PLATELET DISORDERS

Platelet function testing in cardiovascular diseases

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Keywords: platelet, tests, aspirin, clopidogrel, GPIIb-IIIa antagonists

Case Presentation 1: Mr. F. is a 60 year old man with unstable angina who takes aspirin 81 mg each 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 and clopidogrel 75 mg each 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 [1]. 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 (e.g. CD40 ligand [CD40L]) that are released upon platelet activation.

Platelet function testing in cardiovascular diseases

Platelets have an increasingly well-defined, critical role in coronary artery thrombosis [2] and in other common cardiovascular diseases including stroke, peripheral vascular disease, and diabetes mellitus [1]. Although the role of platelets in thrombosis is well characterized, platelets may also have a role in the pathogenesis of the underlying atherosclerotic process [2]. Platelet function tests have been studied in cardiovascular diseases as a means to predict clinical outcomes and to monitor antiplatelet drugs. Table I 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) [3]. Increased platelet surface P-selectin is also a risk factor for silent cerebral infarction in patients with atrial fibrillation [4]. However, circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin in the clinical settings of stable coronary artery disease, [5] PCI, [6] and acute myocardial infarction [6]. Furthermore, circulating monocyte-platelet aggregates are an early marker of acute myocardial infarction [7]. 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 has a much greater clinical benefit from abciximab treatment [8]. High plasma concentrations of sCD40L may be associated with increased cardiovascular risk in apparently healthy women [9]. In patients with stable angina, the PFA-100® closure time may predict the presence or absence of coronary artery stenoses at angiography, thereby potentially avoiding further diagnostic investigations [10]. PFA-100® closure time may also be predictive of the severity of myocardial damage in acute myocardial infarction [11].
Although a number of studies have demonstrated that platelet function tests can predict MACE in cardiovascular diseases, none of these assays have yet 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 ~25% [12]. However, 10–20% of patients with an arterial thrombotic event who are treated with aspirin have a recurrent arterial thrombotic event during long term follow-up [12]. Failure of aspirin to prevent an arterial thrombotic event has been termed “aspirin resistance”. Failure of clopidogrel to prevent an arterial thrombotic event has been termed “clopidogrel resistance”. Similarly, the term “GPIIb-IIIa antagonist resistance” could be used. However, 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 non-compliance with aspirin and/or clopidogrel 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, [13–18] thienopyridines, [19] and GPIIb-IIIa antagonists [20]. 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, and/or GPIIb-IIIa antagonists predict clinical “resistance” to these drugs (i.e. MACE)? Clinically meaningful definitions of aspirin, clopidogrel, and GPIIb-IIIa “resistance” can only be based 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 (COX)-1, resulting in inhibition of thromboxane A₂ (TxA₂), ADP, and other platelet agonists. Platelet-to-platelet aggregation is primarily mediated by activation of platelet surface GPIIb-IIIa (integrin α₃β₃) (not shown). A fibrin meshwork (shown in green) helps to perpetuate and stabilize the platelet aggregate. Reproduced with permission from ref. [24].

Figure 1. Steps in platelet plug formation. A) Prior to vascular injury, platelets are maintained in a resting state by endothelial inhibitory factors: prostacyclin (PGI₂), nitric oxide (NO), and CD39. B) The platelet plug is initiated by exposure of collagen and local generation of thrombin. This causes platelets to adhere via collagen and von Willebrand factor (vWF) and spread. C) The platelet plug is extended via the release of thromboxane A₂ (TxA₂), ADP, and other platelet agonists. Platelet-to-platelet aggregation is primarily mediated by activation of platelet surface GPIIb-IIIa (integrin α₃β₃) (not shown). D) A fibrin meshwork (shown in green) helps to perpetuate and stabilize the platelet aggregate. Reproduced with permission from ref. [24].
<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 GPIb/IIIa Antagonists*</th>
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</thead>
<tbody>
<tr>
<td>In vivo cessation of blood flow by a platelet plug</td>
<td>Bleeding time</td>
<td>In vivo test. Physiological.</td>
<td>Non-specific. Insensitive. High inter-operator CV. Can leave scar.</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>In vivo cessation of high shear blood flow by a platelet plug</td>
<td>PFA-100&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Simple and rapid. Low sample volume. High shear. No sample preparation. Whole blood assay.</td>
<td>Dependent on von Willebrand factor and hematocrit. No instrument adjustment.</td>
<td>Yes [10,11,18]</td>
<td>Yes</td>
<td>Not recommended</td>
<td>Not recommended</td>
</tr>
<tr>
<td></td>
<td>VerifyNow&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Simple and rapid. Point-of-care. Low sample volume. No sample preparation. Whole blood assay.</td>
<td>No instrument adjustment.</td>
<td>Yes [17,20]</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&lt;sup&gt;®&lt;/sup&gt;</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>
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### Table I (Continued)

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<td><strong>Activation-dependent signaling</strong></td>
<td>VASP phosphorylation state (flow cytometry)</td>
<td>Directly dependent on clopidogrel’s target: P2Y12. Low sample volume. Whole blood assay.</td>
<td>Sample preparation. Requires flow cytometer and experienced technician.</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Activation-dependent release from platelets</strong></td>
<td>Platelet-derived microparticles (flow cytometry)</td>
<td>Low sample volume. Whole blood assay.</td>
<td>Sample preparation. Requires flow cytometer and experienced technician.</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td></td>
<td>Serum thromboxane B₂</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></td>
<td>Plasma sCD40L</td>
<td>Majority of plasma sCD40L is platelet-derived.</td>
<td>Separation of plasma can result in artifactual platelet activation.</td>
<td>Yes [8,9]</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Plasma GPV</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></td>
<td>α granule constituents in plasma: platelet factor 4, β-thromboglobulin, soluble P-selectin</td>
<td>Reflect platelet secretion.</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>

**Abbreviations:** COX-1, cyclooxygenase 1; PFA-100, platelet function analyzer-100; sCD40L, soluble CD40 ligand; TRAP, thrombin receptor activating peptide; VASP, vasodilator stimulated phosphoprotein.

*No published studies address the clinical effectiveness of altering therapy based on a laboratory finding of "resistance" to aspirin, clopidogrel, or GPIIb-IIIa antagonists. For further information on these tests see also refs. [1],[25].
Thienopyridines

The thienopyridines (clopidogrel [Plavix®] and ticlopidine [Ticlid®]) inhibit ADP binding to its platelet surface P2Y_{12} receptor. Possible mechanisms of clopidogrel “resistance” are listed in Table II. There is evidence from one trial that an in vitro test of clopidogrel “resistance” (ADP-induced platelet aggregation) predicts MACE, but the number of MACE was again low (Figure 2B) [19]. The P2Y_{12} H2 haplotype is reported to be associated with peripheral artery disease [21].

GPIIb-IIIa Antagonists

GPIIb-IIIa antagonists (abciximab [ReoPro®], eptifibatide [Integrilin®], tirofiban [Aggrastat®]) inhibit fibrinogen binding to platelet surface GPIIb-IIIa (integrin \( \alpha_{IIb}\beta_{3} \)), the final common pathway of platelet aggregation. Although the term “resistance” has not been used in the literature with regard to GPIIb-IIIa antagonists, there is substantial patient-to-patient variability in the degree of inhibition of platelet function by GPIIb-IIIa antagonists.[20] Furthermore, there is evidence that an in vitro test of abciximab “resistance” (VerifyNow®) predicts MACE (Figure 2C) [20].

Treatment of “resistance” to antiplatelet agents

Although some clinicians change treatment based on platelet function testing, [22] the correct treatment, if any, of “aspirin resistance” is unknown. Non-com-
pliance should be considered. Increasing the dose of aspirin is unlikely to be helpful [12]. Addition of a thienopyridine may be useful, with [23] or without continued aspirin therapy. However, increased antiplatelet therapy may increase the risk of bleeding and other side effects. Most importantly, no published studies address the clinical effectiveness of altering therapy based on a laboratory finding of “resistance” to aspirin, clopidogrel, or GPIIb-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 based on such tests.

References