Case presentation 1: Mr F. is a 60-year-old man with unstable angina who takes aspirin, 81 mg/day. A platelet function test demonstrates that his platelets are “resistant” to aspirin. Should his treatment be changed?

Case presentation 2: Mr K. is a 60-year-old man with unstable angina who takes aspirin, 81 mg/day, and clopidogrel, 75 mg/day. A platelet function test demonstrates that his platelets are “resistant” to clopidogrel. Should his treatment be changed?

Normal Platelet Function
Platelets are small cells of great importance in thrombosis, hemorrhage, and inflammation. Formation of the hemostatic plug at sites of vascular injury is described in Figure 1. Platelets localize, amplify, and sustain the coagulant response at the injury site and release procoagulant platelet-derived microparticles. Platelets contain a variety of inflammatory modulators (eg, CD40 ligand [CD40L]) that are released on platelet activation.

Platelet Function Testing in Cardiovascular Diseases
Platelets have an increasingly well-defined critical role in coronary artery thrombosis and in other common cardiovascular diseases, including stroke, peripheral vascular disease, and diabetes mellitus. Although the role of platelets in thrombosis is well characterized, platelets may also have a role in the pathogenesis of the underlying atherosclerotic process. Platelet function tests have been studied in cardiovascular disease as a means to predict clinical outcomes and to monitor antiplatelet drugs. Table 1 summarizes these tests.

Use of Platelet Function Tests to Predict Clinical Outcomes
In acute coronary syndromes and after coronary stenting, flow cytometric analysis of platelet activation–dependent markers predicts major adverse cardiac events (MACE). Increased platelet surface P-selectin is also a risk factor for silent cerebral infarction in patients with atrial fibrillation. However, circulating monocyte–platelet aggregates are a more sensitive marker of in vivo platelet activation than is platelet surface P-selectin in the clinical settings of stable coronary artery disease, human percutaneous coronary intervention, and acute myocardial infarction. Furthermore, circulating monocyte–platelet aggregates are an early marker of acute myocardial infarction. Measurement of plasma CD40L in the first 12 hours after the onset of ischemic symptoms in patients with unstable angina identifies a subgroup of patients that gains a much greater clinical benefit from abciximab treatment. High plasma concentrations of sCD40L may be associated with increased cardiovascular risk in apparently healthy women. In patients with stable angina, the Platelet Function Analyzer-100 (PFA-100; Dade Behring) closure time may predict the presence or absence of coronary artery stenoses at angiography, thereby potentially avoiding further diagnostic investigations. PFA-100 closure time may also be predictive of the severity of myocardial damage in acute myocardial infarction. In summary, although a number of studies have demonstrated that platelet function tests can predict MACE in cardiovascular diseases, none of these assays have been sufficiently studied in large clinical trials to become part of standard clinical care.

Use of Platelet Function Tests to Monitor Antiplatelet Drugs
Aspirin reduces the odds of a serious arterial thrombotic event in high-risk patients by \( \approx 25\% \). However, 10% to
20% of patients with an arterial thrombotic event who are treated with aspirin have a recurrent arterial thrombotic event during long-term follow-up. The failure of aspirin to prevent an arterial thrombotic event has been termed aspirin resistance. The failure of clopidogrel to prevent an arterial thrombotic event has been termed clopidogrel resistance. Similarly, the term GP IIb/IIIa antagonist resistance could be used. Because arterial thrombosis is multifactorial, an adverse arterial thrombotic outcome in a patient may often reflect treatment failure rather than resistance to an antiplatelet drug. Furthermore, patient noncompliance with aspirin, clopidogrel, or both is a frequent and hard-to-detect confounding problem. There is well-documented variability between patients (and “normal” volunteers) with regard to laboratory test responses to aspirin, thienopyridines, and GP IIb/IIIa antagonists. This variability in laboratory test response has also been termed “resistance” to antiplatelet agents. The key question is: Do laboratory tests of resistance to aspirin, clopidogrel, or GP IIb/IIIa antagonists predict clinical resistance to these drugs (ie, MACE)? Clinically meaningful definitions of aspirin, clopidogrel, and GP IIb/IIIa antagonist resistance can be based only on data linking drug-dependent laboratory tests to clinical outcomes in patients. Until such links are clearly established, MACE that occur despite an antiplatelet agent should not be termed drug resistance.

**Aspirin**

Aspirin irreversibly acetylates serine 530 of cyclooxygenase-1 (COX-1), resulting in the inhibition of thromboxane A₂ release from platelets and prostacyclin from endothelial cells. Because platelets lack the synthetic machinery to generate significant amounts of new COX, aspirin-induced COX-1 inhibition lasts for the lifetime of the platelet. In contrast, endothelial cells retain their capacity to generate new COX and recover normal function shortly after exposure to aspirin. Possible mechanisms of aspirin resistance are listed in Table 2. There is evidence that MACE in the settings of acute coronary syndromes, stroke/transient ischemic attacks, and peripheral arterial disease can be predicted by the following in vitro tests of aspirin resistance: arachidonic acid- and ADP-induced platelet aggregation (turbidometric), ADP- and collagen-induced platelet aggregation (impedance), VerifyNow (Accumetrics), PFA-100, or urinary 11-dehydrothromboxane B₂ (Figure 1).
<table>
<thead>
<tr>
<th>Basis of Test</th>
<th>Name of Test</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reported to Predict Clinical Outcomes</th>
<th>Monitoring of Aspirin*</th>
<th>Monitoring of Thienopyridines*</th>
<th>Monitoring of GP Iib/IIa Antagonists*</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo cessation of blood flow by platelet plug</td>
<td>Bleeding time</td>
<td>In vivo test; physiological</td>
<td>Nonspecific; insensitive; high interoperator CV; can leave scar</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>In vitro cessation of high shear blood flow by</td>
<td>PFA-100</td>
<td>Simple, rapid; low sample volume; no sample preparation; whole blood assay</td>
<td>Dependent on von Willebrand factor, hematocrit; no instrument adjustment</td>
<td>Yes12,13,20</td>
<td>Yes</td>
<td>Not recommended</td>
<td>Not recommended</td>
</tr>
<tr>
<td>platelet plug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shear-induced platelet adhesion</td>
<td>IMPACT (cone and platelet analyzer, DiaMed)</td>
<td>Simple, rapid; point-of-care; low sample volume; high shear; whole blood assay</td>
<td>Instrument not yet widely available</td>
<td>No</td>
<td>Under development</td>
<td>Under development</td>
<td>Not recommended</td>
</tr>
<tr>
<td>Platelet-to-platelet aggregation</td>
<td>Aggregometry (turbidometric)</td>
<td>Historical gold standard</td>
<td>Poor reproducibility; high sample volume; sample preparation; time consuming; expensive</td>
<td>Yes14,15</td>
<td>Yes</td>
<td>(with arachidonic acid and ADP)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Aggregometry (impedance)</td>
<td>Whole blood assay</td>
<td>High sample volume; sample preparation; time consuming; expensive</td>
<td>Yes16</td>
<td>Yes</td>
<td>(with arachidonic acid and ADP)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>VerifyNow (ultegra RPA)</td>
<td>Simple, rapid; point-of-care; low sample volume; no sample preparation; whole blood assay</td>
<td>No instrument adjustment</td>
<td>Yes12,122</td>
<td>Yes (with arachidonic acid or propyl gallate cartridge)</td>
<td>Yes (with pending ADP cartridge)</td>
<td>Yes (with TRAP cartridge)</td>
</tr>
<tr>
<td></td>
<td>Plateletworks (Helena Laboratories)</td>
<td>Minimal sample preparation; whole blood assay</td>
<td>Not well studied</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Activation-dependent changes in platelet surface</td>
<td>Platelet surface P-selectin, platelet surface activated GP IIb/IIIa, leukocyte-platelet aggregates (flow cytometry)</td>
<td>Low sample volume; whole blood assay</td>
<td>Sample preparation; expensive; requires flow cytometer and experienced operator</td>
<td>Yes14</td>
<td>Yes (with arachidonic acid)</td>
<td>Yes (with ADP)</td>
<td>Yes</td>
</tr>
<tr>
<td>Activation-dependent signaling</td>
<td>VASP phosphorylation state (flow cytometry)</td>
<td>Directly dependent on clotidopla’s target, P2Y&lt;sub&gt;12&lt;/sub&gt;; low sample volume; whole blood assay</td>
<td>Sample preparation; expensive; requires flow cytometer and experienced technician</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Activation-dependent release from platelets</td>
<td>Platelet-derived microparticles (flow cytometry)</td>
<td>Low sample volume; whole blood assay</td>
<td>Sample preparation; expensive; requires flow cytometer and experienced technician</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Serum thromboxane B&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
<td>Directly dependent on aspirin’s target, COX-1</td>
<td>Indirect measure; not platelet specific</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Urinary 11-dehydrothromboxane B&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td>Directly dependent on aspirin’s target, COX-1</td>
<td>Indirect measure; not platelet specific dependent on renal function</td>
<td>Yes17</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Plasma sCD40L</td>
<td></td>
<td>Majority of plasma sCD40L is platelet-derived</td>
<td>Separation of plasma can result in artifactual platelet activation</td>
<td>Yes11,11</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Plasma GPV</td>
<td></td>
<td>Platelet specific</td>
<td>Separation of plasma can result in artifactual platelet activation; reflects only thrombin-mediated platelet activation</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>α-Granule constituents in plasma: platelet factor 4, β-thromboglobulin, soluble P-selectin</td>
<td>Reflect platelet secretion</td>
<td></td>
<td>Separation of plasma can result in artifactual platelet activation; plasma soluble P-selectin also originates from endothelial cells</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

TRAP indicates thrombin receptor-activating peptide; VASP, vasodilator-stimulated phosphoprotein; CV, coefficient of variation; GPV, glycoprotein V; and RPFA, rapid platelet function analyzer (Accumetrics). For further information on these tests, see reference 1.

*No published studies address the clinical effectiveness of altering therapy based on a laboratory finding of resistance to aspirin, clopidogrel, or GP Iib/IIa antagonists.
TABLE 2. Possible Mechanisms of Aspirin and Clopidogrel Resistance

<table>
<thead>
<tr>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncompliance</td>
</tr>
<tr>
<td>Underdosing</td>
</tr>
<tr>
<td>Poor absorption (enteric-coated aspirin)</td>
</tr>
<tr>
<td>Interference</td>
</tr>
<tr>
<td>NSAID coadministration (competes with aspirin for serine 530 of COX-1)</td>
</tr>
<tr>
<td>Atorvastatin (interferes with cytochrome P450-mediated metabolism of clopidogrel)</td>
</tr>
</tbody>
</table>

**Platelet function**

Incomplete suppression of thromboxane A2 generation (aspirin)

Accelerated platelet turnover, with introduction into bloodstream of newly formed, drug-unaffected platelets

Stress-induced COX-2 in platelets (aspirin)

Increased platelet sensitivity to ADP and collagen

**Single-nucleotide polymorphisms**

Receptors: P2Y12 H2 haplotype (clopidogrel), GP IIb/IIIa, collagen receptor, thromboxane receptor, etc

Enzymes: COX-1, COX-2, thromboxane A2 synthase, etc (aspirin)

**Platelet interactions with other blood cells**

Endothelial cells and monocytes provide PGH2 to platelets (bypassing COX-1) and synthesize their own thromboxane A2 (aspirin)

**Other factors**

Smoking, hypercholesterolemia, etc

**Rather than resistance, is it:**

Aspirin or clopidogrel response variability?

Platelet response variability?

Treatment failure (because arterial thrombosis is multifactorial)?

PGH2 indicates prostaglandin H2.

2A).15–20 However, in all of these studies, the number of MACE was low.

**Thienopyridines**

The thienopyridines clopidogrel (Plavix, Bristol-Myers Squibb/Sanofi Aventis) and ticlopidine (Ticlid, Bristol-Myers Squibb/Sanofi Aventis) inhibit ADP from binding to its platelet surface P2Y12 receptor. Possible mechanisms of clopidogrel resistance are listed in Table 2. Matetzky et al found evidence that an in vitro test of clopidogrel resistance (ADP-induced platelet aggregation) predicts MACE, but the number of MACE was again low (Figure 2B).21 The P2Y12 H2 haplotype is reported to be associated with peripheral artery disease.23

**GP IIb/IIIa Antagonists**

The GP IIb/IIIa antagonists abciximab (ReoPro, Eli Lilly/Centocor), eptifibatide (Integrilin, Millennium Pharmaceuticals), and tirofiban (Aggrastat, Merck) inhibit fibrinogen from binding to platelet surface GP IIb/IIIa (integrin αIIbβ3), the final common pathway of platelet aggregation. Although the term resistance has not been used in the literature with regard to GP IIb/IIIa antagonists, there is substantial patient-to-patient variability in the degree of inhibition of platelet function by GP IIb/IIIa antagonists.22 Furthermore, there is evidence that an in vitro test of abciximab resistance (VerifyNow) predicts MACE (Figure 2C).22

**Treatment for Resistance to Antiplatelet Agents**

Although some clinicians change treatment on the basis of platelet function testing,24 the correct treatment, if any, of aspirin resistance is unknown. Non-compliance should be considered. Increasing the dose of aspirin is unlikely to be helpful.14 Addition of a thienopyridine may be useful, with25 or without continued aspirin therapy. However, increased antiplatelet therapy may increase the risk of bleeding and other side effects. Most important, no published studies address the clinical effectiveness of altering therapy on the basis of a laboratory finding of resistance to aspirin, clopidogrel, or GP IIb/IIIa antagonists. In summary, therefore, other than in research trials, it is not currently appropriate to test for resistance in patients or to change therapy on the basis of such tests.

**Disclosure**

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**References**


4. Deleted in proof.


Figure 2. Evidence that in vitro tests of resistance to antiplatelet drugs predict MACE. A, Aspirin resistance was determined by higher quartiles of urinary 11-dehydrothromboxane B2. P indicates trend of association. B, Clopidogrel resistance in study patients (Pts) was determined by quartiles of inhibition of ADP-induced platelet aggregation. C, Abciximab resistance was determined by VerifyNow 8 h after abciximab bolus but during infusion. Clinical follow-up was (A) 5 y, (B) 6 mo, and (C) 7 d. Reproduced with permission from Circulation. 2001, 2002, 2004, American Heart Association.