Aspirin: The 1899 Wonder Drug

Monitoring Aspirin’s Antiplatelet Property

George A Fritsma MS, MLS
The Fritsma Factor, Your interactive Hemostasis Resource
Precision BioLogic Inc, Dartmouth, Nova Scotia
www.fritsmafactor.com
Aspirin Therapy; The Participant…

- Diagrams the biochemical pathways of aspirin's anti-platelet and anti-inflammatory effects.
- Employs aspirin to reduce risk of cardiovascular disease.
- Reviews effect of aspirin on platelet activation in inflammatory disease, cancer, and depression.
- Orders aspirin assays for compliance, efficacy, and dosage.

Please Silence Your Phone
Systemic Sarcoidosis Symptoms

• Chronic inflammation with granulomas in lungs, spleen, throughout body
  – Persistent intense dry cough, hoarseness
  – Tender reddish bumps or patches on the skin
  – Red and teary eyes or blurred vision
  – Swollen and painful joints
  – Enlarged lymph glands in neck, armpits, groin
  – Enlarged lymph glands in chest and around lungs

• Cardiac sarcoid--fatal

• Rx: low-dose prednisone, plaquenil, azathioprine, tumor necrosis factor
Chronic Inflammation Test

Date of Testing: 06/08/18
Date of Collection: 05/25/18
Healthcare Practitioner: N/A

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Test Score*</th>
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<tbody>
<tr>
<td>Chronic Inflammation Test</td>
<td>993</td>
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<tr>
<td>Urinary 11-dehydrothromboxane B₂</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Test Score Range</th>
<th>Category</th>
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<tbody>
<tr>
<td>&lt;141</td>
<td>No apparent inflammation*</td>
</tr>
<tr>
<td>141 – 421</td>
<td>Apparent inflammation</td>
</tr>
<tr>
<td>&gt;421</td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>Strong aspirin effect</td>
</tr>
<tr>
<td>100 – 150</td>
<td>Apparent aspirin effect</td>
</tr>
<tr>
<td>&gt;150</td>
<td>Aspirin not effective</td>
</tr>
</tbody>
</table>

*Test score is calculated by dividing pg 11-dehydrothromboxane B₂/mg creatinine by 10

*Test score valid for individuals 18 years of age and older
**Chronic Inflammation Test with Aspirin**

<table>
<thead>
<tr>
<th>Collected</th>
<th>ASA mg/day</th>
<th>Score</th>
<th>RI</th>
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<tbody>
<tr>
<td>5/25/18</td>
<td>0</td>
<td>993</td>
<td>&lt;421</td>
</tr>
<tr>
<td>5/29/18</td>
<td></td>
<td>134</td>
<td></td>
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<tr>
<td>12/28/18</td>
<td>81</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>1/8/19</td>
<td></td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>2/1/19</td>
<td></td>
<td>131</td>
<td></td>
</tr>
</tbody>
</table>

Aspirin resistant

*Have we resolved the inflammation or just suppressed the platelets?*
Inflammatory Markers

- Proinflammatory cytokines
  - Tumor necrosis factor $\alpha$
  - Interleukin 1 $\beta$
  - Interleukin 6
  - Interferon $\gamma$

- Prostaglandins and leukotrienes
  - Thromboxane A$_2$
  - Thromboxane B$_2$
  - 11-dehydro thromboxane B$_2$

- Acute phase reactants: CRP, VWF, factor VIII, prothrombin, fibrinogen, complement factors, ferritin, ceruloplasmin, serum amyloid A and haptoglobin

- Hundreds more
Felix Hoffman; 1897

- “Willow-bark Salix” appears in 1534 BC Egyptian papyri
- 1800s: Spirea (Meadowsweet) leaves
  - Salicylic acid—required large quantities and caused gastric pain
- 8/10/1897: Felix Hoffman synthesized pure, stable acetyl salicylic acid at Bayer Labs in Leverkusen, Germany
  - Aspirin: a = acetyl; spir = Spirea
- 1899: Bayer lab mixes ASA with starch: made the first tablet
- No scrip: 5 grains [~325 mg], WHO essential medicine list
- 2017: 40,000 tons of aspirin produced, 50,000,000 people
- Uruguayan stamp shows Hoffman, a willow branch, and his signature from the Bayer lab record.

Mann CC, Plummer ML. The Aspirin Wars: Money, Medicine, and 100 Years of Rampant Competition. New York: Knopf 1991.
Dr. Lawrence Craven: 1948

- California PCP documented 400 men on aspirin had no MIs from 1948–50.
  - Recorded Aspergum related to post-T&A bleeding
  - Extended studies to 8000 men
  - Recommended an aspirin a day to reduce risk of heart attacks, was largely ignored
  - Died of a heart attack at age 74

- 1971: JB Smith demonstrated aspirin’s inhibition of prostaglandin synthesis

BAYER Pharmaceutical Products

HEROIN—HYDROCHLORIDE

is pre-eminently adapted for the manufacture of cough elixirs, cough balsams, cough drops, cough lozenges, and cough medicines of any kind. Price in 1 oz. packages, $4.85 per ounce; less in larger quantities. The efficient dose being very small (<1/48 to 1/24 gr.), it is

The Cheapest Specific for the Relief of Coughs

(In bronchitis, phthisis, whooping cough, etc., etc.)

WRITE FOR LITERATURE TO

FARBENFABRIKEN OF ELBERFELD COMPANY
SELLING AGENTS

P. O. Box 2160
40 Stone Street, NEW YORK
Irreversible Acetylation of Cyclooxygenase-1

- PLT dense tubular system COX-1 acetylated at ser\textsubscript{529}
  - Blocks arachidonic acid’s access to reactive “tunnel”
  - Active site amino acid tyr\textsubscript{385} unmodified by acetyl group but blocked

- Platelet loses COX-1 activation pathway
  - AKA eicosanoid synthesis pathway or prostaglandin pathway
  - Total function recovery ~10%/day as new platelets are produced

- Adhesion and shear-induced aggregation remain
  - VWF-dependent

Acetylation of Cyclooxygenase-1

Acetylsalicylic Acid

\[
\text{O} \quad \text{O-C-CH}_3
\]

Salicylic Acid

\[
\text{C-OH} \quad \text{C-OH}
\]

Esterase

Acetylated COX-1

\[
\text{R-CH}_2-\text{NH}_2-\text{C-CH}_3
\]

Serine 529

Aspirin acetylates COX-1 and reduces TXA$_2$ production

1. **Aspirin**
2. **Phospholipase A$_2$**
3. **Arachidonic Acid**
4. **Cyclooxygenase-1**
5. **PGG$_2$/PGH$_2$**
6. **TXA Synthase**
7. **Thromboxane A$_2$ activates platelet**
8. **Prostaglandins**
9. **Aspirin acetylates COX-1 and reduces TXA$_2$ production**
10. **Thromboxane B$_2$ is a stable, measurable plasma product that is reduced in ASA therapy, converts to water-soluble urinary 11-dehydro TXB$_2$**

**Diagram Notes:**
- **Agonist binds membrane receptor**
- **Phosphatidyl Inositol**
- **O**
COX-1 Inhibited by Aspirin

Aspirin

Arachidonic acid (5, 8, 11, 14 eicosatetraenoic acid)

Ser$_{529}$

PGG$_2$/PGH$_2$

TXA$_2$

Acetylated Ser$_{529}$

AA blocked

Cox 1

Cox 1

Tyr$_{385}$

The Fritsma Factor
Aspirin Pharmacology

- 50% absorbed from stomach, duodenum
- Peak plasma levels at 15 minutes
- Hydrolyzed by esterase in gut, liver and RBCs
- Acetyl group hydrolyzed in 20–30 m, leave salicylic acid (measurable salicylate)
  - Platelet COX-1 acetylation occurs in the pre-systemic (portal) circulation of gut and liver
- Reduces plasma TXB₂ levels beginning within 5 m
  - Max reduction in 30 m
- Reduces urinary 11-dehydrothromboxane B2 in 2 h
Aspirin Efficacy in Secondary Prevention: ISIS-2

1988: ISIS-2 demonstrates a 0.78 incidence of death after MI with streptokinase + ASA vs SK + placebo, FDA clears ASA to reduce risk of secondary MI or a first MI in acute angina.

2002 Antiplatelet Trialists’ Collaboration

Meta-analysis of 287 trials w/ 100,000 high-risk patients: composite 32% decrease in death, MI, ischemic stroke in vascular patients on 75–150 mg aspirin daily:

Aspirin Dosage per Indication

• 75 mg (or baby aspirin—81 mg)
  – Primary and 2º MI and peripheral artery disease prevention, stroke prevention in atrial fibrillation, 2º prevention of TIA and stroke
  – Prevent pre-eclampsia, support fetal retention in primary antiphospholipid syndrome (with low MW heparin)

• 300 mg (or adult—325 mg) subsequent to:
  – MI, acute unstable angina, acute TIA, acute ischemic stroke

Aspirin Efficacy in Primary Prevention

- **Physician’s Health Study 1982–96 (♂’s only)**
  - 1086 healthy ♂ physicians, 40–84
  - 325 mg aspirin on alternate days versus placebo
  - 44% reduction of fatal or nonfatal first MIs
  - Ethical termination at 60 months, 1987
  - ASA cleared in 1988 to prevent TIAs and strokes in healthy ♂’s >50

- **Women’s Health Study 1991–2000 (♀’s only)**
  - 39,876 healthy health care ♀’s over 45
  - 100 mg aspirin on alternate days versus placebo
  - 25% reduction in fatal or non-fatal first MIs
  - But 50% reduction in smokers, hypertensives, hypercholesterolemia, greatest effect >65 YO


# 2018: Weight-based ASA Dosages Affect Odds Ratio of Primary CAD

<table>
<thead>
<tr>
<th>Mass</th>
<th>ASA mg/d</th>
<th>Primary CAD OR</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>50–69 kg</td>
<td>75–100</td>
<td>0.75 (P= .007)</td>
<td>&gt;100 mg ASA raises CAD risk!</td>
</tr>
<tr>
<td>&gt;70 kg</td>
<td>75–100</td>
<td>0.95 (non-sig)</td>
<td>75–100 mg raises CAD risk to 1.33 (P= .0082)!</td>
</tr>
<tr>
<td></td>
<td>&gt;325</td>
<td>↓ (P= .017)</td>
<td></td>
</tr>
</tbody>
</table>

Height data match weight data, findings similar in ♂ and ♀.

Worldwide, 80% of men and 50% of women are >70 kg.

In >70 YO, ASA raised 3Y Ca risk by OR 1.2 (P= .02) more later.

9/22/18: ASA Offers No Protection

- Measured vascular events: CAD, PAD, ischemic stroke
- Questionable protection for those with thrombotic risk factors
- No protection for those without cardiac indications
- Bleeding risk outweighs protection

ASCEND, ARRIVE, and ASPREE Trials

- NEJM 9/16/18: 20,000 >70 in US & Australia
- Primary outcome composite of death, dementia, and disability—no effect
- ASA actually worse in…
  - All-cause mortality
  - Cancer-related death

**Why the change?**

- Modern CAD outcomes negate ASA value?
- CAD redefined?

What is Lacking in Aspirin Studies?

Labs! Why?

• No guideline advocating for aspirin lab assay
• Lab assays are surrogates for outcomes
• VerifyNow and PFA-100 results not reproducible
• Lab assay platform results differ among patients
• But what if you dosed on lab results?
THE DUTCH BOY'S LEAD PARTY

A Paint Book for Girls and Boys

With which is bound
COLOR HARMONY IN THE HOME
A Booklet for the Grown-ups
Aspirin Resistance Monitoring

• Aspirin resistance
  – Laboratory phenomenon: suboptimal platelet function suppression

• Aspirin failure
  – Secondary stroke, MI, peripheral artery disease while on ASA Rx
  – Adverse thrombotic events such as fetal loss in antiphospholipid syndrome despite aspirin therapy
Light Transmittance Aggregometry (LTA) Specimen Preparation

- Collect 9–12 mL whole blood
  - 3–4 2.7 mL tubes + 0.3 citrate
- Centrifuge at 50g 30” (PRP)
- Wait 30 m for “platelet shock”
- Dispense to cuvette
- Test within 4 hours
- Pipette agonist, record absorbance by photometry
- Attempt to record secretion by analyzing “lag phase”
The Fritsma Factor

Resting Platelets

Shape Change

0% Transmittance

Light Transmittance Aggregometry

Primary Aggregation

Secondary Aggregation

Agonist

100% Transmittance

5 minutes

Courtesy of Kathy Jacobs, Chronolog, Inc
Impedance-based Whole Blood Aggregometry (WBA)

- Collect 9 mL blood, do not centrifuge
  - 3 tubes each 2.7 mL + 0.3 citrate
- Aliquot, dilute 1:1 with saline
- Pipette agonist, timer starts
- Electrodes lowered into suspension
WBA: Impedance

- Aggregating platelets form layer on electrodes
- Platelet layer impedes current
  - Resistance in ohms (Ω)
  - 0 Ω = no aggregation
  - Aggregation proportional to Ω
  - Same pattern as LTA

Courtesy of Kathy Jacobs, Chronolog, Inc
Aspirin Efficacy Agonists

- 0.5 mM arachidonic acid (AA)
  - Directly activates eicosanoid synthesis pathway to produce TXA₂
  - TXA₂ activates platelet by binding internal receptors TPα or TPβ
  - Response reduced by aspirin

- 1–5 µg/mL collagen
  - Binds receptors GP Ia/IIa (integrin α2β1), GP IV, GP VI
  - Response reduced by aspirin
  - May bypass aspirin effect, aggregation via alternate pathways

- 5–10 µM ADP
  - ADP binds P₂Y₁₂ receptor
  - Response reduced by thienopyridines like Plavix, Brilinta
  - May bypass aspirin effect, aggregation via alternate pathways
Secretion Response Using The “Firefly” Reaction

- Luciferin
- Chromo-lume® firefly luciferase reagent
- Proportional to ATP release in nM

\[ \text{ATP} \xrightarrow{\text{Luciferin}} \text{Luciferyl:AMP} \xrightarrow{\text{O}_2} \text{Oxyluciferin + AMP} \]

Luminescence

Courtesy of Kathy Jacobs, Chronolog, Inc
Normal Whole Blood Impedance Lumiaggregometry

5 µg/mL collagen, 0.5 mM arachidonic acid, 5 µM ADP
Aspirin or “Aspirin-like Disorder” (Signaling Defect)

The Fritsma Factor

Impedance

0

20 Ω

40 Ω

0.5 mM arachidonic acid

5 Minutes

10 Minutes

ATP Release

3.0 μM

1.5 μM

No (< 5 Ω) Aggregation

No (< 0.1 nM) Release

Courtesy of Margaret Fritsma, UAB
<table>
<thead>
<tr>
<th>Agonist</th>
<th>Collagen 5 μg/mL</th>
</tr>
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<tbody>
<tr>
<td>Tracing</td>
<td>2-Control</td>
</tr>
<tr>
<td>Luminescence</td>
<td>0.83ηm</td>
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<tr>
<td>(Amplitude)</td>
<td>0.81ηm</td>
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<tr>
<td>Aggregation</td>
<td>38Ω</td>
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<tr>
<td>(Impedance)</td>
<td>37Ω</td>
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<table>
<thead>
<tr>
<th>Agonist</th>
<th>Arachidonic Acid 0.5 mM</th>
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<td>Luminescence</td>
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<tr>
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<tr>
<td>(Impedance)</td>
<td>25Ω</td>
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</table>

Courtesy of Patti Tichenor, UAB
Coagulation; using AGGRO/LINK®
<table>
<thead>
<tr>
<th>Agonist</th>
<th>Collagen 5 µg/mL</th>
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<tr>
<td>Tracing</td>
<td>2-Control 4-Patient</td>
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<tr>
<td>Luminescence (Amplitude)</td>
<td>1.69ηm 0.57ηm</td>
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<tr>
<td>Tracing</td>
<td>1 Control 3-Patient</td>
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<td>Aggregation (Impedance)</td>
<td>39Ω 26Ω</td>
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<table>
<thead>
<tr>
<th>Agonist</th>
<th>Arachidonic Acid 0.5 mM</th>
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<td>2-Control 4-Patient</td>
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<tr>
<td>Luminescence (Amplitude)</td>
<td>1.42ηm 0.57ηm</td>
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<tr>
<td>Tracing</td>
<td>1 Control 3-Patient</td>
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<td>Aggregation (Impedance)</td>
<td>26Ω 0Ω</td>
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</table>

Aspirin

Courtesy of Laura Taylor, UAB
Coagulation; using AGGRO/LINK®
Siemens (Dade-Behring) PFA-100

- Transfer 800 μL citrated whole blood to each of two cartridges, load and run to closure time (CT)
  - Coated membrane: agonists
- Collagen and epinephrine (Col/Epi, CEPI)
  - “Weak:” normal CT 98–185 s
  - Initial cartridge
- Collagen and ADP (Col/ADP, CADP)
  - 50 μM ADP
  - “Strong:” normal CT 77–133 s
  - Confirmatory cartridge
PFA-100 Aspirin Response Assay

- Col/Epi Cartridge
  - CT > 185s
  - Col/ADP Cartridge
    - CT > 125s
      - CT < 125s
        - Normal or aspirin resistance

- If ASA present and CEPI CT is < 185s, aspirin resistance

- PFA-100 does not use arachidonic acid

- CT < 185s

- CT > 125s
  - Severe VWD
  - Severe PLT dysfunction

- • Aspirin, NSAIDs
  • Reduced HCT or PLT count
  • Mild PLT dysfunction
  • Mild VWD

Normal or aspirin resistance
Werfen VerifyNow®
Aspirin Resistance Units (ARU)

Whole blood light transmittance rises as platelets aggregate to arachidonic acid. Aggregation suppressed by aspirin:

- ARUs <550: function inhibited = aspirin sensitive
- ARUs >550: function normal = aspirin resistance
Werfen VerifyNow Reaction Chamber

ARUs measure aspirin resistance
VerifyNow Principle

Light source → Mixing chamber → Increase in light transmittance with agglutination of beads; rate and extent of change measured

GP IIb/IIIa integrin → Platelets in whole blood activated by specific agonist in mixing chamber

Fibrinogen-coated beads → Agglutinated beads fall out of the solution
Werfen VerifyNow and PFA-100 Limitations

• Cartridges ~$5.00 each
• Whole blood specimen volume 800 µL/assay
• Precision: CVs >10%, often requires duplication
• Must test whole blood within four hours
• Variable effects of…
  – von Willebrand factor, factor VIII, fibrinogen
  – Platelet count and hematocrit

• Urinary 11-dehydrothromboxane B₂ (11-DHTB₂)
  – Liver-produce metabolite of plasma thromboxane B₂

• Platelet is the primary source for 11-DHTB₂
  – Also renal endothelial cells, monocytes

• Random urine specimen: store and ship
  – Normalized to urine creatinine: Pg 11-DHTB₂/mg creatinine

• Liver and renal disease limitations
2002 HOPE Study: Aspirin Resistance

- Nested (quartile) retrospective case-control sample
  - 488 aspirin-treated CAD patients: end point was 5-yr secondary MI, stroke, or CV death
  - 488 age- and sex-matched controls taking aspirin who did not have an MI, stroke, or CV death

- In aspirin-treated CAD patients, 11-DHTB<sub>2</sub> predicts risk of MI or CV death: fourth quartile 11-DHTB<sub>2</sub> = OR 3.5 for CV death

<table>
<thead>
<tr>
<th>Pg 11-DHTB&lt;sub&gt;2&lt;/sub&gt;/mg creatinine</th>
<th>Quartile</th>
<th>Odds Ratio</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MI</td>
</tr>
<tr>
<td>&lt;134</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>134–193</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>194–298</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>&gt;298</td>
<td>4</td>
<td>2.0</td>
</tr>
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</table>

CHARISMA Trial: Dual Antiplatelet Therapy

- Randomized double-blind prospective trial of 3261 clopidogrel Vs. placebo in patients on aspirin at high risk of athero-thrombosis
  - Tested 1 month after starting clopidogrel, assigned quartiles
  - 144 with one-year stroke, MI, or CV death
  - 3117 with no adverse event
- Fourth quartile 11-DHT$_2$ composite OR=1.66

Kaplan–Meier curves for composite of stroke, MI, or CV death by quartiles

CHARISMA Trial Conclusions

- Fourth quartile normal or elevated $11\text-DHTB}_2$
  - Signifies aspirin resistance
  - Age, $♀$, PAD Hx, smoking, oral hypoglycemic Rx, ACE-inhibitor Rx

- Reduced $11\text-DHTB}_2$
  - Aspirin Rx $>150$ mg/d, NSAIDs, hypercholesterolemia, statin Rx

- Randomization to clopidogrel or placebo did not reduce risk ratio for CV events in patients in the fourth $11\text-DHTB}_2$ quartile

- $11\text-DHTB}_2$ level is potentially modifiable
# Aspirin Resistance Prevalence

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
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<tbody>
<tr>
<td>By definition</td>
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<tr>
<td>PFA-100</td>
<td>29.0%</td>
<td></td>
</tr>
<tr>
<td>Ultegra VerifyNow</td>
<td>26.2%</td>
<td></td>
</tr>
<tr>
<td>LTA</td>
<td>21.3%</td>
<td></td>
</tr>
<tr>
<td>By population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>22.9%</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>32.1%</td>
<td></td>
</tr>
<tr>
<td>By dose</td>
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<td></td>
</tr>
<tr>
<td>&lt; 100 mg/d</td>
<td>35.6%</td>
<td></td>
</tr>
<tr>
<td>101–299 mg/d</td>
<td>28.2%</td>
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<tr>
<td>&gt; 300 mg/d</td>
<td>18.6%</td>
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</table>

# Aspirin Resistance and Adverse Events Review

<table>
<thead>
<tr>
<th>Type</th>
<th>Percutaneous Intervention (Cath)</th>
<th>Stable CAD</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>151</td>
<td>106</td>
</tr>
<tr>
<td>% AR</td>
<td>19.2%</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>VerifyNow</td>
<td>Light Transmittance Aggregometry</td>
</tr>
<tr>
<td>Results</td>
<td>Elevated creatine kinase and troponin I in AR</td>
<td>4th quartile ADP response associated with OR for CV events = 22.4</td>
</tr>
</tbody>
</table>

AR= ASA resistance; CAD= coronary artery disease; OR= odds ratio; CV= cardiovascular; MI= myocardial infarction; CVA= cerebrovascular event
# Siemens PFA-100 and Aspirin Resistance

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum PA, JACC 2003;41:961</td>
<td>9.5% aspirin resistance by CEPI closure time, poor correlation with LTA</td>
</tr>
<tr>
<td>Hézard N, Thromb Res 2002;108:43</td>
<td>Poor aspirin resistance correlation among LTA, CEPI closure time, and flow cytometry (P-selectin)</td>
</tr>
<tr>
<td>Sane DC. Thromb Haemost 2002;88:711</td>
<td>No CEPI closure time difference between aspirin resistance and aspirin sensitive</td>
</tr>
<tr>
<td>Ten Berg JM, Thromb Res 2002;105:385</td>
<td>CEPI closure time does not distinguish low dose from high dose aspirin</td>
</tr>
<tr>
<td>Grundmann K, J Neurol 2003;250:63</td>
<td>53 patients on aspirin for stroke prevention: CEPI closure time significantly shorter in 12/35 patients with recurrent stroke (p &lt;0.01)</td>
</tr>
</tbody>
</table>

CEPI = collagen-epinephrine cartridge  
LTA = light transmittance aggregometry
Variation in Lab Detection of Aspirin Resistance

<table>
<thead>
<tr>
<th>Assay</th>
<th>Aspirin Resistance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Werfen VerifyNow Aspirin</td>
<td>17</td>
</tr>
<tr>
<td>Siemens PFA-100 CEPI</td>
<td>22</td>
</tr>
<tr>
<td>LTA</td>
<td>5</td>
</tr>
<tr>
<td>All tests abnormal per subject</td>
<td>2</td>
</tr>
</tbody>
</table>

24-h Response to 81 & 325 mg ASA

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

Marked dosage difference
Aspirin Seven Days: Test Efficacy Comparison to Whole Blood Aggregometry

<table>
<thead>
<tr>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage</td>
<td>81 mg</td>
</tr>
<tr>
<td>11 DehydroTXB₂</td>
<td>74.3%</td>
</tr>
<tr>
<td>PFA-100 CEPI</td>
<td>81.3%</td>
</tr>
<tr>
<td>VerifyNow Aspirin</td>
<td>72.7%</td>
</tr>
<tr>
<td></td>
<td>51.9%</td>
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</tbody>
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“Laboratory measures of PLT activity are suppressed by ASA Rx, and are affected by the dosage and duration of therapy. Determinations of aspirin response should be made after at least 7 days of treatment. Laboratory test platform results do not closely reflect each other, thus application of laboratory platforms should be made consistently.”

7-d Response to 81 & 325 mg

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

Little dosage difference but patient response is greater
Aspirin Resistance Study Limitations

- Inter-assay variation
- Biological variation over time
- Failure to adjust for race, age and sex
- Failure to confirm compliance
  - Serum salicylate?
  - Non-compliance and early withdrawal may account for most AR
- Failure to separate confounding conditions
  - Hypertension, diabetes, peripheral vascular disease, smoking, and inflammation may contribute to aspirin resistance, while independently raising vascular risk
Proposed Mechanisms of Aspirin Resistance

- Increased platelet turnover: >10%/d
- Activation of alternate platelet pathways not blocked by aspirin
  - Diacylglycerol pathway activated through G-protein
  - Adhesion molecules: collagen (GPIa/IIa) & VWF receptors (GPIb/IX/V)
  - Activation by shear stress in atherosclerosis
- Aspirin-mediated reduction of PLT-inhibiting prostacyclins from vascular endothelial cells
- Elevated VWF, fibrinogen activity level
- Polypharmacy (> 4 drugs)

More Proposed Mechanisms of Aspirin Resistance

- NSAIDs compete for Ser$_{529}$ site
  - Naprosyn, ibuprofen reversible bonds block ASA

- COX-2 Induction most likely
  - Non-constitutive, COX-2 response to cytokines and inflammation
  - Smoking, diabetes, heart failure and hyperlipidemia
  - COX-2 in megakaryocytes, monocytes, macrophages, vascular endothelial cells and newly released platelets
  - After bypass surgery, 16-fold increase of COX-2 causing transient aspirin resistance
  - Acetylation of COX-2 ser$_{529}$ incompletely hinders arachidonic acid’s access to reactive site

COX-2 200x Less Inhibited by Aspirin

Aspirin

Acetylated Ser$_{529}$

COX-2

PGG$_2$/PGH$_2$

Tyr$_{385}$

TXA$_2$

AA partially blocked

Arachidonic Acid

The Fritsma Factor
Dual Antiplatelet Therapy

- Aspirin, 81 or 325 mg + clopidogrel, 75 mg; or prasugrel, ticagrelor critical in stents, reduce secondary MI 20%
- Clopidogrel resistance 15–63% detected by molecular or phenotypic tests, predicts risk of secondary MI
- Little variability in response to prasugrel or ticagrelor
- Ticagrelor is more effective than clopidogrel in ACS with and without PCI. Similar rates of bleeding as clopidogrel
- Typical Rx duration: aspirin indefinite, clopidogrel 1–2 y
- Triple therapy: ASA & clopidogrel + Coumadin or DOAC: benefit in mechanical valves, bleeding up 43%, mortality down 57%
  - Clinical management is tricky
  - Laboratory monitoring may be confounded by polypharmacy
Bridging Dual Antiplatelet Therapy During Surgery

- Discontinue: 20% incidence of ischemia
- Continue: 35% increased bleeding
- ISTH 2019 guidelines
  - Harmonize risks relative to procedure
  - Assess surgical procedure bleeding risk: min, low, mod, high
  - Assess patient thrombosis risk: low, mod, high

So, do we test for aspirin resistance?


Dr. Kristi Smock, ARUP: “I think it is a problem of using different definitions for aspirin resistance and measuring it with tests that have different sensitivities and specificities.” “Moreover,” she adds, “testing for this condition is not generally recommended because it is not known what the treatment changes would be.”

Response: Two Meta-Analyses

• Mean prevalence of laboratory ASA resistance among all methods is 27%. Resistance predicts 3.8 OR for adverse cardiovascular outcomes. No concordance among methods.

• 20 studies totaling 2930 patients with cardiovascular disease. Classified 28% as aspirin resistant. Resistance confers a 3.85 OR for any adverse cardiovascular outcome including a 5.99 OR for death and a 2.96 OR for acute coronary syndrome. No concordance among methods.

• Snoep JD, Hovens MMC, Eikenboom JCJ, et al. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis Arch Inter. Arch Intern Med 2007;167:1593-9

Platelet-Tumor Crosstalk

“Tumor-educated Platelets” (TEP) AKA Tumor Cell-induced Platelet Aggregation (TCIPA)

Tumor receptor $\alpha V\beta 3$ binds PLT $\alpha IIb\beta 3$ (IIb-IIIa) via fibrinogen, aggregates activate PLTs, generate fibrin, activate nearby PLTs and inflammatory WBCs.

Tumor receptor ADAM9 binds PLT $\alpha 6\beta 1$, causes tumor cell extravasation.

Tumors synthesize VWF, binds PLT Ib$\alpha$, mediates metastasis.

May be mitigated by Anfibatide, a venom anti-Ib$\alpha$ polypeptide.

Activated PLTs secrete vascular endothelial GF, platelet-derived GF, trigger tumor angiogenesis. PF4 mitigates tumor hemorrhage by binding heparan sulfate.

PF4 triggers thrombocytopoiesis; thrombocytosis predicts cancer risk, thrombocytopenia reduces risk and enhances chemotherapy.

Activated PLTs generate microparticles that stimulate lung tumors, also a lab tumor marker.
“Tumor-educated Platelets” (TEP)
Tumors Alter Platelet RNA

- PLTs gain tumor markers
- Platelet mRNA sequencing identifies cancer with 96% accuracy
  - Assay is simplified without nuclear DNA or mitochondria
- Sequencing IDs glioblastoma, colorectal, pancreatic, hepatobiliary, breast, and non-small-cell lung cancer with 71% accuracy
- Tumors can likewise "adopt" PLT RNA and markers to evade immunosurveillance.
Platelets Support Tumor Growth and Metastasis

**PLT transforming growth factor β (TGFβ)**
- **PLT** proliferates ovarian tumors
- **PLTs** transform leukemia cells to resist apoptosis
- **PLTs** transform epithelial cells so they invade and seed nearby tissues

Hetero-aggregates of tumor cells, PLTs, and WBCs evade shear damage & immunosurveillance, support extravasation and seeding. Tumor cells “hitch a ride” on PLTs and WBCs to metastasize.
Aspirin Protects Against Cancer

• In 2016, US Preventive Service Task Force recommended 75–100 mg/d ASA for ages 50–69 to reduce risk of cardiovascular disease and colorectal cancer
• COX-2 is overexpressed in colorectal, breast, gastric, lung, pancreatic cancer, and melanoma. COX-2 expression is upregulated by nearby activated platelets.
• PGE2, produced in the eicosanoid synthesis pathway enhances tumor proliferation, angiogenesis, differentiation, inflammation, and immune escape.
• Slow-release 75 mg ASA sufficient to reduce incidence, metastasis, mortality, but…
• Doses up to 600 mg/d needed to suppress COX-2

Savitz JB, Teague TK, Misaki M, et al. Treatment of bipolar depression with minocycline and/or aspirin: an adaptive, 2Å~2 double-blind, randomized, placebo controlled, phase IIA clinical trial. Translational Psychiatry 2018;8
Inflammation, activated platelets and thromboxane A2 generation in occurrence, progression and metastasis of cancer.

- Nuclear Factor kappa B (NFkB)
- IkB
- Cyclooxygenase-2 (COX-2)
- Cyclooxygenase -1 (COX-1)
- Prostaglandin H2
- Thromboxane Synthase
- Thromboxane B2
- Thromboxane A2 **
- Urinary 11-dehydrothromboxane B2 (Chronic Inflammation Test)
- Activated Platelet
- Platelet
- Cancer
Activated Platelets and Cancer References


Activated Platelets and Cancer References


Thanks for Listening