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This speaker has nothing to disclose.
Optimizing Warfarin Dose: Tools and Strategies for Today and Tomorrow

Pathology Grand Rounds
December 19, 2008

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Director, University Thrombosis Service
Co-Director, Hospitalist Program
General Internal Medicine
University of Utah
Objectives

- Become familiar with clinical challenges of warfarin dosing
- Learn about laboratory and clinical tools available to guide selection of warfarin dose
- Understand strengths and limitations of algorithms designed to optimize warfarin dose through consideration of both clinical and laboratory data.
The Storied History of Warfarin
Warfarin / Anticoagulant (AC) Facts

- Over 31 million warfarin prescriptions in U.S. in 2004
  - Atrial fibrillation
  - Venous thromboembolism
  - Mechanical heart valve
  - Other

- With aging population use is increasing:
  - 45% ↑ in warfarin RX 1998-2004

Budnitz et al. JAMA 2006; 296(15): 1858-1866
Warfarin: Mechanism of Action

CYP3A4, 1A2, 1A1

Inactive Warfarin

CYP2C9

Inactive Warfarin

NAD

Oxidized VK

Reduced VK

VKORC1

GGCX

Functional factors

F2

F10

F7

F9

C

S

Non-functional factors

F2

F7

F10

F9

C

S

1x

3-5x
Warfarin: Narrow Therapeutic Range

- Effect assessed by international normalized ratio (INR)
- Dosing titrated to INR target range (usual INR range 2-3)

Hylek et al. NEJM 2003; 349: 1019-1026
## Warfarin: Time in Therapeutic Range (TTR)

(Indirect comparative data)

<table>
<thead>
<tr>
<th></th>
<th>TTR (%)</th>
<th>Above (%)</th>
<th>Below (%)</th>
</tr>
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<tbody>
<tr>
<td>Usual Care</td>
<td>33-64</td>
<td>8-30</td>
<td>20-50</td>
</tr>
<tr>
<td>AC service</td>
<td>59-92</td>
<td>6-16</td>
<td>7-31</td>
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<tr>
<td>Clinical Trial</td>
<td>48-83</td>
<td>1-24</td>
<td>8-40</td>
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</tbody>
</table>

Ansell et al. *CHEST* 2004; 126: 204s-233s

30-60% NOT Therapeutic
## Warfarin Bleeding

<table>
<thead>
<tr>
<th></th>
<th>1st 3 mos.</th>
<th>&gt;3mos</th>
</tr>
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<tbody>
<tr>
<td>Major Bleed</td>
<td>2%</td>
<td>2.7%/yr</td>
</tr>
<tr>
<td>ICH</td>
<td>1.5%</td>
<td>0.7%/yr</td>
</tr>
<tr>
<td>Fatal Bleed</td>
<td>0.4%</td>
<td>0.6%/yr</td>
</tr>
</tbody>
</table>

Over 30,000 major bleeds/yr
Over 3,500 intracranial bleeds/yr

Linkins et al. *Annal Int Med* 2003; 139: 893- 900
Fang et al. *J Am Geri Society* 2006; 54: 1231-1236
Warfarin Initiation

- Individual dose response is highly variable:
  - Maintenance dose may range from 1-40 mg/day

- Standard initiation schemes better than usual care:
  - INR therapeutic by warfarin day#5 = 45-85%
  - INR ≥ 4.0 during initiation = 5-30%

- Limited translation to daily practice
A 32yo, 135kg, male presents with L Leg pain & swelling. PMHX: HTN

Exam: pitting edema of L leg to thigh.

Ultrasound: L Common femoral DVT

Labs: Normal PT and PTT. NI CBC. NI CMP
A Case of Mr. A (continued)

Acute DVT Diagnosed
Started on Fondaparinux 10mg SC daily & Warfarin 5mg daily:

<table>
<thead>
<tr>
<th>Day</th>
<th>Dose</th>
<th>INR</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>7.5</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>7.5</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>7.5</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>7.5</td>
<td>4.8</td>
</tr>
</tbody>
</table>
A Case of Mrs. L

A 77yo, 52kg, female presents with acute severe abdominal pain. PMHX: afib

Exam: Abdomen with peritoneal signs

CTA: SMA thrombosis + ischemic bowel

Labs: NI PT and PTT. NI CBC. Albumin 2.9
Patient went to OR for emergent thrombectomy and partial small bowel resection.

Post-op begun on heparin gtt.

Warfarin 5mg x2 begun → INR >15

Rectus hematoma developed. Given 5mg Vit K. Stabilized.
A Case of Mrs. L (continued)

INR 1.7 → warfarin 2mg x1
   → warfarin 1mg x1
INR 9.0 → warfarin held x3, Vit k 5mg
INR 2.3 → warfarin 1mg q Tues and Sat
INR 2.6 → warfarin 1mg q Tues and Sat
INR 2.4
Can pharmacogenetics take the “guessing” out of warfarin initiation and improve outcomes?
Coumadin® label (Bristol-Myers Squibb Company) was revised August, 2007

- Suggests lower doses may be required for individuals with
  - VKORC1 variants (especially the -1639G>A)
  - CYP2C9*2 and *3

- Questions
  - What are these variants?
  - How should genetic results be used?
  - Will the testing improve patient care?
  - Should this testing be routine? How? When? Where?
Warfarin acts via VKOR

VKORC1
VKOR

Drug response =
sensitive (low dose)
typical (~5mg/d)
resistant (high dose)

Coagulation factors
II, VII, IX, X, etc.

Vitamin K Oxidized
Vitamin K Reduced

r,s-warfarin
Chromosome 16

Highly polymorphic, >30 SNPs known

Associated with
- Multiple coagulation factor deficiency Type 2
- Warfarin sensitivity and resistance

Haplotypes for warfarin sensitivity were defined: *2, Group A

Promoter SNP appears causitive due to effects of expression
Effect of VKORC1 -1639 genotype on warfarin dose

VKORC1 promoter variant affects expression and accounts for ~30% of warfarin dose requirement

Yuan et al. Human Mol Genetics, 14(13):1745-51, 2005
s-Warfarin is inactivated by CYP2C9

- CYP2C9 status affects:
  - drug clearance, $t_{1/2}$
  - time to steady state

Drug-drug interactions
CYP2C9

- Chromosome 10
- Enzyme catalyzes many drug metabolizing reactions for substrates such as: s-warfarin, phenytoin, NSAIDs, and tolbutamide
- Highly polymorphic, >30 alleles described, many other SNPs with unknown significance
- Most common alleles in Caucasians are the *2 and *3 alleles
- Other alleles may be important to other ethnic groups:
  *4 (Asians), *5 (African), *6 (non-functional but rare)
CYP2C9*2 and warfarin dose

S. Sanderson et al.  Genet Med. 2005 Feb;97-104
CYP2C9*3 and warfarin dose

S. Sanderson et al. Genet Med. 2005 Feb;97-104
Effective warfarin doses by CYP2C9 genotype

CYP2C9*2 and *3 account for ~20% of warfarin dose requirement

Higashi MK et al, JAMA 2002; 287:1690
More than just the dose...

Clearance (mL/min/kg)
from drug label, mean (s)
~0.065 (0.025) with 0 variants
~0.041 (0.021) with 1
~0.020 (0.011) with 2

Time to steady-state:
~3-5 days with 0 variants
~6-9 days with 1
~12-15 days with 2

Impacts dose equilibration, time to interpret INRs and making dose adjustments = risk to patient
Summary of common *CYP2C9* and *VKORC1* variants associated with warfarin sensitivity

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant (nucleotide)</th>
<th>* Allele</th>
<th>Protein change</th>
<th>Effect</th>
<th>Allele frequency (Caucasian)</th>
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<tbody>
<tr>
<td>VKORC1</td>
<td>c.-1639 G&gt;A</td>
<td></td>
<td>none</td>
<td>Decreased</td>
<td>0.42</td>
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<tr>
<td>CYP2C9</td>
<td>c.430 C&gt;T</td>
<td>*2</td>
<td>R144C</td>
<td>Modest reduction in activity</td>
<td>0.08-0.13</td>
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<tr>
<td></td>
<td></td>
<td>*3</td>
<td>I345L</td>
<td>Dramatic reduction in enzyme</td>
<td>0.06-0.10</td>
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</table>

**Dose selection**

**Dose management, predict maintenance dose**
Sqrt(Dose) =

0.628 – 0.0135(Age) – 0.240(CYP2C9*2) –
0.370 (CYP2C9*3) – 0.241(VKORC1) +
0.0162(Height)

• Age: input years
• CYP2C9: input 0, 1, or 2 based on # of variant alleles
• VKORC1 -1639G>A : input 1 for GG, 2 for GA, and 3 for AA
• Height: input cms

Sconce et al. Blood 106(7):2329-33, 2005
## Genotype-based warfarin doses

55 yr old, 6’ tall man

<table>
<thead>
<tr>
<th>CYP2C9 variants</th>
<th>VKORC1 GG calculated initial dose</th>
<th>% reduced from 6.8</th>
<th>VKORC1 AG calculated initial dose</th>
<th>% reduced from 6.8</th>
<th>VKORC1 AA calculated initial dose</th>
<th>% reduced from 6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7</td>
<td></td>
<td>6</td>
<td>18%</td>
<td>5</td>
<td>34%</td>
</tr>
<tr>
<td>CYP2C9*2</td>
<td>6</td>
<td>18%</td>
<td>5</td>
<td>34%</td>
<td>4</td>
<td>47%</td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>5</td>
<td>26%</td>
<td>4</td>
<td>41%</td>
<td>3</td>
<td>54%</td>
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<tr>
<td>CYP2C9*2/*2</td>
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<td>34%</td>
<td>4</td>
<td>47%</td>
<td>3</td>
<td>60%</td>
</tr>
<tr>
<td>CYP2C9*2/3</td>
<td>4</td>
<td>41%</td>
<td>3</td>
<td>56%</td>
<td>2</td>
<td>66%</td>
</tr>
<tr>
<td>CYP2C9*3/*3</td>
<td>4</td>
<td>49%</td>
<td>3</td>
<td>62%</td>
<td>2</td>
<td>72%</td>
</tr>
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</table>
Welcome to WarfarinDosing.org, a free Web site to help doctors and other clinicians begin warfarin therapy by estimating the therapeutic dose in patients new to warfarin. This site is supported by the Barnes-Jewish Hospital at Washington University Medical Center, the NIH, and donations. Estimates are based on clinical factors and (when available) genotypes of two genes: cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKORC1).

Recommendations on this Web site are based on data from over 1000 patients. Once information is entered onto the next page, the initial estimate of therapeutic dose explains 53% of the variability in a warfarin dose. If you return to the Web site and enter an INR value after 3 and/or 4 warfarin doses, the dose refinement is even more accurate.
User gets dose, but has to round

Estimated therapeutic dose: **2.8 mg/day.**

Today’s prescribed dose: 3.0 mg.

Automatic check for agreement w/ estimate

Patient number*: WU-001-JD

Caution

You should not decrease the frequency of INR monitoring based on the above estimate. We check the INR after 3 warfarin doses and modify the dose when clinically indicated.

Additional Information

Email the results to*: WelbyM@GeneralHospital.edu

Emails doctor or pharmacist

Address email to: Dr. First Name: Marcus Last Name: Welby

Email copy to: Florence Nightingale

Emails study nurse with all info.

Text to accompany email: John Doe is in bed 7340

Private text is not stored in database

When would you like an email to remind you to check the INR: In 70 hours.

* This email address is required to save and to access this record. Information entered into this site will not be disclosed to any 3rd party nor used for commercial purposes.
Comparison of published algorithms

Grice et al. JCOM 2008;14(4):183-90
Limitations of algorithms

- Don’t predict warfarin resistance
- Origin
  Retrospective (i.e., maintenance doses) analysis of specific populations (i.e., Caucasians) using various statistical tools
- Not standardized
- Don’t consider dosing vs. pill size available for Rx
- Don’t account for, or address management of kinetic differences
  - Frequency of INRs
  - Frequency of dosing/adjustments
Does application of warfarin genotyping to dose selection improve patient care?

- Let’s see!

- Investigators from ARUP (Dept of Pathology), Dept of Internal Medicine, and the University Orthopaedic Center

- Vendor support from Third Wave, Tm Biosciences, Idaho Technology, and Autogenomics

- Protocol approved and active August, 2006-2008
Study flow

Pre-Op Clinic Visit: Consecutive THA/TKA Patients
Consent & Labs drawn for CYP2C9 & VKORC1

Randomization
Day of Surgery

Study group
Warfarin dosed by “Sconce” corrected

Control Group
Validated control dosing
Nomogram
(3-5 mg warfarin/day)

Post-Hospital Management
Anticoagulation service management
2 to 4 week warfarin duration (pre-specified)

90 day clinical follow up
Adverse Events recorded with predefined criteria and blinded adjudication

263 patients enrolled
229 patients underwent surgery & VKA used
Complete follow-up
Pharmacogenetic Warfarin Initiation Trial
UHOSP Orthopedic Joint Replacement Patients

229 patients undergoing joint replacement surgery: Pharmacogenetics dosing versus Standard initiation protocol

<table>
<thead>
<tr>
<th></th>
<th>Genetic</th>
<th>Standard</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean</td>
<td>59</td>
<td>60</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg, mean</td>
<td>87.3</td>
<td>91.1</td>
<td>NS</td>
</tr>
<tr>
<td>Any allele variant</td>
<td>79%</td>
<td>74%</td>
<td>NS</td>
</tr>
<tr>
<td>Doses to INR goal</td>
<td>3.7</td>
<td>3.4</td>
<td>NS</td>
</tr>
<tr>
<td>INR &gt;2.9 d14</td>
<td>45.6%</td>
<td>43%</td>
<td>NS</td>
</tr>
<tr>
<td>Mean dose adjustments</td>
<td>8.4</td>
<td>7.0</td>
<td>NS</td>
</tr>
<tr>
<td>VTE (n)</td>
<td>0</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Major Bleed (n)</td>
<td>2</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Minor Bleed (n)</td>
<td>1</td>
<td>6</td>
<td>NS</td>
</tr>
</tbody>
</table>

McMillin et al. Data on File
Variant – Dose Relationship

Final VKA dose (mg/d) vs. Number of variants
Cumulative percent of patients achieving a therapeutic INR (1.8-2.9)

Time to therapeutic INR

Dose of warfarin

No variants
variant 1
variants 2
variants 3
Accuracy of Algorithm Predicted Dose
Pharmacogenetic Warfarin Initiation Trial
(Couma-Gen Investigators)

- Pharmacogenetic (n=101) vs. Nomogram/Anticoag service (n=99)
- Excluded: Advanced age, Cr >2.5 mg/dL, hepatic insufficiency

<table>
<thead>
<tr>
<th></th>
<th>Genetic</th>
<th>Standard</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean</td>
<td>63.2</td>
<td>56.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Weight, kg, mean</td>
<td>92.1</td>
<td>94.7</td>
<td>-</td>
</tr>
<tr>
<td>Any allele variant</td>
<td>61.0%</td>
<td>79.6%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>INR out of range</td>
<td>30.7%</td>
<td>33.1%</td>
<td>0.47</td>
</tr>
<tr>
<td>Therapeutic INR d8</td>
<td>68.8%</td>
<td>63%</td>
<td>0.41</td>
</tr>
<tr>
<td>Adverse event*</td>
<td>34.7%</td>
<td>42.4%</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Anderson et al, Circulation 116, 2007

* Clinical event + INR ≥ 4.0
Pharmacogenetic Warfarin Initiation Trial
(Couma-Gen Investigators)

- Gene-based dosing
  - Predicted actual dose requirements more accurately than standard dosing protocols
  - Resulted in fewer and smaller dose changes (3.0 vs. 3.6)
  - Resulted in fewer INR test requests (7.2 vs. 8.1)

- Those patients with no variants or multiple variants showed promising reductions in the number of out-of-range INRs, but the effects were not statistically significant

Anderson et al, Circulation 116, 2007
Warfarin gene-based dosing
(The Caraco Study)

- N=191 warfarin-naïve inpatients treated for AFIB, DVT, or PE at the Hadassah University Hospital (Israel)

- Target INR was 2-3

- Dosing: validated computer predicted dosing model first 8 days versus pharmacogenetic modeled dosing

- Primary endpoints: time to first INR>2, and time for stable anticoagulation

Warfarin gene-based dosing
(The Caraco Study)

Findings:
- Time to first therapeutic INR was 2.7 days earlier in the study group
- Stable anticoagulation was attained 18.1 days earlier in the study group
- The study group was therapeutic 80% of the time, versus 63% of the time in the control group
- Incidence of minor bleeding was lower (3.2%) in the study group than in the control group (12.5%)

Conclusions:
- Dosing based on CYP2C9 improves patient care

Perspective: Pharmacogenetic Warfarin Dosing

- Genetics explains 30-50% of warfarin dose variability
- Clinical features explain 20-30%
- >20% of dose variability remains unexplained

- The gene-dose relationship is clear & convincing.
- Improving outcomes remains to be proven
- Well-designed, large prospective RCTs are needed
- Validated tools needed to help apply to busy clinical practice
Proposed clinical algorithm for today

Patient has taken warfarin previously

- YES: No testing
- NO:
  - Test TAT < 4 days?
    - YES: Offer testing
    - NO:

Modified from Thacker et al., J Thromb Haemost 6:1445-9, 2008
Test TAT < 4 days?

No

Patient high-risk for bleed INR high?

No

No testing

YES

Offer testing

Modified from Thacker et al., J Thromb Haemost 6:1445-9, 2008
### Commercially available reagents

<table>
<thead>
<tr>
<th>Gene variants</th>
<th>Nucleotide position</th>
<th>Nucleotide exchange</th>
<th>Third Wave</th>
<th>Luminex</th>
<th>Idaho Technology</th>
<th>Autogenomics</th>
<th>Nanogen</th>
<th>Osmetech</th>
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<td><strong>CYP2C9</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>*2</td>
<td>430</td>
<td>C&gt;T</td>
<td>■</td>
<td>■</td>
<td>■</td>
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<tr>
<td>*6</td>
<td>818</td>
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<td>■</td>
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<td><strong>VKORC1</strong></td>
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<td>3673</td>
<td>(-) 1639G&gt;A</td>
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<td></td>
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<tr>
<td>5808</td>
<td>173+324</td>
<td>T&gt;G</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td></td>
<td>■</td>
</tr>
<tr>
<td>6009</td>
<td>173+525</td>
<td>C&gt;T</td>
<td>■</td>
<td></td>
<td></td>
<td>■</td>
<td></td>
<td>■</td>
</tr>
<tr>
<td>6484</td>
<td>173+1000</td>
<td>C&gt;T</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td></td>
<td>■</td>
</tr>
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Idaho Technology, Inc. ASRs

- DNA extracted from whole blood
- PCR set up
  - Primers
  - Probes (SimpleProbe® with fluorophore hybridization specific to point mutations)
  - Mastermix
- PCR and Melting
  - 35 minutes, single protocol for all 3 assays
- Data analysis
**CYP2C9 *2**
- WT Tm = 54°
- Mut Tm = 61°

**CYP2C9 *3**
- WT Tm = 53°
- Mut Tm = 63°

**VKORC1-1639**
- WT Tm = 57°
- Mut Tm = 66°
Laboratory issues related to implementation of warfarin pharmacogenetic testing

- TAT
- $$$
- Interpretation?
- Application tools?
  - Dose initiation
  - Dosing regimen
  - Dose revision
[s-wafarin] prediction $\approx$ CYP2C9

~10 d to Css, too high?

[s-wafarin] target $\approx$ VKORC1
Adding INR information and more clinical information after initial dosing should improve accuracy of dose recommendations (account for ~80% of warfarin dose)

INR₃ dose revision algorithm:

Warfarin dose (mg/day) =

\[
\text{EXP}[1.453 - 1.657 \times \ln(\text{INR}_3) + 0.093 \times \ln(\text{EBL}) \times \\
\ln(\text{INR}_3) - 1.62 \times \text{statin} + 0.070 \times \text{first warfarin dose} + \\
0.061 \times \text{second warfarin dose}]
\]

INR₄ in press, INR₇ in development

Conclusions

- Gene-dose relationships for warfarin are well established
- Combining genotypes with clinical information accounts for 20-80% of the warfarin dose
- Genotyping may improve safety of warfarin, if used to select and manage the dose
- Algorithms may not be useful for patients that do not possess variants in CYP2C9 and VKORC1
- Validated, easy to use tools are needed
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  - Andy Wilson

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