Platelet Function Testing in Practice: A Case Study

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Dual antiplatelet therapy with aspirin and a thienopyridine reduces ischemic cardiovascular events following percutaneous coronary intervention. However, despite this treatment, residual risk of ischemic events persists. Among other factors, enhanced platelet reactivity after thienopyridine therapy is associated with an increased risk of ischemic cardiovascular events. A heterogeneous and variable patient response to the thienopyridine clopidogrel exists and has been attributed to a number of genetic, pharmacologic, and clinical factors. Developments in point-of-care platelet function testing allow for the assessment of on-treatment platelet reactivity after thienopyridine therapy and thus identify poor responders. We report two cases of stent thrombosis in which the bedside rapid platelet function VerifyNow P2Y12 Assay® (Accumetrics, San Diego, CA) was used to determine on-treatment platelet reactivity and identify potential etiologies of the thrombotic events.

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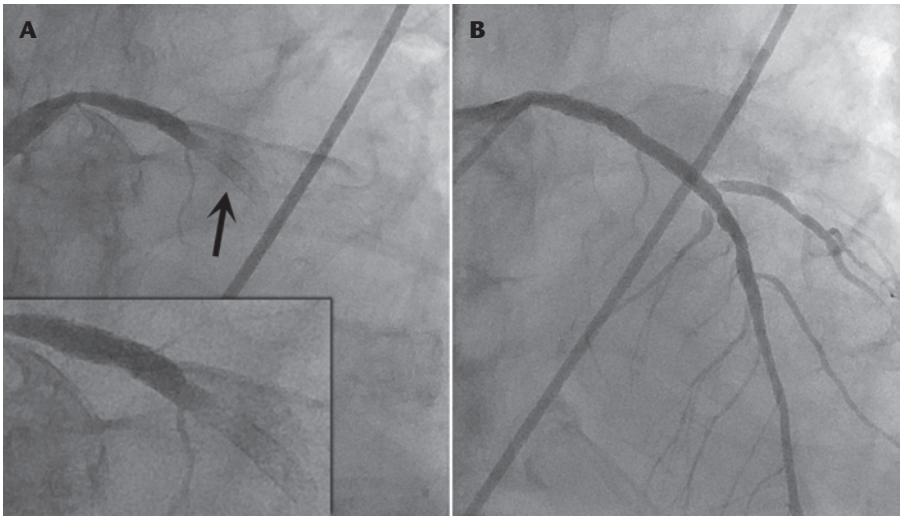
Enhanced platelet reactivity following percutaneous coronary intervention (PCI) is associated with adverse ischemic cardiovascular events including stent thrombosis.[1] Although dual antiplatelet therapy (DAPT) with aspirin and a thienopyridine is used to reduce risk of ischemic cardiovascular events following PCI,[2] high platelet reactivity after clopidogrel therapy is associated with increased morbidity and mortality.[3-6] We report two cases to illustrate contexts in which platelet function testing may be used in clinical practice; an algorithm to guide the use of platelet function testing is also presented.

Case 1

An 87-year-old man with history of non–ST-segment elevation myocardial infarction (NSTEMI) and PCI with placement of a 3.0 × 33 mm Cypher® (Cordis Corp., Miami, FL) drug-eluting stent 11 months prior presented with an acute anterior STEMI. Emergent coronary angiography revealed a proximal left anterior descending (LAD) thrombosis. Platelet function testing with the VerifyNow P2Y12 Assay® (Accumetrics, San Diego, CA) rapid bedside $P2Y_{12}$ assay revealed on-treatment reactivity of 291 $P2Y_{12}$ reaction units (PRUs) and 0% platelet inhibition while the patient was reportedly on DAPT, which included clopidogrel. He was treated with aspirin, 325 mg, clopidogrel, 600 mg, and intravenous (IV) bivalirudin prior to PCI. Following balloon dilatation and extraction thrombectomy, a bare-metal stent was deployed in the proximal LAD. Due to cardiogenic shock, inotropic support and placement of an intra-aortic balloon bump was required. He was discharged on DAPT with aspirin and clopidogrel, 75 mg/d. Platelet function testing a day after the 600-mg loading dose of clopidogrel demonstrated a PRU of 149 (platelet inhibition 37%). Four weeks later the patient presented with the sudden onset of severe left-sided chest pain; electrocardiography revealed an anterior ST-segment elevation myocardial infarction (STEMI). Emergent coronary angiography revealed LAD stent thrombosis (Figure 1A). The VerifyNow P2Y12 Assay revealed a PRU of 280 (0% PI). Balloon dilatation and thrombectomy were performed with excellent angiographic result (Figure 1B). Nursing home facility records were obtained, which documented the patient's refusal of both aspirin and clopidogrel for 2 weeks prior to admission. Recurrent stent thrombosis and low serial PI measurements were considered a result of medication nonadherence. Extensive counseling and patient education resulted in an agreement to continue DAPT. After a repeat loading dose of clopidogrel, a PRU of 163 (platelet inhibition 42%) was observed. The patient remained asymptomatic at 2-month follow-up.

Figure 1. *Anterior-posterior, cranial angiogram of an 87-year-old man with repeated stent thrombosis and clopidogrel noncompliance.* **(A)** *Outline of a previously placed proximal left anterior descending artery (LAD) stent* (arrow and inset) *and total occlusion due to stent thrombosis.* **(B)** *Restored flow to the LAD is observed following balloon angioplasty and aspiration thrombectomy.*



Case 2

A 45-year-old woman with a history of hypertension and hyperlipidemia was directly transferred to the catheterization laboratory after presenting to an outreach hospital with an acute anterior STEMI. She was treated with aspirin, 325 mg, clopidogrel, 600 mg, and IV heparin, and transferred for primary PCI. Coronary angiography revealed total occlusion of the proximal LAD. After balloon dilatation, a 3.0 × 18 mm PROMUS® stent (Boston Scientific, Natick, MA) was deployed at 18 atmospheres. The following morning the patient developed acute recurrence of chest pain and anterior ST-segment elevation. The VerifyNow P2Y12 Assay revealed a PRU of 276 (platelet inhibition 8%), and emergent angiography revealed total occlusion of the proximal LAD due to stent thrombosis (Figure 2A). IV heparin and eptifibatide were initiated, and balloon dilatation and extraction thrombectomy were performed, followed by intravascular ultrasound evaluation of the stented segment. There was no evidence of stent malapposition or edge dissection. Nevertheless, a noncompliant balloon was dilated at high pressure in the stented segment with an excellent final result (Figure 2B). Acute stent thrombosis was thought to be secondary to clopidogrel hyporesponsiveness. Clopidogrel was discontinued, prasugrel was initiated, and her subsequent clinical course has been uneventful.

Discussion

DAPT with aspirin and a thienopyridine reduces adverse cardiovascular events following PCI.[2] However, despite routine use, residual risk of ischemic events persists. The two patients discussed here presented with stent thrombosis and STEMI; potential

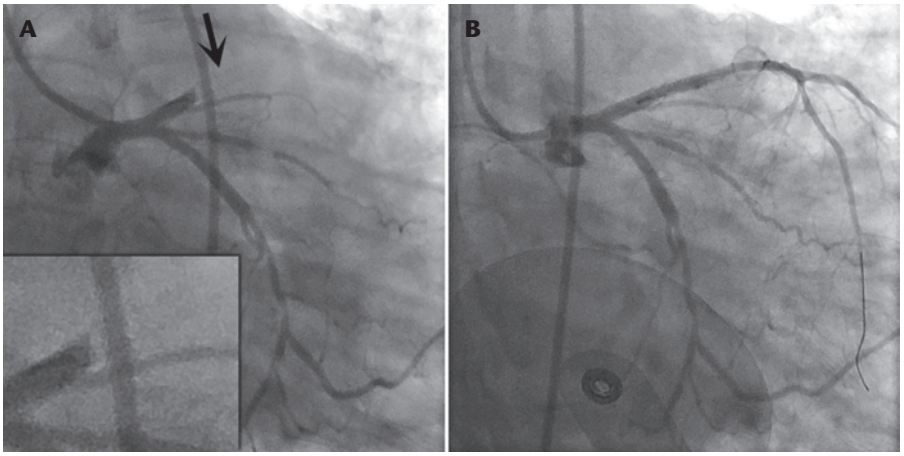


Figure 2. *Anterior-posterior, caudal angiogram of a 45-year-old woman with acute stent thrombosis and clopidogrel hyporesponsiveness.* **(A)** *Outline of previously placed left anterior descending artery (LAD) stent* (arrow and inset) *and total occlusion due to stent thrombosis.* **(B)** *Restored flow to the LAD is observed following balloon angioplasty and aspiration thrombectomy.*

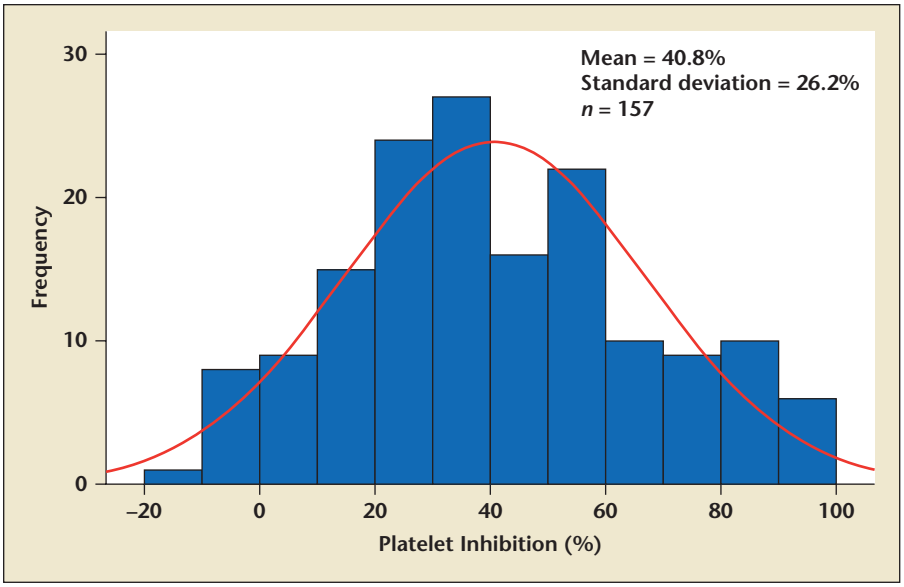
etiologies included clopidogrel nonadherence and hyporesponsiveness, respectively. These etiologies were easily identified by utilizing point-of-care platelet function testing with the VerifyNow P2Y12 Assay.

Premature discontinuation of one or both of the antiplatelet agents is commonly seen in clinical practice and estimated to occur in 14.4% of treated individuals within 12 months of undergoing PCI.[7] Furthermore, recent data show that the risk of very late stent thrombosis is significant beyond the mandated 12 months of DAPT.[8] Because thienopyridine noncompliance is a major contributor to the risk of stent thrombosis and adverse ischemic events, platelet function testing can be useful to assess platelet reactivity both before and after observed medication dosing. Enhanced platelet inhibition following observed dosing supports the diagnosis of nonadherence. As demonstrated in the first case, once nonadherence is identified, patient education and counseling can help improve compliance and clinical outcomes.

In patients compliant with thienopyridine therapy, the phenomenon of non- and hyporesponsiveness to clopidogrel is well recognized.[9] A normally distributed platelet reactivity response to clopidogrel has been demonstrated utilizing traditional light transmission aggregometry (LTA) and bedside platelet function assays (Figure 3).[9,10] The multifactorial etiology for this variability includes clinical and genetic risk factors. We have previously shown that higher body mass index and an elevated serum fibrinogen in patients with diabetes (Figure 4) are both associated with clopidogrel hyporesponsiveness.[9,11,12] Genetic polymorphisms of the hepatic cytochrome P-450 (CYP) 2C19 isoenzyme (*CYP2C19*)[10-12] and intestinal epithelial cell *ABCB1* efflux pump[13,14] have also been implicated in diminished platelet reactivity response to clopidogrel.

A number of testing modalities have been used to quantify platelet function with clopidogrel, and the traditional standard remains LTA. Limitations of LTA include confinement to the clinical laboratory, labor-intensive assays, variability between different operators and institutions, and delayed availability of results.[15,16] Newer, bedside rapid platelet function assays include the VerifyNow P2Y12 Assay, the Multiplate® analyzer (Dynabyte Informationssysteme GmbH, Munich, Germany), Plateletworks® (Helena Laboratories, Beaumont, TX), Platelet Function Analyzer-100

Figure 3. *Platelet inhibition distribution with clopidogrel therapy in patients undergoing percutaneous coronary interventions as measured with the rapid platelet function VerifyNow P2Y12 Assay® (Accumetrics, San Diego, CA). Reprinted from* J Am Coll Cardiol. *Vol. 52, Ang L et al. Elevated plasma fibrinogen and diabetes mellitus are associated with lower inhibition of platelet reactivity with clopidogrel. Pages 1052-1059. © 2008 with permission from Elsevier.*



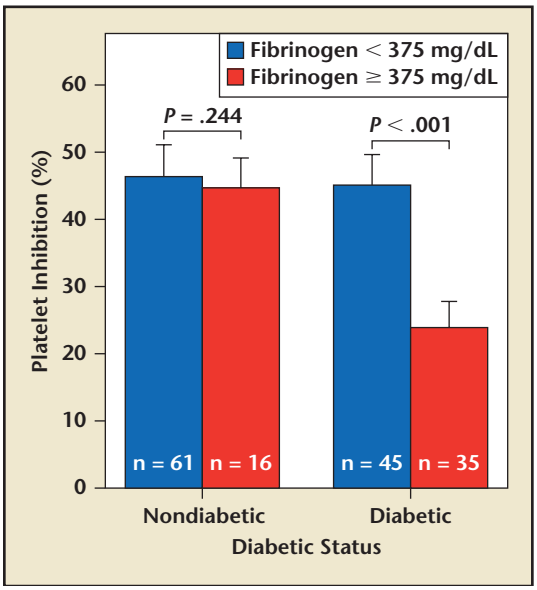


Figure 4. *Patients with diabetes with elevated serum fibrinogen (≥ 375 mg/dL) have significantly lower platelet inhibition with clopidogrel compared with nondiabetic patients or diabetic patients with lower fibrinogen levels. Reprinted from* J Am Coll Cardiol. *Vol. 52, Ang L et al. Elevated plasma fibrinogen and diabetes mellitus are associated with lower inhibition of platelet reactivity with clopidogrel. Pages 1052-1059. © 2008 with permission from Elsevier.*

(Dade Behring Inc., Deerfield, IL), and vasodilator-stimulated phosphoprotein flow cytometry.[9,16,17] However, these assays have also been shown to possess variable ability to predict adverse ischemic events.[18]

The VerifyNow P2Y12 Assay correlates well with the results of traditional LTA. A number of small prospective studies have demonstrated an association between post-treatment PRU ≥ 230 with higher ischemic cardiovascular events.[3-6] Calculated platelet inhibition and PRU are strongly correlated, and platelet inhibition < 30% closely approximates this PRU cutoff.[19] Additional prospective studies have shown an association between platelet inhibition < 30% measured by LTA and higher ischemic events.[1,20,21] The Gauging Responsiveness With a VerifyNow Assay–Impact on Thrombosis and Safety (GRAVITAS) trial tested whether doubling of clopidogrel maintenance dose (150 mg/d) in poor responders with PRU ≥ 230 would lower platelet reactivity and improve clinical outcomes following drug-eluting stent implantation. However, this trial showed no reduction in ischemic cardiovascular events and only a small improvement in level of platelet inhibition with the higher clopidogrel dose.[22] In contrast, prasugrel has consistently been shown to attain a higher level of platelet inhibition compared with clopidogrel.[23] The Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction (TRITON-TIMI) 38 trial showed clinical superiority of prasugrel over standard-dose clopidogrel for the reduction of MI, target vessel revascularization, and stent thrombosis within 15 months following PCI.[24] Consistent with the increased potency and ischemic event reduction with prasugrel, higher bleeding was also observed in prasugrel-treated patients. Similarly, ticagrelor is a reversible, non-thienopyridine antiplatelet agent found to improve ischemic cardiovascular outcomes and level of platelet inhibition compared with standard dose clopidogrel, but with increased bleeding.[25,26] Overall, these trials suggest that hyporesponders to standard clopidogrel therapy might obtain clinical benefit with reduction of adverse ischemic cardiovascular events using a more potent antiplatelet agent rather than increasing the dose of clopidogrel. The second patient presented here was a clopidogrel hyporesponder, but only identified after platelet function testing and an adverse event.

We present an algorithm for use in clinical practice that utilizes platelet function testing to manage poor response to thienopyridine therapy after PCI (Figure 5). Using the VerifyNow P2Y12 Assay, PRU < 230, and platelet inhibition ≥ 30% together suggests adequate medication response and adherence, whereas the presence of either PRU ≥ 230 or platelet inhibition < 30% alone or together suggests either nonadherence or hyporesponsiveness to clopidogrel therapy. Persistent poor response despite observed administration of a clopidogrel loading dose identifies hyporesponders who may benefit from prasugrel or ticagrelor.

Platelet function testing, as demonstrated in the reported cases and suggested treatment algorithm, can be used in clinical practice to guide therapeutic strategies.

Conclusions

Although DAPT with aspirin and a thienopyridine reduces ischemic cardiovascular events following PCI, poor platelet inhibition due to medication noncompliance and hyporesponsiveness exposes patients

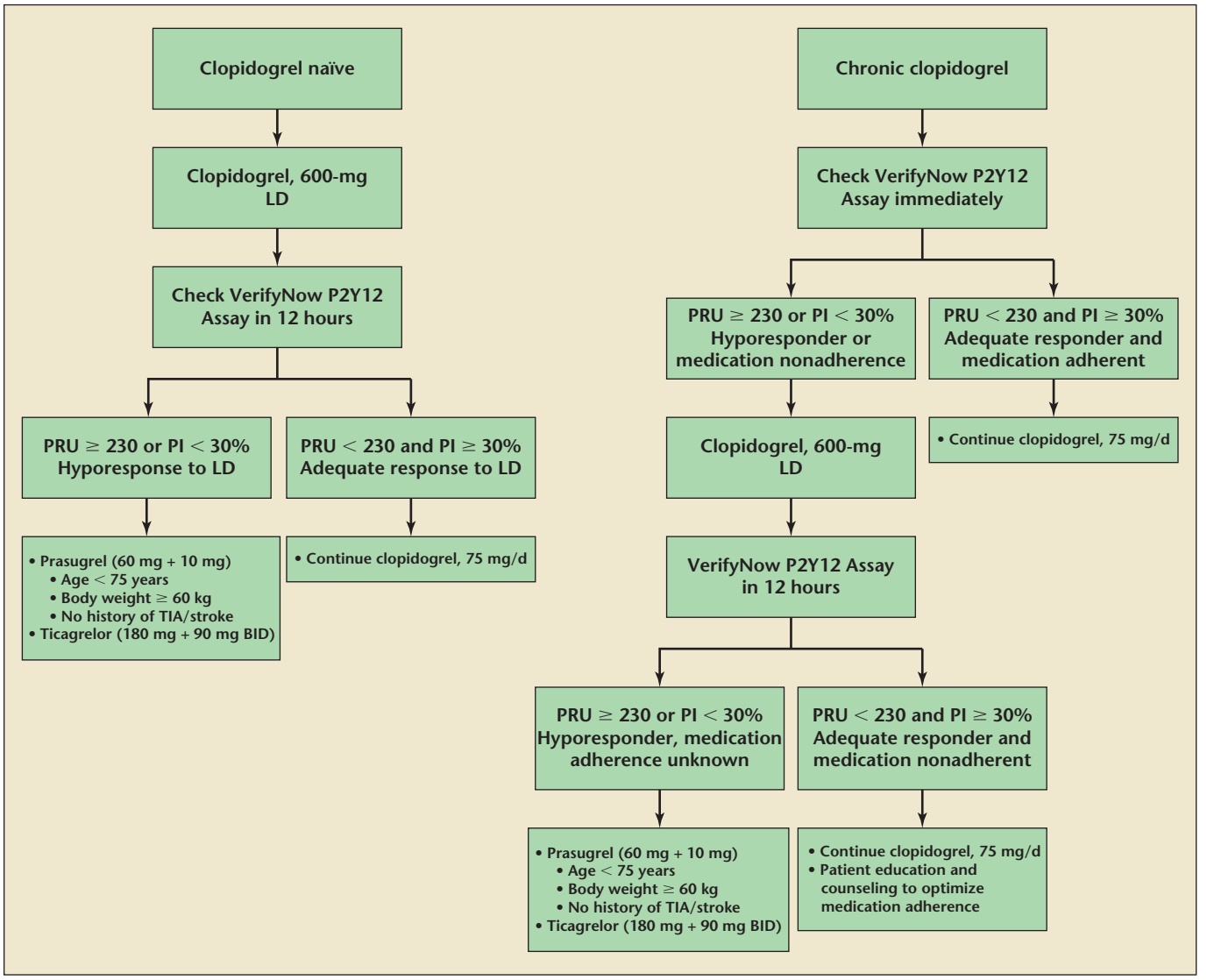


Figure 5. *A suggested algorithm for utilizing platelet function testing using the VerifyNow P2Y12 Assay® (Accumetrics, San Diego, CA) for patients with clopidogrel therapy after percutaneous coronary intervention. BID, twice daily; LD, loading dose; PI, platelet inhibition; PRU, platelet reactivity unit; TIA, transient ischemic attack.*

Main Points

- Nonadherence or hyporesponsiveness to dual antiplatelet therapy, particularly clopidogrel, following percutaneous coronary intervention exposes patients to residual risk of adverse ischemic cardiovascular events.
- Platelet function testing in clinical practice can be used to identify patient nonadherence to antiplatelet therapy and to identify hyporesponsiveness to thienopyridine therapy.
- Stent thrombosis is a catastrophic adverse event; two cases are presented in which rapid platelet function testing was used to identify a potential etiology.
- A suggested algorithm using the VerifyNow P2Y12 Assay to guide therapeutic strategies for clopidogrel noncompliance/hyporesponsiveness is presented.

to residual risk of adverse ischemic events. Platelet function testing, as demonstrated in the reported cases and suggested treatment algorithm, can be used in clinical practice to guide therapeutic strategies. ■

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