Can an old drug be taught new pharmacogenetic tricks?

Elizabeth Silverman

Despite warfarin's continued presence near the top of the FDA's list of adverse drug events and the availability of competing agents, the drug continues to be a mainstay of anticoagulant therapy, particularly among general practitioners. Its narrow therapeutic window and intra- and interpatient variability require regular measurement of the international normalized ratio. This, along with the large genetic component to warfarin response, principally contributed by variants in the genes VKORC1 (–1639G → A) and CYP2C9 (*2 and *3), led many to hypothesize that pharmacogenetics could improve warfarin safety.

The results of three clinical trials designed to test this hypothesis were published in the Dec. 12, 2013 *New England Journal of Medicine* (369[24]: 2283–2293;2294–2303;2304–2312), and their interpretation proved challenging. Choice of patient population, study design, statistical power, the economics of pharmacogenetic testing, and the problems of rare variants, particularly those that may be race based, were among the issues the trials raised and that will likely have an impact on future pharmacogenetic trials.

The U.S.-based COAG trial was a multicenter, double-blind randomized, controlled trial of 1,015 patients assigned to receive a warfarin dose based on a clinical algorithm or on a clinical algorithm that included genotype. After the first three days a dose revision algorithm was used, and after five days doses were adjusted using INR tests. The primary endpoint was the percentage of time the INR was in the therapeutic range from day four or five through day 28.

The second trial (EU-PACT) was conducted in the United Kingdom and Sweden. It enrolled 455 patients and was a single-blind, multicenter, randomized, controlled trial that compared a genotype-guided group with a group that received standard dosing of 10 mg on day one, 5 mg on day two, and 5 mg on day three. Doses thereafter were determined according to local clinical practice. The primary endpoint was percentage of time in the therapeutic range.

The third trial, a component of EU-PACT, did not use warfarin but two other vitamin K antagonists, acenocoumarol and phenprocoumon, that have longer half-lives than warfarin. The 548 patients in this study were from two single-blind randomized trials brought together because of low enrollment. The trial was similar to COAG in that patients in one arm received initial drug doses that were determined using a clinical algorithm that contained genotype information, while patients in the other arm received doses based on the clinical algorithm alone. The endpoint was percentage of time in the therapeutic range in the 12 weeks after initiation of therapy.

There are two other trials: a randomized, controlled trial conducted at the University of North Carolina (Jonas DE, et al. *Pharmacogenomics*. 2013;14[13]:1593–1603) and the still ongoing GIFT trial, the results of which are expected at the end of this year. The North Carolina trial studied 109 patients randomized to receive dosing determined by an algorithm containing genetic plus clinical information or only clinical information. Primary endpoints were time in the therapeutic range and number of anticoagulation visits over 90 days. Secondary endpoints were time to therapeutic dose, INR>4, emergency visits, hospitalizations, hemorrhagic events, thrombotic events, and mortality.

The GIFT trial is similar in design to COAG, but instead of an adjustment of dose on day four or five based on a dose refinement algorithm, GIFT uses the dose refinement algorithm through day 11. This trial includes an additional gene variant, CYP4F2, that affects the rate of vitamin K metabolism and is associated with warfarin resistance.

Contrary to hopes and expectations, the trials that compared use of a clinical algorithm alone to a clinical algorithm plus genotype showed no statistically significant difference between the two arms. However, in the COAG trial there was a difference between dosing strategy and race, with African American patients, who made up

27 percent of each arm, faring worse when genotyping was included. This was thought to be a reflection of the fact that variants specific to African Americans were not included in the algorithm. Though this trial was not powered to detect differences in bleeding or thromboembolic events, no difference between the control and experimental arms was observed in the four-week follow-up period.

These results were echoed by the similarly designed EU-PACT trial that used alternative vitamin K antagonists, with the exception of the African American component, as the European clinical trial subjects were more than 95 percent Caucasian. In the North Carolina study, genotype-guided dosing did not reduce the number of visits or improve time in the therapeutic range. However, the genotyping patients experienced fewer hospitalizations and hemorrhagic and thrombotic events and lower mortality, though the data were not statistically significant. It was only when genotyping was compared with standard dosing in the warfarin EU-PACT trial that a statistically significant difference was found that favored the use of genotyping. There were also fewer incidents of excessive bleeding in the genotype group, and the mean time to reach therapeutic INR was statistically significant at 21 days versus 29 days in the control group.

Taken together, the results of these studies do not appear to favor a role for pharmacogenetics in managing warfarin. However, Charles Eby, MD, a member of the COAG trial steering committee and professor of pathology and immunology and associate chief of the Division of Laboratory and Genomic Medicine, Washington University School of Medicine, says, “Comparing these two warfarin trials [COAG and EU-PACT], with a purely Caucasian population in Europe, pharmacogenetics dosing is superior to standard-of-practice dosing for time within therapeutic range at 30 days, and that standard-of-practice dosing performed just as well as the clinical algorithm dosing in the COAG trial.” At least for Caucasians, then, “Pharmacogenetic dosing may have some benefit for time within therapeutic range.” Whether that would reduce the number of adverse clinical outcomes, he says, would have to be extrapolated.

Dr. Eby

Given the studies’ limitations, others too feel the jury is still out. Alan Wu, PhD, a COAG investigator and professor of laboratory medicine at the University of California, San Francisco, and chief of the clinical chemistry laboratory at San Francisco General Hospital, says the wrong patient population was enrolled because patients at anticoagulation clinics are not the typical patient population. “Much of warfarin is given in primary care by doctors who don’t have the same degree of expertise,” he says. “Therefore, benefit such as seen in the EU-PACT trial where they just gave 5 mg would be more evident in an all-comers general practice environment than what we see in COAG.”

Dr. Wu notes, too, the high degree of compliance in the COAG patient population. “What if you don’t have a compliant patient? What if somebody is just given one shot to get the dose right? In my practice at San Francisco General, that’s the norm.” In similar indigent populations, pharmacogenetics could be of great utility. Comparing pharmacogenetics with a gold standard, such as a clinical algorithm that clearly works, is not the best way to determine utility, in his view.

The design of the control arm was a subject of much debate within the COAG group itself, given that most physicians do not use the clinical algorithm. Tolerance for trial and error is ingrained in warfarin clinical practice, Dr. Eby notes, adding that the steering committee members for the COAG trial had many discussions about trial design. “Ultimately,” he says, “the commitment was to perform a randomized, controlled trial with as few different variables between the control arm and the pharmacogenetics arm, recognizing this was not the standard of

practice but also recognizing it would provide the best scientific information for the additional value of pharmacogenetic testing."

Dr. Johnson

As a result, says Julie Johnson, Pharm D, an author of the COAG paper and dean and distinguished professor of pharmacy, University of Florida College of Pharmacy, the results are not surprising. "The COAG trial was designed in a way that made it extraordinarily difficult to show benefit from genetic-guided therapy. The clinical algorithm gets you closer than a standard dose—we knew that in 2009 from a *New England Journal of Medicine* article on which I was the senior author. The trial also employed frequent INR monitoring, probably more frequently than happens anywhere in the U.S., so between the clinical algorithm and the very, very frequent INR monitoring, it made it hard for the pharmacogenetic arm to show a difference." The external validity of the EU-PACT trial was much higher than the external validity of the COAG trial because it matched a real-world situation much more closely, she agrees. Just as Dr. Wu believes that pharmacogenetic testing for warfarin might give you the biggest bang for the buck in urban settings, Dr. Johnson feels similarly about rural populations, where standard dosing and limited INR testing prevail.

Dr. Weck

University of North Carolina study author Karen Weck, MD, a professor in the UNC Division of Pathology and Laboratory Medicine and director of the medical genetics laboratory, wanted to include a third arm in the UNC study that would include data from historical standard practice. Ultimately, a completely prospective randomized trial design was chosen even though it was recognized that coagulation control is better when a clinical algorithm, rather than standard dosing, is used and when patients are treated in specialized coagulation clinics that perform frequent INR testing. While this level of care does not reflect the experience of most patients, Dr. Weck notes, "The reason our study and the COAG study were designed the way they were is because that's the best way to directly analyze the impact of genetic testing." Another limitation is that the initial dosing period during which pharmacogenetics was used was very short, and it was only a few days before INR results guided dosing. Dr. Weck also notes that diet and compliance cannot easily be controlled for, both of which can have an effect on results. Evidence-based research is important, but "we could be shooting ourselves in the foot because of the way the trials are designed," she says.

Dr. Gage

Brian Gage, MD, principal investigator for the GIFT trial and professor of medicine at Washington University School of Medicine and medical director of the Barnes-Jewish Blood Thinner Clinic, hopes that the slightly different design used in GIFT might yield more definitive and positive results. Given the conflicting results of the COAG and EU-PACT trials and the trend in COAG for fewer bleeds in patients randomized to pharmacogenetics dosing, it's critical, he says, that GIFT, the third multicenter pharmacogenetic trial, titled Genetics Informatics Trial of Warfarin Therapy to Prevent Deep Vein Thrombosis, be completed.

"Like COAG, but unlike EU-PACT, GIFT is a double-blind trial, so preliminary results are not available. GIFT differs from COAG and EU-PACT in that it includes an additional gene, CYP4F2, and up to 11 days of pharmacogenetic dosing, rather than five. Thus," he says, "the putative advantages of pharmacogenetic dosing may be magnified in GIFT."

This raises the question: Why does standard dosing not include the use of a clinical algorithm, particularly with one available free at www.warfarin dosing.org? For one, there's a lack of knowledge about the use and availability of the algorithm, a problem that pathologists can help remedy in their role as consultants to clinicians, Dr. Eby says. He also suggests that the study results might be an opportunity for the FDA to encourage the use of a clinical algorithm. Says Dr. Johnson, "The bottom line—and this is consistent with the editorial in the New England Journal and the commentators at the American Heart Association meeting where the two studies were presented—is that we have to stop this fixed-dose initiation of warfarin. We just have to move to a clinical algorithm."

But clinical practice can be slow to change, she acknowledges, and considering that adverse events are rare (though the numbers are collectively high given the drug's wide use), many physicians are confident in their ability to manage warfarin. Moreover, diminishing reimbursement by public and private payers leaves primary care providers with little appetite for new steps or practices in patient care that consume valuable, nonreimbursable time, especially when there is little perceived patient benefit.

Although the underlying purpose of all the studies was to determine if the use of pharmacogenetics could make warfarin use safer, the rarity of adverse events makes direct measurement of such events challenging. None of the studies was powered to achieve this, and all use time in INR as a surrogate. Says Dr. Wu: "A trial that would demonstrate the utility of pharmacogenetic testing cannot be done. You would have to recruit thousands of doctors, and they are interested in clinical practice, not clinical trials."

In Dr. Eby's view, the trial would be feasible but unfundable. For the newer drugs there are active surveillance programs that are likely to provide more accurate information about bleeding risks, he says. And clinical trials do not mirror daily medical practice or the patients who are cared for daily. "But I don't think there will be that same quality of information about warfarin. It's a generic drug that's been around for more than 50 years." A meta-analysis across trials would be possible, but Dr. Johnson points out that the European studies didn't collect the bleeding data the same way the U.S. trials did, and this highlights the kind of problems encountered when attempting this type of analysis. However, a privately held company, Iverson Genetics, is conducting a trial in which adverse events are the primary endpoint. The trial is a multicenter, randomized trial of 3,300 patients, 65 years and older, that is funded by the Centers for Medicare and Medicaid Services and tests a clinical algorithm with and without the results of the company's genotyping test. Results of this study are due at the end of 2015.

The COAG study demonstrates the weakness in understanding variants that affect African Americans, who did worse when the genetic algorithm was used, most likely because the variants that affect their response to warfarin were not included in the genetic test. "It's still not well understood," Dr. Weck says, "what all the factors are that are associated with the difference in warfarin response in African Americans. This is another limitation of the studies."

Says Dr. Gage: "The pharmacogenetic dosing algorithms used in COAG and EU-PACT were very accurate in non-black participants—the R2 was 0.75 by day four or five. In African American participants the R2 was only 0.4. The pharmacogenetic dosing algorithms do not include rare polymorphisms that might have improved the accuracy of

pharmacogenetic dosing in African Americans: CYP2C9*5, CYP2C9*6, CYP2C9*8, CALU rs339097, and CYP2C19 rs12777823." Individually, he says, each of the SNPs plays only a small role in predicting warfarin dose. "But when considered together, they might improve the accuracy of pharmacogenetic dosing algorithms considerably in African Americans."

Given the diversity of the U.S. population, the inclusion of ethnic-specific variants is an issue likely to recur in future pharmacogenetic and pharmacogenomic studies. It also underscores the difficulty in identifying and studying rare variants in general, race based or not. Though it is the common variants that are the most easily identified and therefore studied, it is the rarer variants that could have the greatest impact on drug safety—another reason the results of the COAG and EU-PACT trials were not surprising. Says Dr. Weck: "I have always felt that we are missing the most important factors, which are the rare genetic variants that have a high impact on warfarin dose response. For example, those would include the rare patients who are CYP2C*3 homozygotes who have extreme warfarin sensitivity and require very low doses of warfarin." Such patients require only 1–2 mg of warfarin per day and are at most risk of adverse events. (The COAG and EU-PACT warfarin trials had one each of these patients and the UNC trial had none.)

"Current clinical trial designs are not optimal for studying rare variants," Dr. Weck says. "We could risk throwing the baby out with the bath water for warfarin genetics and pharmacogenetics in general because we have been focusing so much on the common SNPs associated with a comparatively minor effect on response."
Though the presence of rare variants that cause warfarin sensitivity and raise the risk of bleeding are perhaps of greatest concern, the rare variants associated with warfarin resistance can create a different set of clinical complications. Says Dr. Wu: "If you look at the regression analysis between VKOR and 2C9 and dose, it's only about 50 to 60 percent in terms of an R2 value, which means there is still great variability between predicted and actual dose needed to maintain a stable INR. And, in my opinion, genotyping is effective for predicting warfarin sensitivity, but the remaining variability is due to the absence of genomic markers that predict warfarin resistance, that is, someone who needs more than the standard 5-mg dose." This is ongoing, he adds, and identifying additional genes for an R2 value of 70 to 80 percent may be possible. "That could stimulate a whole other round of trials," he says, "assuming warfarin is still around."

This was considered when the GIFT trial was designed; it will include a marker of warfarin resistance—the first time this variant has been used in a large, prospective trial. Study subjects are hip and knee replacement patients, who have a higher incidence of thrombotic complications than the general anticoagulant population. Nevertheless, rare variants make demonstrating warfarin pharmacogenetic utility a challenge. "The underlying goal is to protect outliers from complications," Dr. Eby says. "That guarantees they are diluted by a larger number of patients whose management is not improved by pharmacogenetics. This issue may always be there for pharmacogenetics. We are trying to benefit the rare patients but can't find the rare patients until we genotype everybody."

Powerful economics are at work that make continued investment in warfarin genetic research unlikely. Warfarin is an inexpensive generic drug, and pharmacogenetic data would have to be compelling to persuade payers to reimburse for a $200 to $300 genetic test before the drug's use. This is especially true in light of the latest studies pointing to a clinical algorithm being equally effective. But the reimbursement issue is not solely related to warfarin's status as a generic. Payers often do pay for a pharmacogenetic test for the generic clopidogrel, Dr. Johnson says. In her center they find that about 28 percent of patients have a genotype that indicates the drug will have reduced efficacy, a percentage that makes testing more cost-effective than the hunt for rare variants. In addition, hospitals facing no Medicare reimbursement for readmissions within 30 days realize they can pay for a lot of clopidogrel genetic testing with the money saved from one readmission they will not be paid for, Dr. Johnson says.

Dr. Laposata

Clopidogrel may have made a better high-profile pharmacogenetics test case than warfarin. Whether genetics are used or not, warfarin dosing is adjusted using INR testing whereas platelet function tests do not carry the same clinical weight. Michael Laposata, MD, PhD, the Edward and Nancy Fody professor of pathology at Vanderbilt University School of Medicine and soon to be chair of pathology, as of July 1, at the University of Texas Medical Branch at Galveston, says a consulting group estimates that the cost of treating a patient who rethrombosed a stent is $25,000, and assuming 60 adverse clopidogrel-related events among a data set of 6,400 interventional cardiology patients, the cost is $1.5 million. As far as clopidogrel is concerned, genetic testing would more or less pay for itself when the price of the serious complication is taken into account. Of warfarin pharmacogenetic testing, Dr. Laposata says everyone was asking the big question: “Is anybody dying because we are not doing it? Does anybody have morbidity or more strokes, or anything like that? So we thought these trials would land on the same side of the street and we could figure this out. They didn’t.”

Despite the difficulty in making a similar cost-effectiveness case for warfarin, Dr. Laposata sees value in the genetic tests. He believes that safety would be greater if patients knew they carried warfarin sensitivity variants, and he cites a hypothetical case of a postsurgical restart in a warfarin-sensitive patient for which a physician might not scrutinize a chart and assume a standard 5-mg dose is safe.
The continued study of warfarin pharmacogenetics and the ability to make a cost-effectiveness argument for testing are hindered also by genetics not being the only factor to have an impact on warfarin safety. Most adverse events occur in the initial dosing period, but warfarin levels long term are influenced also by dietary intake of vitamin K and the addition of other medications.

The introduction of new oral anticoagulants will play a role, too, in how much additional effort will be put into warfarin pharmacogenetics. When COAG was initiated in 2009, none of the new agents had been approved. Unlike warfarin, the genetic influences governing the responses of the newer anticoagulants have not yet been widely investigated. Given the high cost of the newer agents over a patient’s lifetime, it may prove easier to support economic arguments for future pharmacogenetic testing related to their use. Dabigatran was No. 1 on the 2012 FDA drug adverse-event list (warfarin was No. 2), which might bolster the argument for pharmacogenetic studies, and though Boehringer Ingelheim markets the drug as not requiring dose testing, the Feb. 7 New York Times reported an internal company study that supports the role of testing for some patients.

Some physicians, Dr. Laposata among them, believe the newer agents are safer. “The thing that I like best about them,” he says, “is that if you bleed with them, you are less likely to bleed in the head than elsewhere.” Reversal agents are expected to become available in the next couple of years.

Warfarin seems not to be going away any time soon, and its low price is only one of the reasons. Six to nine months ago warfarin was still responsible for 80 percent of anticoagulant prescriptions in the U.S., Dr. Johnson says, and its use has not declined. “We’ve had warfarin for 60 years—it’s a tough drug but there are no surprises. Physicians worry about whether bad things will be uncovered with the new agents as they are used more.” Dr. Laposata, though, sees a declining future for warfarin and predicts the decline will be exponential rather than linear. “In 2009 when COAG started,” he says, “we had no idea where we were going to be with novel oral anticoagulants. So it would be hard to muster a lot of enthusiasm for trying to figure out the best way to do this with warfarin—that is, pharmacogenetics or not—when the population of people using it will be so much smaller.”

But the case for understanding the genetics of warfarin response may not be totally lost. Many pharmacogenetic tests, like that for warfarin, are standalone tests, each costing hundreds of dollars and therefore hard to justify economically. That’s unlikely to remain the case for long. “The cost-effectiveness of identifying rare variants will be

modulated by more efficient, cost-effective genomic testing," Dr. Weck says. "We should be able to identify these really important variants that are the most likely to be associated with extremes in response or adverse events. But we also need to be able to mine those data to look for new variants in different ethnic populations like African Americans or other groups." A whole exome sequencing project is underway at the University of North Carolina, and the list of potential incidental findings to report includes pharmacogenetic variants.

In the meantime, Dr. Laposata says, "Sequencing the entire genome would be a huge plus. It does appear that a lot of drugs cluster around the cytochrome P450s. One can argue that even at this point, if you want to get a pretty good look at a lot of drugs, CYP2D6, CYP2C19, and CYP2C9 account for about 75 percent of drugs pharmacogenomically. If we sequenced even those three genes, we'd have a pretty good sense about dozens and dozens of drugs."

Even today Dr. Johnson sees an increasing number of patients come in with genetic data about themselves, principally from 23andme. "Right now it is about ordering a specific test and being able to justify that the cost of the test is offset by some benefit. Where we're moving eventually is that a lot of people are going to have a lot of genetic information about themselves. At that point it's not so much should I use the information but more can I justify ignoring the information."□□

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