. It is unaffected by the presence of unfractionated heparin [in contrast to the thrombin time] and so is often used to establish the presence of UFH in a sample with a prolonged APTT.</td>
</tr>
</tbody>
</table>