Update on Aspirin and Plavix Sensitivity and Resistance Testing:

David L McGlasson, MS, MLS(ASCP)CM,
59th Clinical Research Division,, Wilford Hall Medical Center, Lackland AFB, TX, 78236-5300

This information is for education only and is not a product endorsement.
Introduction

• Aspirin irreversibly acetylates platelet cyclooxygenase, preventing activation by blocking the prostaglandin pathway
• The platelet inhibiting effect of a single aspirin may be detectable by platelet function assays within 24 hours
• Failure to detect aspirin-induced platelet suppression may indicate physiological aspirin insensitivity, a phenomenon called “aspirin resistance”
• Aspirin resistance is a recognized cause of failed aspirin therapy and may imply increased risk arterial thrombosis.
Why is it important?

- Aspirin is used for prevention of complications of vascular diseases such as heart attack and strokes. Gender issues?

- Studies have shown using Aspirin alone reduced recurrent non-fatal stroke by 18%.

- However, studies have shown about 5-40% (about 1-2 million) of patients taking Aspirin may not be receiving full benefit because of resistance.

- Several studies have suggested a significant increase of major vascular events associated with aspirin resistance. It may be reasonable to alter therapy in the aspirin resistant population rather than continue to take a drug that a test suggests is ineffective.
Mechanism of Action of ASA: Inhibits the prostaglandin-producing enzyme cyclooxygenase which converts arachidonic acid into prostaglandins.
Mechanisms of Action of Oral Antiplatelet Therapies

- clopidogrel bisulfate
- ticlopidine HCl
- ADP
- aspirin
- dipyridamole
- phosphodiesterase
- collagen thrombin TXA₂
- COX
- GP IIb/IIIa (fibrinogen receptor)
- TXA₂
- cAMP

ADP = adenosine diphosphate, TXA₂ = thromboxane A₂, COX = cyclooxygenase.
ASPIRIN RESISTANCE

- **ASA resistance** refers to less than expected suppression of thromboxane A$_2$ production by ASA. Reported to be independently associated with an increased risk of adverse cardiovascular events.

- **Clinical resistance**: inability of ASA to protect subjects from cardiovascular events such as an acute MI.

- **Laboratory ASA resistance**: refers to the lack of anticipated effect of ASA on a laboratory assay of its antiplatelet effect.
POSSIBLE CAUSES OF ASPIRIN RESISTANCE

• Poor compliance by subjects.
• Drug interaction: ibuprofen, naproxen.
• Inadequate ASA dose.
• Increased turnover of platelets.
• Genetic polymorphisms of cyclo-oxygenase-1.
• Up regulation of alternate (non-platelet) pathways of thromboxane production.
• No standardized approach to the diagnosis and there are no proven effective treatments for aspirin resistance that improve outcome. Yet!
Research Background

- Eikelboom J et al: HOPE study: among patients with cardiovascular disease who take aspirin with persistent high 11-dehydro-thromboxane B2, had a 3.5 fold increase in the risk of death from heart attack.

- Grotemeyer K. H et al: two year follow up of aspirin responders and non responders (180 Post-stroke patient): Major end point (CVA, MI, Vascular death) seen in 4.4% of aspirin responders but 40% in aspirin non-responders.

- Gum P., Topol E, et al: A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease among patient with aspirin resistance, 24% experienced death, MI, or CVA compared to 10% of patient who were not resistant.

- Faraday et al: Relation Between Atherosclerosis Risk Factor and Aspirin Resistance in a Primary Prevention Population found that higher 11-DHT B2 levels is the only criteria associated with atherosclerosis risk factors suggesting that this measurement may represent the most relevant approach for identifying asymptomatic subjects who ASA treatment fail.
Research Background

• Patrono et al: Low-Dose Aspirin for the Prevention of Atherothrombosis: Benefits fine for high risk subjects but may be marginal in low risk populations.

• Rjderk PM et al: Women’s Health study in healthy women gave surprising results in that protection from stroke by 17% over men but no reduction in the risk of MI. Reverse effect for men in protection from MI but low protection from stroke.

• Becker DM et al: Women experienced the same or greater decrease in platelet reactivity after ASA therapy, retaining modestly more platelet reactivity compared with men.

• Bhatt DK et al: Overall clopidogrel + ASA was not significantly more effective than ASA alone in reducing MI, stroke and CVA.

• Lordkipanidze M et al: Aspirin resistance: Truth or dare. ASA resistance is poorly understood with testing not equivalent to each other. Like LA testing?
Research Background

- Goodman T, Sharma P, Ferro A. The genetics of aspirin resistance: ASA may not be effective in the prevention of thrombosis, depending on genetic makeup. Genetic testing is not currently useful for predicting the effect of ASA clinically.

- Schwertner HA, McGlasson DL, Christopher M, Bush AC. Effects of different ASA formulations on platelet aggregation times and plasma salicylate concentrations.


Research Background

- Geske et al: Gender Variability of Urinary 11-DHT B2 levels in Diabetes Mellitus. Healthy females had higher levels than males. DM patients had higher levels than healthy controls. Female DM had higher levels than healthy females and DM males. No difference between DM males and healthy males. In response to ASA 325 healthy females levels were higher than healthy males.

- Gengo F et al: Prevalence of platelet non-responsiveness to ASa in patients treated for secondary stroke prophylaxis and in patients with recurrent ischemic events. Prevalence of nonresponsiveness to ASA was statistically higher in patients who suffered recurrent cerebral ischemia while taking ASA compared with patients who remained without new ischemic symptoms.
Introduction

• Assays that measure platelet response to aspirin may predict clinical outcomes
• We compared four methods for monitoring 24-hour platelet inhibition (single dose) and a 7 day dosing regimen in healthy subjects by 81 mg and 325 mg (standard child and adult) dosages
• We anticipated these assays will reveal a greater anti-platelet effect of 325 mg compared to 81 mg of aspirin
• We further anticipated the assays were comparable in their ability to detect aspirin effect
• We further anticipated that the 7-day dosing regimen would reveal a greater anti-platelet effect compared to the 24-hour regimen.
Research Objectives

- To measure platelet response to aspirin using four commercially available assays to determine:
  1) Whether results of these assays compare and validate each other
  2) Whether the degree of platelet inhibition under different single doses of Aspirin (81 and 325mg) are similar
Assays

Four commercially available assays were used in this study

• Whole blood aggregometry: examines platelet aggregation by using platelet agonists Collagen, ADP, Arachidonic Acid.

• PFA-100: tests platelet aggregation by measuring time to occlude an aperture. (Closure time)

• Verify/Now Accumetrics: studies platelet function by using arachidonic acid reagent. ASA inhibits platelet function and does not react to AA. Platelet aggregation is quantified as ARU (aspirin resistance units).

• Aspirin-works: Measure level of urine 11-Dehydrothromboxane (metabolite of Thromboxane A2) in pg/mg of creatinine.
Significance

• If these platelet function assays are found to be comparable, we may be able to choose the most time efficient, cost-effective approach to obtain this information.

• Data obtained can be used to distinguish aspirin resistant and aspirin sensitive individuals.

• If aspirin resistance is associated with increased risk of recurrent stroke, CVA, MI etc., then using platelet function assays could detect such individuals (who could then be offered other anti-thrombotic therapy?)
Plan for Data Analysis

- Whole blood platelet aggregation (WBPA) using Chronolog- 570Vs aggregometer was the gold standard test

- The other 3 assays results were compared to WBPA to validate equivalency
• Records whole blood platelet activation by platelet aggregation impedance
• Whole blood platelet aggregation is the reference method for aspirin detection
• 10 µL of aggregation agonists 1.0 µg/mL collagen (Coll) and 0.5 mM arachidonic acid (AA) were added to 1:1 saline/whole blood suspensions
• Aggregation impedance ≤ 8 ohms indicates aspirin effect
PLATELET AGGREGATION
- Platelet rich plasma (light transmission aggregometry) LTA

- Measures change in light transmission upon addition of agonist

- Considered by some the gold standard

- Labor intensive, not specific

- Sensitivity variable

- Correlates with clinical events
WHOLE BLOOD AGGREGATION

- Measures impedance: Superior to PRP?
- Evaluates platelets in a physiologic milieu in the presence of RBC and WBC which are known to modulate platelet function.
- Faster and uses less specimen making it better for children and hard to draw subjects.
- Higher sensitivity to medication responses.
- Does not require centrifugation thus avoiding injury to platelets and loss of giant platelets.
Methods

1. Whole blood sample is diluted with 0.9 % saline, 1:1 in cuvette.

2. Electrode is placed in sample.
Methods

3. Platelets form a monolayer on the electrode

4. Voltage is run through the electrode and resistance baseline is assigned a value of zero ohms

5. Agonist is added to stimulate aggregation
Methods

Amount of aggregation is directly proportional to the change in resistance in ohms.
**Test ID:** APSEY BL 325  
**Name:** APSEY, DOUGLAS  
**ID:** 4043  
**Lab:** CRES  
**Blood Draw Time:** 1000

**TRACE 1**
- **Date:** 3/23/2005  
- **Time:** 11:26:07 AM  
- **Name:** ,  
- **ID:** ,  
- **Lab:** ,  
- **Blood Draw Time:**

**TRACE 2**
- **Date:** 3/23/2005  
- **Time:** 11:26:07 AM  
- **Name:** ,  
- **ID:** ,  
- **Lab:** ,  
- **Blood Draw Time:**

**TRACE 3**
- **Date:** 3/23/2005  
- **Time:** 11:26:07 AM  
- **Name:** ,  
- **ID:** ,  
- **Lab:** ,  
- **Blood Draw Time:**

**TRACE 4**
- **Date:** 3/23/2005  
- **Time:** 11:26:07 AM  
- **Name:** ,  
- **ID:** ,  
- **Lab:** ,  
- **Blood Draw Time:**

<table>
<thead>
<tr>
<th>Instrument Reagent</th>
<th>Trace 1</th>
<th>Trace 2</th>
<th>Trace 3</th>
<th>Trace 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP ADP 10.0UM</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
</tr>
<tr>
<td>IMP COLL 5.0UG</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
</tr>
<tr>
<td>IMP COLL 1.0UG</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
</tr>
<tr>
<td>IMP AA 5.0NM</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amplitude Gain</th>
<th>Trace 1</th>
<th>Trace 2</th>
<th>Trace 3</th>
<th>Trace 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 ohm</td>
<td>11</td>
<td>18</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>32 ohm</td>
<td>11</td>
<td>18</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>19 ohm</td>
<td>11</td>
<td>18</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>16 ohm</td>
<td>11</td>
<td>18</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lag Time Area Under</th>
<th>Trace 1</th>
<th>Trace 2</th>
<th>Trace 3</th>
<th>Trace 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:48</td>
<td>2:04</td>
<td>3:00</td>
<td>2:32</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**BL 325MG ASA**
Test ID: 81694

TRACE 1 Date: 3/15/2007 Time: 11:09:56 AM  
Name: SPARC, STUDY  
ID: 81694  
Lab: CRD  
Blood Draw Time: 1030

TRACE 2 Date: 3/15/2007 Time: 11:09:56 AM  
Name: SPARC, STUDY  
ID: 81694  
Lab: CRD  
Blood Draw Time: 1030

TRACE 3 Date: 3/15/2007 Time: 11:09:56 AM  
Name: SPARC, STUDY  
ID: 81694  
Lab: CRD  
Blood Draw Time: 1030

TRACE 4 Date: 3/15/2007 Time: 11:09:56 AM  
Name: SPARC, STUDY  
ID: 81694  
Lab: CRD  
Blood Draw Time: 1030

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument Reagent</td>
<td>IMP</td>
<td>IMP</td>
<td>IMP</td>
<td>IMP</td>
</tr>
<tr>
<td>ADP</td>
<td>ADP</td>
<td>ADP</td>
<td>ADP</td>
<td>ADP</td>
</tr>
<tr>
<td>10.0UM</td>
<td>10.0UM</td>
<td>10.0UM</td>
<td>10.0UM</td>
<td>10.0UM</td>
</tr>
<tr>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
</tr>
<tr>
<td>Stirrer Gain</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Amplitude</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Slope</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lag Time</td>
<td>4:06</td>
<td>5:00</td>
<td>5:00</td>
<td>5:00</td>
</tr>
<tr>
<td>Area Under</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CATH LAB SUBJECT
Test ID: 33734
TRACE 1 Date: 5/18/2007 Time: 11:34:31 AM
Name: SPARC, STUDY
ID: 33734 Lab: CRD
Blood Draw Time: 0940

TRACE 2 Date: 5/18/2007 Time: 11:34:31 AM
Name: SPARC, STUDY
ID: 33734 Lab: CRD
Blood Draw Time: 0940

TRACE 3 Date: 5/18/2007 Time: 11:34:31 AM
Name: SPARC, STUDY
ID: 33734 Lab: CRD
Blood Draw Time: 0940

TRACE 4 Date: 5/18/2007 Time: 11:34:31 AM
Name: SPARC, STUDY
ID: 33734 Lab: CRD
Blood Draw Time: 0940

<table>
<thead>
<tr>
<th>Instrument Reagent</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP ADP 10.0UM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMP ADP 5.0UM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMP COL 1.0UG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMP AA 5.0NM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stirrer Gain</th>
<th>1200</th>
<th>1200</th>
<th>1200</th>
<th>1200</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Amplitude Slope</th>
<th>2 ohm</th>
<th>3 ohm</th>
<th>13 ohm</th>
<th>11 ohm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lag Time Area Under</th>
<th>1:44</th>
<th>2:40</th>
<th>2:52</th>
<th>3:48</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Comments
CATH LAB SUBJECT
WHOLE BLOOD LUMI-AGGREGOMETRY vs OPTICAL-LUMI

After 3 Days of Aspirin Treatment @ 325 mg

Whole Blood Aggregation

Optical Aggregation

Courtesy of: Anna M. Dyszkiewicz-Korpanty, MD, University of Texas Southwestern Medical Center at Dallas, Department of Medicine
WHOLE BLOOD [Impedance] AGGREGOMETRY

and the

Effect of ASA on Platelets, RBC’s and WBC’s (L)

Inhibition of Collagen Induced Aggregation

WB | PRP | PRP + RBC | PRP + WBC

50 | **
40 | 30 | * p<0.02
20 | 10 | * p<0.001

Reference: Platelet Aggregation in Human Whole Blood After Chronic Administration of Aspirin, De La Cruz et al, Thrombosis Res 46;133-140, 1987
Dade-Behring PFA-100®

- Records platelet-induced whole blood interval to occlusion of an aperture in a biochemically active membrane cartridge producing “closure time” (CT). Alternative to Ivy Bleeding Time.
- Specimens first assayed with ADP/collagen impregnated cartridges
- If ADP/collagen CT ≤ 145 s, aspirin effect was assessed by epinephrine/collagen (EPI/COLL) impregnated cartridges
- CT ≥ 175 seconds is anticipated aspirin response
Collagen/Epinephrine (CEPI) is the primary screening cartridge. Collagen/ADP (CADP) indicates if a platelet dysfunction observed with CEPI is due to ASA or may reflect Plavix effect.
PFA - 100

- Requires PFA -100 instrument

- Uses cartridges coated with collagen and epinephrine or collagen and ADP

- Rapid, easy to perform

- Whole blood – platelet count dependent. Hematocrit dependent. May be affected by high fibrinogen and vWF.

- Sensitivity variable

- Clinical outcomes studies limited

- Qualitative – results measured in closure time (sec)
PFA-100® Test Procedure

STEP 1

Pipette 800 µL of citrated whole blood into the sample reservoir of the test cartridge(s).

STEP 1

Place the cassette onto the carousel of the analyzer.

STEP 1

Using the integrated keypad, initiate the test run.
PFA-100® Test Principle

occlusion process

final result and print-out

PFA-100
REV. 2.00 S/N:
00370

14/07/97 14:01

ID#: 23456.17
Test Type: Collagen/EPI
SAMPLE A: 110 SEC
The Solution: A diagnostic test that can help physicians determine if aspirin therapy is working for their patients.

Verify Now by Accumetrics

- **RAPID**
  - Result available in less than 10 minutes

- **EASY**
  - Whole blood - no sample preparation
  - Automatic sampling from closed tube
  - Factory calibrated reagents
  - CLIA - moderately complex; filed for waived status
  - FDA Cleared
  - Reimbursement/ CPT code

- **ACCURATE**
  - A quantitative reference point measured in Aspirin Reactive Units (ARU)

*Correlates to optical platelet aggregometry*
Insert assay device
Add blood sample
Result in minutes

Clinical Lab
Cardiac Cath Lab
Point of Care
Doctors’ Office
Ultegra® Aspirin Test Results

If a patient result is <550 ARU, then platelet dysfunction has been detected, indicating that Aspirin IS working.

If a patient result is ≥550 ARU, then no platelet dysfunction has been detected, indicating that the anti-platelet effect may not have been achieved or Aspirin IS NOT Working.
Verify Now

- Verify/Now Accumetrics Ultegra instrument
- Cartridge containing fibrinogen-coated microparticles in a proprietary tube using Arachidonic Acid as agonist.
- Whole blood
- Rapid, easy to perform
- Sensitivity and specificity variable
- Clinical outcomes studies limited
- Qualitative – results measured as aspirin response units
Mapping of ARU to % Inhibition

% Inhibition

ARU
VerifyNow® P2Y12

- **RAPID**
  - Result available in <3 minutes
- **EASY**
  - Whole blood - no sample preparation
  - Automatic sampling from closed tube
  - Factory calibrated reagents
- **ACCURATE**
  - More specific than optical aggregometry
  - Can measure % platelet inhibition without weaning patient off drug
- **COST-EFFECTIVE**
  - Reimbursement
  - CPT code 85576 (2 times)
  - FDA cleared
Results are based on the rate and extent of platelet aggregation and are reported in P2Y12 Reaction Units (PRU) and % platelet inhibition.

- PRU result is ‘P2Y12-mediated platelet aggregation’ via adenosine diphosphate (ADP) pathway
- Base result is ‘Maximal platelet aggregation’ via Thrombin Receptor Activating Peptide (TRAP) pathway which is independent of aspirin and clopidogrel
VerifyNow® P2Y12 Advantages

- Greater specificity for clopidogrel than test methods using ADP alone, e.g., optical aggregometry
- Ability to measure % platelet inhibition in patients on clopidogrel without first withdrawing clopidogrel
- Rapid - Time to result <3 minutes
ADP activates platelets via two ADP receptors: P2Y12 and P2Y1...
Tests using ADP alone measure ADP-induced platelet aggregation via both P2Y12 & P2Y1 receptors...

which may over-estimate the degree of aggregation, by as much as 25%
PGE1 minimizes contribution of P2Y1 aggregation
VerifyNow® P2Y12 Result Calculations

ADP-mediated platelet activation determines the PRU value

TRAP-mediated platelet activation approximates Baseline PRU

Clopidogrel-induced % platelet inhibition = Baseline PRU – Post-PRU Baseline PRU X 100
Urinary 11-dehydrothromboxane B$_2$
AspirinWorks
Urinary 11-dehydrothromboxane B$_2$
AspirinWorks

- Requires ELISA equipment and urinary creatinine result
- Random urine specimen that can be frozen until ready for testing.
- Sensitivity good
- Specificity uncertain
- Labor intense, not rapid. Two hour specimen incubation. Recently FDA approved. Established test in optimized format
- Quantitative - Results reported as pg 11-dehydrothromboxane B$_2$/mg creatinine
- May be used to guide incremental aspirin therapy
VASP.P2Y12
Vasodilator Stimulated Phosphoprotein

- Dedicated to the monitoring of specific platelet ADP receptor (P2Y12) antagonists: Thienopyrdines
- Regulated by cAMP cascade
- cAMP activated by PGE1 (1)
- Inhibited by ADP through P2Y12 receptors (2)
- VASP phosphorylation correlates with P2Y12 receptor inhibition. Non-phosphorylation state correlates with the active form of P2Y12 receptor.
- Thienopyrdines can be demonstrated with PLT VASP/P2Y12 (3). Performed by flow cytometry on citrated blood.
The aim of the assay is to evaluate the efficacy of Plavix therapy.

Uses the PRI or platelet reactivity index expressed as a percentage to measure the difference in VASP fluorescence intensity between resting +PGE1 and activated +ADP platelets.

Aleil B et al: J Thromb Haemost, 2005; 1:85-92 measuring VASP for clopidogrel resistance in patients with ischemic cardiovascular diseases found the following:

- 85.8 to 6.6% PRI with the 85.8 being poor responders and 6.6% good responders. 30% of treated subjects were in range with bad responders. (PLAVIX RESISTANCE?)
Tests Requiring Blood Specimen

• Advantages
  – Point of Care
  – Rapid results

• Disadvantages
  – Preanalytical variables
  – Lack of standardization
  – Test must be run within 3-4 hours
  – Limited clinical outcomes data
     (except platelet aggregation)
Tests Requiring Blood Specimen
Additional considerations

- Platelet function tests requiring whole blood may be impacted by:
  - Platelet count
  - Hematocrit
  - Fibrinogen - elevated levels (Lower fibrinogen levels have shown greater ASA response). Values above 380 mg/dl have been shown to affect assay.
  - Factor VIII – elevated levels
  - vWF – elevated levels
Urinary 11-dehydrothromboxane B$_2$

- Metabolite not formed by platelet
- High concentration
- Longer circulating half-life
- Minimal pre-analytical variables
- Specimen stable 72 hours at room temperature
- Major clinical outcomes study to support the test
- Standardization of test
  - to outcome studies
  - between laboratories
  - Disadvantages: Liver disease, renal disease may affect results
Comparison of The 24-hour Sensitivity of Four Platelet Function Assays to A Single Aspirin

DL McGlasson, G Fritsma, M Chen, Z Knight, M Dobbs, 59th Clinical Research Squadron and Department of Neurology Wilford Hall Medical Center, Lackland AFB, TX and University of Alabama Birmingham, Division of Laboratory Medicine, Birmingham, AL
Aspirin Response Assays

- Assays that measure platelet response to aspirin may predict aspirin’s cardioprotective effect
- We compared four methods for monitoring 24-hour platelet inhibition in healthy subjects by a single 81 mg and 325 mg (standard child and adult) aspirin dose
- We anticipated these assays would reveal a greater 24-hour anti-platelet aspirin effect of 325 mg compared to 81 mg
- We further anticipated that the assays were comparable in their ability to detect the aspirin effect

Introduction
Subjects and Procedure

- Fifty normal healthy volunteers were enrolled. None had taken aspirin or other NSAIDs for $\geq 14$ days
- 20 females, mean age 33.1 (18-51)
- 30 males, mean age 36.6 (20-58)
  1. Baseline citrated whole blood and urine
  2. Subjects observed to ingest a single 81 mg aspirin
  3. Citrated blood and urine obtained 24 hours after dosing
- Process repeated $\geq 14$ days with single 325 mg aspirin
Records whole blood platelet aggregation by impedance

Whole blood platelet aggregation is chosen as the reference method for aspirin response detection

10 uL of aggregation agonists 1.0 µg/mL collagen (Coll) and 0.5 mM arachidonic acid (AA) were added to 1:1 saline/whole blood suspensions

Post-aspirin aggregation impedance ≤ 8 ohms indicates anticipated aspirin response
11-dehydro Thromboxane B₂

- Urine 11-dehydrothromboxane B₂ (11-DHT) is an end product of the platelet arachidonic acid prostaglandin pathway whose urine concentration reflects in vivo platelet activity.
- Aspirin inhibits the prostaglandin pathway and decreases urine 11-DHT production.
- ≥ 50% 11-DHT reduction from baseline indicates aspirin effect.
- Urine 11-DHT is measured using random urine when normalized to urine creatinine.

Materials and Methods
Verify/Now®

- Arachidonic acid (AA)-impregnated cartridge aggregates platelets
- Aggregation time interval expressed as aspirin reaction units (ARUs)
- Post-aspirin aggregation impedance $\leq 550$ ARUs indicates response

Materials and Methods
Dade-Behring PFA-100®

- Records platelet-induced whole blood interval to occlusion of an agonist-impregnated cartridge aperture producing closure time (CT)
- Specimens first assayed with ADP/collagen impregnated cartridges
- If ADP/collagen CT ≤ 145 s, aspirin effect was assessed by epinephrine/collagen (EPI/Coll) impregnated cartridges
- EPI/Coll CT ≥ 175 seconds is anticipated aspirin response

Materials and Methods
24-Hour Response to 81 mg and 325 mg Aspirin: Means

<table>
<thead>
<tr>
<th></th>
<th>Chronolog WBA® Aggregometry Reference Method</th>
<th>11-DHT</th>
<th>VerifyNow®</th>
<th>Dade-Behring PFA-100®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 ug Coll</td>
<td>0.5 mM AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline 81 mg</td>
<td>20.5 Ω</td>
<td>19.1 Ω</td>
<td>978.4 pg/mg</td>
<td>643.7 ARU</td>
</tr>
<tr>
<td>24-h Response to 81 mg</td>
<td>16.1 Ω*</td>
<td>2.1 Ω*</td>
<td>510.7 pg/mg*</td>
<td>600.7 ARU*</td>
</tr>
<tr>
<td>Baseline 325 mg</td>
<td>18.2 Ω</td>
<td>18.1 Ω</td>
<td>884.5 pg/mg</td>
<td>646.2 ARU</td>
</tr>
<tr>
<td>24-h Response to 325 mg</td>
<td>13.6 Ω*</td>
<td>1.9 Ω*</td>
<td>349.1 pg/mg*</td>
<td>465.3 ARU*</td>
</tr>
</tbody>
</table>

In all assays, 81 mg to 325 mg baselines are not significantly different at p < 0.05
*All aspirin responses significant at p < 0.05

Results
## 24-Hour Response to 81 mg and 325 mg Aspirin: Action Limits

<table>
<thead>
<tr>
<th></th>
<th>Chronolog WBA® Aggregometry Reference Method</th>
<th>11-DHT</th>
<th>VerifyNow®</th>
<th>Dade-Behring PFA-100®</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 ug Coll</td>
<td>0.5 mM AA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Action Limit</td>
<td>$\geq 8 \Omega$</td>
<td>$\leq 50%$ Reduction</td>
<td>$\geq 550$ ARU</td>
<td>EPI/COLL CT $\leq 175$ s</td>
</tr>
<tr>
<td>Response to 81 mg Aspirin</td>
<td>11 (22.4%)</td>
<td>12 (24.5%)</td>
<td>23 (46.9%)</td>
<td>10 (20.4%)</td>
</tr>
<tr>
<td>Response to 325 mg Aspirin</td>
<td>44 (89.8%)</td>
<td>44 (89.8%)</td>
<td>39 (80.0%)</td>
<td>43 (87.8%)</td>
</tr>
</tbody>
</table>
24-Hour Response to 81 mg and 325 mg Aspirin: Action Limits

Subjects Responsive to Aspirin By Assay Method (N = 49)

- Agg: 1 ug Coll
- Agg: .5 mM AA
- 11-DHT
- Ultegra
- PFA-100

Results
24-hour Response to 325 mg Aspirin

• There was no significant gender effect at baseline or 24 hours for 11-DHT and VerifyNow in either the 81 or 325 mg arm (data not displayed)
• The systems equivalently recorded an average 85.5% 24-hour individual subject responses to 325 mg aspirin relative to action limits
24-hour Response to 81 mg Aspirin

• The systems recorded a significant mean reduction of platelet function 24 hours after a single dose of 81 or 325 mg aspirin
• The ratio of individual subject responses to 81 mg aspirin relative to action limits averaged 30.2%
• The 11-DHT individual subject responses to 81 mg aspirin, 46.9%, is the most sensitive
• The Dade-Behring PFA-100 individual subject responses to 81 mg aspirin, 36.7%, is the second most sensitive

Discussion
Predictive Values of Methods

• The predictive value of 11-DHT, VerifyNow, and PFA-100 compared to aggregation, averages 39% at 81 mg aspirin
• The predictive values of 11-DHT and VerifyNow compared to aggregation at 325 mg aspirin are 86.8% and 93.0%, respectively
• 11-DHT and VerifyNow duplicate the reference method’s ability to identify the 24-hour platelet response to 325 but not 81 mg aspirin
• These data may be confirmed using a 7-day dosage schedule
Analysis

- Platelet inhibition across 3 assays seems to be dose dependent (81mg vs 325 mg) at 24 hours.

- Out of 38 individuals whose WBPA showed no significant changes at 81 mg, 31 of those individual become responders at 325 mg.

- % of aspirin resistance may be high in this study secondary to one time dose effect. If subjects were to take aspirin on daily basis, % of aspirin resistance may drop.

- Initial responders may develop aspirin tolerance according to some studies when taking aspirin chronically.
Comparison of Four Commercial Platelet Function Assays’ Ability to Detect Response to 7 Days of Aspirin at 81 and 325 mg Doses

DL McGlasson, G Fritsma, M Chen, Z Knight, M Dobbs
59th Clinical Research Squadron and Department of Neurology
Wilford Hall Medical Center, Lackland AFB, TX and
University of Alabama Birmingham
Division of Laboratory Medicine, Birmingham, AL
Aspirin Response

• We compared the ability of four commercial platelet function assays to detect the 7-day aspirin (ASA) response in normal subjects taking 81 or 325 mg.

• Laboratory detection of inadequate ASA-induced platelet suppression may indicate physiological insensitivity, called “aspirin resistance.”

• ASA resistance is a recognized cause of failed ASA therapy and may predict arterial thrombosis risk.

• We anticipated the assays would reveal a dosage effect for 325 mg compared to 81 mg ASA.

• We anticipated the assays are comparable in their ability to detect ASA response.
Materials and Methods

- We consented forty-five normal healthy volunteers. None had taken ASA or other NSAIDs for \( \geq 14 \) days
  - 22 females, mean age 33.1 (18-51)
  - 23 males, mean age 36.6 (20-58)
1. Baseline 3.2% Na citrate whole blood and urine
2. Subjects provided a single 81 mg aspirin for 7 days
3. Na citrate whole blood and urine obtained 24 hours after final dose
- Repeated \( \geq 14 \) days with single 325 mg ASA for 7 days
## Mean Responses to 7-Day ASA at 81 mg and 325 mg

<table>
<thead>
<tr>
<th></th>
<th>Chronolog WBA® WBPA Reference Method</th>
<th>11-DHT</th>
<th>Accumetrics Verify/Now®</th>
<th>Dade-Behring PFA-100®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 ug/mL Coll</td>
<td>500 μM AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline pre-81 mg</td>
<td>20.0 Ω</td>
<td>16.8 Ω</td>
<td>538.0 pg/mg</td>
<td>634.5 ARU</td>
</tr>
<tr>
<td>7-d response to 81 mg</td>
<td>6.0 Ω*</td>
<td>3.2 Ω*</td>
<td>161.7 pg/mg*</td>
<td>436.3 ARU*</td>
</tr>
<tr>
<td>Baseline pre-325 mg</td>
<td>21.3 Ω</td>
<td>19.6 Ω</td>
<td>642.4 pg/mg</td>
<td>647.6 ARU</td>
</tr>
<tr>
<td>7-d response to 325 mg</td>
<td>4.1 Ω*</td>
<td>1.0 Ω*</td>
<td>206.7 pg/mg*</td>
<td>425.8 ARU*</td>
</tr>
</tbody>
</table>

No pre-81 mg to pre-325 mg baselines are significantly different at p < 0.05

*All 7-day responses significant at p < 0.05
### Percent 7-Day Response to 81 mg and 325 mg ASA by Action Limits

<table>
<thead>
<tr>
<th></th>
<th>N and (%) ASA Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N = 45</strong></td>
<td></td>
</tr>
<tr>
<td>Chronolog WBA® WBPA Reference Method</td>
<td>11-DHT</td>
</tr>
<tr>
<td></td>
<td>1 ug/mL Coll</td>
</tr>
<tr>
<td>Action Limit</td>
<td>≥ 8 Ω aggregation</td>
</tr>
<tr>
<td>Response to 81 mg ASA</td>
<td>32 (71.1)</td>
</tr>
<tr>
<td>Response to 325 mg ASA</td>
<td>38 (84.4)</td>
</tr>
</tbody>
</table>
Numerical 7-Day Response to 81 mg and 325 mg ASA by Action Limits

Subjects Responsive to ASA by Assay Method (N = 45)
Discussion

• Mean platelet response to ASA at 81 or 325 mg ASA for 7 days for all platforms were significant
• Verify/Now is the most sensitive to 81 mg and 325 mg ASA
• WBPA using 1.0 µg/mL collagen, 11-DHT and PFA-100 detected the most instances of ASA resistance
• Positive predictive values were comparable for 11-DHT, PFA-100, and Verify/Now at 81 and 325 mg
• These data provide support for these methods to use in clinical settings to distinguish aspirin responders vs. non responders
• *We recommend continued testing on clinical populations to confirm the dosage effect and compare platforms to clinical outcomes*
Potential study limitations

- Other possible mechanisms of clinical aspirin resistance
  - Patient non-compliance and underdosing
  - COX 2 expression inducing production of THX-A2
  - Glycoprotein IIb/IIIa polymorphism
  - Erythrocyte/Leukocyte/platelet interaction
    - Elevated fibrinogen and vonWillebrand’s factor
    - Type II Diabetics do not respond as well to ASA
    - Cigarette smoking and hypercholesterolemia
- Platelet inhibition may not be constant over an extended time with a fixed dose of Aspirin
- Aspirin resistance in the single dose study may be higher because of one time dose effect. Percent of aspirin resistance may be reduced if given aspirin on daily basis.
- Some people might show biochemical platelet inhibition at baseline without administration of antiplatelet drugs.
Clinical Implications

• For individuals who do not respond to 81mg ASA when tested by these methods, titrating up aspirin dose may be needed to achieve sufficient platelet inhibition over several days and retest.

• For those who are aspirin non-responders when testing by these methods with 325 mg aspirin (including urine 11-dehydrothromboxane), alternate anti-platelet therapy may be indicated.

• Initial responders may develop aspirin tolerance according to some studies when taking aspirin chronically.

• There are needs for randomized double blind studies to show that by giving alternate anti-platelet therapy in patients with a history of vascular events on ASA and shown to be biochemically ASA resistant, the risk of further events is decreased when compared to those individuals continued with aspirin.
Clinical Implications

- Caveat emptor
  - There are no clinical studies to date showing that patients who are aspirin resistant *in vitro* do not derive some clinical benefit and protection from taking ASA at any accepted dose
  
  - Because of the complexity of the platelet activation process, one single test is unlikely to adequately reflect all aspects of platelet function that are relevant to clinical events
  
  - We need prospective controlled studies to test the hypothesis that *biochemical* aspirin resistance translates to *clinical* aspirin resistance