1. What do we know about it?

2. How do we define it?

3. What causes it?

4. What do we do about it?
1. What do we know about it?

2. How do we define it?

3. What causes it?

4. What do we do about it?
Long-term Efficacy of ASA in Reducing Death or MI in Patients with Unstable Angina

Wallentin LC et al JACC 1991;18:1587–1593
People still have events while on ASA!

Do all patients respond in the same way?
Inter-Individual Variability in Response to ASA

N=10

Quick AJ.
American Journal of Medical Science
Sept 1966:265-9
Platelet Hyperreactivity Following ACS Predicts 5-Year Outcomes

- Relative risk compared to group with negative aggregation.

*RR=1.6 CI (0.7-3.5)
*RR=3.1 CI (1.6-5.8)
*RR=5.4 CI (2.2-13.4)

## ASA Resistance: Long-term Clinical Studies

<table>
<thead>
<tr>
<th>Pts</th>
<th>ASA dose</th>
<th>Test</th>
<th>F/U</th>
<th>End-point</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke(^1) (n=180)</td>
<td>1500 mg</td>
<td>Plt Reactivity</td>
<td>24 m</td>
<td>Stroke/MI/ Vascular death</td>
<td>10-fold lower risk in ASA responders</td>
</tr>
<tr>
<td>PVD(^2) (n=100)</td>
<td>100 mg</td>
<td>Whole blood Aggregometry</td>
<td>18 m</td>
<td>Arterial Occlusion</td>
<td>87% higher risk in ASA-R</td>
</tr>
<tr>
<td>CVD/CVA(^3) (n=53) TIA</td>
<td>100 mg</td>
<td>PFA-10</td>
<td>&gt;60 m</td>
<td>Recurrent CVA/ TIA</td>
<td>Recurrent CVA 34% ASA-R vs. 0% no recurrent events</td>
</tr>
<tr>
<td>Subgroup HOPE(^4) (n=967)</td>
<td>75-325 mg</td>
<td>Urinary 11-dehydro TX B2</td>
<td>5 yrs</td>
<td>MI/Stroke/ CVDeath</td>
<td>1.8 times higher risk in upper vs. lower quartile</td>
</tr>
<tr>
<td>CVD(^5) (n=326)</td>
<td>325 mg</td>
<td>Optical platelet aggregation</td>
<td>679±185 days</td>
<td>Death/MI/CVA</td>
<td>24% ASA-R vs. 10% ASA-S [HR 3.12 (95% CI 1.1-8.9, p=0.03)]</td>
</tr>
</tbody>
</table>

**Primary Endpoint—MI/Stroke/CV Death**

The primary outcome occurred in 9.3% of pts in the clopidogrel + ASA group and 11.4% in the placebo + ASA group.

*Other standard therapies were used as appropriate.

Clopidogrel Response Variability following Loading Dose Administration

$n = 48$

- **Clopidogrel 300 mg**
- **UFH 100 UI/kg**
- **baseline (ASA)**
- **10 min Post-PCI**
- **4 hrs post-PCI**
- **24 hrs post-PCI**

IPA - LTA (%)

**Median (SD) %**

- 7.8(11.3)
- 25.6(22.3)
- 40.9(26.2)

Individual Response Variability to Dual Antiplatelet Therapy in the Steady State Phase of Treatment

% Platelet Aggregation (LTA-ADP 20 µmol/L)

Bleeding risk
Ischemic risk

Adapted from Angiolillo DJ et al. Am J Cardiol. 2006;97:38-43.
# Clinical Relevance of Clopidogrel Non-responsiveness

## Post-Stent Ischemic Events and Periprocedural Infarction

<table>
<thead>
<tr>
<th>N</th>
<th>Functional Parameter</th>
<th>Clinical Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>↑ platelet aggregation (4th quartile)</td>
<td>Post-primary PCI ischemic events (6 months)</td>
</tr>
<tr>
<td>192</td>
<td>↑ periprocedural platelet aggregation</td>
<td>Post-PCI ischemic events (6 months)</td>
</tr>
<tr>
<td>120</td>
<td>↑ periprocedural platelet aggregation</td>
<td>Myonecrosis and inflammation marker release</td>
</tr>
<tr>
<td>106</td>
<td>↑ platelet aggregation</td>
<td>Post-PCI ischemic events (30 days)</td>
</tr>
<tr>
<td>120</td>
<td>↑ clopidogrel/aspirin-resistant patients</td>
<td>Post PCI-myonecrosis</td>
</tr>
<tr>
<td>292</td>
<td>↑ platelet aggregation</td>
<td>Post-PCI ischemic events (30 days)</td>
</tr>
<tr>
<td>802</td>
<td>↑ platelet aggregation (3rd &amp; 4th quartiles)</td>
<td>Post-PCI ischemic events (30 days)</td>
</tr>
<tr>
<td>379</td>
<td>↓ platelet inhibition</td>
<td>Post-PCI ischemic events (3 months)</td>
</tr>
<tr>
<td>100</td>
<td>↑ platelet aggregation</td>
<td>Post-PCI ischemic events (12 months)</td>
</tr>
<tr>
<td>173</td>
<td>↑ platelet aggregation (4th quartile)</td>
<td>Ischemic events (24 months)</td>
</tr>
</tbody>
</table>

adapted from Angiolillo DJ et al. Am J Cardiov Drugs.
## Clinical Relevance of Clopidogrel Non-responsiveness

### Stent Thrombosis

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Functional Parameter</th>
<th>Clinical Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mueller et al. Thromb Haemost 2003</td>
<td>105</td>
<td>↓ inhibition of platelet aggregation</td>
<td>Stent thrombosis</td>
</tr>
<tr>
<td>Barragan et al. CCI 2003</td>
<td>36</td>
<td>↑P2Y&lt;sub&gt;12&lt;/sub&gt; reactivity ratio (VASP-levels)</td>
<td>Stent thrombosis</td>
</tr>
<tr>
<td>Gurbel et al. JACC 2005</td>
<td>120</td>
<td>↑P2Y&lt;sub&gt;12&lt;/sub&gt; reactivity ratio; ↑ platelet aggregation; ↑ stimulated GPIIb/IIa expression</td>
<td>Stent thrombosis</td>
</tr>
<tr>
<td>Ajzenberg et al. JACC 2005</td>
<td>49</td>
<td>↑ shear-induced platelet aggregation</td>
<td>Stent thrombosis</td>
</tr>
<tr>
<td>Buonamici et al JACC 2007</td>
<td>804</td>
<td>↑ platelet aggregation</td>
<td>Stent thrombosis</td>
</tr>
</tbody>
</table>

adapted from Angiolillo DJ et al. Am J Cardiov Drugs. 2007.
Variability in Responsiveness to Antiplatelet Therapy

1. What do we know about it?

2. How do we define it?

3. What causes it?

4. What do we do about it?
Definition(s) of “APT Resistance?”

The fact that some patients may experience recurrent vascular events despite the use of APT should be properly defined as “treatment failure” rather than “APT resistance” (multiple pathways mediate thrombotic events).

APT Resistance/Non-responsiveness = Failure to inhibit the target

APT Resistance/Non-responsiveness ≠ Clinical failure
Platelet Function Tests

- **Platelet Aggregation**
  - Light transmittance aggregometry (LTA) \(\rightarrow gold\ standard\)
  - Impedance platelet aggregation

- **Flow Cytometry**
  - GPIIb/IIIa receptor activation
  - P-selectin expression
  - Monocyte-platelet aggregates
  - Vasodilator-associated stimulated phosphoprotein (VASP)

- **Point-of-care**
  - Ultegra rapid platelet function analyzer (VerifyNow)
  - Thromboelastagrapb (TEG)
  - PFA-100
  - Plateletworks
  - Cone and plate(let) analyzer (IMPACT)

- **Genetic testing**

adapted from Angiolillo DJ et al. J Am Coll Cardiol. 2007
**Light Transmittance Aggregometry**

**Test of platelet aggregation**

- Agonist (e.g., ADP, collagen, arachidonic acid, epinephrine) added to platelet rich plasma
- Platelet aggregation is monitored by change in light transmittance

![Diagram showing light transmittance over time](chart)

Agonist added at Time=0

% Transmittance

0

100

Time
Light Transmittance Aggregometry

1 mM AA = 2%
2 mM AA = 4%
5 µM ADP = 54%
20 µM ADP = 74%
ASPECT study: Individual platelet aggregation data measured after stimuli by 3 concentrations of AA by LTA at 3 different doses of aspirin.
### ASPECT study: Effects of Assay and Dose on Measurement of Aspirin Resistance

<table>
<thead>
<tr>
<th>Assay</th>
<th>Resistance (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>81 mg</td>
</tr>
<tr>
<td>LTA</td>
<td></td>
</tr>
<tr>
<td>1 mmol/L AA</td>
<td>1</td>
</tr>
<tr>
<td>2 mmol/L AA</td>
<td>2</td>
</tr>
<tr>
<td>5 mmol/L AA</td>
<td>2</td>
</tr>
<tr>
<td>2 µg/mL Collagen</td>
<td>12</td>
</tr>
<tr>
<td>5 µmol/L ADP</td>
<td>19</td>
</tr>
<tr>
<td>TEG-1 mmol/L AA</td>
<td>5</td>
</tr>
<tr>
<td>VerifyNow</td>
<td>7</td>
</tr>
<tr>
<td>Urinary 11-dehydro-Thromboxane B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>31</td>
</tr>
<tr>
<td>PFA-100</td>
<td>32</td>
</tr>
</tbody>
</table>

Clinical Outcomes: Aspirin Responsiveness by Aggregometry And PFA-100

Clinical Outcomes based on PFA-100 Results
CEPI-CT ≤ 193 s

Gum, P. JACC 2003;41:961-5
Inhibition of Platelet Aggregation (IPA)

\[
IPA = \frac{(\text{MPA}_{\text{Pre}} - \text{MPA}_{\text{Post}}) \times 100}{\text{MPA}_{\text{Pre}}}
\]

57% = \frac{(77 - 33) \times 100}{77}

Maximal Platelet Aggregation (MPA)

Light Transmittance Aggregometry
Definitions of Non/Low – Response using LTA

Gurbel PA et al., Circulation 2003
Absolute change in platelet aggregation from baseline < 10%

Müller I et al., Thromb Haemostas 2003
Relative change in platelet aggregation from baseline < 10%

Matetzky S et al., Circulation 2004
Lowest quartile of relative reduction of platelet aggregation

Serebruany VL et al., J Am Coll Cardiol 2005
Platelet aggregation 2 standard deviations below mean

Angiolillo DJ et al., Thromb Res 2005
Relative change in platelet aggregation from baseline < 40%

(Variable results also depending on the concentration of ADP used)
Definitions of Non-Response: Which one should we use? Absolute Change or Relative Change or Post-treatment platelet reactivity?

![Graph A](Responder)

- **Responder**
  - Baseline (Pre)
  - + Treatment (Post)
  - %Transmittance
  - Time (minutes)
  - (80 – 55) X 100%
  - 31% = 80

![Graph B](Low-Responder)

- **Low-Responder**
  - Baseline (Pre)
  - + Treatment (Post)
  - %Transmittance
  - Time (minutes)
  - (50 – 41) X 100%
  - 18% = 50

**Responder**

- Baseline (Pre)
- + Treatment (Post)
- %Transmittance
- Time (minutes)
- (80 – 55) X 100%
- 31% = 80

**Non-Responder**

- Baseline (Pre)
- + Treatment (Post)
- %Transmittance
- Time (minutes)
- (50 – 41) X 100%
- 18% = 50

---

**Absolute Change**

- 31% = (80 – 55) X 100%

**Relative Change**

- 18% = (50 – 41) X 100%

---

**Definitions of Non-Response:**

- **Responder:**
  - Baseline (Pre) to Treatment (Post) transmittance change

- **Non-Responder:**
  - Baseline (Pre) to Treatment (Post) transmittance change

**Comparison:**

- **Responder:**
  - Transmittance change = 31%

- **Non-Responder:**
  - Transmittance change = 18%
Platelet Reactivity in Patients and Recurrent Events Post-Stenting: Results of the PREPARE POST-STENTING Study

Therapeutic target for P2Y_{12} inhibition (?)

Prospective studies with “tailored” treatment regimens warranted!

Optimal ROC determined cut-off value to define MACE in T2DM

MACE (CV death, STEMI, UA/NSTEMI, stroke)

Log Rank, p = 0.0002

HR: 3.35; 95% CI, 1.68-6.66; p= 0.001

Variability in Responsiveness to Antiplatelet Therapy

1. What do we know about it?

2. How do we define it?

3. What causes it?

4. What do we do about it?
Aspirin Resistance – “More Than Just a Laboratory Curiosity”

**Clinical Factors**
- Failure to prescribe
- Non-compliance
- Non-absorption
- Interaction with ibuprofen

**Cellular Factors**
- Insufficient suppression of COX-1
- Over-expression of COX-2 mRNA
- Erythrocyte induced platelet activation
- Increased norepinephrine
- Generation of 8-iso-PGF$_{2\alpha}$

**Genetic Polymorphisms**
- COX-1
- GPIIIa receptor
- Collagen receptor
- vWF receptor

Overestimation of Aspirin Resistance: *Key Role of Compliance*
Platelet function (COX-1 independent) in DM vs non-DM on aspirin

**LTA-ADP**

- **DM:** 60% Platelet aggregation
- **Non-DM:** 20% Platelet aggregation

- **p<0.05**

**PFA-100**

- **DM:** 90% Patients CEPI-CT<300 sec
- **Non-DM:** 10% Patients CEPI-CT<300 sec

- **p<0.01**

*Angiolillo DJ et al. Diabetes 2005; 54:2430-5*

*Angiolillo DJ et al. Am J Cardiol 2006; 97:38-43*
Genetic Factors
• Polymorphisms of CYP
• Polymorphisms of GPIa
• Polymorphisms of P2Y12
• Polymorphisms of GPIIIa

Cellular Factors
• Accelerated platelet turnover
• Reduced CYP3A metabolic activity
• Increased ADP exposure
• Up-regulation of the P2Y12 pathway
• Up-regulation of the P2Y1 pathway
• Up-regulation of P2Y–independent pathways (collagen, epinephrine, TXA2, thrombin)

Clinical Factors
• Failure to prescribe/poor compliance
• Under-dosing
• Poor absorption
• Drug-drug interactions involving CYP3A4
• Acute coronary syndrome
• Diabetes mellitus/insulin resistance
• Elevated body mass index

GP IIb/IIIa receptor (reduced platelet activation)
Modulation of Acute Clopidogrel-induced Antiplatelet Effects

Gene sequence variations of the CYP3A4 enzyme (IVS10+12G>A)

Inhibition of ADP (2µM)–induced GP IIb/IIIa activation following a 300 mg clopidogrel LD

Responders Low-Responders NON-Responders

p=0.003

Angiolillo DJ et al. Arterioscler Thromb Vasc Biol. 2006; 26: 1895-1900
Influence of Diabetes Mellitus on Clopidogrel-induced Antiplatelet Effects


Platelet Function According to Hypoglycemic Treatment

Angiolillo DJ et al. J Am Coll Cardiol 2006; 48: 298-304
Diabetes as Predictor of Stent Thrombosis at One-Year in the Era of DES

OR=2.0 (0.8-4.9)  OR=2.8 (1.7-4.3)  HR=3.7 (1.7-7.9)  HR=2.03 (1.07-3.83)

Variability in Responsiveness to Antiplatelet Therapy

1. What do we know about it?
2. How do we define it?
3. What causes it?
4. What do we do about it?
Aspirin Resistant Patient Management

- Educate patient on importance of compliance
- Eliminate interfering substances (ibuprofen)
- Increase aspirin dose (?) (...*increasing the dose of aspirin does not enhance COX-1 inhibition*)
- Switch to other anti-platelet medications (?) (...*no evidence that switching to alternative treatment strategies improves outcomes*)
Cellular Factors
- Accelerated platelet turnover
- Reduced CYP3A metabolic activity
- Increased ADP exposure
- Up-regulation of the P2Y\textsubscript{12} pathway
- Up-regulation of P2Y\textsubscript{1}–independent pathways (collagen, epinephrine, TXA\textsubscript{2}, thrombin)

Clinical Factors
- Failure to prescribe/poor compliance
- Under-dosing
- Poor absorption
- Drug-drug interactions involving CYP3A4
- Acute coronary syndrome
- Diabetes mellitus/insulin resistance
- Elevated body mass index

Genetic Factors
- Polymorphisms of CYP3A4
- Polymorphisms of GPIa
- Polymorphisms of P2Y\textsubscript{12}
- Polymorphisms of GPIIIa

High Clopidogrel Loading Dose Regimen

ADP-Activated GP IIb/IIIa

300 mg loading dose (n=27)  600 mg loading dose (n=23)

% Positive Cells

p<0.0001  p=0.001 (MANOVA)  p=0.009  p=0.005

Basal  4h  24h  48h  Post - PCI

ISAR-CHOICE
Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect

Platelet Aggregation

Active metabolite

ARMYDA-2 Trial: Primary endpoint

Primary Composite of death, MI, and TVR at 30 days

Clopidogrel pre-treatment 4-8 hrs before PCI

\[ p = 0.041 \]

Patti G et al. Circulation 2005

- 600 mg: 4% (n=126)
- 300 mg: 12% (n=129)
Patients with UA/NSTEMI planned for early invasive strategy, i.e. intend for PCI as early as possible within 24 hrs

**RANDOMIZE**

**Clopidogrel High Dose Group**
Clopidogrel 600mg loading dose Day 1 followed by 150mg from Day 2 to Day 7; 75mg from Day 8 to 30

**ASA low dose group**
At least 300mg Day1; 75–100mg from D2 to D30

**ASA high dose group**
At least 300mg Day1; 300mg–325mg from D2 to D30

**Randomize**

**Clopidogrel Standard Dose Group**
Clopidogrel 300mg (+placebo) Day 1 followed by 75mg (+placebo) from Day 2 to Day 7; 75mg from Day 8 to 30

**ASA low dose group**
At least 300mg Day1; 75–100mg from D2 to D30

**ASA high dose group**
At least 300mg Day1; 300mg–325mg from D2 to D30

**Randomize**

PCI: Percutaneous coronary intervention
UA/NSTEMI: Unstable angina/non-ST-segment elevation myocardial infarction

**CURRENT/OASIS 7**
Clopidogrel optimal loading dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for Interventions
In patients in whom subacute thrombosis may be catastrophic or lethal (unprotected left main, bifurcating left main, or last patent coronary vessel), platelet aggregation studies may be considered and the dose of clopidogrel increased to **150 mg per day** if less than 50% inhibition of platelet aggregation is demonstrated.

OPTIMUS Study: (Optimizing anti-Platelet Therapy In diabetes Mellitus)

Inclusion Criteria
Type 2 diabetes mellitus patients with coronary artery disease on aspirin (81 mg) + clopidogrel (75 mg) therapy for ≥1 month

Study Time Point 1
Platelet function assessment to identify suboptimal and optimal responders

Suboptimal responders *
Randomization
150 mg clopidogrel/day for 30 days (n=20)
75 mg clopidogrel/day for 30 days (n=20)

Optimal responders
Not eligible for randomization

Study Time Point 2
Platelet function assessment

75 mg clopidogrel/day for 30 days

Study Time Point 3
Platelet function assessment

* >50% ADP (20 μmol/L)-induced post-treatment platelet reactivity

OPTIMUS Study:
(Optimizing anti-Platelet Therapy In diabetes Mellitus)

Primary Endpoint: Maximal ADP (20 μmol/L) Platelet Aggregation

Insights into updated ACC/AHA/SCAI 2005 PCI guidelines

Functional impact of 150mg clopidogrel dosing in patients with <50% inhibition defined by the VerifyNow P2Y\textsubscript{12} assay (OPTIMUS substudy)

$p=0.009$

OPTIMUS

Prevalence of Patients Reaching Therapeutic P2Y\textsubscript{12} Target Levels (20 $\mu$mol/L-induced Agg\textsubscript{max} \leq 50\%)

Functional impact of 75mg vs 150mg clopidogrel following elective PCI: Results of a Randomized Study

Triple versus Dual Antiplatelet Therapy

Role for cilostazol in addition to aspirin and clopidogrel?

Stent thrombosis @ 30 days (Lee SW et al JACC 2006)

Triple therapy vs dual therapy

9/1597 (0.5%) vs 1/1415 (0.1%) p=0.024

DECLARE-Long Study: Triple therapy significantly reduced late loss at 6 months after DES implantation and the occurrence of TLR and major adverse cardiac events in patients with long coronary lesions (Lee SW et al Am J Cardiol 2007).
**Primary Endpoint**

P2Y\textsubscript{12} reactivity index (PRI)

\[ p < 0.0001 \]
# Novel P2Y$_{12}$ ADP receptor antagonist

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type</th>
<th>Route</th>
<th>Action</th>
<th>Dose</th>
<th>Mean platelet inhibition (time required)</th>
<th>Trials (phase III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prasugrel (CS-747)</td>
<td>Thienopyridine (3rd generation) - requires hepatic conversion to active metabolite</td>
<td>Oral</td>
<td>Irreversible binding</td>
<td>60 mg loading dose, 10 mg maintenance dose</td>
<td>≈ 70% (&lt; 1 hour)</td>
<td>TRITON</td>
</tr>
<tr>
<td>Cangrelor (ARC-669931MX)</td>
<td>ATP analogue - Direct inhibition</td>
<td>Parenteral</td>
<td>Competitive binding</td>
<td>4 µg/kg/min</td>
<td>≈ 95% (few minutes)</td>
<td>CHAMPION</td>
</tr>
<tr>
<td>AZD-6140</td>
<td>Cyclopetyl-triazolopyrimidine - Direct inhibition</td>
<td>Oral</td>
<td>Competitive binding</td>
<td>90 mg/twice daily</td>
<td>≈ 95% (2-4 hours)</td>
<td>PLATO</td>
</tr>
</tbody>
</table>

More potent and less variability!!

High platelet reactivity, rapid fibrin formation and clot strength are risk factors for ischemic events after PCI. Clot strength is the most predictive. These findings may explain the occurrence of events despite treatment with COX-1 and P2Y$\textsubscript{12}$ inhibitors, suggesting the need to address thrombin inhibition during and after PCI.

Thrombus Formation

Two key elements: **cellular** (platelets) and **plasmatic** (coagulation factors)

- Collagen
- ADP
- TXA₂
- Thrombin
- Fibrinogen → Fibrin
- Platelet activation
- Platelet aggregation
- Tissue Factor
- Plasma Clotting cascade
- Prothrombin cascade
Platelet Stimuli

- GP IIb/IIIa integrin
- ADP
- collagen
- Thrombin
- Serotonin
- Shear rate
- AA
- TxA$_2$
- COX-1

Platelet Aggregation
Thrombin

ADP

TXA

ADP P2Y

fibrinogen receptor

GP IIb/IIIa

COX-1

CAMP

Adaptation

Clopidogrel bisulfate

SCH 530348

Aspirin

TRA-PCI trial