CLINICAL PRACTICE

Warfarin Pharmacogenetics: Ready for Clinical Utility?

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ABBREVIATIONS: CYP2C9 = cytochrome P450 2C9; INR= international normalized ratio; PGx = pharmacogenetic; VKOR = vitamin K epoxide reductase; VKORC1 = vitamin K epoxide reductase, complex 1; AERS = Adverse Event Reporting System

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Despite the Federal Drug Administration's August 2007 relabeling of warfarin to recommend pharmacogenetic (PGx) testing, the clinical application remains controversial. Many questions exist regarding how information gleaned from genetic testing can be applied in warfarin therapy. In particular, does PGx testing lead to a shorter time to stable INR compared to prudent international normalized ratio (INR) monitoring coupled with the consideration of age, BMI, diet, and physical condition? Does it reduce clinical complications? Other topics of uncertainty include whether the correct warfarin dose can be obtained based on genotype, whether PGx testing is cost-effective, and turn-around-time. Despite the reservations about warfarin PGx testing, there are several subsets of patients for whom such testing could be beneficial.

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BACKGROUND

Warfarin sodium is a commonly prescribed anticoagulant used for the prevention of thromboembolic events and treatment of thromboembolic disorders. The annual number of outpatient warfarin prescriptions increased by 45% from 1998 to 2004 in the U.S., from 21.1 million to 30.6 million [1]. With the aging population and projected increased prevalence of atrial fibrillation, the number of warfarin prescriptions is predicted to continue to increase[1,2].

There are many challenges in regulating warfarin dosing. Warfarin has a very narrow therapeutic window and when the prothrombin time or INR falls outside of target range, there is an increased risk for major bleeding or thrombotic complications. Typically, the INR during warfarin treatment should optimally fall between two and three for most patients, although the target INR may vary depending on indication for treatment[3,4]. A large study demonstrated that the minimum number of deaths and brain vessel events occurs at INRs of 2.24 and 2.38, respectively[5]. Warfarin is the most frequent drug implicated in U.S. emergency department visits[6]. From 1993 to 2005, based on data from the FDA's Adverse Event Reporting System (AERS), warfarin-associated cases had rates of 86% for bleeding with serious outcome and 10% fatal bleeding[1]. This is in contrast to all drugs reported to the AERS for that same period in which 30% of the cases had serious outcomes and 7% had fatal outcomes. Thus, compared to other drugs, warfarin has a high frequency of adverse events, with a high frequency of serious and fatal outcomes.

Warfarin therapy often requires multiple titrations to achieve a stable, target INR. There is a large inter-patient range of warfarin dosing requirements, with doses on the low end of the range associated with warfarin sensitivity and doses on the high end of the range associated with warfarin resistance. Age, weight, concomitant medications, co-morbidities, and genetics, play into the variability of warfarin dosing; many of these are related to the metabolism of warfarin.

Genetics is an important factor in warfarin dosing variability, especially as it relates to warfarin sensitivity. Using clinical factors alone, one study demonstrated the ability to control 17–22% of warfarin dosing variability; when genetic factors were added, 53–54% of warfarin dosing variability was abrogated[7]. Other studies have shown the genetic contribution to warfarin dosing variability to be as high as 40%[8]. Because of the relatively high contribution of genetics to warfarin dosing variability, in addition to other factors, the FDA relabeled warfarin in August 2007 to recommend PGx testing for warfarin therapy.

WARFARIN PHARMACOGENETICS

Warfarin is a racemic mixture of S- and R-enantiomers, with S-warfarin providing about 70-80% of its activity[9,10]. Most S-warfarin is metabolized by the cytochrome P450 enzyme CYP2C9, which is encoded for by the *CYP2C9* gene. Two polymorphic regions in *CYP2C9*, *2 (Arg144Cys) and *3 (Ile359Leu), are associated with warfarin sensitivity. These polymorphisms lead to decreased CYP2C9 enzymatic activity, resulting in slower S-warfarin clearance, longer half-life of S-warfarin and a prolonged interval to steady state[11]. As vitamin K antagonists, both R- and S-warfarin inhibit vitamin K epoxide reductase (VKOR), encoded for by the vitamin K epoxide reductase, complex 1 (*VKORC1*) gene. A common *VKORC1* promoter polymorphism at 1639G>A, resulting in decreased promoter activity and decreased production of VKOR, is associated with warfarin sensitivity. Another gene, *CYP4F2*, has recently been described to contribute to an approximately 1 mg/day decrease in necessary warfarin dose between wild type individuals and those with the variant allele[12]. Other genes have additionally been implicated in contributing to warfarin dosing variability, but on a much smaller scale than *CYP2C9* and *VKORC1*. Most of the current state of knowledge regarding warfarin pharmacogenetics relates to warfarin sensitivity attributed to *CYP2C9* and *VKORC1*, and several clinical laboratories now offer PGx testing for warfarin sensitivity based on these polymorphisms.

Meanwhile, interest in the genetic basis for warfarin resistance is gaining momentum. A recent report demonstrated that *VKORC1* variants associated with warfarin resistance occur at a higher frequency than previously thought[13]. With increased evidence for genetic bases for warfarin resistance, PGx testing in this arena may eventually become a reality.

CLINICAL UTILITY OF WARFARIN PGX

Clinical Outcomes. Whether warfarin PGx testing reduces clinical complications and shortens time to stable INR will likely have a major impact on its uptake in the clinical setting. Multiple retrospective analyses have demonstrated associations between *CYP2C9* and/or *VKORC1* variants and bleeding risk[14-19]. Other studies have not shown an association, however, many studies failed to consider the cumulative effect of *CYP2C9* and *VKORC1* variants. Schalekamp et al demonstrated heterozygous carriers of *VKORC1* and *CYP2C9* variants have a much higher risk of severe over-anticoagulation, compared to individuals with none or one variant[20]. The cumulative effect of *CYP2C9* and *VKORC1* variants on time to first INR >4 has also been illustrated[19].

A prospective, randomized controlled trial by Caraco et al, investigated outcomes in 185 patients with *CYP2C9* genotype-informed vs. genotype-uninformed warfarin therapy, from initiation of dosing through stabilization of therapy[21]. Individuals with *CYP2C9* genotype-guided therapy reached a shorter interval to first therapeutic INR (2.73 days, $p<0.001$) and stable anticoagulation (average of 18 days earlier, $p<0.001$), longer time spent in the therapeutic range (80.4 vs. 63.4%, $p<0.001$), and a lower bleeding incidence (3.2 vs. 12.5%, $p<0.02$), compared to the genotype-uniformed group.

Percent out-of-range INRs from another prospective, randomized controlled trial did not differ significantly between genotype-guided vs. genotype-uninformed treatment arms for the entire study population (n=200)[22]. However, results were significantly different between wild-type and multiple variant carriers, and the genotype-guided treatment arm required fewer and smaller dose adjustments and fewer INR measurements compared to the genotype-uninformed treatment arm.

While many studies have demonstrated associations between *CYP2C9* and/or *VKORC1* variants and, for example, bleeding risk or out-of-range INRs, there is still controversy regarding these studies. Additionally, the two randomized controlled trials performed to date had small study populations and had some conflicting results. Thus, it is clear that large, multicenter randomized controlled trials are needed to more accurately determine whether pharmacogenetic-guided warfarin treatment has a significant impact on clinical outcome.

Impact of timing. There is disagreement about whether optimal warfarin dosing should include genotyping prior to initiation of therapy. Variants in *CYP2C9* and *VKORC1* may affect the time for plasma warfarin concentration to achieve therapeutic levels (*VKORC1*) and steady state (*CYP2C9*)[23].

Reynolds et al maintain that it is not critical to incorporate genotyping results into the initial warfarin dose estimate because of delayed time to steady state due to *CYP2C9* variants[23]. However, the impact of the timing of *CYP2C9* and *VKORC1* genotyping is probably not yet fully understood, and whether incorporating genetic information into the initial warfarin dose is ultimately beneficial for the patient remains to be determined. Nonetheless, limits in technology and resources may prohibit many laboratories from providing one-day turnaround for pharmacogenetic tests. Additionally, many hospitals and clinics rely on send-out pharmacogenetic testing. Thus, at the present stage, genotyping results will likely be available on days 2-4 following specimen receipt.

Genotype-guided warfarin dosing. Although *CYP2C9* and *VKORC1* variants are associated with warfarin dose variability, there is no current clinically validated dosing algorithm that incorporates genotype. However, many research-based dosing algorithms that incorporate clinical and genetic factors have been developed and published[24-32]. Some may fail to account for all genotypes (e.g. *CYP2C9* *1/*2 vs. *1/*3), genes (e.g. *CYP2C9* and *VKORC1*); and they may not include logarithmic transformation for warfarin dose[11]. In addition, many dosing algorithms apply to only one ethnic or racial group, reflecting the variable allele frequencies among such groups.

Despite these limitations, warfarin dosing algorithms that incorporate genetic information have been shown to predict up to 62% of the variability in warfarin dosing[30]. By reviewing INRs after three warfarin doses, up to 79% of warfarin dosing variability can be corrected by an algorithm (found at www.warfarindosing.org)[28]. This algorithm was developed on data from 1015 patients and was prospectively validated in 292 additional patients[7]. It is comparatively comprehensive in the clinical and genetic factors it accounts for; further, it allows for the inclusion of INR values with or without genotyping information[11,33].

Studies examining clinical outcomes related to prospective dosing of warfarin based on genetic and non-genetic factors are limited. For example, a recent prospective study evaluating a dosing algorithm incorporating *CYP2C9* and *VKORC1* genotypes and clinical parameters in a warfarin naïve Hans-Chinese population improved time to stable INR and reduced adverse events with the evaluated dosing algorithm[30]. However, this study lacked a control group of non-pharmacogenetic dosed individuals. Thus, while many pharmacogenetic-based dosing algorithms are available, in order to assess whether these algorithms result in improved clinical outcomes, large randomized controlled trials are necessary.

PUTTING WARFARIN PGX TESTING INTO PRACTICE

Despite many of the unanswered questions and gaps in knowledge regarding warfarin PGx testing, there have been many published examples in which such testing has been proven to be beneficial[34-38]. Furthermore, while standard of care is questionable, there are also several subgroups of patients for whom warfarin PGx testing could be considered currently. First are individuals with a family history of difficult warfarin titration. These individuals are more likely to have a genetic predisposition to warfarin sensitivity (or resistance). Second are pre-surgery patients receiving total joint or valve replacement whose circumstances, including altered diet, concomitant medications, and inactivity, could confound warfarin dosing variability in this group of patients. By genotyping these patients, at least approximately 30-40% of the dosing variability could be controlled for. Furthermore, genotyping can be performed prior to initiating warfarin treatment in this set of patients, thereby providing for more optimal pre-treatment counseling. A third group of individuals for whom warfarin PGx testing might be beneficial in its current state are individuals who may have a longer wait until their first INR measurement because of the timing of their initial visit to the coagulation clinic (e.g. before a weekend or holiday). A fourth group of individuals might be non-local patients who will be returning to their local setting where their follow-up care is uncertain. Thus, while limited clinical outcomes data has supported warfarin PGx testing for routing clinical care, certain subpopulations of patients could benefit from genetic testing.

CONCLUSION

As described previously, the three main hurdles to translating validated PGx markers into clinical practice are Reluctance, Regulation, and Reimbursement[39]. Warfarin PGx is certainly no stranger to the three Rs, and until clinical outcomes data and other important issues as described above can be better addressed, it is likely that warfarin PGx testing as standard of care will remain controversial. Proven clinical utility in multiple patient populations using clinically validated dosing algorithms will help determine whether or not warfarin PGx testing will become part of standard clinical practice. In the meantime, genotyping of specific subgroups of patients being initiated on warfarin therapy could prove beneficial.

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