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Overview of Current Evidence for Using Pharmacogenetic Testing to Guide Warfarin Initial Dose Selection

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Warfarin is a vitamin K antagonist (VKA) widely prescribed in the setting of atrial fibrillation, cardiac valve replacements, and secondary to a deep-vein thrombosis (DVT) or pulmonary embolism (PE) for the treatment or prevention of thromboembolic events.[1] It is considered a narrow therapeutic index drug and exhibits a large amount of inter-patient variability in dose requirements, both of which make appropriately dosing the medication a challenge. Warfarin dosing regimens are titrated to a target international normalized ratio (INR) range specific to the indication for anticoagulation. The typical goal INR range for anticoagulation in the setting of atrial fibrillation and secondary prevention of a DVT or PE, for example, is 2-3.[2] Determination of a goal INR range is largely guideline-based, with occasional exceptions in special cases after physician consideration of bleeding or thrombosis risk.[3] It can become more complex in the setting of indications, such as antiphospholipid syndrome (APS), which have evidence suggesting various therapeutic INR ranges. Recent European League Against Rheumatism (EULAR) guidance recommends targeting an INR range of either 2-3 or 3-4 for APS patients with first arterial thrombosis.[4] They leave the ultimate INR goal range up to provider's discretion depending on the patient's risk of adverse events.

Patients on warfarin therapy have their dosing regimens adjusted in order to achieve a therapeutic INR. Standard practice is to adjust dosing regimens by increasing or decreasing a percentage of the total weekly dose, sometimes resulting in complicated regimens and varying strengths being taken throughout the course of a week.[5] In order to allow for this individualization of dosing regimens, warfarin comes in 10 different strength tablets ranging from 1mg to 10mg.[6] Large differences can be observed between patients regarding total weekly dose requirements and time to reach a therapeutic INR; it is not uncommon for patients to spend time out of therapeutic range, especially in the beginning phases of therapy. A large, retrospective cohort study observed that in the first 6 months of therapy nearly 2/3 of warfarin users spend <65% of the time in therapeutic range.[7] Spending time out of goal INR range can be dangerous considering its significant association with negative outcomes such as major bleeding, acute coronary syndrome, and thromboembolism.[8] Subtherapeutic INR values are indicative of under anticoagulation, putting the patient at risk for a thromboembolism. A supratherapeutic INR is indicative of over anticoagulation, putting the patient at risk for a bleeding event. Outside factors can contribute to fluctuating INR values including vitamin K intake, adherence, liver function, concurrent medications or supplements, cigarette smoking, and alcohol.9 Each of these factors affect warfarin anticoagulation in different ways, resulting in either sub- or supratherapeutic INR values. It is estimated that over 60,000 emergency department (ED) visits relating to complications of warfarin therapy are made every year.[10] About 2/3 of these ED visits are reported as a result of acute hemorrhage, and just under 1/3 are a result of an elevated INR with no signs of bleeding. In order to ensure both a safe and efficacious course of therapy, patients should have their INR monitored regularly.

"Evidence-Based Management of Anticoagulant Therapy: Antithrombotic Therapy and Prevention of Thrombosis Guidelines" published by the American College of Chest Physicians in the journal CHEST® are often regarded as the gold-standard of anticoagulation management.[2] Evidence-based dosing recommendations are important to warfarin considering the risks associated with both subtherapeutic and supratherapeutic levels of the medication. Unfortunately, there is no single factor that can be used to determine the best starting dose for individual patients. Initial warfarin doses are currently chosen empirically based on patient-specific factors such as age, weight, underlying conditions, and potentially genetics. Validated warfarin dosing nomograms are often used to help decision making, but they have varying recommendations regarding starting doses. One nomogram proposed by Kovacs et al suggests that a 2-day 10mg loading dose is a safe and effective way to initiate warfarin.[11] Another validated nomogram proposes a 5mg starting dose.[12] Despite conflicting recommendations, all validated warfarin dosing nomograms or computer-based algorithms are supported by the current ACCP/CHEST® antithrombotic therapy guidelines for use as a clinical decision support tool.[2] The guidelines suggests considering lower starting doses of 2mg-3mg per day for patients who are elderly, malnourished, or have liver dysfunction or heart failure. The risk for either a recurrent thromboembolism or major hemorrhage is highest in the initial months of therapy, making choosing an appropriate starting dose crutial.[13,14] Providers use their clinical judgement and published guideline recommendations to evaluate the many rele-

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vant patient-specific factors in determining the most accurate starting dose for individual patients.

Current ACCP/CHEST® guidelines, last updated in 2012, recommend against the use of pharmacogenetic (PGx) testing in initial warfarin starting dose determination due to cost of testing and a lack of evidence behind their impact on therapeutic outcomes.[2] While evidence may have been limited in 2012, there are larger and more robust studies exploring the utilization of pharmacogenetics in warfarin therapy to date. Additionally, pharmacogenetic testing accessibility has improved in the last decade.[15] The purpose of this paper is to present more recent literature investigating pharmacogenetics' role in warfarin dosing, the cost-effectiveness of testing, and its impact on relevant clinical outcomes including bleeding and thromboembolic events.

Determining the Genetic Link

Warfarin is formulated as a racemic mixture, including both the R and S stereoisomers.[16] Both stereoisomers exhibit anticoagulant effects, however, S-warfarin is 3-5x more potent of a VKA than the R-isomer. Numerous cytochrome P-450 (CYP) enzymes are involved in the metabolism of R-warfarin including CYP3A4, CYP2C9, CYP2C8 CYP1A2, and CYP2C19. While CYP2C9 plays a minor role in the metabolism of R-warfarin, it is the main contributor to metabolism of the more potent S-warfarin. Both stereoisomers exhibit inhibition on vitamin K dependent clotting factors by targeting the vitamin K epoxide reductase complex 1 (VKORC1). VKORC1 is responsible for activating vitamin K, therefore its inhibition by warfarin decreases the available vitamin K in the body. Both CYP2C9 and VKORC1 play a significant role in warfarin metabolism and response, but the extent of the impact that genetic variations have on warfarin dosing and clinical outcomes is still largely controversial.[16]

CYP2C9 and VKORC1 are considered polymorphic genes, meaning multiple variant forms of their DNA sequences are expressed in the population as seen in Figures 1& 2.[17] Variant forms are designated with a star allele (*2, *3, *4) once they are identified, with *1 referring to the wild-type DNA sequence. Each patient has a genotype based on the two star alleles they express, one inherited from each parent. The combined activity of each star allele determines a patient's phenotype, which can range anywhere from poor metabolizer to ultra-rapid metabolizer. While many variants do not change the function of the enzyme, others can substantially increase or decrease their activity.

In the case of CYP2C9, the *2 and *3 alleles are well known to be associated with a significant decrease in CYP2C9 activity.[18] Less CYP2C9 activity results in less metabolism of warfarin, and therefore higher levels of the medication in the body. Patients who express CYP2C9 *2 or *3 alleles require lower maintenance doses and are at an increased risk of bleeding during the initiation of the therapy.[19,20] VKORC1 variant 1639 G>A is associated with a decrease in its activity, resulting in less activation of vitamin K.[21] With less circulating active vitamin K in their body, patients expressing at least one variant allele in VKORC1 (GA or AA) have been shown to be more sensitive to the effects of warfarin. They require lower doses and take less time to achieve a therapeutic INR.[22,23]

The relationship between CYP2C9 and VKORC1 genotype and an individual's therapeutic maintenance dose of warfarin has been fairly well established.[24,25] A retrospective study in European-American patients on long-term warfarin therapy found that patients who express reduced function CYP2C9 and/or VKORC1 alleles are associated with lower warfarin dose requirements (2-3mg/day) while patients who do not express any reduced function alleles are associated with higher warfarin dose requirements (>6mg/day).[26] Of the many factors contributing to the large inter-patient differences in warfarin dose requirements, CYP2C9 and VKORC1 polymorphisms are thought to account for 30-50% of this variability.[27] The controversy regarding CYP2C9 and VKORC1's use in clinical practice lies with whether their utilization makes a significant difference on clinical outcomes, and if that difference is worth the cost of testing.[2]

Current Guidance

The American College of Chest Physicians' "Evidence-Based Management of Anticoagulant Therapy: Antithrombotic Therapy and Prevention of Thrombosis Guidelines" provide extensive evidence-based recommendations for clinical decisions. The 9th edition of the ACCP/CHEST® guidelines published in 2012 contain the most recent updates to the management of anticoagulant therapy.[2] The updated guideline addresses 23 common questions with sufficient evidence at the time to formulate a recommendation. They comment on topics such as therapeutic goals, dose and duration of therapy, as well as indications for therapy. Only 2 of the 23 recommendations are categorized as "strong", one being against the use of pharmacogenetic testing to help guide initial warfarin dosing. The guidelines describe the reasons behind this strong recommendation. These reasons, along with any perceived limitations to them, are discussed below.

Time to Therapeutic INR or Time Spent in Goal INR Range

ACCP/CHEST® guidelines suggest that pharmacogenetic-guided warfarin dosing results in no difference in time to achieve therapeutic INR nor time spent in range based on a study conducted by Anderson et al.[28] The study investigated the impact a PGx-guided algorithm for warfarin starting doses has on INR-related outcomes in a largely inpatient population. INR was measured routinely in both groups on days 0, 3, 5, 8, 21, 60, and 90, allowing physicians to adjust warfarin dosing regimens as they normally would. Patients with PGx-guided warfarin initiation spent an average of 30.7% out of therapeutic range, while those who received standard dosing spent an average of 33.1% of the time outside of therapeutic range. By day 5, 69.7% of the PGx-guided group and 68.3% of the standard dosing group achieved a therapeutic INR. Neither difference, time spent out of therapeutic range nor therapeutic INR by day 5, was statistically significant. Anderson et al[28] did observe significant differences in secondary outcomes, however, including the accuracy of initial doses compared to final maintenance doses between PGx-guided and standard algorithms. The PGx-guided arm required smaller and less dose adjustments to achieve a stable and therapeutic INR. PGx-guided algorithms most improved the accuracy of starting doses in patients who expressed wild-type or >1 variant alleles. Patients expressing wild-type CYP2C9 and VKORC1 genotypes required an average dose adjustment of 0.5mg/week to achieve a stable therapeutic INR with PGx-guided initiation, while those who used standard dosing algorithms required an average change of 10mg/week to achieve the same result. Similarly, patients who expressed >1 variant allele required an average adjustment of 0.6mg/week to achieve a stable therapeutic INR with PGx-guided starting doses, and those with standard dosing required an average change of 13.6mg/week. For patients only expressing 1 variant allele in

Figure 1 | Percentage of Population Expressing Reduced Function of CYP2C9 Allele[11]

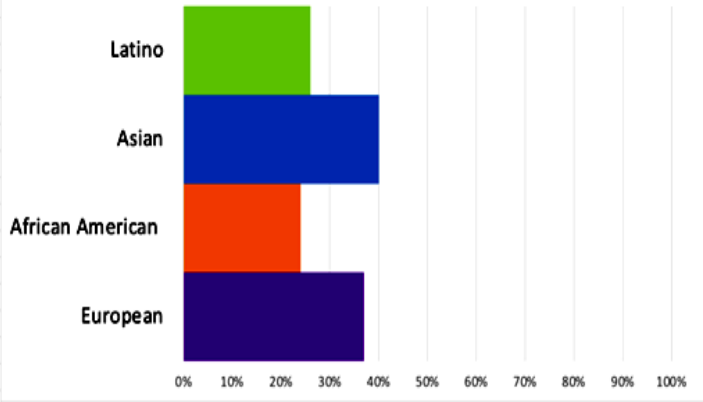
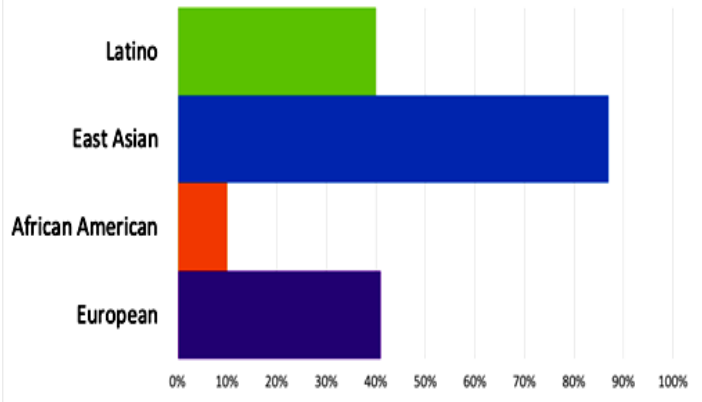


Figure 2 | Percentage of Population Expressing Reduced Function of VKORC1 Allele[11]



CYP2C9 and VKORC1, 41% of the study population, there was no significant difference in the accuracy of starting doses between algorithms.[28]

Thrombotic Events, Major Bleeding, or Survival

Four randomized controlled trials (RCTs) were described in the 2012 guidelines to support their statement that PGx-guided warfarin dosing results in no difference in thrombotic events, major bleeding, or survival.[28,29,30,31] They all compared outcomes in patients who received genotype-guided and standard dosing regimens in new warfarin users. None of the RCTs reported a significant difference in thrombotic events, major bleeding, or survival. One included study conducted by Hillman et al[29] did observe a large difference in instances of hemorrhagic events or DVT/PE in the first month of therapy between the standard and genotype-guided groups (30% vs 11%, respectively). However, this randomized-controlled pilot trial sought out to investigate the feasibility of CYP2C9 genotype-guided warfarin initiation. With only 38 participants, it was admittedly underpowered to make any claims on the efficacy of PGx-guided warfarin dosing.

Anderson et al[28], likely the strongest study of the four, included 200 patients newly initiated on warfarin, with 99 participants receiving PGx-guided warfarin starting doses and 101 participants being dosed based on standard algorithms. Serious clinical adverse events were defined as the use of vitamin K, major bleeding events, thromboembolic events, stroke, myocardial infarction, and death (all cause). The study reported 5 and 4 serious adverse events in the standard and PGx-guided groups, respectively, a difference that did not reach statistical significance. Similarly, Caraco et al[31] did not report a significant difference in serious adverse events. They did, however, find that PGx-guided initial warfarin dosing resulted in significantly less instances of minor bleeding than standard dosing algorithms (3.2% vs 12.5%, $P<0.02$).

Limitations of Evidence

While the RCT's cited by the guidelines may not provide convincing evidence on their own supporting the use of pharmacogenetics in initial warfarin dosing, there are limitations to these studies that should be considered with their interpretation. Only two of the four studies included VKORC1 in addition to CYP2C9 genotyping, as a result their PGx-guided dosing algorithm would inherently be less accurate than ones that incorporate both genotypes.[32] Additionally, all the RCTs were relatively small with a combined study population of 544 participants. Warfarin randomized-controlled trials like that of Anderson et al[28] follow-up with patients regularly by design, allowing for a quick response to out-of-range INR levels. Frequent INR monitoring, especially in the beginning weeks of therapy, is likely to skew INR-related outcomes by minimizing the participant's time spent out of range regardless of which study arm they are in. This ability to frequently monitor INR is not guaranteed in a real-world outpatient setting where many patients manage their warfarin therapy. Clinical trials often report shorter monitoring intervals, more time spent in therapeutic range, and a lower rate of minor bleeding events than that of warfarin patients in the real-world setting.[33] Therefore, randomized controlled trials might underestimate the true impact pharmacogenetics has on the safety and efficacy of warfarin therapy. This suggests that in RCT's like those addressed above, secondary outcomes like those describing the accuracy of starting doses are likely more applicable than time spent in therapeutic INR range to the outpatient setting where regular and often follow-up is not guaranteed.

Cost Effectiveness of Pharmacogenetic Testing

Based on the analysis of three economic evaluations available at the time of publication, the ACCP/CHEST® guidelines determined that using pharmacogenetic testing to guide initial warfarin dosing was not cost-effective.[2] The three evaluations estimated the incremental cost-effectiveness ratio to range from $50,000-$170,000 per quality-adjusted life year (QALY) gained, with a sensitivity analysis from one study estimating this value to be as high as $300,000 per QALY gained.[34] The estimated cost of PGx testing among the three evaluations ranged from $400 to $550. Based on their results, Patrick et al predicted that genotype-guided warfarin dosing would become cost-effective for atrial fibrillation patients if it could reduce out-of-range INR values by 5%-9%.[35] In the economic evaluation performed by Eckman et al it was determined that although PGx-guided warfarin dosing does not appear to be cost-effective for the typical patient initiating warfarin, it may demonstrate cost-effectiveness when used in patients who have a high risk for hemorrhage.[34] Sensitivity analyses performed by Eckman et al predicted that for testing to be cost effective for someone with a typical bleed risk it would have to cost less than $200, be available within 24 hours, and prevent 32% of major bleeding events.[34]

Cost effectiveness in these economic evaluation studies was defined as an incremental cost-effectiveness ratio of <$50,0000 per QALY gained, as is standard in most pharmacoeconomic analyses.[36] It is worth noting that the standard "willingness to pay" threshold in healthcare of $50,000 per QALY gained was established in the early 1990's based on the cost of dialysis in the

1980's.[37] Many experts have suggested this threshold is outdated and should be adjusted to account for over 30 years of inflation and advancements in medical technology.[36,38,39]

UPDATED CLINICAL TRIAL EVIDENCE

Review of Randomized-Controlled Trials

Since the 2012 publication of the ACCP/CHEST® guidelines for anticoagulant management, numerous large and high-quality studies have been published on the use of pharmacogenetic-guided warfarin dosing. The largest of the more recent publications is a multi-center randomized-controlled trial called the Genetic Informatic Trial (GIFT).[40] GIFT reported clinical outcomes and anticoagulation control in 1650 participants undergoing hip or knee arthroplasty receiving perioperative warfarin therapy for the first time. Patients were randomized to receive either genotype-guided or clinically guided warfarin dosing for the first 11 days of therapy. Randomization was stratified based on race due to varying proportions of genotype frequencies in different populations (**Figures 1&2**). Genotype-guided dose recommendations were determined with the utilization of a web-based algorithm (warfarindosing.org) that incorporates clinical factors as well as genetic information into decision making. The primary outcome was defined as a composite of multiple adverse events including major bleeding, INR >4, or death within 30 days, and symptomatic or asymptomatic VTE within 60 days of arthroplasty. Patients were followed up with for a total of 90 days. GIFT found a statistically significant absolute reduction of 3.9% in the composite primary outcome with genotype-guided warfarin dosing. This reduction was largely driven by the number of patients with an INR of >4 after 30 days. The 1.4% absolute reduction in major bleeding, VTE and death observed in the genotype-guided group did not remain statistically significant when analyzed separately from excessive anticoagulation (INR ≥4).

The Genetic Informatic Trial was a multi-center trial conducted in mostly high-volume academic medical centers. Gage et al predicted that based on their results, genotype-guided warfarin therapy would likely have a larger benefit in low-volume medical centers with higher rates of adverse events.[40] These findings prompted Tse et al to conduct a systematic review and large meta-analysis of randomized-controlled trials comparing genotype-guided and standard warfarin dosing regimens, to which the GIFT trial contributed 20% of the total patient population.[41] The final analysis consisted of 18 RCTs, four of which were the same RCT's referenced by the 2012 ACCP/CHEST® guidelines for anticoagulant management. The remaining 14 were published between 2012 and 2017. This meta-analysis focused only on RCT's investigating newly initiated warfarin rather than maintenance therapy, and follow-up time ranged from 21 to 90 days with the mean between all included trials being 64 days. Dosing regimens between groups varied from trial to trial, although all compared a genotype-guided to a standard algorithm. PGx-guided algorithms included both CYP2C9 and VKORC1 genotyping in 16 of the 18 trials. Standard regimens also varied between trials, with 11 utilizing a "fixed-dose" technique and 7 determining starting doses with a "clinically informed" technique. A total of 2626 patients received genotype-guided warfarin dosing and 2604 received standard-dosing. The diversity of the total patient population, however, was lacking with the vast majority of studies including only Chinese and/or Caucasian patients. Other ethnicities such as African, Hispanic, and Native American were included in low numbers in just four trials.

Tse et al[41] reported a significant improvement in INR-related outcomes with genotype-guided warfarin dosing, resulting in an average improvement of 2.6 and 5.9 days in time to achieve a therapeutic INR and time to stable INR, respectively. Most of the included trials individually did not find a statistically significant reduction in clinical adverse events with genotype-guided therapy. Once the results were compiled the meta-analysis revealed that groups who received genotype-guided warfarin dosing were 18% less likely to experience a bleeding event; this now reached statistical significance. No difference was observed in the rate of thromboembolism or mortality between groups. A summary of the findings can be found in **Table 1**.

The investigators were able to use the absolute risk differences and calculate the number of patients needed to receive genotyping to reduce the number of adverse events by one. This was estimated to be only 40 patients for major bleeding, but 238 for a thromboembolic event.

Real-World Study

In addition to RCT's and meta-analysis discussed above, one 2019 retrospective cohort study conducted by Zhang et al[42] investigated a genotype-based warfarin dosing algorithm's effect on anticoagulation endpoints in a real-world setting. The 844 included patients were newly initiated on warfarin therapy within the enrollment period and had available CYP2C9 and VKORC1 ge-

Table 1 | Summary of Outcomes Comparing Genotype-Guided Warfarin Dosing to Standard Dosing Regimens[41]

Outcome	Mean Difference	P-Value	Clinical Interpretation (in terms of Genotype-Guided Therapy)
Time to Reach Therapeutic INR	-2.6 ± 0.3 days	<0.0001	Achieves therapeutic INR faster
Time to Reach Stable INR	-5.9 ± 2.0 days	0.01	Achieves a stable INR faster
Total Time in Range	+3.1 ± 1.2%	0.011	Spend more time in INR range
Excessive Anticoagulation (INR ≥4)	RR[a]: 0.87 CI[b]: 0.78—0.98	0.026	Less likely to be over anticoagulated
Bleeding Event	RR[a]: 0.82 CI[b]: 0.69—0.98	0.012	Less likely to have a bleeding event
Thromboembolism	RR[a]: 0.84 CI[b]: 0.56—1.26	0.4	Does not reduce risk of thromboembolism
Mortality	RR[a]: 1.16 CI[b]: 0.46—2.90	0.76	Does not reduce risk of mortality

[a]Relative Risk; [b]95% Confidence Interval

Table 2 | Summary of Outcomes Comparing Genotype-Guided Dosing to Standard Dosing Regimen in the Outpatient Setting[42]

Outcome	Genotype-Guided Dosing (n=413)	Clinically Fixed Dosing (n=431)	Mean Difference	P-Value
Time to Reach Therapeutic INR	10.21 ± 4.68 days	14.31 ± 8.26 days	-4.1 days	<0.0001
Total Time in Range (Days 4-14)	54.28 ± 21.90%	47.01 ± 26.25%	+7.27%	0.012
Total Time in Range (Days 15-28)	59.60 ± 20.12%	51.71 ± 18.96%	+7.9%	0.001
Excessive Anticoagulation (INR ≥4)	3.4%	5.1%	-1.7%	0.218
Major Bleeding Event[a]	0%	1.2%	-1.2%	0.062
Non-major Bleeding Event	0.2%	7.9%	-7.7%	<0.001
Thromboembolism	0%	1.2%	-1.2%	0.062

[a]Major bleeding events were those that resulted in death, caused chronic sequelae or consumed major healthcare resources

netic results. Initial warfarin doses were determined with a genotype-guided algorithm in 413 patients, and 431 received standard clinically fixed dosing. The study collected data for 90 days following the initiation of warfarin therapy.

Primary outcomes included days to reach a therapeutic INR range and total time spent in range. The genotype-guided cohort achieved a therapeutic INR an average of 4 days faster than the standard dosing cohort. Additionally, the total time spent in range in the first month of therapy was significantly higher in the genotype-guided group. Those who received standard clinically fixed warfarin dosing spent an average of 7-8% less time in range in the first month of therapy. Following one month of therapy, there were no significant differences between groups in the total time spent in range.

Secondary outcomes included clinical adverse events such as major bleeding, non-major bleeding, an INR ≥4, and thromboembolism. Major bleeding was defined as any bleed that resulted in death, was life threatening, or could cause chronic sequelae or consume major health-care resources. A total of 5 patients suffered from a major bleed and 5 patients suffered from a thromboembolism in the 90-day follow-up period, all of these patients were in the standard dosing cohort. However, the observed difference in thromboembolism and major bleed was not found to be statistically significant. Non-major bleeding was the most commonly reported adverse event. Only one patient in the genotype-guided cohort reported a non-major bleed, while 34 patients reported a non-major bleed in the standard dosing group. This observed difference in non-major bleeding did reach statistical significance. A summary of the findings can be seen in **Table 2.**

A limitation to the interpretation of this study is that the standard starting dose in Chinese healthcare settings, 2.5mg/day, is lower than what is considered standard practice in the United States.[2] A large proportion of Chinese patients show increased warfarin sensitivity and require lower maintenance doses than Caucasians.[43] The necessity of lower starting doses might be explained by the vast majority of the East Asian population expressing a VKORC1 genotype of AA or GA (**Figure 2**), which aligns with what was observed in the study population.[42] Despite the necessity in this population, the use of lower starting doses in standard dosing arms may limit the generalizability of this study to more diverse populations.

Cost Effectiveness of Pharmacogenetic Testing to Guide Warfarin Initiation

A systematic review performed by Zhu et al[44] in 2020 summarized literature evaluating the cost-effectiveness of pharmacogenetic testing in the setting of cardiovascular diseases. Warfarin and CYP2C9/VKORC1 were evaluated in 34.7% of the total included articles in the systematic review, six of which can be considered relevant to this paper. These six economic evaluations looked at initial warfarin dosing, were published in the last decade, and included both CYP2C9 and VKORC1 genotyping in their evaluation. The majority of the studies (66%) determined that pharmacogenetic testing in the setting of warfarin therapy was a cost-effective option for patients. The use of direct acting oral anticoagulants (DOACs) was compared to both PGx-guided and standard warfarin dosing regimens in three of the studies, and while two of the three concluded PGx-guided warfarin therapy was cost-effective compared to standard warfarin therapy, none found it more cost effective than DOACs.

When compared to standard warfarin dosing, Nshimyumukiza et al[49] found that PGx-guided dosing results in an incremental cost-effectiveness ratio (ICER) of $54,118. With a willingness to pay threshold of $50,000, this was not shown to be a cost-effective option for their patient population. Alternatively, You et al[50] found that PGx-guided warfarin initiation results in lower healthcare costs over the course of 25 years and gained more QALYs than standard warfarin therapy. This meant that standard warfarin dosing was dominated by PGx-guided dosing in their analysis. The economic evaluation was based on a decision analysis model (Markov Model) that incorporated evidence-based probabilities of warfarin related outcomes occurring in 65 year old newly diagnosed atrial fibrillation patients. Possible outcomes included remaining healthy or developing various complications such as major and minor bleeding or thromboembolism. Direct medical costs were estimated based on the cost of genetic testing, warfarin tablets, monthly clinic visits, and the one-time cost of a major event if applicable. A summary of all their conclusions can be seen in **Table 3**.

Previous economic evaluations referenced by the ACCP/

Table 3 | Conclusions from Cost-Effectiveness Evaluations[45-50]

Study (*Country*)	Comparison	WTP[a] Threshold	ICER[b]	Cost Effective for Genotype-Guided Dosing?[c]
Verhoef et al, 2016[45] *(United Kingdom & Sweden)*	Standard dosing vs. PGx[d] guided dosing	**UK:** £20,000-30,000 **Sweden:** 500,000 SEK[f]	**UK:** £6,702 **Sweden:** 253,848 SEK	Yes
Mitropoulou et al, 2015[46] *(Croatia)*		£40,000- 50,000	£31,225	Yes
Chong et al, 2014[48] *(Thailand)*		160,000 THB[g] ($5,333)	1,473,852 THB ($49,234)	No
Pink et al, 2014[47] *(United Kingdom)*	Standard dosing vs. PGx guided dosing vs. DOAC[e]	£20,000-30,000	£13,226	Yes, but not compared to DOAC therapy
Nshimyumukiza, et. al., 2013[49] *(Canada)*		$50,000	$54,118	No
You et al, 2012[50] *(United States and Canada)*		$50,000	Dominant	Yes, but not compared to DOAC therapy

[a]Willingness to pay; [b]Incremental cost-effectiveness ratio per quality adjusted life-year gained; [c]Compared to standard warfarin dosing; [d]Pharmacogenetic; [e]Direct-acting oral anticoagulant; [f]Swedish Kroner; [g]Thai Baht

CHEST® guidelines determined pharmacogenomic testing was not cost-effective for new warfarin users. These conclusions, however, were based on pharmacogenetic testing costing patients up to $550 out of pocket. In the last decade, accessibility to PGx testing has improved largely as a result of third party direct-to-consumer testing kits. Patients are now able to order large panels of relevant pharmacogenomic genes through online services. Cost varies depending on the company, and almost all take major health insurances although coverage is not common outside of the psychiatry setting.[51] Invitae© provides consumers with a pharmacogenetic panel of 38 genes, including CYP2C9 and VKORC1, for $250.[52] This is less than half of the predicted price in previous cost-effective analyses. GeneSight© allows consumers to order various pharmacogenetic panels depending on their needs. The Psychotropic Panel includes CYP2C9 genetic testing and can be provided to Medicare Part B, and sometimes Medicaid, beneficiaries for $0 out of pocket.[53] Genesight© partners with commercial health insurance companies, as well, although coverage varies. They report over 95% of patients paying $330 or less out-of-pocket for testing. In addition to the expansion of direct-to-consumer PGx testing, large health systems have also begun the implementation of pre-emptive pharmacogenetic panel testing.[54,55] Pre-emptive PGx panel testing, as well as the expansion of third-party PGx testing, have potential to increase the number of patients newly initiating warfarin with relevant pharmacogenetic results already available for use.

Clinical Discussion

Determination of warfarin starting doses is notoriously complicated by the many factors at play influencing response to therapy. Additionally, there are various guideline recommendations available for appropriate starting dose regimens. Regardless of the complexities, providers attempt to consider all relevant information and use their best clinical judgement to predict a starting dose that will allow the individual to achieve a therapeutic INR in a safe and effective manner. As of 2012, the American College of Chest Physicians recommended against the use of pharmacogenomics in the setting of warfarin therapy, citing a lack of evidence and not being cost-effective. With a decade of medical advancements and improved accessibility to testing it was important to reevaluate the evidence behind this recommendation.

Genotype-guided warfarin dosing is widely studied and seemingly validated in majority white and Asian populations. There continues to be a lack of diversity in more recent studies investigating CYP2C9 and VKORC1's use in practice, which can limit the scope of their application.[56] CPIC's dosing nomogram takes the lack of representation into account in their 2017 guidelines for pharmacogenetic guided warfarin dosing.[17] This is a unique approach intended to modify the application of genotype-guided warfarin therapy in hopes of improving equity in patient populations where the evidence is lacking at this time. The feasibility for integrating genetic information into warfarin dosing doesn't require a robust background in genetics. An online web-based algorithm (warfarindosing.org) allows providers to input genotypes, along with other relevant clinical factors, to calculate an appropriate starting dose for their individual patients.

Presence of a single nucleotide polymorphism in the CYP2C cluster (rs12777823) genotype and expression of CYP4F2*3 have more recently been proposed as potential contributors to inter-patient variances in warfarin dose requirements. CYP4F2 is thought to account for 1-2% of observed variances, much less than that of CYP2C9 and VKORC1. A recent systematic review and meta-analysis did not find CYP4F2 to be associated with varying warfarin dose requirements in African patients but did find rs12777823 the be associated with inter-patient variability in the same patient population.[57] While warfarindosing.org allows users to input CYP4F2 and rs12777823 genotype if available, there does not seem to be enough evidence at this time to recommend their widespread use in clinical practice.

Randomized-controlled trials investigating the impact of genotype-guided therapy cannot take into account the many variables that exist in a real-world outpatient setting. This includes factors such as missed doctor appointments, longer follow-up periods, and worse adherence which are seen at higher rates outside of a controlled trial setting. These factors may be even more relevant to narrow therapeutic index drugs, such as warfarin, that

require regular monitoring. Patients who are not followed up with regularly would likely benefit more from an accurate starting dose than what results from a randomized-controlled trial would show. Real-world studies like that of Zhang et al[42] are crucial to investigate the true impact of implementing genotype-guided warfarin dosing in practice. Results reported by Zhang et al show a larger improvement in warfarin therapy management and non-major bleeding outcomes with genotype-guided dosing than what was seen in RCTs. This seems to suggest that controlled trials underestimate the effect of genotype-guided dosing in new warfarin users, but there is a need for additional studies of similar methodology to confirm these findings in more diverse populations.

Based on recent RCT's as well as a real-world study surrogate outcomes such as time to therapeutic INR and time spent in range seem to be improved from using pharmacogenetic guided warfarin initiation. Not only are these important considering they are predictive of clinical outcomes, but they also can impact a patient's quality of life on their own. Taking a longer time to achieve a therapeutic INR range results in more clinic visits and more dose adjustments. This can cause an increased burden on the patient in the form of lost wages and unpleasant side effects, as well as an increased burden on the clinic from frequent follow-up appointments. Time spent in therapeutic range is most crucial in the first 30 days of therapy; this time frame is also when genotype-guided dosing appears to have the most pronounced impact on outcomes for new warfarin users.

Conclusion

Conflicting evidence exists regarding the use of CYP2C9 and VKORC1 genotyping to determine warfarin starting doses. The correlation between genotype-guided warfarin therapy and INR-related outcomes such as time to reach therapeutic levels or time spent in range appears to be well established based on review of recent literature. Recent data suggests that it may improve time to therapeutic range by 2.6-4 days. The association between genotype-guided dosing and a reduction in adverse events, on the other hand, remains to have conflicting evidence existing in the literature. A recent meta-analysis indicates that genotype-guided warfarin dosing may significantly reduce the relative risk of bleeding events by about 18% compared to standard dosing (**Table 1**). Evidence supporting this reduction was consistent in a real-world outpatient setting with 0.2% and 7.9% of patients in the genotype guided and standard dosing groups reporting a non-major bleed (**Table 2**). With bleeding being reported as the most common side effect of warfarin users, and major bleeding occurring in up to 5% of patients, this is certainly significant.[58] Despite the trend in decreasing major or minor bleeding events with the use of genotype-guided warfarin therapy, this does not seem to be the case for all clinical adverse outcomes. Evidence showing a decrease in thrombotic events or overall mortality is still lacking. There appears to be no association between genotype-guided warfarin dosing and an improvement in these outcomes based on the current literature.

Historically, genotype-guided warfarin therapy has not been considered cost-effective by published guidelines. Although there appears to be more evidence supporting the cost-effectiveness of CYP2C9 and VKORC1 testing for warfarin therapy to date, there are still conflicting conclusions in the more recent literature. There is a need for cost effectiveness analyses in diverse populations before definitively determining whether ordering genetic testing prior to starting warfarin is cost-effective for the general population. It may be beneficial to reevaluate the cost-effectiveness of genotype-guided warfarin therapy in the United States. With the expansion of third party and pre-emptive panel testing, however, there is an increasing chance that genetic information might already be available. In these scenarios, cost would not be relevant to the evaluation of their use in practice.

Pharmacogenomics is not the only factor, but it is undeniably a relevant factor, for providers to consider when initiating warfarin. Recent literature reports no evidence of an increase in harm to patients after using genotype-guided warfarin dosing, shows there is likely a benefit in INR management, and might reduce the instance of bleeding events. Available evidence suggests no reason to date for not using CYP2C9 and VKORC1 genotypes to help guide warfarin dosing if already available or affordable to the patient. With the changes in genetic testing availability and current evidence, it would be reasonable for the ACCP/CHEST® guidelines to consider softening the language against the use of CYP2C9 and VKORC1 genotyping to help guide warfarin dosing.

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