Antiplatelet Resistance—Does it Exist and How to Measure it?

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Abstract: Aspirin and clopidogrel are the most commonly used antiplatelet agents in patients with coronary artery disease. The existence of resistance to these agents has been a controversial issue and new drugs are being developed to overcome this problem. Laboratory tests, which can identify resistance and correlate this with clinical outcome, are being studied in order to identify patients at risk of future thrombotic events. We discuss the evidence for the existence of antiplatelet resistance—both in the laboratory and in the clinical setting. So far, platelet aggregometry has been considered the gold standard test, but is very operator dependant, time consuming, and has shown little correlation with other available tests of antiplatelet resistance. We discuss the available tests of platelet function, their limitations, and evidence for their use. A simple, rapid, near-patient test, which is affordable and useful in the clinical (not just laboratory) setting, could allow risk stratification of patients and individualization of antiplatelet medication to improve outcome.

Keywords: antiplatelet, resistance
Introduction

Platelets are the key players in pathological thrombus formation, that leads to myocardial infarction, ischaemic stroke, and peripheral vascular disease. Aspirin, the oldest antiplatelet agent has shown a significant benefit in the secondary prevention of these ischaemic events. Platelets can be activated through a number of pathways, and antiplatelet agents aim to block one or more of these.

Aspirin and clopidogrel are the antiplatelet agents most commonly used in patients with coronary artery disease. However, some patients continue to experience thrombotic events despite treatment with these agents, and this phenomenon has been termed antiplatelet “resistance”. The exact cause or mechanism that underlies such resistance is unknown; furthermore, the existence of “resistance” has been challenged and remains an issue of much contention.

Nevertheless, it has fuelled the pharmaceutical industry to develop newer drugs, which will be able to “overcome this resistance”. Recent results from the TRITON-TIMI 38 and DISPERSE-2 studies provide promising results for the newcomer antiplatelet agents Prasugrel and AZD6140.

In addition, it has also resulted in a search for a laboratory test to identify patients who exhibit “resistance” to antiplatelet medication, in order to detect those at risk of future thrombotic events.

So far, light transmittance aggregometry has been considered the gold standard test of platelet function. However, this method is highly operator-dependent and has shown little correlation with other available tests of antiplatelet resistance. The ideal test to assess antiplatelet medication should 1) use physiologically relevant agonists to induce platelet activation, 2) be easy to perform (by clinicians), 3) give rapid results within a clinically-relevant timeframe, 4) correlate closely with clinical events, 5) have a high sensitivity and 6) be affordable. None of the available techniques currently fulfils all these criteria.

In this paper, we present the clinical evidence for the existence of antiplatelet resistance, describe the techniques used to date to identify antiplatelet resistance in the laboratory and their relative merits and shortcomings.

Mechanisms of Action of Antiplatelet Drugs

Aspirin

The major cyclooxygenase product in platelets is thromboxane A$_2$ (TxA$_2$) which induces platelet aggregation and acts as a potent vasoconstrictor. Aspirin blocks production of TxA$_2$ by acetylation of COX1, the enzyme that produces the cyclic endoperoxide precursor of thromboxane A2. Since platelets do not synthesize new proteins, the action of aspirin on platelet cyclooxygenase is permanent, lasting for the lifetime of the platelet (7–10 days) and repeated doses of aspirin produce a cumulative effect on platelet function. However, aspirin is considered a suboptimal antiplatelet agent since it antagonizes only one particular pathway of platelet activation, leaving several other important pathways unaffected.

Clopidogrel

Clopidogrel is a thienopyridine derivative. It is a prodrug, oxidized by the hepatic cytochrome P450 system to its active metabolite which irreversibly binds to the ADP-coupled P$_2$Y$_12$ receptor. P$_2$Y$_12$ inhibition thus inhibits ADP-induced platelet activation and resultant aggregation. There is no doubt that clopidogrel is an effective antiplatelet agent, and when added to aspirin, significantly reduces the occurrence of thrombotic events. Importantly, no direct head-to-head comparisons of aspirin and clopidogrel have been performed in clinical trials. Instead, trials of clopidogrel have assessed its efficacy as an “add-on” therapy to aspirin, presumably to reduce thrombotic events in those patients in whom aspirin may not be totally preventive.

Ticlopidine

Ticlopidine is another thienopyridine that permanently inhibits the P$_2$Y$_12$ receptor. It is a prodrug that requires conversion to the active metabolite by the hepatic cytochrome P450 enzyme. It is rapidly absorbed, highly bioavailable and has a prolonged effect. However, its unfavourable side-effect profile with risk of bone marrow suppression has placed it second position with regard to clopidogrel and led to the withdrawal of this drug in some countries (e.g. United Kingdom).
Prasugrel
Prasugrel is a new oral thienopyridine derivative that produces more potent and irreversible P2Y12 receptor blockade, with a rapid onset of action. Its active metabolite is R-138727 and it is deemed to be 10 times more potent than currently available thienopyridine derivatives. The JUMBO-TIMI 26 study showed improved platelet inhibition, MACE and reduction in ischemic events with prasugrel compared to clopidogrel. Recent results from the TRITON-TIMI 38 study comparing clopidogrel with prasugrel in 13,608 patients has shown reduced interpatient variability, a 19% relative reduction in the primary endpoint of MACE \( (p = 0.0004) \), 24% reduction in myocardial infarction and 52% relative reduction in in-stent restenosis in the prasugrel group. However, bleeding complications were also more frequent with prasugrel, especially intracranial hemorrhage in patients with prior CVA/TIA.

AZD6140
AZD6140 is an oral and reversible P2Y12 receptor blocker that does not require hepatic conversion to an active metabolite and produces an overall superior ADP-induced platelet inhibition with less response variability than clopidogrel. It belongs to the cyclopentyltriazolopyrimidine group and has rapid onset and offset of action, which may make it particularly useful in patients who have to undergo imminent surgery. Results from the DISPERSE 2 study showed superior platelet inhibition with AZD6140 when compared to standard dose of clopidogrel in 990 patients with acute coronary syndromes, although this did not translate into a significant reduction in the rates of myocardial infarction.\(^6,7\) The PLATO study will compare AZD6140 with clopidogrel in 18,000 patients with ACS.\(^2\) AZD6140 is still not licensed for clinical use.

Cangrelor
This is a potent parenteral P2Y12 receptor antagonist. It is an ATP analogue with a very rapid onset and a short half-life, with recovery of platelet function in 20–50 minutes after discontinuation of the drug. Its rapid onset of action makes it an attractive option for patients undergoing emergent PCI even if they then need to go on to have bypass surgery. The ongoing CHAMPION study is comparing clopidogrel with cangrelor in patients undergoing PCI.\(^6\) This drug is still not licensed for clinical use.

Glycoprotein IIb/IIIa inhibitors
Glycoprotein IIb/IIIa receptors on the platelet surface bind fibrinogen, and are the final common pathway of platelet activation. The GP IIb/IIIa receptor may be activated by any platelet agonist and consequently inhibition of binding to this receptor blocks platelet aggregation induced by any agonist. Three agents approved for use at present are abciximab, eptifibatide and tirofiban. All are effective but need to be given intravenously and are only approved for short-term use.

Dipyridamole
Dipyridamole interferes with platelet function by increasing the cellular concentration of cyclic AMP. This effect is mediated by inhibition of cyclic nucleotide phosphodiesterase and/or by blockade of available uptake of adenosine, which acts at adenosine A2 receptors to stimulate platelet adenylyl cyclase. It has little or no benefit as an antithrombotic drug. In trials in which a regimen of dipyridamole plus aspirin was compared with aspirin alone, dipyridamole provided no additional beneficial effect.\(^8\) A single study suggests that dipyridamole plus aspirin reduces strokes in patients with prior stroke or transient ischemic attack.\(^9\)

Cilostazol
Cilostazol is a reversible cAMP phosphodiesterase inhibitor with antiplatelet, antithrombotic and vasodilatory effects. Compared to either placebo or pentoxifylline in six double-blind randomised controlled trials, it has been shown to be effective in reducing intermittent claudication in patients with peripheral arterial disease.\(^10\)

BM573
BM573 is a thromboxane A2 synthase inhibitor and receptor antagonist. It has been shown to reduce atherosclerosis in LDL-deficient mice, suggesting it may have a role to play in preventing progression of atheroma. It is still in development.\(^11\)
Laboratory Tests for Monitoring Antiplatelet Therapy

Platelet function tests were devised to detect patients with abnormal platelet reactivity, which may be inborn or acquired. A number of tests are currently available to assess platelet function, some laboratory based and some near patient point of care tests. They have been used in a number of research studies to detect the effect of antiplatelet medication, however none are in routine clinical use as the available tests demonstrate a large variability in the response to antiplatelet medication, with variable prevalence of “resistance”. The most frequently performed platelet function test is platelet or whole blood aggregation induced by ADP or collagen. Platelet aggregometry has been described as the “gold standard” platelet function test, against which other platelet function tests are compared. Flow cytometry is also frequently used to measure platelet activation, and has additional advantages over global tests of platelet function in providing detailed analysis of the surface markers on the platelet. This provides greater insight into the pathomechanism of platelet activation, but may provide less detailed information on platelet activation. Near patient point of care tests are more convenient and provide more readily available test results for clinicians. However, the Scientific and Standardization Committee and the International Society on Thrombosis and Haemostasis do not recommend use of platelet function testing outside research trials, as there is inadequate data addressing the clinical effectiveness of tailoring antiplatelet therapy based on laboratory results of antiplatelet resistance.12

Bleeding time

Dating back as far as 1901, this simple test measures the time it takes for a small skin cut to stop bleeding. It has very poor reproducibility and no study so far has shown it to correlate with bleeding or thrombotic risk.

Light transmittance aggregometry

Regarded as the gold standard test for assessing platelet reactivity and for validating other, newer tests. Baseline light transmittance is performed on whole blood or platelet fraction, and compared with transmittance following the addition of platelet agonists, such as arachidonate, ADP, thrombin receptor activating peptide, collagen, or epinephrine. Platelets clump in response to these agents and an increase in light transmittance is noted. Subjects whose platelet aggregation is more than 20% with arachidonate are considered aspirin resistant. It is relatively expensive, time consuming, performed on anticoagulated blood and variability in results has been reported.

Flow cytometry

Here, blood cells are labelled with a fluorescently conjugated monoclonal antibody and are then passed through a flow cytometer, at 1000 to 10 000 cells per minute. They then pass through an active laser light, which activates the fluorophore that is conjugated to the monoclonal antibody. The intensity of fluorescence is directly proportional to the antigen being studied. P selectin (CD62) is expressed on the surface of activated platelets, and helps in formation of the monocyte-platelet aggregates, which are considered to be the most sensitive marker of platelet activation.

Urinary thromboxane

Urinary thromboxane is a simple test to assess platelet activation through urinary metabolites. Activated platelets synthesize 11-dihydroxy thromboxane B2, an active metabolite of TxA2, and this is detected in urine with an ELISA assay. However, although detection of 11-dihydroxy thromboxane B2 in urine reflects systemic TxA2 formation, 30% is derived from non-platelet sources and thus falsely high readings may be observed in inflammatory conditions.13

PFA-100

The PFA-100™ System (Dade Behring, Germany) is a semi-automated dual channel device. Blood is drawn into a tube containing 3.2% citrate and allowed to stand for between 30 min and 4 h, after which 800 µl of citrated whole blood is added to each of two pre-prepared cartridges to wet the filters. Both cartridges contain a membrane coated with type I equine collagen together with an agonist to induce platelet aggregation. In one cartridge the membrane is coated with 10 µM epinephrine and the other with 10 µM ADP. The measurement begins by drawing the blood through a capillary tube and a single aperture (150 µm diameter) into a collagen coated cellulose-acetate filter. This results in the platelets being pre-activated by shear stress of 190 dynes/cm² even before reaching the filters and the agonists. As platelets come into contact with the collagen, they adhere, aggregate and
form the primary hemostatic plug, which occludes the aperture (closure time, CT). The greater the platelet inhibition, the longer the closure time.

Verify now
The Verify Now system (Accumetrics, California) is a platelet function assay utilizing light source to detect the amount of platelet aggregation. Platelets adhere to the fibrinogen coated beads in the tube, aggregate and fall out of solution, changing the extent and rate of light transmittance. Light transmittance is inversely related to the amount of platelet aggregation. There are 3 types of assays available. The Aspirin assay utilizes arachidonic acid as an agonist to assess the antiplatelet effect of aspirin. Similarly, the P2Y12 assay utilizes ADP as an agonist to assess the effect of clopidogrel and the IIbIIIa assay utilizes a thrombin receptor activating peptide as an agonist to assess the response to IIbIIIa inhibitors.

TEG 5000 thromboelastograph haemostasis system
Thrombelastography (Haemoscope, USA) measures all phases of haemostasis from clot formation to clot lysis. Blood is held in a cylindrical cup which oscillates through an angle of approximately 5 degrees. A pin is suspended into this blood by a torsion wire, and monitored for motion. The strength of the fibrin platelet bond during clot formation affects the magnitude of the pin motion, giving an idea of overall haemostasis. TEG Platelet Mapping technology allows estimation of the percentage inhibition of platelet aggregation by aspirin, clopidogrel or GPIIb/IIIa inhibitors, thus allowing tailoring of individual antiplatelet therapy.

Global Thrombosis Test (GTT)
The Global Thrombosis Test (GTT) (Montrose Diagnostics Ltd, UK) is a novel platelet function test, which is currently the most physiological test of platelet reactivity, in that the technique is performed on non-anticoagulated, native blood, without added external agonists. In this technique, an occlusive thrombus is formed using high shear stress, analogous to that in a stenosed coronary artery. This first phase of the test creates an occlusive thrombus under conditions of high shear and is used as marker of platelet function, the more reactive the platelets, the faster the occlusion will occur (occlusion time, seconds). The restart of blood flow following occlusion is due to spontaneous thrombolysis (lysis time, seconds). This is a near-patient test, which provides a result within 10 minutes on the patient’s thrombotic status, and is thus highly applicable to acute clinical situations, as well as more general screening. Studies correlating GTT with clinical outcomes are currently in progress and early results suggest it may have an important role in clinical practice.14

Limitations of Platelet Function Tests
Most tests lack sensitivity, have low positive predictive value for clinical events and are difficult to perform in the clinical setting. Furthermore, most tests are performed on anticoagulated blood or use supra-high doses of agonists to induce platelet aggregation, so the physiological relevance of such tests remains questionable. There is clearly a need for a truly physiological, reliable, reproducible and clinically relevant test.15 Tests such as aggregometry and flow-cytometry are time consuming, need special expertise to perform and are not applicable to providing a rapid result in the clinical setting to influence practice. A common drawback with many platelet function tests has been the lack of serial measurements. Furthermore, most studies have been carried out on a small number of patients and there has been no published study suggesting an improvement in clinical outcome with tailoring of antiplatelet medication based on the results of platelet function tests. Recent studies comparing the results with different platelet function tests noted there was wide variability and poor correlation amongst them.15,16

Evidence that Antiplatelet Resistance Exists
Definition of “resistance”
The term “resistance” as applied to antiplatelet medication implies an endogenous mechanism in certain individuals, which prevents the drug from exerting its full antithrombotic effects. This is however, a misnomer. Almost all drugs known to man exert varying effects in different individuals and this should not be termed resistance. The causes for this are plentiful, and listed below. The proposed mechanisms underlying antiplatelet resistance to aspirin and clopidogrel are summarised in Table 1.
From a clinical point of view, the term “resistance” has been used to describe the ongoing thrombotic events that occur in some individuals despite taking antiplatelet medication. However, the laboratory phenomenon of resistance is based on the results of platelet function tests, which show incomplete inhibition of aggregation by the medication in question. We will now discuss the evidence for existence of “antiplatelet resistance”.

| Laboratory Evidence of Antiplatelet Resistance |
|------------------------------------------------|
| The prevalence of aspirin “resistance” is considered to be between 5%–60%, with a similar prevalence of clopidogrel resistance. Studies reporting aspirin resistance are summarized in Table 2. Studies reporting on the phenomenon of clopidogrel resistance are summarized in Table 3. The prevalence of antiplatelet resistance varies with the laboratory method used, the drug studied, the drug dose and with the disease state. As shown in Tables 1 and 2, there is wide variation in the reported prevalence of antiplatelet resistance and lack of consistency between studies. |

| Prevalence of Antiplatelet Resistance in Different Populations |
|---------------------------------------------------------------|
| Stable coronary artery disease |
| A prospective study of 326 patients with stable CAD revealed the incidence of aspirin resistance... |
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was 5.5% when assessed by LTA and 9.5% when assessed using the PFA-100.\textsuperscript{19,20} Another study of 98 subjects showed 30% resistance in patients on aspirin 160 mg/d using the PFA-100,\textsuperscript{21} with another study using the PFA-100 reporting a 27% incidence.\textsuperscript{22}

### Acute coronary syndrome
Among 104 ACS patients tested with the PFA-100, the incidence of aspirin resistance was found to be 40%.\textsuperscript{22} The Warfarin Aspirin Reinfraction II Study (WARIS-II) study allocated 202 patients to receive aspirin, warfarin or both. Aspirin resistance was observed in 35% of subjects taking aspirin alone and in 40% taking aspirin and warfarin. Major adverse cardiac events occurred more frequently in aspirin non-responders compared to responders (36% vs. 24%, p = 0.28).\textsuperscript{23}

### Elective PCI
The VerifyNow assay was used to detect aspirin resistance in 151 Asian patients undergoing elective PCI. All patients had been taking 80–325 mg aspirin for at least a week prior to the procedure, yet 19% were found to be aspirin resistant.\textsuperscript{24}

### In-stent restenosis
In a study of 204 patients, 31% patients with in-stent restenosis were found to be aspirin resistant using PFA-100, compared to 11% of those with patent stents (p < 0.001).\textsuperscript{25}

### Clinical Significance of Antiplatelet Resistance
The concept of antiplatelet resistance is variably defined. It is not clear whether the definition of antiplatelet resistance should be based on laboratory results or clinical outcomes. However, several recent studies suggest that antiplatelet therapy resistance is associated with an increase in the risk of adverse cardiovascular outcomes in patients with CAD, CVA or PVD (Table 4 and Table 5).

### Stable CAD
A study by Gum and co-workers (using optical platelet aggregation) evaluated 326 patients with stable CAD receiving aspirin for greater than 7 days. Aspirin resistance was identified if the aggregation was greater than 70% in response to 10 micromolar ADP or greater than 20% with 0.5 mg/mL of arachidonic acid. Based on this definition, 5.5% of patients were “resistant” and

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**Table 2. Summary of laboratory tests reporting Aspirin resistance.**

| Study | n  | Type of subjects | Aspirin dose | Platelet function test | Prevalence of resistance (%) |
|-------|----|------------------|--------------|------------------------|-----------------------------|
| Gum et al\textsuperscript{20} | 325 | Stable CAD       | 325 mg       | ADP and AA induced optical aggregation | 5.2 |
| Mueller et al\textsuperscript{23} | 100 | PAD              | 100 mg       | Corrected whole blood aggregometry | 60 |
| Grotemeyer et al\textsuperscript{35} | 180 | CVA              | 1500 mg      | Platelet reactivity | 33 |
| Chen et al\textsuperscript{44} | 151 | Elective PCI     | 80–325 mg    | RPFA                  | 19 |
| Andersen et al\textsuperscript{23} | 202 | Post MI          | 160 mg Aspirin vs. 75 mg Aspirin plus warfarin | PFA-100 | 35% in patients taking aspirin only, vs. 40% in patients taking aspirin and warfarin |
| Macchi et al\textsuperscript{21} | 98  | Stable CAD       | 160 mg       | PFA-100                | 29% |
| Helgason et al\textsuperscript{51} | 306 | CVA              | 300–325 mg   | ADP induced platelet aggregation | 25%
| Hobikoglu et al\textsuperscript{22} | 204 | ACS: 104 Stable CAD: 100 | 80–300 mg | PFA-100 | 40% in ACS 27% in Stable CAD |
| Grundmann et al\textsuperscript{52} | 53  | CVA/TIA in prev 3 days 35 | 100 mg | PFA-100 | 34% in symptomatic patients 0% in asymptomatic patients |
| Alberts et al\textsuperscript{53} | 129 | CVA              | 81 mg vs. 325 mg | PFA-100 | 37% overall, with 56% in patients on 81 mg vs. 28% in those on 325 mg aspirin. |
had a significant increase in the combined endpoint of death, MI or stroke over a follow up period of nearly 2 years, compared to responders.26

**Elective PCI**

The VerifyNow assay was used to detect antiplatelet resistance to GPIIb/IIIa inhibitors in 485 patients undergoing elective PCI. Patients whose platelet function was inhibited by 90% or more had an event rate of 2% compared with 10% for patients with inhibition of less than 90%.27

Holzholter measured platelet reactivity in 802 patients undergoing elective PCI after 600 mg clopidogrel loading and concluded that patients with high platelet reactivity had a worse clinical outcome at 30 days.28

**Stent thrombosis**

Gurbel and coworkers compared 20 patients with subacute stent thrombosis (SAT) to 100 patients undergoing PCI who did not experience SAT. Using LTA to assess platelet reactivity and VASP to assess clopidogrel effect, the results suggested that high post treatment platelet reactivity and incomplete inhibition of P2Y12 are risk factors for stent thrombosis.30

In another study of 105 patients undergoing elective PCI, 5%–11% were identified as being clopidogrel resistance. Table 3 summaries the laboratory tests reporting clopidogrel resistance.

### Table 3: Summary of laboratory tests reporting clopidogrel resistance.

| Study                  | n    | Condition studied | Loading dose clopidogrel | Maintenance dose clopidogrel | Platelet function test | Prevalence of resistance |
|------------------------|------|-------------------|--------------------------|------------------------------|------------------------|--------------------------|
| Gurbet al64             | 92   | PCI               | 300 mg                   | 75 mg                        | LTA                    | 31%–35%                  |
| Angiolillo et al55      | 52   | Diabetes          | 300 mg                   | 75 mg                        | LTA and Flow cytometry | 38% in DM, 8% in non-DM  |
| Angiolillo et al56      | 48   | PCI               | 300 mg                   | 75 mg                        | LTA                    | 44%                      |
| Lepantalo et al57       | 50   | PCI               | 300 mg                   | 75 mg                        | LTA and PFA 100        | 40%                      |
| Jaremo et al58          | 18   | PCI               | 300 mg                   | 75 mg                        | LTA                    | 28%                      |
| Lev El et al69          | 150  | PCI               | 300 mg                   | –                            | LTA                    | 24%                      |
| Mobely et al60          | 50   | PCI               | 300 mg                   | 75 mg                        | LTA                    | 30%                      |
| Muller et al61          | 115  | PCI               | 600 mg                   | 75 mg                        | LTA                    | 5%–11%                   |
| Barragan et al62        | 48   | ISR vs no ISR22   | Clop 75 mg B.I.D. vs. Tirodipine 250 mg B.I.D. | Flow cytometry | 63% (ISR) vs. 40% (no ISR) |
| Bounamici et al32       | 804  | ISR               | 600 mg                   | 75 mg                        | ADP induced platelet aggregation | 13%                      |
| Ajzenberg et al83       | 32   | ISR vs. no ISR22  | 300 mg                   | 75 mg                        | Shear induced platelet aggregation (SiPA) | 41% (cases) vs. 18% (controls) at shear rate of 200/s 57% (cases) vs. 23% (controls) at shear rate of 4000/s |
| Matetzky et al29        | 60   | STEMI             | 300 mg                   | 75 mg                        | LTA                    | 25%                      |
| Dziewierz et al64       | 31   | CAD               | 300 mg                   | –                            | LTA                    | 23%                      |

**Primary angioplasty**

Reduction in platelet aggregation in response to high dose loading treatment with aspirin and clopidogrel was assessed in 60 patients presenting with acute ST elevation MI undergoing primary PCI. They were divided into 4 quartiles based on reduction in platelet aggregation using the cone and plate aggregometer. Patients in the first quartile had platelet aggregation 103% ± 8%, whereas those in the 2nd, 3rd and 4th quartile had platelet aggregation of 69, 58 and 33% of their respective baselines. At 6 months’ follow-up, 7 patients in the first quartile and 1 patient in the 2nd quartile had experienced a cardiovascular event.29

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non-responders and among these patients, 2 SATs were documented.\(^{31}\)

In a well-conducted large, prospective study, Bounamici and co-workers measured platelet aggregation in response to ADP in 804 patients undergoing coronary intervention with a drug eluting stent and followed them up for 6 months. The incidence of SAT was 8.6% in non-responders (105 patients) and 2.3% in responders suggesting a strong correlation between stent thrombosis and clopidogrel resistance.\(^ {32}\)

Another small study showed that 10 patients with SAT had significantly greater shear-induced platelet aggregation compared to PCI patients who had not experienced SAT or compared to normal controls, indicating that resistance to antiplatelet therapy and increased shear induced platelet aggregation correlated well with stent thrombosis.\(^ {33}\)

The interval between SAT and assessment of antiplatelet resistance in all the above studies was variable. Also, the patient population was diverse hence further trials are required to assess the relation between stent thrombosis and antiplatelet resistance mechanisms.

### Cerebrovascular disease

A small study compared 35 patients with symptoms (ischemic stroke or TIA) in the preceding 3 days to 18 patients without symptoms (no CVA symptom for \(\geq 24\) months), all of who had been taking aspirin for at least 5 months. Using the PFA-100, 34% of symptomatic patients were identified as aspirin resistant compared to none of the asymptomatic patients.\(^ {34}\)

Among 180 stroke patients, aspirin resistance was identified in 33%. All patients were followed up for 2 years and major end points were observed in 40% of aspirin resistant patients compared with 4% of aspirin responders \((p < 0.0001)\).\(^ {35}\)

### Diabetes

Angiolillo and colleagues assessed platelet reactivity using LTA and flow cytometry in 173 type 2 diabetics

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**Table 4. Summary of recent studies reporting on the clinical correlates of laboratory antiplatelet resistance to aspirin.**

| Study                  | Population                          | Follow-up | Method                                      | Primary endpoint | Clinical implications                                           |
|------------------------|-------------------------------------|-----------|---------------------------------------------|------------------|---------------------------------------------------------------|
| Gum et al\(^ {26}\)    | Stable CAD \((n = 326)\)            | 2 yrs     | Optical aggregometry                        | MACE             | 5.2% resistance, associated with increased risk \((hazard ratio 3.12)\) of CV death, MI or stroke |
| Substudy of HOPE\(^ {77}\) | Patients with MI, stroke or CV death \((n = 488)\) | 5 y       | Urinary thromboxane metabolite levels       | MACE             | Patients in the upper quartile had 1.8 times higher risk than those in the lower quartile \((p = 0.009)\) |
| Mueller et al\(^ {78}\) | Intermittent claudication undergoing peripheral angioplasty \((n = 100)\) | 18 m      | Corrected whole blood aggregometry          | ISR              | Risk of reocclusion at the site of angioplasty was 87\% higher in patients with failed inhibition of aggregation to collagen and ADP |
| Pamukcu et al\(^ {79}\) | ACS \((n = 105)\)                   | 1 y       | PFA-100                                     | MACE             | MACE occurred in 45\% of patients with aspirin resistance and in 12\% in aspirin-sensitive patients |
| Grotemeyer et al\(^ {80}\) | Cerebrovascular disease \((n = 174)\) | 2 y       | Platelet reactivity test- Residual number of platelets in supernatant of centrifuged samples\(^ {81}\) | MACE             | Recurrent stroke, MI or vascular death was more likely to occur in aspirin non-responders compared with responders \((40 \text{ vs. } 4.4\%, p < 0.001)\) |
Table 5. Summary of recent studies reporting on the clinical correlates of laboratory antiplatelet resistance to clopidogrel.

| Study                  | Population                             | Follow-up  | Method                                                | Endpoint | Clinical relevance |
|------------------------|----------------------------------------|------------|                                                      |          |                   |
| Matetzky et al⁵²       | STEMI undergoing primary PCI (n = 60)   | 6 months   | ADP induced aggregation using LTA                   | MACE     | Patients with recurrent cardiac events had lower percentage reduction of ADP-induced platelet aggregation 91 ± 21 vs. 62% ± 21% percent of baseline, p < 0.001 (day 3) and 90 ± 16 vs. 64% ± 27%, p < 0.001 (day 6) |
| Barragan et al⁵³      | SAT (n = 16) vs. No SAT (n = 30)       | Retrospective | Enhanced platelet reactivity using VASP assay | NA       | Significant difference in VASP assay between SAT (63.28% ± 9.56%) vs. no SAT (39.80% ± 10.9%) P < 0.0001 |
| Ajzenberg et al⁵⁴     | N = 49 NoSAT = 22 Healthy = 17        | Retrospective | Shear induced platelet aggregation (SIPA) | NA       | SIPA higher in cases than in controls 41 ± 12 vs. 18 ± 8%, p = 0.013 at shear rate of 200/s and 57 ± 16 vs 23% ± 21%, p = 0.009 at shear rate of 400/s |
| CREST⁵⁵               | N = 120 SAT = 20 NO SAT = 100         | Retrospective | High post-treatment reactivity assessed by LTA and incomplete P2Y12 receptor inhibition assessed by VASP | NA       | Increased platelet reactivity in patients with SAT 49 ± 4 vs. 33% ± 2% for 5 µmol/l ADP-induced aggregation, p < 0.05 and 65 ± 3 vs. 51% ± 2% for 20 µmol/l ADP-induced aggregation, p < 0.001 |
| Cuisset et al⁵⁶        | 106 ACS patients undergoing PCI       | 1 month    | LTA assessed at the time of the intervention       | MACE     | Patients with recurrent CV events had a significantly higher ADP-induced platelet aggregation (p < 0.0001) |
| EXCELSIOR⁵⁷           | 802 undergoing elective PCI pre-treated with 600 mg loading dose clopidogrel | 1 month    | ADP-induced platelet aggregation assessed by LTA   | MACE     | MACE increased with quartiles of ADP-induced platelet aggregation, i.e. 0.5% in the 2 quartiles with the lowest platelet aggregation 3.1% in the third quartile 3.5% in the highest quartile (p = 0.034). |
| Gurbel et al (PREPARE POST-STENTING)⁵⁸ | 192 patients undergoing non emergent PCI 36-UA 11-ACS 145-Stable IHD | 6 months   | ADP induced platelet aggregation assessed by LTA   | MACE     | Post treatment ADP-induced aggregation by LTA was greater in those patients with recurrent events compared to event free patients (63 ± 12 vs. 56% ± 15%, p = 0.02) |
| Lev et al⁵⁹           | 150 patients undergoing elective PCI  | –          | LTA                                                 | –        | The percentage of patients with high post-clopidogrel ADP-induced aggregation (>75th percentile) was higher among aspirin-resistant than aspirin-sensitive patients (5 µmol/l ADP: 79 vs. 18%, p = 0.001; 20 µmol/l ADP: 73 vs. 19%, p = 0.001). |
| Geisler et al⁶⁰       | 379 PCI patients (206 stable CAD and 173 with ACS) treated with 600 mg clopidogrel loading | 3 months   | Assessment of response to clopidogrel using LTA   | MACE     | MACE more frequent in clopidogrel non-responders than in those sensitive to clopidogrel (23 vs. 6%; p = 0.004). |
with CAD on dual antiplatelet therapy, and showed that patients with high platelet reactivity (HPR) had an increased risk of MACE with a hazard ratio of 3.35. High platelet aggregation in diabetic patients may be secondary to various factors such as decreased nitric oxide production, increased sensitivity to ADP and overproduction of leptin receptors secondary to obesity.\textsuperscript{36} The OPTIMUS study showed HPR in 60% of diabetic patients despite treatment with 150 mg daily clopidogrel, suggesting that high doses of clopidogrel may not overcome the increased platelet reactivity in certain population subgroups.\textsuperscript{37}

Peripheral vascular disease
A study of 100 patients with intermittent claudication undergoing elective iliofemoral balloon angioplasty assessed for aspirin resistance using whole blood aggregometry at baseline and at regular intervals for up to a year post angioplasty. Reocclusion at the site of angioplasty during follow up occurred exclusively in patients who had been identified as being aspirin resistant.\textsuperscript{38}

Management of Antiplatelet Resistance
The clinician is currently able to partially improve the responsiveness to antiplatelet therapy by acting on extrinsic factors, involved in the aetiology of resistance, including compliance to treatment, drug-drug interactions and good control of blood pressure, glycaemia and lipid levels. Several studies have shown that clopidogrel loading with 600 mg has a stronger and faster inhibitory effect on platelet reactivity than the 300 mg loading dose.\textsuperscript{39,40} Increasing the loading dose to 900 mg has not been shown to be of benefit, indicating a threshold to the platelet inhibitory effect of clopidogrel.\textsuperscript{41,42}

The CLEAR-PLATELETS study showed that Clopidogrel loading combined with epifibatide resulted in reduced myocardial necrosis compared to standard or high loading dose of clopidogrel alone.\textsuperscript{43}

The ISAR-CHOICE-2 study demonstrated the beneficial effect on platelet inhibition of increasing the maintenance dose of clopidogrel to 150 mg.\textsuperscript{44}

In the ARMYDA-4 study, reloading with 600 mg clopidogrel pre PCI did not confer any additional benefit in patients on chronic clopidogrel therapy. The ARMYDA-5 study, which compared Clopidogrel loading with 600 mg “in lab” vs. 4–8 hours pre PCI, did not show any significant difference in outcome in the two groups, but this study was underpowered to detect a significant difference.\textsuperscript{45,46}

Results from the recent ARMYDA-PRO study suggest high pre PCI platelet reactivity using the VerifyNow P2Y12 assay may predict MACE at 30 days.\textsuperscript{47} Use of point of care platelet function tests may help in identification of these high-risk patients, and assist the clinician in optimising their antiplatelet medications.

However, there still remains controversy over the benefit and the safety of high loading and maintenance doses of clopidogrel.

Several studies are focusing on this issue, including the CURRENT/OASIS-7 trial. This large population-based study will evaluate whether high dose clopidogrel and/or aspirin improves clinical outcome or increases bleeding risk.

Alternative antiplatelet drugs, including novel P\textsubscript{2}Y\textsubscript{12} ADP receptor antagonists are currently under clinical investigations. Results from the recent TRITON-TIMI 38 study show that prasugrel significantly reduced the rates of recurrent ischemic events, including stent thrombosis, although this was offset by an increase in major bleeding.\textsuperscript{1} The GRAVITAS study is currently underway to assess whether tailoring the dose of antiplatelet medication based on the results of the VerifyNow assay improves clinical outcomes.

Whether higher doses of aspirin and/or clopidogrel are sufficient to overcome the “resistance” seen in some individuals on low doses of these drugs, is not known. How higher doses of aspirin and/or clopidogrel compare to novel antiplatelet drugs with respect to their antiplatelet effects and specifically, in patients who are “resistant” to low dose aspirin/clopidogrel is again, unknown. The relative bleeding risks with these regimens has also not been evaluated.

Conclusion
There is no doubt that the laboratory phenomenon of “resistance” to antiplatelet medication exists. There are many tests to assess platelet reactivity and these have demonstrated a large variability in the response to antiplatelet medication, with variable prevalence of “resistance”. The definition of “resistance” is fraught with difficulty as the different methods report different prevalences, depending
on the test used, the cut-off value used to define resistance, the timing with respect to medication and the population studied. A meta-analysis by Hovens et al found heterogeneity in the prevalence of aspirin resistance, and this was due to the variability in results using different platelet function tests.48

It is extremely difficult for clinicians to determine which method to use to assess platelet function and how to interpret the results. There has been no good correlation so far amongst the various different platelet function tests. Many are time consuming, and not applicable to a clinical setting. None fulfils the “ideal” criteria described in our introduction. Furthermore, to date, there is very little data to suggest that altering antiplatelet medication based on the results of laboratory tests of “resistance” improves clinical outcomes.

A meta-analysis by Snoep et al49 suggests patients with laboratory aspirin resistance are more likely to experience adverse cardiac events, but it is important to point out that no prospective, well-powered clinical trial has assessed the benefit of tailoring antiplatelet medication specifically to populations with increased platelet reactivity. This is partly because we do not know which test or tests best define antiplatelet resistance and which medication(s) best improve outcome in these patient populations. The approach to the problem of antiplatelet resistance has been to develop newer drugs to further inhibit platelet reactivity or increase the dose and timing of treatment with currently available antiplatelet agents. However, both these approaches have been targeted at “allcomers”, rather than specifically tailoring either of these approaches to those patients identified as being non-responders. Importantly, the common side-effect of bleeding with all antiplatelet medications means that the risk vs. benefit ratio needs to be carefully balanced, and it may be more important to individualize such medication to subjects identified as “resistant” rather than giving stronger medication or higher doses to allcomers. Furthermore, the prevalence of “resistance” to these newly developed antiplatelet medications has not been evaluated.

We believe a simple, rapid, near-patient test, which is affordable and useful in the clinical (not just laboratory) setting needs to be validated in a large scale clinical trial, to identify patients with impaired response to antiplatelet medication. This would allow risk stratification and individualization of antiplatelet medication to improve outcome in these patients, with novel treatments or optimised doses of currently available drugs.

**Abbreviations**

ACS, acute coronary syndrome; ADP, adenosine diphosphate; CAD, coronary artery disease; CVA, cerebrovascular accident; DM, diabetes mellitus; GP, glycoprotein; IHD, ischaemic heart disease; ISR, in-stent restenosis; LTA, light transmission aggregometry; MACE, major adverse cardiac events; MI, myocardial infarction; PCI, percutaneous coronary intervention; PFA-100, platelet function analyser 100; PVD, peripheral vascular disease; SAT, subacute stent thrombosis; STEMI, ST elevation myocardial infarction; TRAP, thrombin receptor activating peptide

**Disclosures**

The authors report no conflicts of interest.

**References**

1. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2007;357(20):2001–15.
2. Cannon CP, Husted S, Harrington RA, et al. Safety, tolerability, and initial efficacy of AZD6140, the first reversible oral adenosine diphosphate receptor antagonist, compared with clopidogrel, in patients with non-ST-segment elevation acute coronary syndrome: primary results of the DISPERSE-2 trial. J Am Coll Cardiol. 2007;50(19):1844–51.
3. Savi P, Pereillo JM, Urzabitia MF, Combalbert J, Picard C, Maffrand JP, et al. Identification and biological activity of the active metabolite of clopidogrel. Throm Haemostast. 2000;113:340–5.
4. The Clopidogrel in Unstable Angina to prevent recurrent events trial Investigators. Effects of Clopidogrel in Addition to Aspirin in Patients with Acute Coronary Syndromes without ST-Segment Elevation. N Engl J Med. 2001;345:494–502.
5. Stephen D, Wiviott Elliott M, Antman Kenneth J, Winters, et al. Eugene Braunwald for the JUMBO–TIMI 26 Investigators. Randomized Comparison of Prasugrel (CS-747, LY640315), a Novel Thienopyridine P2Y12 Antagonist, With Clopidogrel in Percutaneous Coronary Intervention: Results of the Joint Utilization of Medications to Block Platelets Optimally (JUMBO)–TIMI 26 Trial. Circulation. 2005;111:3366–73.
6. Wiviott SD. Clopidogrel response variability, resistance, or both? Am J Cardiol. 2006;98:18–24.
7. Cannon CP, Husted S, Storey RF, et al; for the DISPERSE 2 Investigators. The DISPERSE 2 Trial:Safety, tolerability and preliminary efficacy of AZD6140, the first oral reversible ADP receptor antagonist, compared with clopidogrel in patients with non-ST segment elevation acute coronary syndrome. Circulation. 2005;112.
8. Antithrombotic Trialists Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ. 2002;324:71–86.
9. Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A. European Stroke Prevention Study 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. J Neurol Sci. 1996;143(1–2):113.
10. Chapman TM, Gao KL. Cilostazol: A Review of its Use in Intermittent Claudication. American Journal of Cardiovascular Drugs. 2003;22:117–38.
11. Tillmann C, Yao Y, Ding T, Michel J, Domenico P. A novel thromboxane receptor antagonist and synthase inhibitor, BM-573, reduces development and progression of atherosclerosis in LDL receptor deficient mice. *Eur J Pharmacol*. 2007;561:105–11.

12. Michelson AD, Cattaneo M, Eikelboom JW, et al. Aspirin resistance: position paper of the Working Group on Aspirin Resistance, Platelet Physiology Subcommittee of the Scientific and Standardization Committee, International Society on Thrombosis and Haemostasis. *J Thromb Haemost*. 2005;3:1309–11.

13. Ohmori T, Yatomi Y, Nonaka T, et al. Aspirin resistance detected with aggregometry with aggregometry cannot be explained by cyclooxygenase-activity-involvement of other signalling pathways in cardiovascular events of aspirin treated patients. *J Thromb Haemost*. 2006;4:1271–8.

14. Yoshida J, Yamada T, Ikari H, et al. GTP: a global in-vitro test of platelet function and thrombolyis. *Blood Coag Fibrinolysis*. 2003;14:31–9.

15. Lordkipanidze M, Pharrand C, Schampaert E, Turgeon J, Palisaitis DA, et al. Aspirin resistance in patients with cardiovascular disease. *European Heart Journal*. 2007;28:1702–8.

16. Lordkipanidze M, Pharrand C, Nguyen TA, Schampaert E, Palisaitis DA, et al. Comparison of four tests to assess inhibition of platelet function by clopidogrel in stable coronary artery disease patients. *European Heart Journal*. DOI: 10.1093/eurheartj/ehn419.

17. Burns TL, Mooss MD, Hilleman DL. Antiplatelet Drug Resistance: Not Ready for Prime Time. *Pharmacotherapy*. 2005;25(11):1621–8.

18. PAMUKU B. A review of aspirin resistance; definition, possible mechanisms, detection with platelet function tests, and its clinical outcomes. *J Thromb Thrombolysis*. 2007;23:23–22.

19. Guin PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol*. 2001;88:230–5.

20. Antiplatelet trials collaboration. Collaborative overview of randomised trials of antiplatelet therapy: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ*. 1994;308:81–106.

21. Macchi L, Christiaens L, Brabant S, et al. Resistance in vitro to low dose aspirin is associated with platelet PLA2 (GPIIIa) polymorphism but not with C807T (GP 1a/IIa) and C-5T Kozak (GP IIb/IIIa) polymorphisms. *J Am Coll Cardiol*. 2003;42:1115–9.

22. Hobbikoglu GF, Norgaz T, Aksu H, et al. High frequency of aspirin resistance in patients with acute coronary syndrome. *Tohoku J Exp Med*. 2005;207:59–64.

23. Anderssen K, Hurlen M, Arnesen H, Seljeifot I. Aspirin non-responsiveness as measured by PFA-100 in patients with coronary artery disease. *Thromb Res*. 2002;108:37–42.

24. Chen WH, Lee PY, Ng W, Tse HF, Lau CP. Aspirin resistance is associated with platelet PI A1 (GPIIIa) polymorphism but not with C807T (GP 1a/IIa) polymorphism. *J Thromb Thrombolysis*. 2003;14:31–9.

25. Steinhubl S, Talley DJ, Braden GA, et al. Point of care measured platelet inhibition correlates with a reduced risk of an adverse cardiac event after percutaneous coronary intervention. Results of the GOLDS (AU-Assessing Ultegra) Multicentre study. *Circulation*. 2001;103:2572–8.

26. Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol*. 2006;48(9):1742–50.

27. Matetzky S, Shenkman B, Guetta V, Schechter M, Bienart R, Goldenberg I, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation*. 2004;109:3171–5.

28. Gurbel PA, Bledin KP, Samara W, et al. Clopidogrel effects on platelet reactivity in patients with stent thrombosis. Results of the CREST study. *J Am Coll Cardiol*. 2005;46:1827–32.

29. Muller I, Besta F, Schulz C, Massberg S, Schonig, Gavazw M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost*. 2003;89:783–7.

30. Buonomici P, Marucci R, Migliorini A, et al. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. *J Am Coll Cardiol*. 2007;49(24):2312–7.

31. Ajzenberg N, Aubry P, Huisse MG, Cachier A, El Amara W, Feldman LJ, et al. Enhanced shear induced platelet aggregation in patients who experience subacute stent thrombosis: A case control study. *J Am Coll Cardiol*. 2005;45:1753–6.

32. Grundmann K, Jaschonek K, Kleine B, Dichgans J, Topka H. Aspirin non responder status in patients with recurrent cerebral ischaemic attacks. *J Neuros*. 2003;250:63–6.

33. Grotenemeyer KH, Scharafinski HW, Husstedt IW. Two year follow up of aspirin responder and aspirin non responder. A pilot study including 180 post stroke patients. *Thromb Res*. 1993;71:397–403.

34. Angiolillo DJ, Bernardo E, Sabaté M, et al. Impact of platelet reactivity on cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Coll Cardiol*. 2007;50(16):1541–7.

35. Angiolillo DJ, Shoemaker SB, Desai B, et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. *Circulation*. 2007;115(6):708–16.

36. Mueller MR, Salat A, Stangl P, et al. Variable platelet response to low dose aspirin and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. *Thromb Haemost*. 1997;76:1003–7.

37. Patti G, Colonna G, Pasceri V, et al. Randomised trial of high loading dose of clopidogrel for reduction of periprocedural myocardial infarction in patients undergoing coronary intervention: results from the ARMYDA-2 (Antiplatelet therapy for reduction of Myocardial damage during angioplasty) study. *Circulation*. 2005;111:2099–106.

38. Cuisset T, Frere C, Quilici J, et al. Benefit of 600-loading dose of clopidogrel on platelet reactivity and clinical outcomes in patients with non-SG segment elevation acute coronary syndrome undergoing coronary stenting. *J Am Coll Cardiol*. 2006;48:1339–45.

39. Von Beckherter N, Taubert D, Pogasta-Murray G, et al. Absorption, metabolism, and antiplatelet effects of 300, 600 and 900 mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel effect) Trial. *Circulation*. 2005;112:2946–50.

40. Montalescot G, Sideris G, Meuleman C, et al. A randomized comparison of high clopidogrel loading-doses in patients with non-ST-elevation acute coronary syndromes: the ALBION trial. *J Am Coll Cardiol*. 2006;48:931–8.

41. Gurbel PA, Bledin K, Zaman KA, Yoho J, Hayes KM, Tantry U. Results of the Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets (CLEAR PLATELETS) Study. *Circulation*. 2005;111:1153–9.

42. Von Beckherter N, Kastrati A, Wieczorek G, Pogasta-Murray G, Sibbing D, Schoemig A. A double blind randomized comparison between two clopidogrel maintenance doses after percutaneous coronary intervention (ISAR-CHOICE 2 Trial). *Eur Heart J*. 2006;27:5039.

43. Di Sciascio G, for the ARMYDA-4 Investigators. ARMYDA-4 (Antiplatelet therapy for Reduction of Myocardial Damage during Angioplasty) Study. Prospective, multicenter, randomized, double blind trial investigating influence on PCI outcome of additional 600 mg clopidogrel load in patients on chronic therapy-ARMYDA-RELOAD. Presented at: Transcatheter Cardiovascular Therapeutics (TCT); 2007, October 23; Washington, DC.

44. Di Sciascio G, for the ARMYDA-5 Investigators. ARMYDA-5 (Antiplatelet therapy for Reduction of Myocardial Damage during Angioplasty) Study. Prospective, multicenter, randomized trial investigating influence on outcome of in-lab 600 mg clopidogrel loading vs. 6-hour pre-PCI treatment-ARMYDA-Preload. Presented at: Transcatheter Cardiovascular Therapeutics (TCT); 2007, October 23; Washington, DC.
63. Ajzenberg N, Aubry P, Huisse MG, et al. Enhanced shear induced platelet
62. Barragan P, Bouvier JL, Roquebert PO, et al. Resistance to thienopyridines:
61. Muller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. Prevalence
59. Lev EL, Patel RT, Maresh KJ, Guthikonda S, Granada J, DeLao T, et al.
58. Lepnatalo A, Virtanen KS, Heikkila J, Wartiovaara U, Lassila R. Limited
53. Alberts MJ, Bergman DL, Molner E, Jovanic BD, Ushiwata I, Teruya J.
52. Helgason CM, Bolin KM, Hoff JA, et al. Development of aspirin resistance
51. Helgason CM, Bolin KM, Hoff JA, et al. Development of aspirin resistance
50. Mobley JE, Bresee SJ, Wortham DC, Craft RM, Snider CC, Carroll RC.
49. Snoep JD, Hovens MM, Eikenboom JC, Van der Bom JG, Huisman MV.
48. Hoven MM, Snoep JD, Eikenboom JC, Van der Bom JG, Mertens BJ,
47. Patti G, Nusca A, Mangiapacca F, Gatto L, D’Ambrosio A, Di Sciascio G.

Interindividual variability in response to clopidogrel in patients with
scheduled for elective coronary stent placement.

2003;59:295–302.

Catheter Cardiof Interv. 2003;59:295–302.

Diabetic Care. 2003;26:3264–72.

Blanche D. Involvement of hydrogen and lipid peroxides in acute tobacco
smoking induced platelet hyperreactivity. Am J Physiol. 1995;268:H679–85.

Ichiki K, Ikeda H, Haramaki N, et al. Long term smoking impairs platelet
derived nitric oxide release. Circulation. 1996;94:3109–14.

Davis JW, Hartman CR, Lewis HD, et al. Cigarette smoking induced
enhancement of platelet function: lack of prevention by aspirin in men with
coronary artery disease. J Lab Clin Med. 1985;105:479–83.

Santos MT, Valles J, Marcus AJ, et al. Enhancement of platelet reactivity
and modulation of eicosanoid production by intact erythrocytes. J Clin
Invest. 1991;87:571–80.

Valles J, Santos MT, Aznar J, et al. Erythrocytes metabolically enhance
collagen-induced platelet responsiveness via thromboxane production, ADP
release, and recruitment. Blood. 1991;78:154–62.

Sacco M, Pellegrini F, Roncaglioni MC, et al. Primary prevention of
recurrent cardiovascular events with low dose aspirin and vitamin E in type 2 diabetes.

Diabetic Care. 2003;26:3264–72.

Gurbel PA, Bleden KP, Hiatt BL, O’Connor CM. Clopidogrel for coronary
therapy undergoing percuatneous coronary interventions.

2006;46:1827–32.

Pamukcu B, OFlaz H, Acor RD, et al. The role of exercise on platelet
reactivity. Thromb Res. 2000;99:887–94.

Pamukcu B, OFlaz H, Acor RD, et al. The role of exercise on platelet
aggregation in patients with stable angina pectoris and healthy
controls. Eur Heart J. 1997;18:807–15.

Larson PT, Wallen NH, Hjemdahl P. Norepinephrine induced human
plateletactivation in vivo is only partly counteracted by aspirin. Circulation.
1994;89:1951–7.

Urben M, SeljeIot I, Arnesen H. Increased platelet aggregability during
exercise in patients with previous myocardial infarction. Lack of inhibition
by aspirin. Thromb Res. 2000;99:887–94.

Pamukcu B, OFlaz H, Acor RD, et al. The role of exercise on platelet
aggregation in patients with stable coronary artery disease: exercise
induces aspirin resistant platelet activation. J Thromb Thrombolysis.
2005;20:17–22.

Eikelboom JW, Hirsh J, Weitz J, et al. Aspirin resistant thromboxane
biosynthesis and the risk of myocardial infarction, stroke or cardiovascular
death in patients at high risk of cardiovascular events. Circulation.
2002;105:1650–5.

Muller MR, Salat A, Stangl P, et al. Variable platelet response to low-dose
ASA and the risk of limb deterioration in patients submitted to peripheral
arterial angioplasty. Thromb Haemost. 1997;78:1003–7.

Pamukcu B, OFlaz H, Oncul A, et al. The role of aspirin resistance on outcome
in patients with acute coronary syndrome and the effect of clopidogrel
therapy in the prevention of major cardiovascular events. J Thromb
Thrombolysis. 2006;22(2):103–10.

Grotmeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of
aspirin responder and aspirin non-responder. A pilot study including 180
post-stroke patients. Thromb Res. 1993;71:397–403.

Grotmeyer KH. The platelet-reactivity-test-A useful byproduct of the blood
sampling procedure? Thrombosis Res. 1991;61:423–31.

Matetzky S, Shenkmen B, Guetta V, et al. Clopidogrel resistance is associated
with increased risk of recurrent atherothrombotic events in patients with
acute myocardial infarction. Circulation. 2004;109:3171–5.

Barragan P, Bouvier JL, Roquebert PO, et al. Resistance to thiopopyridines:
clinical detection of coronary stent thrombosis by monitoring of vasodilator-
stimulated phosphoprotein phosphorylation. Catheter Cardiovasc Interv.
2003;59:295–302.

Ajzenberg N, Aubry P, Huisse MG, et al. Enhanced shear induced platelet
aggregation in patients who experience subacute stent thrombosis: a
case control study. J Am Coll Cardiol. 2005;45:1753–6.

Dziewierz A, Dudek D, Helb G, Rakowski T, Mielecki W, Dubiel JS.
Interindividual variability in response to clopidogrel in patients with
coronary artery disease. Kardiol Pol. 2005;62:108–17.

Sacco M, Pellegrini F, Roncaglioni MC, et al. Primary prevention of
cardiovascular events with low dose aspirin and vitamin E in type 2 diabetes.
Antiplatelet resistance—does it exist and how to measure it?

87. Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree for peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. J Am Coll Cardiol. 2006;48:1742–50.

88. Gurbel PA, Bilden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol. 2005;46:1820–6.

89. Lev EI, Patel RT, Maresh KJ, et al. Aspirin and Clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. J Am Coll Cardiol. 2006;47:27–33.

90. Geisler T, langer H, Wydymus M, et al. Low response to Clopidogrel is associated with cardiovascular outcome after coronary stent implantation. Eur Heart J. 2006;27:2420–5.