Vulnerable Blood:
Clinical Utility of Genotyping and Platelet Function Testing

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## Disclosures

### Research Grants/Support
- Nanosphere
- Haemonetics
- Daiichi Sankyo/Lilly
- CSL Pharmaceuticals
- HCRI
- NIH

### Honoraria/Consulting
- Pozen
- Astra Zeneca
- Daiichi Sankyo/Lilly
- Accumetrics
- Nanosphere
- Boehringer
- Merck
- Medtronic
- CSL
- t2 Biosystems

**Dr. Gurbel has patents in the field of platelet function testing**
What is (was) the Common Link Between These Men?

Highly Reactive Platelets - Inadequate Antiplatelet Therapy

CORONARY THROMBOSIS = Myocardial Infarction
What Are the Main Fears We Have As Interventionalists?

**Biggest Fear:** Stent Thrombosis

**Second Biggest Fear:** Bleeding
What Makes the Patient “High-Risk”?

**Demographic Variables**
Women, Age, BMI, DM, Smoking, Hypertension, Renal failure

**Procedural Variables:**
Stent Length, diameter, type, complex anatomy

**High Thrombogenicity**
High platelet reactivity, Prothrombotic factors
Hypercoaguability, Inflammation
DM, women, age, smoking, race, renal failure
Clopidogrel- LoF Carriers, CCB’s, PPI’s, no-smoking

**Poor LV Function**
Last vessel to viable myocardium
A distinct pathophysiological state of heightened platelet reactivity to ADP, platelet activation, inflammation and hypercoagulability, marks the development of symptomatic cardiovascular disease from chronic stable disease.
Post-PCI/MI Thrombotic Events- A “Plateletcentric” Problem!!!!


- PCI/ACS
- Platelet Adhesion /Activation
- Platelet Aggregation
- Sustained GPIIb/IIIa Activation
- Hypercoagulability
- Inflammation
- Ischemic Events/Stent Thrombosis
- ADP
- P2Y₁² Blockers
- TxA₂
- Thrombin
- Aspirin

Platelet Inhibition by Clopidogrel is **UNPREDICTABLE**

24 Hours

- Resistance = 31%
- % Inhibition

5 Days

- Resistance = 31%
- % Inhibition

30 Days

- Resistance = 15%
- % Inhibition


On-treatment Platelet Reactivity is **UNPREDICTABLE**

- On-Treatment Platelet Aggregation at 24h (5 μM ADP)

Gurbel PA et al. *J Am Coll Cardiol*. 2005;45:1392-6
Until now, we did nothing to assess antiplatelet drug responsiveness:

WHY?

• Earlier platelet function studies were criticized for:
  - laboratory artifacts
  - assay variability

• Results regarded as “unconvincing”
  - “the tests are crude substitutes for the …. interactions … in vivo”,
  - “failed to satisfy … the minimal criteria to establish a causal relation … between the results of the .. test and …. a thromboembolic event”.

POC and Near POC Tools to Measure Platelet Function

**VerifyNow**
- Turbidometric based
- Activated platelets to bind fibrinogen-coated beads
- No pipetting, whole blood
- P2Y$_{12}$ and Aspirin Assays
- Most widely linked to outcome

**Thrombleastography**
- Measures physical properties of platelet-fibrin clot:
  - Platelet-fibrin clot strength
- Platelet Mapping Assay
  - Aspirin and P2Y$_{12}$ receptor blocker response
  - Labor intensive, cost

**Multiplatelet Analyzer**
- Measures electrical resistance between electrodes as activated platelets adhere
- Antiplatelet effect of aspirin, P2Y$_{12}$ receptor blockers, and GPIIb/IIIa antagonists
Emergence of a Therapeutic Target
For P2Y\textsubscript{12} Inhibitors
Point-of-Care testing has markedly advanced the field:

- Easier to study platelet reactivity

- Facilitation of translational research

 VerifyNow
ADAPT-DES Registry: Stent Thrombosis by HPR

Multivariable Propensity Score Adjusted Risk of VerifyNow PRU >208
1-year Adverse Events

<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>2.49 [1.43, 4.31]</td>
<td>0.001</td>
</tr>
<tr>
<td>- Definite</td>
<td>3.05 [1.62, 5.75]</td>
<td>0.0006</td>
</tr>
<tr>
<td>MI</td>
<td>1.42 [1.09, 1.86]</td>
<td>0.01</td>
</tr>
<tr>
<td>Major bleeding</td>
<td>0.73 [0.61, 0.89]</td>
<td>0.002</td>
</tr>
<tr>
<td>Death, all-cause</td>
<td>1.20 [0.85, 1.70]</td>
<td>0.30</td>
</tr>
</tbody>
</table>


N=8349

HR [95% CI]=3.89 [1.90, 7.98]
P <0.001

4x increased risk
We now have data in tens of thousands of patients:

**HPR is a major risk factor** for post-PCI thrombotic event occurrence.
There Have Been 2 Major Prospective Trials of Personalized Antiplatelet Therapy Using Platelet Function Testing in the PCI Patient.
GRAVITAS Study (n=2,214)

Elective or Urgent PCI with DES

VerifyNow P2Y12 Test 12-24 hours post-PCI

PRU ≥ 230

High-Dose Clopidogrel
clopidogrel 600-mg, then
clopidogrel 150-mg daily X 6 months

Standard-Dose Clopidogrel
clopidogrel 75-mg daily X 6 months

Primary Efficacy Endpoint: CV Death, Non-Fatal MI, Stent Thrombosis at 6 mo (5% Predicted Event Rate)

Key Safety Endpoint: GUSTO Moderate or Severe Bleeding at 6 mo

All patients received aspirin (81-162mg daily)

Price MJ et al. JAMA. 2011;3051097-105
- Low risk patients
- Suboptimal remedy for HPR (high dose clopidogrel)
- Low post-D/C event rates – inadequately sized for post- D/C event occurrence
- Incomplete protocol following (ARCTIC)
- Inadequate to refute the utility of personalization
- The challenge for a future randomized trial:
  Adequate n, Funding
### Efficacy of Personalized Antiplatelet Therapy in PCI patients: Systematic review and meta-analysis

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Tailored arm</th>
<th>Control</th>
<th>Odds Ratio</th>
<th>M-H, Random, 95% CI</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
<td>Total</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>2097</td>
<td>2116</td>
<td>100.0%</td>
<td>0.38 [0.18, 0.78]</td>
</tr>
<tr>
<td>Total events</td>
<td>9</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: Tau² = 0.00; Chi² = 1.36, df = 4 (P = 0.85); I² = 0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 2.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Cardiovascular Death

- **(P = 0.006)**

### STENT Thrombosis

- **(P = 0.006)**

### Major Bleeding

- **(P = 0.44)**

### Major and Minor Bleeding

- **(P = 0.24)**

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Aradi D et al. *Int J Cardiol.* 2012 Jun 15. [Epub ahead of print]
Role of POC Testing: Bleeding Risk in Clopidogrel-Treated Patients

VerifyNow Assay

Multiplatele Analyzer

Thrombelastography
Gurbel PA et al. Am Heart J. 2010;160
The Platelet Function Therapeutic Window and the Concept of “Thrombosis Immunity”

**Figure 3**

Post-PCI Ischemic/Thrombotic Clinical Events

- **Immunity** Thresholds:
  - ~170 PRU
  - ~50% VASP-PRI
  - ~35% 5 μM ADP
  - ~46% 20 μM ADP
  - ~416 AU* MULTIPLATE
  - ~65 mm MA_{KH}-TEG

The sigmoid cumulative frequency curve in patients with post- percutaneous coronary intervention ischemic/thrombotic clinical events relative to platelet reactivity to adenosine diphosphate. These data support the concept of a therapeutic window for P2Y_{12} blockade. Adapted, with permission, from Gurbel et al. (7). Abbreviation as in Figure 1.

Genetic Testing
Influence of Genetics on Clopidogrel Efficacy

FDA Drug Safety Communication: Reduced effectiveness of Plavix (clopidogrel) in patients who are poor metabolizers of the drug

PLAVIX (clopidogrel bisulfate) tablets
Initial U.S. Approval: 1997

WARNING: DIMINISHED EFFECTIVENESS IN POOR METABOLIZERS

See full prescribing information for complete boxed warning.

- Effectiveness of Plavix depends on activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19. (5.1)
- Poor metabolizers treated with Plavix at recommended doses exhibit higher cardiovascular event rates following acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI) than patients with normal CYP2C19 function. (12.5)
- Tests are available to identify a patient's CYP2C19 genotype and can be used as an aid in determining therapeutic strategy. (12.5)
- Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers. (2.3, 5.1)

-----------------------------RECENT MAJOR CHANGES-----------------------------

Boxed Warning 03/2010
Dosage and Administration (2.3) 03/2010
Warnings and Precautions (5.1, 5.2, 5.3) 03/2010
A genetic locus unequivocally associated with clopidogrel response variability

Genome Wide Association Study ~ 500,000 SNP’s

- Healthy Amish subjects (n=429) with extensive family relationships treated with 75mg x7d clopidogrel
- Contribution of genetic component to clopidogrel response variability ~70%
- Contribution of CYP2C19 locus to clopidogrel response variability is only ~12%
- Majority of clopidogrel response variability remains unexplained
  (rare/other genetic variants that escaped detection with GWAS)

Relation of \textit{CYP2C19*2} Allele to PD Response and Clinical Outcome

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Box plots showing platelet aggregation before and after clopidogrel treatment.}
\end{figure}


HR = 2.42; 95\% CI, 1.18-4.99; \(P=0.02\)
CYP2C19 LoF = ~ 30% Americans & ~ 2% are homozygotes

Platelet reactivity in the clopidogrel-treated homzygotes is very high
- a subject of FDA “boxed warning”

Is it rational take this 2% chance
when we have the capability of easily detecting 2C19 LoF?
Near Point-of-Care Genotyping: Verigene System

Bench top instrumentation

Single-use disposable cartridges

Results available in 3 hours

Verigene® CYP2C19 Test Performance

<table>
<thead>
<tr>
<th>Blinded Methods Comparison Study</th>
<th>Bi-Directional DNA Sequencing</th>
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<tbody>
<tr>
<td></td>
<td>*1/*1</td>
</tr>
<tr>
<td>Verigene® Test</td>
<td></td>
</tr>
<tr>
<td>(*2-*10, *13, *17 alleles)</td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td></td>
</tr>
<tr>
<td>*2/*1</td>
<td></td>
</tr>
<tr>
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<td>*17/*1</td>
<td></td>
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<td>*17/*17</td>
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</table>


100% concordance with conventional sequencing
Spartan RX CYP2C19 System

- Sensitivity – 100%
- Specificity – 99.4%

Performance Characteristics of Rapid Testing vs. Direct DNA Sequencing

- Buccal Swab/Real Time PCR
- 60 minutes to identify:
  - CYP2C19*2 carrier status
  - Heterozygous vs. Homozygous

### How Do Genotyping and Phenotyping Differ?

<table>
<thead>
<tr>
<th>Genotyping</th>
<th>Platelet Function Testing</th>
</tr>
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<tbody>
<tr>
<td>- Stable Risk Factor</td>
<td>- Labile Risk Factor</td>
</tr>
<tr>
<td>- No Method Variability</td>
<td>- Method Variability</td>
</tr>
<tr>
<td>- Assists in Choosing Initial Rx</td>
<td>- <strong>No</strong> Assistance in Choosing Initial Rx</td>
</tr>
<tr>
<td>- Provides “Yes” or “No” Readout</td>
<td>- Provides Continuous Readout</td>
</tr>
<tr>
<td>- Supported by Multicenter Trial Data</td>
<td>- Supported by Mostly Registry Data</td>
</tr>
<tr>
<td>- No Proven Prospective Evidence</td>
<td>- No Proven Prospective Evidence</td>
</tr>
<tr>
<td>- Addressed in Guidelines</td>
<td>- Addressed in Guidelines</td>
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</table>
Guidelines for Platelet Function and Genetic Testing

ACCF/AHA FOCUSED UPDATE

2011 ACCF/AHA Focused Update of the Guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction (Updating the 2007 Guideline)

PRACTICE GUIDELINE

2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention

ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation

Hamm CW et al. Eur Heart J. 2011;32:2999-3054

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Grade</th>
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<tbody>
<tr>
<td>Increasing the maintenance dose of clopidogrel based on platelet function testing is not advised as routine, but may be considered in selected cases.</td>
<td>IIb</td>
</tr>
<tr>
<td>Genotyping and/or platelet function testing may be considered in selected cases when clopidogrel is used.</td>
<td>IIb</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
</tbody>
</table>
Role of POC Testing in Clopidogrel-Treated Patients Undergoing CABG

TARGET-CABG Study

On clopidogrel (n=96)
- Clopidogrel naïve (n=95)

CATHETERIZATION

TEG MA_{ADP}

<35mm
Wait 5 d

35-50mm
Wait 3-5 d

>50mm
< 1 d

Primary Endpoint: 24 hrs chest output
Secondary Endpoint: Hospital duration

Primary Endpoint:

24 Hr Chest Output

Secondary Endpoint:

~ 50% shorter waiting time than recommended in the current guidelines.

2012 Update to The Society of Thoracic Surgeons Guideline on Use of Antiplatelet Drugs in Patients Having Cardiac and Noncardiac Operations

G. Treatment Options for Patients Taking Antiplatelet Drugs Who Require Urgent Operations

Class IIa Recommendation

a. For patients who require urgent operation while on dual antiplatelet therapy, delay of even a day or two before operation is reasonable to decrease bleeding risk and minimize thrombotic risk in patients with ACS. (Level of evidence B)

b. For patients on dual antiplatelet therapy, it is reasonable to make decisions about surgical delay based on tests of platelet inhibition rather than arbitrary use of a specified period of surgical delay. (Level of evidence B)
Conclusions

1) Clopidogrel resistance is a pharmacodynamic event associated with a high risk of post-PCI thrombosis.

2) HPR is an established risk factor- the most potent of all.

3) HPR is reliably overcome by ticagrelor and prasugrel.

4) Platelet reactivity easily and rapidly detected with POC.

5) Irrational to give placebo to prevent a catastrophic thrombotic event.

6) Irrational to give expensive drugs associated with more bleeding to all patients - when clopidogrel works in ~ 2/3 of patients.
Who is the Optimal Patient for Testing?

- Selective testing in **High risk PCI** patients on clopidogrel-phenotyping - can they safely stay on it?
- Genotyping - which drug to start?

What is “high risk”?

**Variables mostly associated with increased risk of ST, MI, HPR:**

- ACS (current or prior)
- H/O stent thrombosis, TVR
- Poor LV function
- Multivessel stenting
- Complex anatomy (e.g. bifurcation, long, small stents)
- BMI, DM, PPI
Approach to Determining Blood Vulnerability

Future:

Thinking Man’s Strategy