Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Clopidogrel Pharmacogenetics

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Slide 1: Title Slide
Hello, my name is Bonny Lewis Van. I am the Laboratory Director of the Marion County Public Health Department in Indianapolis, IN. Welcome to this Pearl of Laboratory Medicine on "Clopidogrel Pharmacogenetics."

Slide 2: Overview
In this presentation I will define Pharmacogenetics, followed by a brief review of the role of platelets in blood clot formation. This will be important when I discuss the mechanism of action of our main topic for this presentation, Clopidogrel. We will briefly review genetic variation in CYP2C19 before we discuss the role variation in this gene has on clopidogrel pharmacodynamics. I will also present to you the findings from some large clinical trials on clopidogrel pharmacogenetics, and finally, I will present the current clinical guidelines on using CYP2C19 genotyping to guide clopidogrel dosing.

Slide 3: Pharmacogenetics
Pharmacogenetics is one part of a larger approach to medical care called Personalized Medicine, in which treatment of disease is tailored to an individual's need, as much as possible, through the judicious use of lab tests to better describe patient status, needs, and responses to therapy.

Lab tests that use genetics to personalize pharmacotherapy in particular are called pharmacogenetic tests. These tests may be assessing natural inherited variation in the patient, called alleles. They may also be looking for somatic mutations which arise due to disease processes such as cancer. Or pharmacogenetic tests may be assessing particular markers of virulence or resistance to medications in infectious agents such as in MRSA or in HIV.

As an aside, previously specific molecular testing procedure codes did not exist. Instead, the test was accounted for by stacking codes which accounted for the specific steps used to arrive at a result. For instance, codes for DNA isolation, PCR, and allele discrimination would all be used together, and may be used in multiples to account for the technique used. In 2013, this coding has changed and many of the most common molecular tests now have unique CPT codes.

Slide 4: Platelet Plug Formation

In order to understand clopidogrel pharmacogenetics, we must first review the role of platelets in clot formation. Within the coagulation cascade, the platelet plug is an important step to stop blood flow after an injury (or pro-thrombotic signal) occurs. The platelet plug forms in 3 steps. First, in platelet adhesion, the platelets stick to exposed collagen underlying damaged endothelial cells in the vessel wall. Second, the platelets become activated by the adhesion and extend projections to make contact with each other. They then release ADP, and vasoconstrictors called thromboxane A2 and serotonin (further decreasing blood flow). The extensions and the activators cause stickiness, thereby activating other platelets. And finally, in the third and final platelet aggregation step, the activated platelets stick together and activate new platelets to form a mass called a platelet plug. The platelet plug is reinforced by fibrin threads formed during clotting process.

Slide 5: Measures of Platelet Activity

The goal of anticoagulation therapy is to dial down this platelet plug formation in an attempt to limit inappropriate intravascular clot formation. In order to assess the effectiveness of this therapy, several assays of platelet activity are available. The results of these assays are expressed as a Platelet Reactivity Index (PRI). There are several assays available including point-of-care, activity mediator measurement, and direct functional assays of platelet aggregation in vitro. I have listed some assays of each type. So why do we concern ourselves with genetic variation at all? Why do we not simply rely on PRI to inform us of the patient phenotype in relatively real-time? It is precisely because there are so many ways of measuring PRI, and the measures are not directly related to each other, that present a problem. The different assays may provide different information and are subject to intra- and inter-individual variation. Also, if a patient is cared for by multiple providers, those providers may use different assays. This may make it difficult to assess the effectiveness of treatment over time.

Slide 6: Clopidogrel

Clopidogrel bisulfate was initially available as Plavix but is now available as a generic formulation. It is one of a class of anticoagulants known as thienopyridine class inhibitor of P2Y12 ADP platelet receptors. These receptors are important in the second step of the platelet plug formation, in that they cause the platelets to aggregate in response to ADP released by the initial platelet response to injury. Clopidogrel may be given as monotherapy, or together with aspirin, to downregulate platelet reactivity.

Slide 7: Mechanism of Action

In this slide, we see a diagrammatic representation of the action of clopidogrel and aspirin on signaling in the platelet. The two stimuli, activated thromboxane, and ADP are irreversibly blocked by aspirin and clopidogrel, respectively. Clopidogrel specifically binds to the P2Y12 receptor, thereby attenuating the signaling effect of ADP.

Slide 8: Metabolism via CYP2C19

Clopidogrel is administered orally as a prodrug. After intestinal absorption, the majority of the dose is inactivated by esterases in the GI and the liver. The remaining 15% of the drug is metabolized by a number of enzymes in a two-step process resulting in up to 5% of the original dose available as an active metabolite. The enzyme CYP2C19 oxidizes both steps of the activation of pro-drug to active drug.

Slide 9: Genetic Variation CYP2C19

Cytochrome P450 2C19 is one of a large and diverse class of oxidizing enzymes located in the endoplasmic reticulum. The enzymes are responsible for metabolizing endogenous compounds such as lipids and steroid hormones, and they also are involved in eliminating exogenous toxic substances from the body by making them more water soluble. Like all CYP enzymes, CYP2C19 is genetically variable, and both reduced and enhanced function alleles exist in the human population. The most common reduced function alleles are designated CYP2C19*2 and CYP2C19*3. These alleles are quite common, with 3-5% of the Caucasian population and 15-20% of the Asian population having 2 copies resulting in a predicted poor metabolizer (PM) phenotype. The CYP2C19*17 allele results in increased protein expression, and therefore increased enzymatic activity and may be found in 3-5% of the Asian population and 18%-27% in African and Caucasian populations, respectively. When present, the CYP2C19*17 allele may result in an ultra-rapid metabolizer (UM) phenotype depending on with which allele it is paired. The majority of the population are either extensive metabolizers (EM) or intermediate metabolizers (IM) having no or one reduced function allele, respectively. There are many more variations in this gene, and many of them have been shown to have an effect on catalytic activity of the enzyme in vitro. There is not yet enough clinical information to include more genetic information in clopidogrel dosing guidelines at this time.

Slide 10: CYP2C19 Clinical Testing

The CPT code for clinical testing for inherited variation in CYP2C19 is 81225. There are currently two FDA-cleared devices which are specific for CYP2C19, from Autogenomics and from Nanosphere. These assays are limited to the determination of CYP2C19 metabolic status only, and are not cleared for the prediction of drug-dosing. Many labs that offer CYP2C19 genotyping services are using a laboratory developed test. Whether evaluating a molecular LDT or completing a method validation of a cleared assay, doing so has important differences from traditional clinical chemistry tests. I have included on this slide a reference by Mattocks and others which describes a standard framework for designing and completing molecular method validations and verifications.

Slide 11: Clinical Trials

There have been many clinical studies looking at the impact of CYP2C19 variation on clopidogrel dosing. I have highlighted three recent publications in the field here.

It had been hypothesized that increasing the dose in reduced metabolizers would overcome the reduced formation of active metabolites. A genetic substudy of the GRAVITAS trial data on clopidogrel dosing showed no benefit from doubling the maintenance dose from 75mg to 150mg in patients with one or more copies of CYP2C19 reduced function alleles *2 or *3.

A meta-analysis by Holmes and colleagues published in JAMA in 2011 combined the results for over 40,000 patients in 32 studies and assessed the effect of CYP2C19 genotype and clopidogrel response. The authors concluded that the data do not support the use of genotyping in clinical practice. This finding was controversial and caused a large response, and much debate.

And finally, preliminary results from the ELEVATE-TIMI 56 study evaluated the effect of dose escalation of clopidogrel maintenance dose in order to assess if the decreased phenotypic PRI response in IM individuals and PM patients could be overcome.

Slide 12: ELEVATE-TIMI 56

Looking more closely at the ELEVATE-TIMI 56 study design and results, we see that as reported from GRAVITAS, doubling the maintenance dose did not overcome the apparent active compound formation deficit seen in CYP2C19 heterozygous patients. However, a tripling of the dose to 225mg was able to attain the same level of PRI repression as 75mg in wild-types. However, the CYP2C19 PM patients were never able to achieve EM level of PRI even with 300mg of clopidogrel.

Slide 13: ELEVATE –TIMI 56 (cont.)

The pharmacodynamics response observed in this dose escalation study demonstrated for the first time that intermediate metabolizer status of clopidogrel could be overcome by tripling the dose. The study also reinforced the observation that CYP2C19 poor metabolizer status could not be overcome by increasing the maintenance dose of clopidogrel. There remains an open question as to what effect this dosing strategy may have on outcomes including MI or mortality.

Slide 14: Clinical Dosing Guidelines

The ELEVATE-TIMI 56 results have not been confirmed, or tested prospectively in a clinical dosing trial. Therefore, they have not been incorporated into the recently updated clinical dosing algorithm for CYP2C19 with clopidogrel in ACS/PCI patients. Depending on the genotyping results, there are two suggested clinical actions from this algorithm; standard dosing or consider alternative therapies, either prasugrel or ticagrelor.

Slide 15: Conclusion

In conclusion, this presentation has only scratched the surface of the role of clopidogrel pharmacogenetics. We have briefly reviewed the role of genetic variation on clopidogrel pharmacotherapy, including results from recent trials and reviews. The current phenotyping assays were presented, and the FDA-cleared genotyping tests. The current clinical guidelines were also presented. As of June 2013, the adoption of CYP2C19 genotyping for clopidogrel is low, and reimbursement is not universal.

Slide 16: Resources

For more information on this topic, please refer to the references imbedded in the presentation. I would also like to direct your attention to sources of more general information on this and other pharmacogenetics issues at the Pharmacogenomics Knowledge Source <http://www.pharmgkb.org> and the Personalized Medicine Division of AACC<http://www.aacc.org/members/divisions/personalized/pages/default.aspx>. I have also included a link to the clopidogrel prescribing information <http://packageinserts.bms.com/pi/pi_plavix.pdf>.

Slide 17: References

Slide 18: Disclosures

In addition to my role as Laboratory Director for the Marion County Public Health Department, I am also employed as a Technical Consultant for Harmonyx Diagnostics, which offers CYP2C19 testing for Clopidogrel dosing.

Slide 19: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "Clopidogrel Pharmacogenetics." I am Bonny Lewis Van.