**Impact of Age, CYP2C9 Genotype and Concomitant Medication on the Rate of Rise for Prothrombin Time During the First 30 Days of Warfarin Therapy**

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**Objectives:** To characterize the impact of several important clinical variables on the rate of anticoagulation during warfarin initiation (i.e., the first 30 days).

**Design:** Retrospective study.

**Setting:** An anticoagulation service of a large horizontally integrated, multispecialty group practice in central and northern Wisconsin.

**Participants:** Patients with sufficient laboratory data obtained during the initiation phase of warfarin treatment.

**Methods:** Patients were consented and genotyped for cytochrome P450 (CYP) 2C9 polymorphisms. Anticoagulation laboratory data were then electronically abstracted and fitted to a logistic growth model. Rate of anticoagulation was compared between groups.

**Results:** During warfarin initiation, the mean slope for rise in International Normalized Ratio (INR) of prothrombin time was significantly associated with age (p=0.03, n=166). Because a relationship between diabetes and warfarin dosing has been suggested previously, we assessed the impact of this comorbidity in our model as well. Diabetes showed relatively little impact, but concomitant treatment with an anti-diabetic sulfonylurea medication was associated with an increase in slope (3-fold, p<0.05). Since this drug interaction may occur at the level of CYP2C9, we also assessed the impact of CYP2C9 genotype in our model. The impact of CYP2C9 genotype was marginally significant (p=0.119, non-pooled dataset; p=0.053, data pooled for CYP2C9 *2/*2, *2/*3 and *3/*3).

**Conclusions:** Age and concomitant sulfonylurea therapy alter the rate of anticoagulation during the first 30 days of warfarin therapy.

**Keywords:** Pharmacogenetics, Pharmacokinetics, Drug interactions, Logistic modeling, Prothrombin time
factors appear to influence the decision-making process when a patient and their physician assess the risks and benefits of anticoagulation.7,8 These factors include the patient’s unique risk of thromboembolism (based on comorbidities), appropriate concern regarding the narrow therapeutic index of warfarin and the potential severity of warfarin-related adverse bleeding events.

We recently characterized the relative impact of clinical covariates (including age, gender, body composition, comorbidity and concomitant medication) on stable maintenance warfarin dose, in the context of cytochrome P450 (CYP) 2C9 genotype, in a large retrospective patient cohort followed regularly in our outpatient anticoagulation clinic.9 Interestingly, these data revealed a complex multivariate interaction between diabetes and genotype, with respect to overall final maintenance dose of warfarin.9 Although diabetes is known to be associated with hypercoagulable states, the pathogenetic mechanism appears to involve platelet dysfunction rather than a disruption of clotting factor homeostasis.4,10-18 Other factors (e.g., comorbidity or concomitant medication) are therefore likely to contribute to this interaction.

The majority of warfarin-related adverse bleeding events occur during the first 30 days of warfarin therapy.8,19 We therefore opted to extend our analysis of the original retrospective cohort to gain additional mechanistic insight into the clinical factors associated with rate of warfarin anticoagulation, specifically during the period of drug initiation. Using a strategy that employs electronic data abstraction and mathematical modeling, we now report the impact of age, CYP2C9 genotype and concomitant medication on the rate of warfarin anticoagulation.

**Methods**

**Study design**

We utilized electronic data abstraction to retrospectively query the clinical medical records of the anticoagulation population characterized previously in our cohort study of warfarin maintenance dose.9 Recruitment strategies have been summarized below, along with a specific description of inclusion and exclusion criteria. The goal of the present study was to identify a subset of patients with sufficient laboratory data during the initiation phase of warfarin treatment for use in modeling and analysis of factors influencing the initial rate of anticoagulation. Consent was obtained according to institutional guidelines. The genotyping methods were published previously.9

**Patient recruitment**

Patients were recruited from the anticoagulation service of a large horizontally integrated, multispecialty group practice in central and northern Wisconsin. This service is staffed by registered nurses using physician-approved protocols to provide a standardized approach to patient assessment, warfarin dosage, laboratory testing and medical record documentation while in communication with the anticoagulation medical director or the patient’s referring provider. Patients were excluded from this study if they were known to have underlying conditions that may influence drug dosage, such as cancer, renal or hepatic insufficiency, or heart failure. Conditions were identified based on disease-specific clinical and/or laboratory indicators, such as increased serum creatinine in the case of renal insufficiency and increased circulating transaminase levels in the case of hepatic insufficiency, as well as the diagnostic codes assigned.

Eligible patients were recruited and enrolled after providing informed consent according to an institutional protocol approved by the Institutional Review Board of Marshfield Clinic Research Foundation. Six hundred eligible patients were approached; 453 agreed to participate. Medical charts, anticoagulation data and prescribed medications were abstracted from the clinical records of all 453 consenting patients, and blood samples were collected for genotype analysis. Investigators performing the DNA analysis were blinded to any clinical data. The genotyping data was published previously.9

**Electronic abstraction of clinical laboratory data**

A computer program was constructed to abstract the International Normalized Ratios (INRs) for prothrombin time during the first 30 days of warfarin therapy. Since some patients from the original cohort of 453 may have initiated warfarin at an outside institution or their initial laboratory data were otherwise unavailable, the overall number of patients in the final electronically abstracted dataset was less than the original 453.

Only patients who started warfarin after 1995 were considered. Prior to this date, adequate INR data were available inconsistently in the electronic medical record. This reduced the dataset from 453 to 354. A selection algorithm was then used to flag patients who had a normal pre-treatment INR (value ≤1.5) and four or more INR results available electronically within the initial 30-day exposure period. A total of 236 patients met these selection criteria and were considered for additional detailed medical record review. Of these 236 records, dosing data was available for 195 patients.

Data for these 195 patients were abstracted and entered into a study database. All available INR results were considered through 30 days following initiation of warfarin therapy. For any given patient, the data string was truncated if an INR decline of 33% or more was identified. In total, 1,235 INR test results were modeled with a median of 6 results per patient.

**Kinetic modeling**

The relationship between the initiation of multiple oral dosing of warfarin and the rise in INR in any given patient is not direct and is potentially influenced by a number of genetic and environmental factors.20 Clinically there is a lag between
the first pulses of oral warfarin and the beginning of a rise in INR after which the INR rises more rapidly. A sigmoidal dose-response curve best describes this behavior and, therefore, a logistic function was used to model each subject’s initial response to warfarin. This relationship was found to be consistent with the pattern observed in most subjects (figure 1). The following logistic growth model was applied.\(^{21}\)

\[
\text{INR} = 1 + \frac{(0.1 + 0.1e^b)}{(1.0 + e^{b/c})} \quad \text{Equation 1.}
\]

By definition, the INR is constrained to be 1.1 (our observed median) on the day of initiation (day 0), and the INR increases over time to an asymptote represented by $1.1 + 0.1e^b$. Primary interest was in the initial rate of rise (slope). Analysis of the slope was therefore restricted to a time domain limited by the inflection point (b/c) (figure 2). The slope within this time domain can be expressed in terms of the model parameters as follows:

\[
\text{Slope-to-inflection} = 0.05c (e^b - 1.0) / b \quad \text{Equation 2.}
\]

**Statistical Analysis**

The model was applied separately to data for 195 patients and converged (i.e., the statistical software was able to calculate parameter estimates) for 166 individuals (85%). Various clinical factors were then evaluated for possible association with the slope-to-inflection. The Spearman rank correlation was used to characterize associations among quantitative variates, and the Kruskal-Wallis test was used for comparisons among groups. Analysis of covariance (ANCOVA) was used to further evaluate factors while adjusting for dose after a log transformation to help normalize the skewed distribution of the slope estimates. Warfarin dose was incorporated in the ANCOVA models using two separate terms: (1) initial dose for the first day on drug and (2) average dose over the remaining 30-day study period. Results were deemed significant at the 5% level (i.e., $p<0.05$).

**Results**

**Impact of age**

The demographics of the 166 patients with model results are shown in table 1. The gender distribution was 77 females (46%) and 89 males (54%), and the distribution of clinical comorbidity and indication for warfarin therapy were similar to those for the entire population.\(^9\) The overall median slope was 0.21 INR units per day (median inflection point 5.0 days). Clinical variables were first tested for association with slope in univariate analyses. Age, initial dose and mean dose were all significantly correlated with this slope parameter (table 1).

**Impact of concomitant medication**

Based upon our previously reported association between maintenance warfarin dose and diabetes, we also tested the rate of anticoagulation (warfarin initiation) for association with the presence of diabetes and for association with the concomitant use of a sulfonylurea medication. The results are shown in table 2. Only concomitant sulfonylurea medication was found to have a significant association with slope. The INR was found to rise faster in patients using this class of medications (3-fold increase in the median). This observation was true even relative to the other 16 patients with diabetes who were not using sulfonylurea medication ($p=0.014$).

Most sulfonylurea medications are metabolized by CYP2C9. Due to the substantial impact of sulfonylureas, the impact of other medications known to interact with CYP2C9 was assessed (table 3). For this analysis, medications were categorized as (A) transcriptional inducer, (B) mechanism-based inhibitor or (C) competitive substrate, according to a nationally recognized web-based resource for drug interactions (http://medicine.iupui.edu/flockhart/). Mean INR slopes were then compared between groups. In situations where patients were taking more than one of these medications, patients were assigned to the category with highest potential for transcriptional impact on the CYP2C9
gene. When analyzed according to category, medications interacting with CYP2C9 showed no association with the initial rate of warfarin anticoagulation (p=0.547). In this analysis, the most commonly observed classes of CYP2C9 inhibitors were proton pump inhibitors (n = 7 subjects), fibric acid derivatives (n = 6 subjects) and serotonin selective reuptake inhibitors (n = 3 subjects). The most commonly observed classes of CYP2C9 substrates were cyclooxygenase-II inhibitors (n = 9 subjects), angiotensin-II receptor antagonists (n = 9 subjects) and sulfonylureas (n = 6 subjects). Individual drug effects were not tested.

Impact of genotype
We also attempted to gain insight into the potential impact of congenital variation in CYP2C9 enzyme activity. In our study population, most of the genetic variation in the CYP2C9 gene can be attributed to either CYP2C9*2 or CYP2C9*3 with approximately one-third of these patients expressing at least one copy of these variant alleles. The initial anticoagulation rate in our model is presented for each allele group in table 4. Little association was detected between CYP2C9 genotype and slope. Power was limited, however, with only 59 patients in the non-wild type genotype.

Post hoc adjustment for physician prescribing practices
Lastly, analyses adjusting for dose were used to control for differences in warfarin dosing strategies. Although the initial warfarin dose was positively correlated with rate of rise of INR, mean dose for the remainder of the 30-day period was negatively correlated (table 1). The combination of these two terms may reflect common discrepancies between the initial dose and the optimal dose as determined over time. Therefore, key comparisons were subsequently adjusted for both dosing terms (table 5). To control for genotype, this analysis was conducted only on patients expressing the homozygous wild type genotype (CYP2C9*1/*1; n=107).

| Table 1. Patient demographics and correlation with slope. |
|---------------------------------|----------------|----------------|----------------|-----------------|
|                                | Number | Mean | S.D. | Spearman Correlation Coefficient | p-value |
| Age (years)                    | 166    | 68.4 | 11.7 | 0.18                          | 0.031   |
| Weight (kg)                    | 163    | 86.9 | 21.5 | -0.04                         | 0.657   |
| Height (cm)                    | 156    | 170.0| 10.9 | -0.08                         | 0.293   |
| BSA (m²)                       | 156    | 1.97 | 0.26 | -0.05                         | 0.556   |
| Initial dose (mg)              | 166    | 6.2  | 2.6  | 0.32                          | <0.001  |
| Mean dose (mg) days 2 through 28 | 166 | 4.8  | 1.8  | -0.33                         | <0.001  |

BSA: body surface area; S.D.: standard deviation

| Table 2. Analysis of slope by gender, diabetes and sulfonylurea co-medication. |
|---------------------------------|----------------|----------------|----------------|-----------------|
|                                | Number | Mean | S.D. | Median | p-value |
| Gender                         |        |      |      |        |        |
| Female                         | 77     | 0.29 | 0.25 | 0.23   | 0.157   |
| Male                           | 89     | 0.23 | 0.15 | 0.19   |
| Diabetes                       |        |      |      |        |        |
| With                           | 22     | 0.34 | 0.25 | 0.25   | 0.057   |
| Without                        | 144    | 0.24 | 0.19 | 0.20   |
| Sulfonylurea                   |        |      |      |        |        |
| With                           | 6      | 0.58 | 0.35 | 0.61   | 0.018   |
| Without                        | 160    | 0.24 | 0.19 | 0.20   |

S.D.: standard deviation
Using this approach, the association with age was attenuated after the data were adjusted for dose (table 5). This is consistent with the fact that physicians tend to use lower initial warfarin doses in older patients (p=0.011, data not shown). Conversely, the association with sulfonylureas appeared stronger when the data were adjusted for dose suggesting, in general, that physicians had not considered the potential kinetic impact of this drug-drug interaction when initiating warfarin therapy in diabetic patients.

Discussion

We previously demonstrated the presence of a multivariate association between the maintenance dose of warfarin and the following clinical variables: age, gender, body size, comorbidity, concomitant medication and CYP2C9 genotype. The relationship between warfarin dose and age, in particular, has long been understood in the context of stable maintenance dose. The results generated by the current study further our understanding of this relationship by extending it back to the period of warfarin initiation (i.e., prior to determination of the maintenance dose).

This association between age and initial rate of anticoagulation is particularly important, since data have clearly documented that the risk of serious warfarin-related bleeding complications increases with age. Although our initial dataset revealed that age was highly associated with mean INR slope, this association was attenuated when the data were adjusted for warfarin dose (Post Hoc, table 5). As stated earlier, the most likely interpretation for this observation would be that physicians had appropriately considered age when choosing an initial warfarin dose.

The active enantiomer of warfarin (S-warfarin) is metabolized primarily by CYP2C9. An unequivocal association has been shown between CYP2C9 gene polymorphisms and warfarin-related clinical outcomes. The relationship between CYP2C9 gene polymorphisms and warfarin maintenance dose has since been characterized in the context of a variety of salient clinical covariates. More recently, others have suggested that CYP2C9 genotype may impact warfarin initiation dose as well. Therefore, it is odd that we did not observe a more robust relationship between CYP2C9 genotype and rate of anticoagulation in the current study. One potential explanation includes a lack of statistical power with respect to patients expressing variant alleles (n=166 for the entire cohort, n=107 for homozygous wild type, and n=59 for the subset expressing at least one variant CYP2C9 allele). Larger, prospective studies are needed to clarify whether CYP2C9 genotype alters the initial rate of warfarin anticoagulation.

Like warfarin, most sulfonylureas are also metabolized by CYP2C9. Sulfonylurea use is known to inhibit the metabolism of other drugs in a competitive fashion and sulfonylureas are often used as probe substrates for CYP2C9 activity. The potential for a warfarin-sulfonylurea drug interaction is therefore substantial. Data generated in the

| Table 3. Analysis of INR slope by CYP2C9 co-medication interaction category.† |
|---------------------------------|--------|------|--------|
| Interaction Category            | Number | Mean | S.D.   | Median |
| CYP2C9 inducer                  | 2      | 0.44 | 0.28   | 0.44   |
| CYP2C9 inhibitor                | 21     | 0.23 | 0.15   | 0.16   |
| CYP2C9 substrate                | 25     | 0.34 | 0.28   | 0.23   |
| No CYP2C9 medications           | 118    | 0.24 | 0.19   | 0.21   |

*p=0.547: Group comparison excluding two patients on CYP2C9 inducer medications. INR: International Normalized Ratio; S.D.: standard deviation

| Table 4. Analysis of INR slope by CYP2C9 genotype.† |
|---------------------------------|--------|------|--------|
| Genotype | Number | Mean | S.D.   | Median |
| *1/*1    | 107    | 0.26 | 0.24   | 0.19   |
| *1/*2    | 35     | 0.21 | 0.09   | 0.21   |
| *1/*3    | 17     | 0.25 | 0.12   | 0.25   |
| *2/*2    | 5      | 0.32 | 0.14   | 0.29   |
| *2/*3    | 1      | 0.56 | —      | 0.56   |
| *3/*3    | 1      | 0.68 | —      | 0.68   |

†p=0.119 comparing all genotypes, p=0.053 for comparison pooling *2/*2, *2/*3, and *3/*3, and p=0.441 for comparison excluding *2/*2, *2/*3, and *3/*3 due to small numbers. INR: International Normalized Ratio; S.D.: standard deviation
Table 5. Estimates of relative INR slope adjusting for warfarin dose.*

<table>
<thead>
<tr>
<th>Clinical Factor</th>
<th>Slope Ratio</th>
<th>95% Confidence Limits</th>
<th>p-value</th>
<th>Full Model† p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.003</td>
<td>0.990 – 1.017</td>
<td>0.624</td>
<td>0.779</td>
</tr>
<tr>
<td>Sulfonlurea</td>
<td>3.727</td>
<td>1.914 – 7.257</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.505</td>
<td>0.974 – 2.326</td>
<td>0.065</td>
<td>0.733</td>
</tr>
</tbody>
</table>

*Results from analysis of covariance models with a single clinical factor and warfarin dose (initial and subsequent mean) in patients with the *1/*1 genotype only. Estimates represent the multiplicative effect of the factor and, for age, the effect is relative to a 1-year increase in age.
†Results including age, diabetes, and sulfonlurea simultaneously in the model.

INR = International Normalized Ratio.

The current study reveal that the association between sulfonlurea use and rate of warfarin anticoagulation is statistically significant (p=0.018). Since the effect size for this relationship is quite large (i.e., sulfonlureas were associated with a 3-fold increase in the median INR slope), physicians may want to consider prescribing a reduced starting dose when initiating warfarin therapy in their diabetic patients requiring anticoagulation. Although our results were significant even upon adjustment for other factors, this evidence is limited by the small number using sulfonlurea medication (6 patients), and we must acknowledge the possibility that other unmeasured differences in that group may be involved. Furthermore, since no significant association was observed between slope and CYP2C9 substrates (as a class), it is conceivable that the interaction observed between slope and sulfonlurea may be independent of CYP2C9. Larger studies are needed to clarify the nature of this interaction.

Currently, efforts are being made to standardize the process of model-based warfarin dosing.30,35-37 Rational dosing models that include a combination of genetic and clinical information are likely to lead to improved physician confidence in situations requiring the use of warfarin anticoagulation.

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