

USE OF GENETIC TESTING TO GUIDE THE INITIATION OF WARFARIN THERAPY

A Technology Assessment

INTRODUCTION

The California Technology Assessment Forum is requested to review the scientific evidence for the use of genetic testing to guide the initial dosing of warfarin when initiating therapy for anticoagulation.

BACKGROUND

On August 16, 2007, the U.S. Food and Drug Administration (FDA) issued a labeling change on the product packaging of warfarin highlighting the “the opportunity for healthcare providers to use genetic tests to improve their initial estimate of what is a reasonable warfarin dose for individual patients.”¹ This is the first FDA recommendation to consider genetic testing when initiating a commonly prescribed medication and may set a precedent for the future use of genetic technologies in clinical practice. Given the high risk for drug-related adverse events with warfarin and the rapid increase in prescriptions for warfarin in the United States with over 31 million written in 2004², there has been tremendous interest in applying “personalized medicine” through genetic testing to the problem of warfarin dosing.

The use of warfarin

Warfarin is primarily used to prevent blood clotting in patients at high risk for forming clots or to treat patients who already have a clot. The most common indication for warfarin is the prevention of strokes associated with atrial fibrillation. Warfarin is also commonly used for patients with deep venous thromboses (DVT) or pulmonary emboli (PE), artificial heart valves, severe congestive heart failure, strokes and patients undergoing orthopedic procedures that require prolonged immobility.

The half-life of warfarin is between 36 and 42 hours.³ It is strongly bound to albumin, so drugs that displace it from albumin affect its biological activity. It is primarily metabolized by the cytochrome P450 enzyme *CYP2C9* in the liver, although at least four other P-450 enzymes play a role. Thus, drugs that affect *CYP2C9* will also affect the activity of warfarin.

Warfarin acts by inhibiting the synthesis of coagulation factors II, VII, IX, and X, thus decreasing the tendency of the blood to form clots. However, warfarin also reduces levels of the natural anticoagulants protein C and protein S, which increases clotting risk in the short term. On balance, when warfarin is fully active clotting is inhibited. Vitamin K is an essential co-factor in the carboxylation reactions that are inhibited by warfarin. Warfarin inhibits the vitamin K epoxide reductase (*VKORC1*) protein complex, the key enzyme required to regenerate the reduced (active) form of vitamin K.

Monitoring of the efficacy.

Therapeutic dosing reduces the active clotting factors by 30% to 50% of their usual level. However, levels of these clotting factors are not routinely measured. Instead, a standardized measure of prothrombin time (PT), the International Normalized Ratio (INR), is monitored. The INR primarily reflects the activity of factors II, VII, and X. For most indications, the therapeutic range is an INR between 2.0 and 3.0. When the INR rises above four, there is no additional efficacy in the prevention of clot formation, but the risk of major bleeding increases markedly.⁴⁻⁹ Most patients require between 2 mg. and 10 mg of warfarin daily to achieve a stable INR in the therapeutic range. During the initiation phase, the INR is monitored every two to three days to guide therapy. Because of variability in vitamin K intake, other dietary factors, the effect of intercurrent illness, and variations in warfarin metabolism due to other factors, frequent monitoring of the INR is required even for patients on a stable dose of warfarin for months. Patients monitored less frequently than every four weeks have higher rates of significant bleeding than those monitored more frequently.¹⁰

The primary adverse event that limits the use of warfarin is major bleeding. This is usually defined as fatal bleeding, symptomatic bleeding in a critical organ (central nervous system, pericardium, retroperitoneum), or bleeding resulting in a drop of hemoglobin of 2 mg/dL or that requires transfusion of two or more units of blood. Variability in the definition of major bleeding has made it difficult to compare bleeding rates across studies. An international consensus conference agreed upon a standard definition to be used in future studies.¹¹ In a pooled analysis of the five large randomized trials of warfarin therapy for atrial fibrillation, the annual incidence of major bleeding for patients on warfarin was 1.3%, with differences between the warfarin and control arms of only 0.3%.¹² This undoubtedly underestimates the true excess risk of major bleeding associated with warfarin therapy because patients enrolled in clinical trials are likely to be healthier and more carefully monitored than the average patients in routine clinical practice. Of note, an earlier systematic review of randomized trials, estimated the risk of major bleeding to be 3% (95% CI 2.6% to 3.4%)¹³, suggesting that current management of warfarin therapy may be improving. There has been a trend towards

lower goal INR's because of randomized trials demonstrating that a target INR between 2.0 and 3.0 is safer and equally efficacious when compared with higher targets. The annual risk for major bleeding reported in these studies ranges from zero percent to greater than ten percent, although the vast majority reports less than a five percent risk.¹⁴

Many risk factors for major bleeding complications of warfarin therapy have been identified including age, intensity of anticoagulation, variability of INR, sex, cancer, history of GI bleeding, history of stroke and a number of other co-morbidities.¹⁴ Most studies have reported that the incidence of major bleeding is highest during the first several weeks of therapy,¹⁵⁻²² although there are exceptions.^{23, 24}

There is no widely agreed upon standard for initiating warfarin therapy. Large loading doses (≥ 20 mg) are contraindicated as they reduce the level of protein C more rapidly than those of the clotting factors. This creates a potentially hypercoaguable state contrary to the goals of therapy. One small randomized trial in outpatients demonstrated more rapid achievement of a stable INR in the therapeutic range starting with 10 mg of warfarin for the first two doses and then dropping to 5 mg per day and adjusting subsequent dosing based on the INR.²⁵ There was no increase in the rate of excessive anticoagulation, bleeding or clotting as a consequence of the higher initial dose and a therapeutic INR was achieved an average of 1.4 days earlier. However, two other trials among inpatients did not demonstrate a significant benefit to starting with 10 mg and suggested the potential for excessive anticoagulation.^{26, 27} Additional studies have shown a strong effect of age on the risk for excessive anticoagulation²⁸⁻³¹ with some groups suggesting that it is prudent for older patients to receive initial doses lower than 5 mg.^{10, 32} Even though studies have shown that age, sex, race, and measures of body size (height, weight, body mass index, body surface area) are all significant factors in models predicting the steady state warfarin dose³³⁻³⁵, no model is currently used widely to guide initial warfarin therapy. One proprietary commercial algorithm to guide overall management of warfarin therapy has been shown to be beneficial in two small randomized trials^{36, 37}, but it is not in widespread use. Two other algorithms have been studied, but again are not widely used.^{38, 39} Most of these algorithms utilize the INR measured after two to four doses to predict the final warfarin requirements rather than utilizing other clinical information about the patient.

Genetics and warfarin

CYP2C9

The primary hepatic cytochrome P450 enzyme responsible for warfarin metabolism is *CYP2C9*. The common genetic form (wild type) of *CYP2C9* with normal enzyme activity is called *CYP2C9*1*. Although

there are greater than 100 polymorphic alleles constituting at least 37 haplotypes at *CYP2C9*, most are rare. Studies indicate that the common *CYP2C9**2 and *CYP2C9**3 genetic variations are important because patients with these variations metabolize warfarin slower than patients with *CYP2C9**1 (Table 1). Many studies have demonstrated that groups of patients with variant alleles require lower average doses of warfarin to maintain a therapeutic INR.⁴⁰⁻⁶² However, the range of doses observed for patients with specific genotypes is almost as broad as that seen for the population as a whole. For example, one study found that the stable dose for patients with the *1 / *1 genotype ranged from 1 mg. to 10 mg per day; similarly the stable dose for patients with both the *1 / *2 and *2 / *3 genotypes ranged from 1 mg. to 8 mg per day.⁶³ A systematic review funded by the American College of Medical Genetics (ACMG) summarized the relative reduction in warfarin dose from 11 of these studies using meta-analysis. Their results are summarized in Table 1. Many studies suggest that the prevalence of *CYP2C9* genetic variations differs across ethnic groups.⁶⁴⁻⁷⁴

Table 1. *CYP2C9* Allele Frequency and Dose Estimation among a Caucasian Population

Genotype	Warfarin metabolism rate	Frequency (%)	Warfarin dose compared with *1/*1	Average dose†
*1/*1	Normal	63.8	100%	5.6 mg
*1/*2	Intermediate	19.5	78%	4.9 mg
*1/*3	Intermediate	12.6	64%	3.3 mg
*2/*2	Intermediate	1.9	57%	4.1 mg
*2/*3	Slow	1.5	47%	2.3 mg
*3/*3	Slow	0.6	24%	1.6 mg

*1 is “wildtype”; *2 and *3 demonstrated reduced activity.

† The average dose is taken from the study of Higashi et al.⁴⁷

Some studies have suggested that patients with *CYP2C9**2 and *CYP2C9**3 genetic variations are at an increased risk for bleeding with warfarin therapy.^{40, 47, 54, 55, 75} In these studies, patients with these variants generally required lower doses of warfarin to maintain a therapeutic INR level. The potential to reduce major bleeding by avoiding excessive anti-coagulation in these patients is the major potential benefit from genotyping. However, the excess risk for bleeding may be due to a longer time to achieve steady state warfarin levels in the blood. In one study, patients with the *1 / *1 genotype achieve a steady state in three to five days, while patients with the *1 / *3 genotype took 12 to 15 days.⁷⁶

VKORC1

VKORC1 is the primary target for warfarin activity. *VKORC1* reduces vitamin K 2, 3-epoxide to active vitamin K required for coagulation factor synthesis and this redox reaction is inhibited by warfarin. Several non-coding single nucleotide polymorphisms (SNPs) in complete linkage disequilibrium form a variant haplotype that is associated with reduced levels of epoxide reductase, and, thus, increased sensitivity to warfarin (Table 2). The variant haplotype is particularly common among Japanese (89.1%) compared to Caucasians (42.2%) or African-Americans (8.6%).^{59, 77, 78} There are fewer studies evaluating *VKORC1* genetic variations than for *CYP2C9*. Many different nomenclatures have been proposed to describe the variations, in part because several different genetic changes have been identified that are in strong linkage disequilibrium. For this review, we have adopted the haplotype naming system used by Rieder et al⁷⁹ in their New England Journal of Medicine article, which was also used in the ACMG systematic review.⁸⁰ The genotype of patients with two wild type copies of the *VKORC1* gene is labeled BB, those with one variant haplotype AB, and those with two copies of the variant haplotype AA. Many studies have demonstrated that the variant *VKORC1* haplotypes affect the dose of warfarin needed to maintain the INR in the therapeutic range.^{41-43, 46, 56, 59, 62, 77, 79, 81-96} The ACMG review⁸⁰ estimated the relative dose effect of these variants by combining the results of six of these studies (see Table 2).

Table 2. *VKORC1* Haplotype Frequency and Dose Estimation Among a Caucasian Population

Haplotype	<i>VKORC1</i> enzyme production	Frequency (%)	Warfarin dose compared with BB	Average dose†
BB	Low	35	100%	6.1 mg
AB	Medium	47	72%	4.4 mg
AA	High	18	50%	3.2 mg

N is “normal”, V is variant.

†Average dose taken from the study of Rieder et al.⁷⁹

Contribution of genotyping to the variability in warfarin dose

Several research groups have created models including *CYP2C9* and *VKORC1* genetic variants that predict the steady state dose of warfarin required by patients.^{42, 43, 56, 59, 79, 81, 82, 96-98} Some of the models also included age, sex, race, some measure of body size (height, weight, body mass index, or body surface area), indication for therapy, smoking, vitamin K intake, current INR, goal INR, INR after the third warfarin dose, additional genetic polymorphisms, and drugs known to affect warfarin metabolism in their calculations. The percent of variability in warfarin dosing explained by the complete models ranges from about 50% to

60%. Summary estimates in the ACMG review suggest that about 23% of the variance is explained by *VKORC1* genotypes, 17% by *CYP2C9* variants, 9% by weight, 7% by age, and 44% remains unexplained.⁸⁰ A substantial proportion of the variability in dose requirements remained unexplained in all models. The ACMG review criticized these models for not using the logarithmic transformation of the warfarin dose as the dependant variable because the dose is not normally distributed. The authors also note that many of the models do not differentiate between the *1 / *2 and *1 / *3 genotypes.

It is clear from these models, that on average they predict the final warfarin dose well, although significant variability remains at the individual level. However, it remains unclear whether use of these models to guide initial warfarin dose determination improves clinical outcomes. The most important clinical outcome is significant bleeding events, which occur most frequently during the initiation phase of warfarin. Clinical trials would only need to follow patients for the first one to two months following initiation of therapy to evaluate efficacy. Genotyping is unlikely to have any clinical utility after the first week of therapy because the empiric response of the patient to warfarin should be sufficient to guide subsequent dose adjustments. Other clinical outcomes which would improve quality of life for patients and would likely reduce costs include the time to reach a therapeutic INR level and the time to reach stable warfarin dosing. Shorter times would translate into fewer days using heparins or other anticoagulants offering protection from clotting during the period when warfarin was not effective and less frequent blood draws to check INR.

Summary

Ideally physicians will incorporate the genetic information on *CYP2C9* and *VKORC1*, along with various clinical considerations (vitamin K intake, liver disease, medication use) and patient characteristics (age, sex, body weight) to better estimate the initial warfarin doses for patients. This would not replace regular INR testing, but would increase the likelihood that the dose chosen is close to that actually required by the patient. Theoretically, more appropriate dosing could reduce the risk for excessive anticoagulation among patients requiring lower doses of warfarin, resulting in fewer serious bleeding events. Similarly, more appropriate dosing could reduce the risk for undertreatment among patients requiring higher doses, resulting in a more rapid achievement of a therapeutic INR and stable dosing and consequently fewer dosing adjustments and fewer blood draws. This could improve patient quality of life, as well as reduce the overall expense associated with warfarin therapy.

It is clear from the literature that variants in the *CYP2C9* gene and the *VKORC1* gene affect the dose required to achieve a therapeutic INR and that patients carrying some of the variants are at increased risk

for bleeding complications with warfarin. The question that remains is whether testing for the presence of these genetic variants improves management of patients initiating warfarin therapy so that they suffer fewer major bleeding events. The following review focuses on whether genotype guided warfarin therapy decreases the rate of major bleeding and surrogate markers for bleeding compared with usual INR monitoring.

TECHNOLOGY ASSESSMENT (TA)

TA Criterion 1: The technology must have final approval from the appropriate government regulatory bodies.

On September 17, 2007 the Verigene Warfarin Metabolism Nucleic Acid Test, Verigene System (Nanosphere, Inc., Northbrook, IL) was cleared by the FDA through the 510(k) premarket process.

On January 28, 2008 the FDA cleared the Infiniti 2C9-VKORC1 Multiplex Assay (AutoGenomics, Inc, Carlsbad CA) for detection of Warfarin sensitivity.

TA Criterion 1 is met.

TA Criterion 2: The scientific evidence must permit conclusions concerning the effectiveness of the technology regarding health outcomes.

We searched the English language literature through December, 2007 with PUBMED, EMBASE, Cochrane clinical trials database, Cochrane reviews database, the Database of Abstracts of Reviews of Effects (DARE) and the International Pharmaceutical Abstracts (IPA). A highly sensitive search strategy was conducted by exploding and combining the following Medical Subject Heading (MeSH) terms: warfarin, genotype, pharmacogenetics, genetics, *CYP2C9*, and *VKORC1*. We included the following terms in the Title word (TI) search: warfarin, genotype, *CYP2C9*, and *VKORC1*. We combined the exploded Medical Subject Heading and Title word warfarin with the combination of the other MeSH and TI terms. Our search strategy was similar for the other databases. In order to appraise clinical trials underway we searched www.clinicaltrials.gov on January 6, 2008 exploding and combining the following terms: warfarin, coumadin, genotype, gene, pharmacogenetic. Our search strategy yielded 315 PUBMED, 501 EMBASE, and 117 IPA references. Of the combined 933 references, 208 were duplicates for a total list of 725. Our clinical trial

search yielded five additional clinical trials currently accruing patients. We examined the reference lists of included articles and professional reviews (ACMG) to evaluate the comprehensiveness of our search. We then asked an expert to review our bibliography to ensure completeness of our list.

We included clinical trials that compared pharmacogenetic dosing of warfarin using common genetic variants of *CYP2C9* and/or *VKORC1* versus a standard-dosing algorithm in adult, warfarin-naïve patients and reported essential outcomes (bleeding, thromboembolic events, and death). Secondary outcomes of interest include the frequency of excessive anticoagulation (INR > 3 or INR >4), time to first therapeutic INR, time to stable dose, number of blood draws to achieve stable dose, and percent time out-of-range INR were secondary outcomes of interest. Only 3/725 studies met the inclusion and exclusion criteria (423 patients). At least six additional randomized trials are currently accruing patients (total n > 3500). We also included one study that prospectively evaluated the outcomes among 48 patients whose anticoagulation was guided by an algorithm incorporating *CYP2C9* polymorphisms even though there was no comparison arm.⁹⁹

Two authors independently abstracted data from all studies. The standardized data abstraction form included the following information: number of participants (total and number in each intervention arm), study quality, length of follow-up period, intervention and control dosing algorithms, and outcomes. We resolved all disagreements by discussion and consensus. We contacted the authors of the studies for additional information and to answer specific questions about study results when clarification was needed. We assessed study quality using a validated instrument described by Jadad, et al.¹⁰⁰ This score ranges from 0 to 5 where a higher number describes better study design. Criteria used in this instrument are based on presence of randomization, blinding and appropriate description of withdrawals and dropouts. The study design, quality assessment and study strengths and weaknesses are presented in Table 4

The three randomized trials differed substantially in their quality, interventions and outcome measures (see Tables 3 and 4). Two studies evaluated the contribution of *CYP2C9* variants; one assessed both *CYP2C9* and *VKORC1* variants.

Level of Evidence: 1, 2, 5

TA Criterion 2 is met.

TA Criterion 3: The technology must improve net health outcomes.

As noted above, the health outcome of primary interest is major bleeding. Genetic testing for *CYP2C9* and *VKORC1* common variants has been touted as a relatively simple way to reduce the risk of bleeding in patients on warfarin. Because it usually takes less than a month to reach a stable warfarin dose, the reduction should be measurable with short follow-up. However, the risk of bleeding is relatively low, so large numbers of patients would need to be studied to demonstrate a statistically significant difference. Thus, investigators have focused on other surrogate markers thought to reflect the risk for major bleeding. These include the frequency and percentage of time that patients have supratherapeutic INR measurements (usually defined as an INR > 3.0 or an INR > 4.0), the length of time it takes to achieve a stable warfarin dose (therapeutic without change in dose for at least seven days), and the percentage of time spent outside the target therapeutic range. The goal of warfarin therapy is to prevent thromboembolic events, so these important health outcomes should be evaluated as well.

Prospective cohort – no comparison

Voora et al⁹⁹ reported on the only cohort of patients prospectively treated with warfarin based on an algorithm developed by Gage et al.³⁴ This algorithm predicts the maintenance warfarin dose based on the number of *CYP2C9*2* and *CYP2C9*2* alleles as well as age, sex, race, body surface area, use of amiodarone, the use of statins, and the target INR. Note that *VKORC1* genotyping was not included in this algorithm. The study evaluated the effect of the dosing scheme on the time to stable INR and adverse outcomes (INR > 4, major bleeding, or venous thromboembolism) in 48 consecutive patients scheduled for total hip or knee arthroplasty at a university-based hospital in St. Louis, Missouri. *CYP2C9* variants were present in 33% (16/48) of patients. There was no difference in time to stable anticoagulation between the two groups (14 days vs. 13 days, $p=0.40$), but patients with a *CYP2C9* variant were three times more likely to have an adverse event (63% vs. 22%, hazard ratio 3.6, $p=0.01$). This was primarily due to an increase in INR > 4.0 among those with variant alleles (HR 4.6, $p < 0.01$ despite dosing based on the presence of those alleles. There were no major bleeding episodes and thromboembolic event rates were similar (13% vs. 9%, $p=0.55$). Interestingly, the algorithm did a good job predicting the warfarin maintenance dose (difference < 1 mg/day) and the patient with the lowest requirement (1.5 mg/day) was predicted to have the lowest requirement (1.8 mg/day). This study demonstrates that knowledge about the *CYP2C9* variant alleles does not necessarily reduce the excess risk for bleeding associated with those alleles and highlights the need for high quality randomized trials demonstrating the efficacy of a new approach to initiating warfarin therapy before it is widely adopted.

Randomized clinical trials

This first published randomized trial by Hillman et al¹⁰¹ was designed as a pilot trial comparing pharmacogenetic dosing to standard dosing in patients initiating warfarin therapy who had not previously been treated with warfarin. The intervention arm of the trial received their warfarin dose based on an algorithm developed previously⁴⁸ that included age, sex, body surface area, concomitant medications, comorbidities, clinical indication, and the patient's *CYP2C9* genotype. The study randomized 38 patients to pharmacogenetic dosing (n=18) or standard dosing (n=20). Patients in the standard dosing arm received 5 mg per day initially. Dose adjustments in both arms were made according to a standard algorithm by the anticoagulation service. All patients were followed for four weeks (28 days). Outcomes were similar in the two groups. The percentage of patients with an INR > 4.0 was 33% in the pharmacogenetic dosing group and 30% in the standard dosing group. The percent time that the INR was in range was 41.7% in the pharmacogenetic dosing group and 41.5% in the standard dosing group. There were fewer warfarin-related adverse events in the pharmacogenetic dosing group (two versus five), though it is unclear whether any of these represented major bleeding events. Both events were gastrointestinal (GI) bleeds in the pharmacogenetic group compared with one GI bleed, one episode of hematuria, one nose bleed, one DVT and one PE in the standard dosing group. The authors concluded that trials of pharmacogenetic dosing are feasible and that their initial results suggest that there may be important benefits in preventing adverse events.

The second trial by Caraco et al¹⁰² allocated 142 patients to the pharmacogenetic dosing group and 141 patients to the standard dosing group (total n = 283). However only 191 patients completed the warfarin induction phase of the study (first eight days) because some never started warfarin (n=31), some stopped warfarin early (n=20), and some violated the study protocol by missing INR measurements or not taking the appropriate dose of warfarin (n=25). An additional six patients dropped out before a stable warfarin dose was achieved. There was no comparison of the two treatment groups as randomized, though the groups as analyzed had similar demographic and clinical characteristics, similar indications for warfarin, similar medication use, and a similar distribution of *CYP2C9* genotypes. The pharmacogenetic arm received initial warfarin dosing based on a new, never validated algorithm based on *CYP2C9* genotypes and the use of amiodarone. The standard dosing arm followed a previously validated computer-based algorithm for warfarin therapy.^{36, 37} The primary endpoints of the trial were time to first therapeutic INR (INR > 2) and the time to reach stable anticoagulation. Additional endpoints included the percentage of time within the therapeutic range, thromboembolic events, and major and minor bleeding events. Patients in the

pharmacogenetic group received higher initial doses of warfarin (8.55 vs. 6.67 mg, $p < 0.001$) and reached at therapeutic INR faster (4.80 vs. 7.53 days, $p < 0.001$) compared with the standard dosing group. During the induction phase of the study (first eight days), patients in the pharmacogenetic group spent more time in the therapeutic range (45.4% vs. 24.5%, $p < 0.001$), although there were no differences in the time spent with an INR > 3 (0.41 vs. 0.44 days, $p = 0.55$). The length of the stabilization phase of the study (days 9 through achievement of stable anticoagulation) was significantly shorter in the pharmacogenetic group (14.1 vs. 32.2 days, $p < 0.001$). They also had fewer days at INR > 3 (1.77 vs. 6.58, $p < 0.001$) although this partially is due to the longer follow-up in the standard dosing arm. Similarly, the number of days with INR < 2 was lower in the pharmacogenetic group (2.01 vs. 8.00 days, $p < 0.001$). During stabilization phase, patients in the pharmacogenetic group also spent more time in the therapeutic range (80.4% vs. 63.4%, $p < 0.001$). The incidence of bleeding was lower in the pharmacogenetic group (3.2% vs. 12.5%, $p < 0.02$), although this may not be a true incidence as it was not adjusted for the differential length of follow-up between groups. Only one of the bleeding events was classified as a major bleed: it occurred on day nine in a patient randomized to the standard dosing arm whose INR was 1.74 when the bleeding occurred. There were no new thromboembolic events during the study period. The lack of true randomization and allocation concealment, the high loss to follow-up, the differential length of follow-up in the two groups, and the lack of a true intention to treat analysis call into question the internal validity of this study.

Anderson et al¹⁰³ published the most recent randomized clinical trial. They randomized 206 patients, but six patients withdrew either before starting warfarin or before the first INR measurement. The pharmacogenetic group ($n=101$) received warfarin based on a previously validated algorithm⁴² that incorporated both *CYP2C9* and *VKORC1* genetic variants as well as age, sex, weight. The initial dose was doubled on the first two days of treatment. The standard dosing group ($n=99$) received warfarin at 10 mg per day for the first two days followed by 5 mg per day, which has been demonstrated to be superior to standard 5 mg per day dosing in a small randomized trial (Kovacs et al²⁵). Patients were followed for three months or until the end of warfarin therapy (one month for orthopedic patients). Their primary outcome was the percentage of out of range INR values. There was no difference in out of range INR values between the two groups in the study (31% vs. 33%, $p=0.47$). The pharmacogenetic dosing group required fewer dose adjustments per patient (3.0 vs. 3.6, $p=0.035$) and there was a trend towards fewer INR blood draws (7.2 vs. 8.1, $p = 0.06$). Time within the therapeutic ranges was 69.7% for the pharmacogenetic group and 68.3% for the standard dosing group. The number of clinical adverse events plus INR ≥ 4 (35% vs. 42%, $p=0.26$) and serious adverse events (4.0 vs. 5.1%, $p=0.71$) did not differ between groups. None of the serious adverse events were related to out of range INRs. Unfortunately, the authors did not describe the adverse events in detail in the study and no data

on bleeding events was presented. This study was the highest quality trial published to date. It suggested that the primary benefit of pharmacogenetic dosing may be a reduction in the number of blood draws and dose adjustments needed to achieve a stable INR, but that there may not be a large impact on the time to stable INR or the frequency of out of range INRs. The study was not powered to evaluate important clinical outcomes, but there was a hint that adverse events may be reduced.

Table 3. Study Characteristics

Author Year	Location, Setting, Ethnicity	Study arm	Number randomized	Losses following randomization	Mean Age, years	Male Sex, %	Follow-up, days	Gene(s) tested	PG-dosing algorithm	S-dosing algorithm
Hillman 2005	USA AC and IP, Caucasian	PG	18	0	70.5	45	28	<i>CYP2C9</i>	Hillman model ⁴⁸	Marshfield algorithm ¹⁰⁴
		S	20	0	68.8	44	28			
Caraco 2007	Israel AC Caucasian	PG	142	47	57.6	46	22	<i>CYP2C9</i>	Algorithm constructed de novo ¹⁰²	DAWN AC computer algorithm ³⁶
		S	141	45	59.7	42	40			
Anderson 2007	USA IP Caucasian 94%	PG	101*	0*	63.2	50	46	<i>CYP2C9</i> , <i>VKORC1</i>	Carlquist regression equation ⁴²	10 mg x 2 days, then 5 mg ²⁵
		S	99	0	58.9	57				

PG: pharmacogenetic arm; S: standard dosing arm; AC: anticoagulation clinic; IP: inpatient.

* Data were not presented by study group. 206 patients were randomized; 6 dropped out: 3 because surgery was canceled, 3 stopped warfarin before 1st INR drawn.

Table 4. Summary of Study Design Strengths and Weaknesses

Study	Randomization	Allocation concealment	Comparable groups at baseline	Loss to follow-up equivalent	Blinded outcome assessment	Patient blinding	Co-interventions equivalent	ITT analysis	Quality *
Hillman 2005	Yes	Yes	Yes	Yes	NR	Yes	Yes	Yes	3
Caraco 2007	Pseudo	No	NR	Yes†	Yes	No	No	No	1
Anderson 2007	Yes	Yes	No‡	Yes	Yes	Yes	Yes	Yes	5

ITT: intention to treat; NR: Not reported.

* Jadad score (0-2 poor, 3-4 good, 5 excellent)

† Equivalent, but very high loss to follow-up post-randomization, and there was unequal length of follow-up time between the two groups

‡ Higher number of variant alleles in the standard dosing arm (79.6% versus 61.0%, $p < 0.01$); more hypertensives in the pharmacogenetic arm ($p < 0.02$).

Table 5. Primary Outcomes

Study	Study group	N analyzed	Starting does, mg	Time to therapeutic INR, days	Time to stable warfarin dose	% time INR in range	INR > 4, %	Blood draw for INR	Minor bleeds, %	Major bleeds, %	Thromboembolic events, %
Hillman 2005	PG	18	4.6	NR	NR	41.7	33	NR	0	11†	0
	S	20	5			41.5	30		10	5	10
Caraco 2007	PG	95	8.55*	4.8*	14.1*	45.4*	NR	4.9*	3.2	0	0
	S	96	6.67	7.5	32.2	24.5		10.7	11.4	1	0
Anderson 2007	PG	101	10.2	NR	NR	69.7	NR	7.2	NR	NR‡	NR
	S	99	10			68.6		8.1			

PG: pharmacogenetic arm; S: standard dosing arm.

* $p < 0.05$ between PG and S groups. For the Caraco study, the reported percentage of time to INR in range is for the initiation phase (first eight days). During the maintenance phase the percentages were 80.4 and 63.4 respectively.

† Gastrointestinal bleeding in paper; not clear if meet criteria for major bleed

‡ Serious adverse events were similar in the two groups (4.0% vs. 5.1%, $p = 0.71$) as was the sum of adverse events plus $INR \geq 4$ (35% vs. 42%, $p = 0.26$).

Table 6. Estimates Used for Meta-analysis of the Average Time Spent in the Therapeutic Range

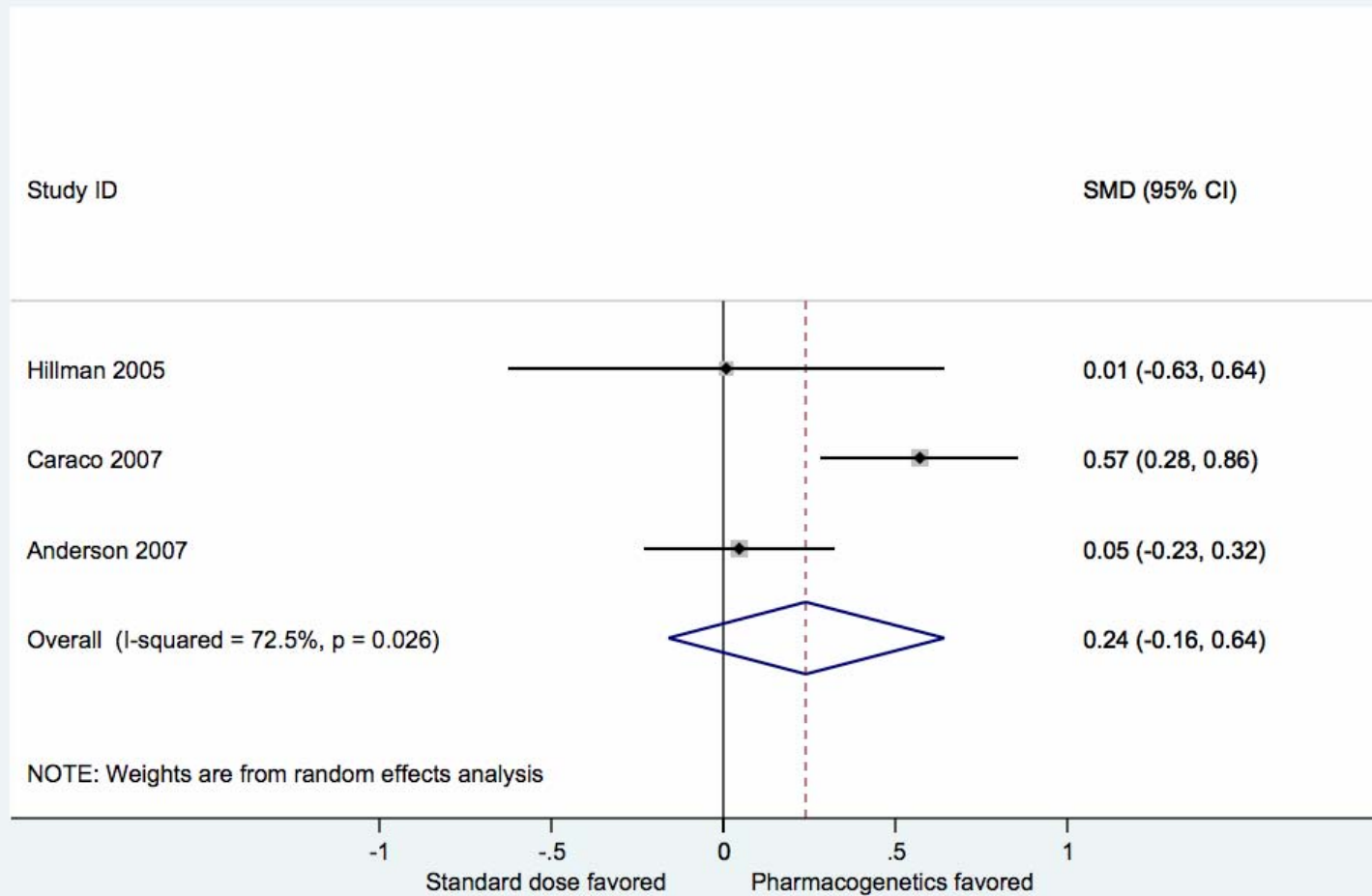
Study	Study group	N analyzed	Average time, %	Standard deviation	Length of follow-up
Hillman 2005	PG	18	41.7	25.4	28
	S	20	41.5	24.9	28
Caraco 2007	PG	95	67.7	20.0	22
	S	96	55.7	22.1	40
Anderson 2007	PG	101	69.7	23.4	46
	S	99	68.6	24.3	46

Table 7: Ongoing Clinical Trials of Warfarin Pharmacogenetics

PI	Title	Location	N	Indication	Outcome	Expected completion
Caldwell	Modeling Genotype and Other Factors to Enhance the Safety of Coumadin Prescribing	US – Marshfield clinic	260	Initiating warfarin therapy	1°: INR in range 2°: time to stable dose, time to INR>4, bleeding and thromboembolic events	5/2008
Caraco	Warfarin Induction Regimen Based Upon CYP2C9, VKORC1 Factor VII Genotyping, PMR and INR Monitoring, as Compared to the Conventional Regimen: a Prospective Controlled Study	Israel	500	Venous Thrombosis; Pulmonary Embolism; Atrial Fibrillation	Time to therapeutic INR, % INR in range, bleeding events	NR
Creager	CRreating an Optimal Warfarin Nomogram (CROWN) Trial	US- Multi-center	500	Initiating warfarin therapy	1°: INR in range 2°: bleeding and thromboembolic events	1/2009
Destache	Warfarin Dosing: Pharmacogenetic Algorithm Compared to Pharmacist's Dosing	US - Creighton	250	Initiating warfarin therapy	Dose comparison	NR
Johnson	PRospective Evaluation Comparing Initiation of Warfarin StrategiEs (PRECISE): Pharmacogenetic-Guided Versus Usual Care	US – Univ. Florida	500	Initiating warfarin therapy	1°: dose accuracy 2°: Time to therapeutic INR, % INR in range	NR
Limdi	Pharmacogenetic optimization of anticoagulation study		500	Stroke	Out of range INR, hemorrhagic and thromboembolic complications	2008
McMillin		US	1000	Hip and knee replacement patients	1°: major bleed, thrombosis, death. 2°: time to therapeutic INR	NR
Pirmohamed	Variability in response to warfarin	UK	2400	NR	Algorithm for dosing	NR
Stein	Genotyping to optimize individualized drug therapy	US	NR	NR	“Clinical outcomes”	NR
Wei	The association of Warfarin Dosage and Plasma Enantiomer Concentration with the Gene polymorphisms of CYP and VKOR	Taiwan	120	Taiwanese patients on warfarin therapy	NR	6/2006

NR: Not reported.

Figure: Meta-analysis of the Average Time Spent in the Therapeutic Range



There was significant heterogeneity among the three randomized trials in terms of design quality, length of follow-up, predictor and outcome measures (Tables 3 – 5). The Anderson study¹⁰³ was the only excellent quality study (score = 5 using the Jadad scoring system¹⁰⁰), while the Caraco study¹⁰² was of poor quality (score = 1) and should not truly be considered a randomized study as group assignment was determined by the patient's identity number (even versus odd). Follow-up ranged from 22 days in the pharmacogenetic arm of the Caraco study¹⁰² to an average of 46 days in the Anderson study.¹⁰³ Hillman¹⁰¹ and Caraco¹⁰² used dosing models that included *CYP2C9* variants; Anderson¹⁰³ was the only study that incorporated both *CYP2C9* and *VKORC1* variants in their dosing model. Each of the three studies used different dosing models for their pharmacogenetic arms and for their "standard" dosing arms. This is reflected in the differing average initial doses across the three studies in both the pharmacogenetic arms (4.7, 8.55, and 10.2 mg/day) and the standard arms (5, 6.67, and 10 mg/day). As suggested by previous observational studies, all three studies demonstrated improved prediction of warfarin maintenance dose in the pharmacogenetic arm. However, only the poorest quality study (Caraco et al¹⁰²) found that pharmacogenetic dosing improved surrogate endpoints for bleeding such as percentage time INR in-range and percentage of time with elevated INR. The tremendous variability in these measures in the standard dosing arms across studies calls into question their utility as surrogate markers. For instance, the percentage time that the INR was within the therapeutic range in the standard arm varied from 24.5% in the initiation phases of the Caraco study¹⁰² to 68.6% in the Anderson study¹⁰³ (Table 5). Finally, there was insufficient power to evaluate the effect of the pharmacogenetic dosing models on the most important outcome, major bleeding, although the trend towards fewer adverse events reported by Hillman¹⁰¹ and Caraco¹⁰² was not replicated by Anderson.¹⁰³

The only outcome reported by all three studies was the average time spent within the therapeutic range. Caraco reported separate statistics for the first eight days (initiation phase) and for days nine through the end of follow-up (stabilization phase). We estimated an average across the study period for the Caraco study in order to perform a meta-analysis across studies (see Table 6 and the Figure). There was significant heterogeneity across the studies ($I^2 = 72.5\%$, $p=0.026$). This may be due to the different treatment algorithms used, the differences in the lengths of the follow-up periods, or differences in the quality of the studies. The two higher quality studies reported almost no difference between the pharmacogenetic and standard dosing arms as is evident in the Forrest plot.

TA Criterion 3 is not met.

TA Criterion 4: The technology must be as beneficial as any established alternatives.

The established alternative to genotyping for *CYP2C9* and *VKORC1* is either dosing based on clinical experience or following one of the treatment algorithms that have been evaluated in the published literature.^{25, 26, 36-39} When compared to standard dosing, the randomized trials described under TA criterion 3¹⁰¹⁻¹⁰³ have not demonstrated that use of genetic information changes management in ways that reduce the bleeding complications associated with warfarin therapy, nor have they convincingly demonstrated a large improvement in potential surrogate markers for bleeding such as the frequency of excessive anticoagulation or the time spent with INR values out of the therapeutic range.

TA Criterion 4 is not met.

TA Criterion 5: The improvement must be attainable outside of the investigational setting.

At least 12 laboratories in the United States offer genotyping for *CYP2C9* and *VKORC1*.⁸⁰ However, none of the clinical studies used a central laboratory, because of the time delay. In order to be clinically useful, the genotyping information must be available within a few hours of the identification of the need for anticoagulation. Otherwise, warfarin therapy will have to be initiated without the value of the new information. There is also no consensus on the best algorithm to use when incorporating genotype information into the initial dosing and management of warfarin therapy. Until these issues are resolved, genotype testing should not be widely promoted.

Furthermore, the clinical utility of genetic testing for this indication has not yet been demonstrated in the investigational setting, so no conclusions can be reached about the effectiveness of the device in a community setting.

TA Criterion 5 is not met.

CONCLUSION

Genotyping studies of patients at a stable, therapeutic dose of warfarin clearly demonstrate that patients who have *CYP2C9**2 and *CYP2C9**3 require lower doses of warfarin on average than patients with the

more common *CYP2C9*1* allele.⁸⁰ Similarly, patients with the A haplotype of *VKORC1* require lower doses of warfarin on average than patients with the B haplotype.⁸⁰ Moreover, these genetic variations have been shown to predict an increased risk of excessive anticoagulation and major bleeding among patients prescribed warfarin. Several investigators have developed statistical models to predict the dose of warfarin needed to achieve stable anticoagulation in the hope that initiating therapy with a more accurate dose would decrease the incidence of major bleeding that occurs at a higher rate during the initiation of warfarin therapy.

The first small case series to test this hypothesis found that their model did reasonably well at predicting the warfarin dose, but that patients with the *CYP2C9*2* and *CYP2C9*3* alleles were still at more than a four-fold risk of excessive anticoagulation.⁹⁹ Subsequently, three relatively small, randomized clinical trials have been published.¹⁰¹⁻¹⁰³ There is a hint that there may be benefit to warfarin dosing based on models incorporating the patient's genotype, but the data are not yet convincing. Only one of these four studies incorporated genotyping information from both *CYP2C9* and *VKORC1*; that study found almost no difference in outcomes between patients receiving warfarin using a pharmacogenetic model and those treated according to a standard approach.¹⁰³ It may be that careful dose adjustment based on the INR response to warfarin therapy is far more important to safe anticoagulation than choosing the correct initial dose.

Significant uncertainty remains in the field. There is no widely accepted, standard pharmacogenetic model to determine the starting dose of warfarin and new models are being developed in 2008. Current models only explain 50% to 60% of the variability in warfarin dosing and the remaining variability is unexplained. Only one of the randomized trials used a model incorporating information from both genes with variants known to influence warfarin dosing. Furthermore, all of the trials to day have been underpowered to meaningfully evaluate the effect of genotyping on major bleeding, the most important clinical outcome. Several large clinical trials are ongoing in both the United States and Europe and should provide important new results within one year that will help to clarify the role of genetic testing in warfarin management (Table 7).

DRAFT RECOMMENDATION

It is recommended that the use of genetic testing to guide initial warfarin dosing does not meet Technology Assessment Criteria 3 through 5 for safety, effectiveness and improvement in health outcomes.

March 5, 2008

RECOMMENDATIONS OF OTHERS

BLUE CROSS BLUE SHIELD ASSOCIATION (BCBSA)

The BCBSA Technology Evaluation Center has not conducted a review of this test.

CENTERS FOR MEDICARE AND MEDICAID SERVICES (CMS)

A policy specific to the use of this test was not found on the CMS web site.

AMERICAN COLLEGE OF MEDICAL GENETICS (ACMG)

The ACMG has been invited to provide an opinion and to attend the meeting. A systematic review of the evidence can be found at:

<http://www.acmg.net/AM/Template.cfm?Section=Home3&Template=/CM/ContentDisplay.cfm&ContentID=263>

AMERICAN COLLEGE OF CARDIOLOGY, CALIFORNIA CHAPTER (CA ACC)

The CA ACC has been invited to provide an opinion and participation at the meeting.

AMERICAN COLLEGE OF PHYSICIANS, NORTHERN CALIFORNIA CHAPTER (ACP)

The ACP, Northern California Chapter has been invited to provide an opinion and participation at the meeting.

AMERICAN SOCIETY OF HEMATOLOGY (ASH)

The ASH has been invited to provide an opinion and participation at the meeting.

CALIFORNIA SOCIETY OF PATHOLOGISTS (CSP).

The CSP has been invited to provide an opinion and participation at the meeting.

ABBREVIATIONS USED IN THIS REVIEW

FDA	U.S. Food and Drug Administration
DVT	Deep venous thrombosis
PE	Pulmonary emboli
<i>VKORC1</i>	Vitamin K epoxide reductase
PT	Prothrombin time
INR	International normalized ratio
ACMG	American College of Medical Genetics
SNPs	Single nucleotide polymorphisms
DARE	Database of Abstracts of Reviews of Effects
IPA	International Pharmaceutical Abstracts
MeSH	Medical Subject Heading
TI	Title word
GI	gastrointestinal

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