Whatever Happened to the PT and PTT?

How to Monitor the New Antithrombotics
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The Fritsma Factor, Your interactive Hemostasis Resource
Sponsored by Precision BioLogic, Dartmouth, Nova Scotia
www.fritsmafactor.com

Whatever Happened?
What’s new in antithrombotics? Everything. We now monitor antiplatelet drugs aspirin, clopidogrel, and in 2009, prasugrel. What do we do about fondaparinux, and the 2011 oral anticoagulants rivaroxaban, apixaban and dabigatran? And we still don’t know how to monitor direct thrombin inhibitors. Are ecarin time, chromogenic X and chromogenic anti-Xa the answer?

Objectives:
1. Brief summary of current antithrombotics
2. Monitor antiplatelet drugs, fondaparinux, direct thrombin inhibitors, and 2010 oral anticoagulants
3. Employ chromogenic X and chromogenic anti-Xa to monitor several new anticoagulants

71 YO Female, Atrial Fibrillation
30 Years of 7.5 mg/day Warfarin

- Monday: INR 11, no bleeding symptoms
  - Target range 2–3
  - Hx: when INR 5–6: bruising, bleeding gums, epistaxis
  - Just started on statin
    - Total cholesterol: 263 mg/dL
    - Triglycerides: 319 mg/dL
- Tuesday: INR 11
  - Vitamin K 10 mg IV push, D/C warfarin
- Thursday: INR 1.5
  - Fasting, resume warfarin 7.5 mg/day
- Monday: INR 2.5

What Happened?
• Dietary change, increased vitamin K?
• Do statins, other drugs interfere with metabolism?
  - Lovastatin and fluvastatin metabolized by CYP450
• Age-related change in warfarin sensitivity?
• Optical coagulometer, lipemia?

The Coumarins

Coumadin (Warfarin) Indications
• Cardiac insufficiency secondary to acute coronary syndrome
  - Ejection fraction < 30%
• Venous thromboembolism
  - Deep venous thrombosis (DVT)
  - Pulmonary embolism (PE)
• Atrial fibrillation
  - Prevent secondary stroke
• Prosthetic heart valves
Properties of Coumadin

- Vitamin K antagonist
- Block \( \gamma \)-carboxylation of glutamic acid in the vitamin K-dependent coagulation factors
  - Factors II, VII, IX, X
  - Control proteins C, S and Z
- Metabolized by cytochrome P450 pathway

Coumadin Dose & Pharmacodynamics

- Start 5 mg/d, adjust to PT-based international normalized ratio (INR) 2–3
  - When over 70, start at 2 mg/d
  - Screen for high risk polymorphisms CYP2C9*2 and *3 and VKORC1 to start at lower dosage
- Onset of action 8-12 hours
- Requires 4 to 5 days to achieve stability
- Daily INRs until two match in Rx range
- Observe two INRs/week for first two weeks
  - Confirm stability
- Then every four weeks for duration

Is the PT/INR All it Could Be?

- INR invalid in first five days of therapy
- Optical coagulometers affected by lipemia
- PT falsely prolonged by lupus anticoagulant
- INR invalid during transition from direct thrombin inhibitors (argatroban) to coumadin
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Reporting Prothrombin Times (Protimes, PTs) The Old Way

- Report PT in seconds compared to the control or the mean of normal range
  - Typical RI: 10.3-13.1 seconds
- This didn’t permit for normalization among laboratories
  - Variation in reagent responsiveness
  - Variation in instrument sensitivity
- Used when screening for coagulopathy

International Normalized Ratio

- Perform prothrombin time (protime, PT)
- PT is normalized world-wide by applying the INR formula:
  - \[ \text{INR} = \left( \frac{\text{PT}_{\text{patient}}}{\text{PT}_{\text{normal mean}}} \right)^{\text{ISI}} \]
  - INR = international normalized ratio
  - \( \text{PT}_{\text{patient}} \) = PT of patient plasma
  - \( \text{PT}_{\text{normal mean}} \) = PT of mean of normal range
  - ISI = international sensitivity index assigned by manufacturer based on comparison to international thromboplastin reagent
- The laboratory computes the formula and reports all PTs in seconds and as INRs

Recommended INRs

<table>
<thead>
<tr>
<th>Indication</th>
<th>Range</th>
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<tbody>
<tr>
<td>Prophylaxis and therapy for DVT, PE, atrial fibrillation, cardiac insufficiency</td>
<td>2–3</td>
</tr>
<tr>
<td>Therapy for mechanical valves, complicated or recurrent AMI</td>
<td>2.5–3.5</td>
</tr>
<tr>
<td>Risk of hemorrhage critical value</td>
<td>&gt; 5.0</td>
</tr>
</tbody>
</table>

International Sensitivity Index (ISI)

- Manufacturers assay 20 normal and 60 stable Coumadin plasmas using:
  - A WHO international reference preparation (IRP) of thromboplastin
  - Their commercial lot number of reagent
  - The manual tilt-tube technique in multiple expert labs
- They generate instrument-specific ISIs for each thromboplastin/instrument combination by regression

PT Variation Among Four Thromboplastins

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Mean (sec)</th>
<th>Example Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16.1</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>67.4</td>
<td>31.3</td>
</tr>
<tr>
<td>B</td>
<td>16.9</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>81.9</td>
<td>8.7</td>
</tr>
<tr>
<td>C</td>
<td>13.7</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>169.0</td>
<td>15.4</td>
</tr>
<tr>
<td>D</td>
<td>17.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>78.3</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Despite normalization, the INR varies significantly as a function of thromboplastin sensitivity. Quarles LA and Harmon SL, personal communication, 1997

Orthogonal regression
Whatever Happened to the PT and PTT?

The Fritsma Factor
Chromogenic Factor X Assay

Russell viper venom + Ca²⁺

Releases yellow pNA, measure at 405 nm

Chromogenic Factor X Assay


The data suggest the CFX can be a useful tool for monitoring oral anticoagulation in patients in which INR confounders are present.

Warfarin Limitations

• These supply vitamin K and reduce Coumadin efficacy:
  – Green vegetables, avocados, liver, nutrition drinks like Ensure, dietary supplements like ginkgo biloba, parenteral nutrition formulations
  – Over 80 drugs unpredictably influence CYP2C9 cytochrome oxidase pathway
  – Warfarin allergy with anaphylaxis
  – PT interference: lupus anticoagulant, coagulopathies
  – Substitute chromogenic X assay

Dosage Anomalies

• Polymorphisms
  – CYP2C9*2 and CYP2C9*3
  – VKORC1
  – Increased warfarin sensitivity
  – Screen and start with 2 mg/d

• Warfarin receptor insufficiency
  – Hereditary warfarin resistance
  – Require dosages of 25 mg/d or more
  – CYP4F2 variant raises dosage 1 mg/d (Feb 08)

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Coumadin Therapeutic Window


Warfarin Overdose

• Most common cause of ER hemorrhage
• Oral, IM, or IV vitamin K provides 6-hour reversal
• Frozen plasma, prothrombin complex concentrate, NovoSeven® (VIIa) provide immediate reversal

Cumulative Adverse Outcomes in VTE Patients on Anticoagulation

<table>
<thead>
<tr>
<th>Outcome</th>
<th>30-d</th>
<th>1-y</th>
<th>3-y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major bleed</td>
<td>9.4%</td>
<td>11.6%</td>
<td>15.8%</td>
</tr>
<tr>
<td>Recurrent VTE</td>
<td>3.5%</td>
<td>10.7%</td>
<td>15.0%</td>
</tr>
<tr>
<td>Mortality</td>
<td>13.0%</td>
<td>26.0%</td>
<td>35.3%</td>
</tr>
</tbody>
</table>


Summary of Warfarin Disadvantages

• Need for monitoring
• Narrow therapeutic window
  – Risk of hemorrhage
• Inter-individual dosing differences
  – Age and diet
  – Polymorphisms for sensitivity
  – Warfarin resistance
• Neutralization by dietary vitamin K
• Interactions with medications that metabolize through cytochrome P-450

Warfarin Limitations

<table>
<thead>
<tr>
<th>Limitation</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five days’ onset of action</td>
<td>Must “bridge” with heparin</td>
</tr>
<tr>
<td>Genetic metabolism variation: CYP2C9*2, *3; VKORC1</td>
<td>Must reduce dose for safety</td>
</tr>
<tr>
<td>Food and drug interactions</td>
<td>Must monitor daily; INR unreliable</td>
</tr>
<tr>
<td>Narrow therapeutic range</td>
<td>Monthly INR monitoring</td>
</tr>
<tr>
<td>Pediatric sensitivity</td>
<td>Geriatric sensitivity</td>
</tr>
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51 YO Male

Acute Myocardial Infection

• Crushing substernal pain upon retiring
• 325 mg ASA PO at home
  – Reduce platelet activation and white clot formation
• Transported to cardiac catheterization lab within 90 minutes for percutaneous intervention (PCI, angioplasty and stent)
  – If >3 hours in transport, start thrombolytic therapy
  • Tissue plasminogen activator (TPA, Alteplase)

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Platelet Membrane
Glycoprotein IIb/IIa Inhibitors (GPIs)
• GP IIb/IIa is a membrane arginine-glycine-aspartate (RGD) sequence receptor
  – Binds fibrinogen and VWF; supports aggregation
• Eptifibatide (Integrilin®) RGD mimetic
  – Use with aspirin or clopidogrel (Plavix®) and heparin
  – IV loading dose of 180 µg/kg over 1 to 2 minutes
  – Continuous infusion of 2 µg/kg/m up to 72 h

PLT GP IIb/IIa αIIbβ3

GP IIb

GP IIIa

Eptifibatide

Platelet Membrane
Glycoprotein IIb/IIa Inhibitors (GPIs)
• Abciximab (ReoPro®) anti-IIb/IIa antibody
  – 0.25 mg/kg/1 minute, then 0.125 µg/kg/m to 10 µg/m max
  – Plasma half life 30 minutes
• Tirofiban (Aggrastat®) peptide inhibitor
  – Analogue of Echinus carinatus venom component
  – Use with aspirin or clopidogrel (Plavix®) and heparin
  – IV 0.4 µg/kg/m 30 minutes, then 0.1 µg/kg/m up to 48 h

Heparin: Crude Extract of Porcine Mucosa
Unbranched sulfated mucopolysaccharide glycosaminoglycan

GPI Dosing and Thrombocytopenia
• Weight-adjusted GPI dose without monitoring is ineffective
  – Poor platelet suppression risks thrombosis
  – Monitor with platelet aggregometry using thrombin receptor activation peptide (TRAP)
  – POC: VerifyNow IIb/IIa assay
  – POC: Multiplate analyzer
• Risk of profound thrombocytopenia
  – Daily platelet counts

Coronary Bypass Graft
Unfractionated Heparin (UFH)
• UFH bolus: 5000–10,000 IUs
  – Two hours after termination of thrombolytic therapy
  – Simultaneous with GPIs
• Maintenance dosage: 1600 IUs/hour
• Terminate at discharge, max 5 days

PTT prolonged by heparin, LA and XII, XI, IX, X, V, II, Fg deficiencies

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Monitoring UFH Therapy
Standard Schedule

- Perform “baseline” PTT to rule out factor deficiency, inhibitors, lupus anticoagulant
- Initiate therapy: bolus + continuous infusion
- At least 4–6h after initiation, not >24h, perform second PTT
- Adjust dose to PTT therapeutic range
  - Never use 1.5–2.5 x mean of normal range
  - Use laboratory-published range
- Laboratory generates range using Brill-Edwards ex vivo curve

Limitations of PTT in UFH Monitoring

- Lupus anticoagulant, present in 1-2% of unselected individuals, prolongs PTT
- Coagulopathy prolongs PTT
- Coagulation factor inhibitor prolongs PTT
- Elevated FVIII renders PTT insensitive to heparin
- Reagent variations require recalibration to the anti-Xa heparin assay, new target ranges with each lot
- Brill-Edwards curve
- Antithrombin deficiency or consumption renders PTT non-responsive, “heparin resistance”

Chromogenic Anti-Xa Heparin Assay

- Separate curves for UFH and LMWH?
- Hybrid curve: one curve fits all
- Different LMWH formulations
  - Aventis 5/1/09 loses Lovenox patent
- Separate curve for fondaparinux?
  - Synthetic pentasaccharide
  - Marilyn Johnston, McMaster: uses same curve as LMWH

Synthetic Pentasaccharide, Fondaparinux

- Glucosamine
- Glucuronic acid
- Iduronic acid

Pentasaccharide and Antithrombin
- Sulfate residues critical to high-affinity AT binding
- Conformational change in AT raises Xa affinity 300X

Monitoring Fondaparinux
- Chromogenic anti-Xa calibrated with pentasaccharide in mg/L
  - No international standard
  - Standards available from Beckman-Coulter and Aniara for their kits
- SC (never IM) injection: 2.5 mg
  - Peak plasma level at 3 h: 0.4–0.5 mg/L
  - Minimum steady state 0.14–0.19
- No current therapeutic range

Pentasaccharide Advantages
- Efficacy
  - 50% reduction in venographic DVT
  - Frequency of repeat DVT 11 days after surgery 6.8%
    - Compared to 13.7% for LMWH (p=10^{-17})
  - Fatal thromboembolic events 1% at day 49, same as LMWH
- Half-life 17 h; single 2.5 mg SC/24 h

Pentasaccharide Contraindications
- Renal disease: kidney only excretion route
  - Creatinine clearance < 30 mL/min
- Weight less than 50 kg
- Over 75 years old; not included in studies
- Bleeding Hx
  - Congenital or acquired coagulopathies
  - Ulcerative gastrointestinal disease
  - Hemorrhagic stroke


Heit JA. The potential role of fondaparinux as venous thromboembolism prophylaxis after total hip or knee replacement of hip fracture surgery. Arch Intern Med 2002; 162: 1806–1808
Whatever Happened to the PT and PTT?

**Rivaroxaban (Xarelto®)**

- An oxazolininone derivative direct anti-Xa
- Safety and efficacy exceed Lovenox in three out of four phase III trials


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**Prophylaxis: Four Phase III Studies**

- Rivaroxaban at 10 mg po/daily is effective as VTE prophylaxis
- Mean risk of major and total VTE recurrence < 3%

**Treatment: EINSTEIN-DVT 2010**

Rivaroxaban almost superior to usual care in treatment of DVT


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**Rivaroxaban inhibits free, fibrin-bound Xa, and IXa-bound Xa, requires no AT**

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**Crosslinked Fibrin**

Fibrinogen

Extrinsic

Intrinsic

Fibrinogen

Fibrin Polymer

Crosslinked Fibrin

---

**Fibrin**

Active Protease Site

TF

Fibrin

Fibrinogen
The Fritsma Factor

Dose Response to Rivaroxaban by FXa, Prothrombinase (Xa/Va)

Rivaroxaban

- Oral dose: 10 mg/day; steady state at 4 hours
- Neutralizes free, clot-bound, and Xa-bound Xa
  - Interacts with other serine proteases
- Excretion: 66% renal, 28% fecal
- Monitoring: none required?
  - Doubles PT interval at 230 nM
  - Doubles PTT interval at 690 nM
  - Neutralizes Xa
- No outcomes-based laboratory therapeutic range established
- Cleared for prophylaxis; Canada & Europe 5/2009


Rivaroxaban Interactions

- No food interactions
- P-glycoprotein inhibitors
  - PGPs are enteric pathways that protect from toxins
  - Inhibitors include azole antimycotics (ketoconazole)
- P450 3A4 inhibitors
  - HIV protease inhibitors (ritonavir)
- NSAIDS, aspirin, and clopidogrel
- OTC supplements such as St. John’s Wort, platelet inhibitors


Apixiban in VTE Prophylaxis

- 12 days, 2.5 mg twice a day
- Comparator: enoxaparin
- Primary safety: major bleeding
- Primary efficacy: composite VTE

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Enox</th>
<th>Safety</th>
<th>Efficacy</th>
</tr>
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<tbody>
<tr>
<td>ADVANCE 1</td>
<td>3195</td>
<td>30 mg</td>
<td>0.7 V 1.4%</td>
<td>9% V 8.9%</td>
</tr>
<tr>
<td>ADVANCE 2</td>
<td>1973</td>
<td>40 mg</td>
<td>0.6 V 0.93%</td>
<td>15% V 24%</td>
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</tbody>
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Apixiban V Aspirin: AVERROES

- Safety: major, minor, intracranial, and fatal bleeding: 13% increase (NS)
**Direct Thrombin Inhibitors (DTIs)**

- **Indication:** heparin-induced thrombocytopenia (HIT)
- **Do not generate or bind anti-heparin-PF4**
  - Platelet counts recover within three days
- **Rapidly reduce thrombin production in HIT**
  - Warfarin too slow
  - LMWH may cross-react
  - Fondaparinux OK


**Argatroban (Novastan®)**

- **Raises nitric oxide, causing vasodilatation**
- **Metabolized and excreted by liver CYP450**

**Argatroban Administration and Monitoring**

- **IV:** 2 mcg/kg/m: immed. steady state
  - 5–7 d
  - Maintain PTT 1.5–3 x MRI
  - Linear to 40 mcg/kg/m
  - Ecarin clotting time
  - Prolongs PT
  - Doubles INR when bridging to Warfarin
- **During PCI**
  - Bolus 350 mcg/kg
  - Continuous infusion 15–40 mcg/kg/m
  - Maintain ACT 300–450 seconds

**Argatroban Comments**

- **Safe to use in renal disease**
- **Liver disease**
  - Reduce to 0.5 µg/kg/h and monitor with PTT
  - Major bleeds 5.3%, minor 14.4%
  - No antidote, but half-life is 40 minutes
  - Inhibits free and fibrin-bound thrombin

**Hirudin: Lepirudin**

- **Inhibits free, not bound thrombin**
- **Metabolized and excreted by kidney**
  - Monitor in kidney disease

**Hirudo medicinalis**

Structure of Lepirudin

- 7000 D, 65 aa polypeptide
Lepirudin Administration
- IV bolus: 0.4 mg/kg/h
- Infusion 0.1–0.15 mg/kg/h 11–14 d
- Steady state within 2.5 hours
- Maintain PTT at 1.5–3 × MRI
- Clearance half-life 20 minutes
- Coronary bypass: 0.25 mg/kg/h
  - ACT > 350 s
  - ECT > 250 s

Bivalirudin
- Neutralizes free and bound thrombin
- FDA-cleared spring, 2008
  - Reduced major hemorrhage by 41% to 61%
  - Proven antithrombotic effect
  - Use with aspirin only
- Bolus 0.75 mg/kg plus 1.75 mg/kg/h
- Renal excretion, 25 m half-life

Chromogenic X When Transitioning from a Direct Thrombin Inhibitor to Warfarin

Bivalirudin in Renal Disease
- If creatinine clearance is <30 mL/minute, reduce infusion to 1 mg/kg/h
  - No reduction in bolus
- If a patient is on hemodialysis, reduce infusion to 0.25 mg/kg/h
- Monitor with PTT or ACT
  - Therapeutic range not defined

Dabigatran (Pradaxa®)

- Oral DTI cleared for prophylaxis in Canada and Europe
  - Application to US FDA 2008
- Indication: post-surgical VTE prevention
- Dose 110 mg/d with wide safety range
  - Immediate steady state
  - No laboratory monitoring

Dabigatran Efficacy and Safety In Three Phase III Trials

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<tr>
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Dabigatran Vs Warfarin in Atrial Fibrillation

Laboratory Assessment

<table>
<thead>
<tr>
<th>Anti-coagulant</th>
<th>Dose</th>
<th>ECT</th>
<th>Anti-Xa</th>
<th>TCT</th>
<th>PT</th>
<th>PTT</th>
<th>Chromo II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran</td>
<td>200 mg TID</td>
<td>5.2x</td>
<td>NE</td>
<td>27x</td>
<td>NR</td>
<td>2.3x</td>
<td>NR</td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>30 mg BID</td>
<td>NE</td>
<td>68%</td>
<td>NE</td>
<td>2.6x</td>
<td>1.8x</td>
<td>NR</td>
</tr>
<tr>
<td>Apixaban</td>
<td>25 mg BID</td>
<td>NE</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>1.2x</td>
<td>NR</td>
</tr>
</tbody>
</table>

ECT, ecarin clotting time; Anti-Xa, chromogenic anti-Xa heparin; PT, prothrombin time; PTT, partial thromboplastin time; TID, three times a day; BID, twice a day; v, fold increase from baseline at peak concentration; NE, no effect; NR, not reported