Do We Need Platelet Function Assays?

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The Antiplatelet Effect of Clopidogrel Varies Widely Among Individuals

Factors Contributing to Inter-Individual Variability in Clopidogrel Response

- **Generation of active metabolite**
  - Polymorphisms of CYP2C19
  - Drug-drug interactions involving CYP2C19
  - Epigenetic influences on CYP2C19 function

- **Intestinal Absorption**

- **Genetic and environmental influences on P2Y12 receptor density and function**

- **Non-compliance**

- **Clinical characteristics**

- **Drug-distribution**
CYP2C19 Genotype Is the Strongest Of Many Contributors to High On-Treatment Reactivity

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>*Partial $\eta^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study arm</td>
<td>0.150</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CYP2C19 genotype†</td>
<td>0.097</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.0183</td>
<td>0.0004</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.0123</td>
<td>0.0037</td>
</tr>
<tr>
<td>History of congestive heart failure</td>
<td>0.0127</td>
<td>0.0031</td>
</tr>
<tr>
<td>Age</td>
<td>0.0094</td>
<td>0.0112</td>
</tr>
<tr>
<td>Creatinine clearance &lt; 60 ml/min</td>
<td>0.0077</td>
<td>0.0215</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.0065</td>
<td>0.0344</td>
</tr>
</tbody>
</table>

Clopidogrel is a Pro-Drug that Undergoes CYP450-Dependent Biotransformation Into an Active Metabolite

Clopidogrel

Hydrolysis by HCE-1 to inactive metabolite

2-Step Oxidation (Cytochrome P450)

CYP2C19: 45% of 1st, 20% of 2nd

CYP1A2, CYP3A4, CYP3A5, CYP2C9, CYP2B6

Active Metabolite

Platelet function testing to determine platelet inhibitory response in patients with UA/NSTEMI (or, after ACS and PCI) on P2Y12 receptor inhibitor therapy may be considered if results of testing may alter management.

Genotyping for a CYP2C19 loss of function variant in patients with UA/NSTEMI (or, after ACS and with PCI) on P2Y12 receptor inhibitor therapy might be considered if results of testing may alter management.

Class IIb: Benefit ≥ Risk; *Treatment may be considered*
Additional studies w/broad objectives needed; additional registry data would be helpful.
Measuring Platelet Reactivity: Receptor Function, Expression, and Consequences

**Receptor Function**
- VASP PRI

**Aggregation**
- LTA
- TEG
- VerifyNow

**Activated GP IIb/IIIa**
- P-selectin
- Monoclonal Antibody

**Flow Cytometry**

**PLATELET**

**Receptor Expression**
- p-selectin
- GpIIb/IIIa

Adapted from Paul Gurbel, MD, Baltimore, MD.
LTA – Principle

Baseline Light Transmission – the unaggregated platelets in plasma create a turbid solution that absorbs light.

Light transmission increases as platelets aggregate and fall to the bottom of the tube.

Agonist

- Arachidonate
- ADP
- TRAP
- Collagen
- Epinephrine

Aggregate Clumping

Platelet Rich Plasma (PRP)
Mechanism of Vasophosphoprotein (VASP) Phosphorylation Analysis To Assess P2Y12 Receptor Activity

Phosphorylation status of VASP after administration of ADP and PGE1 reflects P2Y12 receptor activity (more dephosphorylated = more activity)

Angiolillo D et al, Eur Heart J 2008; 29:2202-2211
Measuring Platelet Reactivity with the VerifyNow Test

Platelet reactivity is measured as a function of an increase in light transmission through whole blood as platelets are activated by ADP: “PRU”

Greater PRU = higher reactivity
Uses of Platelet Function Testing

**DIAGNOSTIC TEST**

Outcome has happened
- Is there evidence of a P2Y\textsubscript{12} antagonist effect?

**PROGNOSTIC TEST**

Outcome has not yet occurred
- What is the risk of a CV event?

Assessment of diagnostic and prognostic utility differ!!
## What We Talk About When We Talk About Risk

<table>
<thead>
<tr>
<th>Type of use</th>
<th>Measure Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnostic Testing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test characteristics</td>
<td>Sensitivity and specificity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive predictive value</td>
<td></td>
</tr>
<tr>
<td>Discrimination</td>
<td>ROC curve (AUC, c-statistic)</td>
<td></td>
</tr>
<tr>
<td>Likelihood ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Risk Prediction</strong></td>
<td>Association</td>
<td>Odds ratio, Hazard ratio, Relative risk</td>
</tr>
<tr>
<td>Discrimination</td>
<td>ROC curve (AUC, c-statistic)</td>
<td></td>
</tr>
<tr>
<td>Calibration</td>
<td>Hosmer-Lemeshow goodness of fit</td>
<td></td>
</tr>
<tr>
<td>Reclassification</td>
<td>Net reclassification improvement</td>
<td></td>
</tr>
</tbody>
</table>

Pletcher MJ et al Circulation 2011;123:1116-24
On-Clopidogrel Platelet Reactivity & Ischemic Events Post-PCI: A Patient-Level Meta-Analysis

Non-ACS pts with high reactivity: HR 2.47 (1.79–3.40), P<0.0001

N=3,041

ADAPT-DES High On-Treatment Reactivity is Associated with Ischemic Events

Adjusted Risk of PRU>208 for 1 year events (N=8583)

<table>
<thead>
<tr>
<th>Event</th>
<th>Adj HR[95% CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST, def/prob</td>
<td>2.51 [1.45, 4.37]</td>
<td>0.001</td>
</tr>
<tr>
<td>- Definite</td>
<td>3.12 [1.65, 5.90]</td>
<td>0.0004</td>
</tr>
<tr>
<td>MI</td>
<td>1.40 [1.07, 1.83]</td>
<td>0.01</td>
</tr>
<tr>
<td>Major bleeding</td>
<td>0.75 [0.62, 0.91]</td>
<td>0.004</td>
</tr>
<tr>
<td>Death, all-cause</td>
<td>1.24 [0.88, 1.75]</td>
<td>0.23</td>
</tr>
</tbody>
</table>

ADAPT DES at 1 Year: Def/Prob ST by PRU Quintiles

8,449 PCI pts with VerifyNow PRU after clopidogrel loading

ADAPT DES at 1 Year: Def/Prob ST by PRU Quintiles

A. Kirtane and G. Stone, ACC 2013
**GRAVITAS Study Design**

**Elective or Urgent PCI with DES***

**VerifyNow P2Y12 Test 12-24 hours post-PCI**

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**No**

**Normal On-treatment Reactivity**

**Random Selection**

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**Yes**

**PRU ≥ 230**

**High On-treatment Reactivity**

**R**

- **N = 1109**
  - **High-Dose Clopidogrel†**
    - clopidogrel 600-mg, then clopidogrel 150-mg/day

- **N = 1105**
  - **Standard-Dose Clopidogrel†**
    - clopidogrel 75-mg/day

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**N = 586**

- **Standard-Dose Clopidogrel†**
  - clopidogrel 75-mg/day

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**Primary Efficacy Endpoint:** CV Death, Non-Fatal MI, Stent Thrombosis at 6 mo

**Pharmacodynamics:** Repeat VerifyNow P2Y12 at 1 and 6 months

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*Peri-PCI clopidogrel per protocol-mandated criteria to ensure steady-state at 12-24 hrs

†placebo-controlled All patients received aspirin (81-162mg daily)

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**Price MJ et al., JAMA 2011**
Primary Endpoint: CV Death, MI, Stent Thrombosis

Only 10% of patients with +TnI

2.3% vs. 2.3%
HR 1.01 (95% CI 0.58 - 1.76)
p=0.98

Observed event rates are listed; P value by log rank test.

On-treatment reactivity treated as a time-varying covariate

CrCl = creatinine clearance, ACS = acute coronary syndrome, MI = myocardial infarction

**GRAVITAS: Lower Reactivity Over Course of Trial**

Associated with Reduced With CV Death, MI, ST at 60 days

N=2796

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR [95% CI]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRU &lt;208</td>
<td>0.23 [0.05, 0.98]</td>
<td>0.047</td>
</tr>
<tr>
<td>ACS</td>
<td>3.95 [1.83, 8.53]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.49 [1.10, 5.64]</td>
<td>0.028</td>
</tr>
<tr>
<td>Stent Length (per mm)</td>
<td>1.01 [1.01, 1.02]</td>
<td>0.003</td>
</tr>
<tr>
<td>Prior MI</td>
<td>2.16 [0.94, 4.93]</td>
<td>0.068</td>
</tr>
<tr>
<td>Beta Blocker</td>
<td>1.27 [0.42, 3.85]</td>
<td>0.668</td>
</tr>
<tr>
<td>CrCl &lt;60</td>
<td>1.48 [0.69, 3.18]</td>
<td>0.668</td>
</tr>
<tr>
<td>Prior PCI</td>
<td>1.76 [0.74, 4.16]</td>
<td>0.201</td>
</tr>
<tr>
<td>Prior CABG</td>
<td>1.92 [0.87, 4.23]</td>
<td>0.108</td>
</tr>
</tbody>
</table>

*On-treatment reactivity treated as a time-varying covariate
CrCl = creatinine clearance, ACS = acute coronary syndrome, MI = myocardial infarction

Price MJ et al, Circulation 2011;124:1132-1137
Achieved Levels of On-Treatment Reactivity at 30-Days Stratified By Randomized Treatment Arm

→ Less than half of patients with high-dose clopidgrel achieved “therapeutic” PRU

HD group, CV events according to PRU <208: HR 0.48 [95%CI, 0.18 to 1.25], P=0.14

Price MJ et al, Circulation 2011;124:1132-1137
Standard of care

VerifyNow P2Y12 + ASA

Drug (ASA, clopidogrel, prasugrel, GP2b3a I.) and Dose adjustments if high platelet reactivity

Stent-PCI

Drug and Dose adjustments if high platelet reactivity at Day 14

Rd

Coronary angiogram

Standard of care

Stent-PCI

Standard of care

12-month FU

Primary endpoint at 12 months:
- Death, MI, stroke, stent thrombosis, urgent revascularization

Statistical considerations:
- Assuming an annual risk of 9% and a 33% relative risk reduction (α risk at 5% and error β of 20%, bilateral test), 2,466 patients were necessary to demonstrate the superiority of the strategy of monitoring and adjustment

73% elective, 27% stabilized NSTE-ACS (?USA), no STEMI

## In-Lab monitoring and adjustment

<table>
<thead>
<tr>
<th></th>
<th>Conventional (n=1227)</th>
<th>Monitoring (n=1213)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aspirin poor responders - %</strong></td>
<td>NA</td>
<td>7.6</td>
</tr>
<tr>
<td>On-table aspirin loading in poor responders - %</td>
<td>NA</td>
<td>85</td>
</tr>
<tr>
<td><strong>Thienopyridine poor responders - %</strong></td>
<td>NA</td>
<td>35</td>
</tr>
<tr>
<td>On-table clopi. loading in poor responders - %</td>
<td>NA</td>
<td>80</td>
</tr>
<tr>
<td>On-table prasu. loading in poor responders - %</td>
<td>NA</td>
<td>3.3</td>
</tr>
<tr>
<td>On-table GP IIbIIIa loading in poor responders - %</td>
<td>NA</td>
<td>80</td>
</tr>
</tbody>
</table>

Predominant intervention after procedure: double-dose clopidogrel!
Primary Endpoint to 1 year
Death, MI, stroke, stent thrombosis, urgent revascularization

Event Probability (primary end point)

HR = 1.13 [0.98-1.29]
p = 0.096

Endpoint driven by **periprocedural MI**, defined as Tn > 3x ULN 6hrs after procedure
## Current Status of RCTs of Platelet Function Testing in PCI

<table>
<thead>
<tr>
<th>Trial</th>
<th>Primary Endpoint</th>
<th>% biomarker positive</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRAVITAS</td>
<td>D/MI/ST (after procedure)</td>
<td>10%</td>
<td>Clopidogrel 150mg</td>
<td>No benefit</td>
</tr>
<tr>
<td>TRIGGER PCI</td>
<td>D/MI (after procedure)</td>
<td>0%</td>
<td>Prasugrel 10mg</td>
<td>No benefit</td>
</tr>
<tr>
<td>ARCTIC</td>
<td>D/MI/CVA/uTVR/ST (including periprocedural MI)</td>
<td>27% “stabilized” NSTEACS (% biomarker positive not reported)</td>
<td><em>Recommended, but not required, personalized Rx</em>&lt;br&gt;Actual:&lt;br&gt;• mostly GPI during procedure&lt;br&gt;• mostly Clop 150mg post</td>
<td>No Benefit – Endpoint driven by elevation in TnI 6hrs post-PCI (approx 32% of patients)</td>
</tr>
</tbody>
</table>

- Role of PFT in ACS not addressed
Why Not Prasugrel or Ticagrelor for All ACS Patients?

- Expensive
- Increased risk of bleeding
- Patients with low on-treatment reactivity (e.g., PRU<208), are at substantially lower risk of ischemic events compared with patients with higher reactivity
  - Absolute risk reduction will be lower (NNT higher) for patients with good response to clopidogrel
- Can PFT help us select the most appropriate patient for clopidogrel or a newer oral P2Y12 inhibitor?
Summary

- On-treatment reactivity (OTR) is a strong risk factor for post-PCI events

- Elective PCI patients in general have low event rates irrespective of OTR
  - More intensive inhibition with prasugrel or ticagrelor may increase bleeding more than reduce thrombotic events

- RCTs have not addressed “tailored” therapy in ACS
  - Can PFT identify patients who would benefit the least (or most) from ticagrelor or prasugrel – ie, help select the most appropriate oral P2Y$_{12}$ antagonist?
  - Guidelines recommend against “routine” PFT

Price MJ. *Lancet*. 2013;382:583-584