Evaluating Clopidogrel Response: Update on Laboratory-Guided Therapy
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Plavix™ (clopidogrel) is the second-best selling drug globally with $8.6 billion in sales in 2008. In 2010 the FDA issued a “Boxed Warning” on Plavix™:

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**FDA recommends that healthcare professionals should:**

- Be aware that some patients may be poor metabolizers of Plavix. They do not effectively convert Plavix to its active form because of **low CYP 2C19 activity**. The effectiveness of Plavix as a preventive therapy is reduced in these patients.

- Be aware that tests are available to determine patients' CYP2C19 status.

- Consider use of other anti-platelet medications or alternative dosing strategies for Plavix in patients who have been identified as poor metabolizers.

- Be aware that, although a higher dose regimen (600 mg loading dose followed by 150 mg once daily) in poor metabolizers increases antiplatelet response, an appropriate dose regimen for poor metabolizers has not been established in a clinical outcome trial.

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Clopidogrel, a **prodrug**, must be converted to an active drug by the Cytochrome P-450 liver enzyme CYP2C19, which is coded in DNA by the gene CYP2C19. Studies have shown that single nucleotide polymorphisms (SNPs) in the CYP2C19 gene (e.g., the *2 and *3 variants) result in “**loss-of-function**” enzymes. Loss-of-function enzymes yield reduced conversion of the prodrug to an active drug, **resulting in higher rates of adverse thrombotic events**.

Dr. Steinhubl, in a recent commentary, made the important observation that: “**Given that only an estimated 2% of ingested clopidogrel ends up bound to platelets, it is easy to appreciate that small changes in its metabolism may substantially affect platelet P2Y12 inhibition.**"
Factors Affecting Clopidogrel “Active Drug” Concentration

1. Genetic Factors:

The CYP2C19 gene and associated variant forms (e.g., *2, *3) determine the level of functional CYP2C19 enzymes in the liver responsible for converting clopidogrel to an active drug. A patient is classified as an “extensive (normal) drug metabolizer”, “intermediate drug metabolizer”, “poor drug metabolizer”, and in some cases, “ultra-rapid drug metabolizer”. Significant differences exist in the frequency of CYP2C19 gene variants among sub-populations.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Caucasian (n=1356)</th>
<th>African-American (n=966)</th>
<th>Asian (n=573)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive Metabolism *1/*1</td>
<td>72%</td>
<td>67%</td>
<td>36%</td>
</tr>
<tr>
<td>Intermediate Metabolism *1/*2 or *1/*3</td>
<td>26%</td>
<td>29%</td>
<td>50%</td>
</tr>
<tr>
<td>Poor Metabolism *2/*2, *2/*3, *3/*3</td>
<td>2%</td>
<td>4%</td>
<td>14%</td>
</tr>
</tbody>
</table>

Table adapted from: Xie et al., Annual Rev Pharmacol Toxicol 2001: 41: 815-50

Note: Confusion on terminology. The FDA “Boxed Warning” specifically uses the term “poor metabolizer”. However, in the Mega et al. study\(^2\) (which most likely prompted the FDA warning), the authors clearly stated that most of the data was based on *2 heterozygous patients (i.e. intermediate metabolizers) because “there were so few homozygotes”:

“…395 subjects carrying at least one CYP2C19 reduced-function allele (27.1% of the study population) were at significantly higher risk for the primary efficacy outcome of death from cardiovascular causes, myocardial infarction, or stroke than were noncarriers (12.1% vs. 8.0%)…”

2. Non-Genetic Factors:

- Age (significant decline in concentration of Cytochrome P-450 enzymes with age)
- Type II diabetes
- Impaired kidney function
- Reduced left ventricular function
- Acute Coronary Syndrome
- Competition from other drugs [proton pump inhibitors (e.g. Zantac), lipophilic statins, calcium-channel blockers, et al.]
- Variations in blood platelet P2Y12 receptor activity
- Body mass index (height, weight) to account for internal dilution of the drug
- Bioavailability (variations in drug absorption)
- Patient drug compliance
Evaluating Clopidogrel Response by Direct Measurement of Platelet Inhibition

For years, some medical institutions have used a variety of methodologies to attempt to directly measure the degree of platelet inhibition following administration of clopidogrel. Most common has been measurement of platelet aggregation by light transmission. Others, such as thromboelastograph (TEG) and vasodialator-stimulator phosphoprotein (VASP) have also been employed. For many practicing physicians, the concept of “platelet function testing” remains foreign.

In 2009 the Cleveland Clinic held a panel discussion on “Platelet Response in Practice: Applying New Insights and Tools for Testing and Treatment.”

Panel members included:

- Deepak Bhatt, MD, Chief of Cardiology, Brigham and Woman’s Hospital, Boston
- Marc Sabatine, MD, Assistant Professor of Medicine, Harvard Medical School Co-principal investigator of CLARITY-TIMI 28 (Clopidogrel as Adjunctive Reperfusion Therapy – Thrombolysis in Myocardial Infarction 28)
- Kandice Kotte-Marchant, MD, PhD, Chair, Pathology and Laboratory Medicine Institute and Section Head, Hemostasis and Thrombosis, Cleveland Clinic
- John Alexander, MD, Associate Professor of Medicine, Duke University
- W. Frank Peacock, MD, Vice Chair Emergency Medicine, Cleveland Clinic

Dr. Alexander pointed out the important fact that platelet inhibition is “multifactorial”. Dr. Kottke-Marchant suggested a need for a methodology that was “global”, i.e., one that responds to all aspects of platelet function: adhesion, aggregation, and granule release. The panel discussed the issue that present platelet function tests have a “reliability issue”. The reliability concern has recently been documented by several significant studies. In the February issue of JAMA, Nicoline Breet, MD, et al. compared several platelet function assays looking for the ability of these assays to predict outcomes (atherothrombotic events) in coronary stent patients. The authors found that light transmission aggregometry was only modestly useful for predicting thrombotic outcomes, and, in their opinion, at present, clinical practice should not be guided by point-of-care platelet function testing.

One of the problems with the platelet function testing may be its inability to screen for all patients with the cytochrome variants. While vendors promote platelet function assays as global, at least one study has shown that only 56% of the *2 carriers were detected by platelet function testing.
Recognizing the reliability problem with platelet function testing and yet apparently understanding that genotyping, important as it is, cannot evaluate for non-genetic influences, Dr. Marc Sabatine suggested at the Cleveland Clinic panel discussion that, if rapid genotyping became practical, the combination of genotyping and platelet function testing “might be complementary.”

In a 2010 commentary in the Journal of the American College of Cardiology, Dr. Paul A. Gurbel et al. expanded on this idea of combining genotyping and platelet function testing to complement the limitations of each method. The commentary is well written with a long list of research citations to make their point:

“While neither alone adequately describes the global risk profile of an individual patient treated with clopidogrel, point-of-care platelet function testing to identify HPR [high on-treatment platelet reactivity] combined with CYP2C19 genetic testing may be more effective in identifying high-risk patients for alternative therapies than either alone.”

Laboratory scientists have been combining tests to improve estimates for over a quarter of a century. Down Syndrome and open neural tube risk screens, originally calculated based on age and AFP, are now calculated from measuring age, AFP, hCG, estriol, inhibin A, PAPP-A, and fetal ultrasound measurements. The more recent pharmacogenetic-guided dosing of Warfarin uses genotype combined with age, sex, body mass index, smoking, diabetes, liver disease, interfering drugs, and prothrombin time INR to calculate an estimated maintenance dose. Geisler et al. from the University of Tubingen were the first to take this approach with clopidogrel. These researchers developed a nomogram that combined both genetic and non-genetic factors to help direct clopidogrel therapy in stent patients. However, Geisler et al. noted that large prospective trials would be needed before such a nomogram could be implemented.

**A Surprising New Variant: The *17 Gain-of-Function “Ultra-Rapid Metabolizer”**

A new CYP2C19 gene mutation, the *17 variant, will add yet more complexity to the evaluation of clopidogrel therapy. The *17 variant is unique in that it results in an enzyme that is classified as gain-of-function, and the patient is considered an ultra-rapid metabolizer. Dr. Sibbing and associates reported in the February 2010 issue of Circulation that, in 1,524 patients undergoing coronary stent placement, a single *17 gene variant resulted in about a 2-fold increase in the incidence in bleeding within 30 days following stent placement. The incidence of bleeding was 2.5% in patients without *17, but 4% in patients with a single *17 variant. Patients with the double *17/*17 genotype exhibited a dose-response 4-fold increase in bleeding (8%). The most striking finding was that out of the 1,524 patients tested, 35% had the single *17 variant and another 5% had the *17/*17 genotype. We recently tested 16 patients in our laboratory and 7
(44%) contained the *17 variant. The high frequency we found for the *17 variant gene was exactly what Sibbing et al. reported.

If CYP2C19 allele frequencies for the general population are known to be approximately 30% for the *2 loss-of-function gene variant and 40% for the *17 gain-of-function gene variant, then a combination of *2/*17 would be expected to occur regularly. To date, allele frequencies and phenotypic outcome for this genotype have not been reported in the literature. We found one *2/*17 out of the 16 we analyzed (6%). The question we ask, and we know our physicians will ask, is this: What is the observed result of this genotype? If the *2 loss-of-function allele is on the maternal chromosome and the *17 gain-of-function allele is on the paternal chromosome (or vice versa), will the patient’s liver contain a mixture of loss-of-function enzymes and gain-of-function enzymes? Will the gain-of-function enzymes simply compensate for the loss-of-function enzymes and result in the equivalent of normal liver function? There is, as of yet, no answer to this question. However, healthcare providers will need to monitor these patients closely. Perhaps this is why, in the discussion section of the Sibbing paper, the following statement was given:

“For the individual patient undergoing coronary stent placement, the information provided by genetic and platelet function testing may be complementary in improving patients’ outcomes.

The concept of genotyping and platelet function testing as being complementary is supported by the following:

1. Combining laboratory tests and patient data to provide more accurate predictive information for guiding patient treatment is a long-standing, proven, clinical laboratory technique.

2. As pointed out above, in 2009 and 2010, major clopidogrel research scientists have called for a complementary approach for genotyping and platelet function testing.

3. An initial pilot study by Geisler et al. has already demonstrated that a complementary approach works.
References: