

Case Reports

Use of a Thrombelastograph Platelet Mapping Assay for Diagnosis of Clopidogrel Resistance: A Case Report

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Abstract: A 62-year-old woman presented to the emergency department with sudden collapse, intractable ventricular fibrillation, and an inferior wall myocardial infarction (MI). An emergent cardiac catheterization showed a totally occluded right coronary artery (RCA). A bare-metal stent was placed in the stenosis, resulting in thrombolysis in myocardial infarction (TIMI)-III flow with 0% residual stenosis. Four days after stenting, the patient developed chest pain. A repeat cardiac catheterization showed a totally occluded stent. The patient was

subsequently tested using a thrombelastograph (TEG) Platelet Mapping assay to exclude clopidogrel resistance. The assay confirmed the patient to be non-responsive to clopidogrel for the inhibition of platelet ADP receptors. In an attempt to increase ADP inhibition, the ADP antagonist was changed to ticlopidine. Further testing was confounded by the presence of abciximab; however, the patient has remained free of cardiac events. **Keywords:** ADP, clopidogrel, platelet inhibition, TEG, thrombelastograph, ticlopidine. *JECT. 2009;41:32–36*

The benefits of anti-platelet therapy in the treatment of acute coronary syndrome (ACS) as an adjunct to percutaneous coronary intervention (PCI) are well established (1,2). However, the appropriate response to resistance of anti-platelet therapy remains undefined. Because universal standards and universal definitions of resistance have not been established, conventional wisdom discourages routine testing for resistance or change of therapy based on resistance (3,4). The benefit of such testing in the setting of major adverse cardiac events (MACE) is perhaps more justified. Several studies, although small in sample size, suggest a correlation of MACE to anti-platelet therapy resistance (5–7).

CASE REPORT

A 62-year-old obese white woman with a history of hypertension, chronic obstructive pulmonary disease, lupus erythematosus, hyperlipidemia, and tobacco use presented to the emergency department with sudden onset of collapse,

syncope, intractable ventricular fibrillation, and ST elevation in the inferior leads. She was resuscitated, her trachea was intubated, and she was transferred for emergent cardiac catheterization. The cardiac catheterization showed a proximally occluded right coronary artery (RCA) with thrombolysis in myocardial infarction (TIMI)-0 flow, tortuous left anterior descending (LAD), and circumflex coronary (Cx) arteries with mild disease, posterobasal hypokinesis, an ejection fraction of 45%, and a left ventricular end diastolic pressure of 16 mmHg. The RCA stenosis was identified as the culprit vessel for her myocardial infarction (Figure 1). A bolus of 10,000 units unfractionated heparin, a 7.5-mg bolus of eptifibatide, and a 175- μ g/min eptifibatide infusion were administered immediately before PCI. The vessel was pre-dilated and stented with a 2.5 \times 28-mm Mini-Vision (Guidant Corp., Santa Clara, CA) bare-metal stent. The stenosis was reduced to 0% with TIMI-III distal flow (Figure 2). The patient was transferred to the coronary care unit on mechanical ventilation after receiving an additional 7.5-mg bolus of eptifibatide and 300 mg of clopidogrel. The eptifibatide infusion was continued for 20 hours in the coronary care unit. She was maintained on aspirin 81 mg daily and clopidogrel 75 mg daily. The patient was extubated 24 hours after the PCI. She was transferred to a step-down unit 2 days after extubation, with plans for discharge the following morning.

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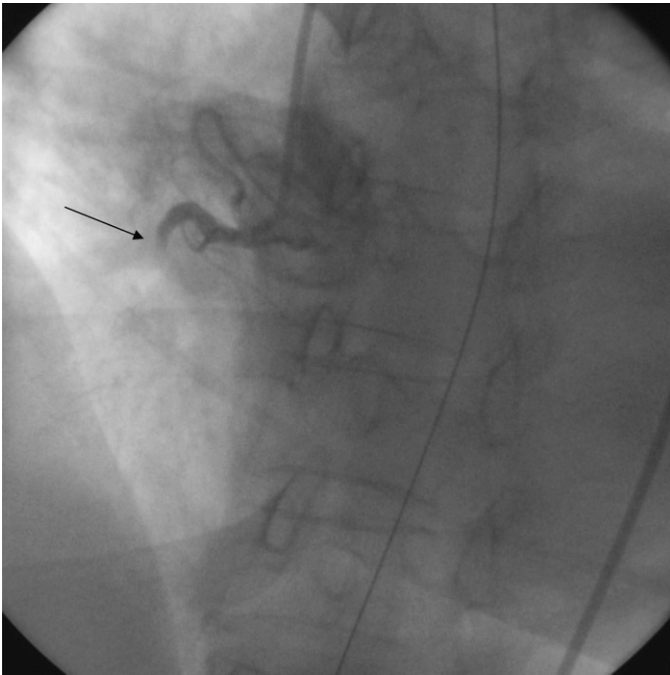


Figure 1. Proximally occluded RCA.

The morning of discharge (4 days after stenting), the patient developed recurrent chest pain and electrocardiogram changes suggestive of stent occlusion. The patient was given 325 mg aspirin in the coronary step-down unit and transferred urgently to the catheterization laboratory.

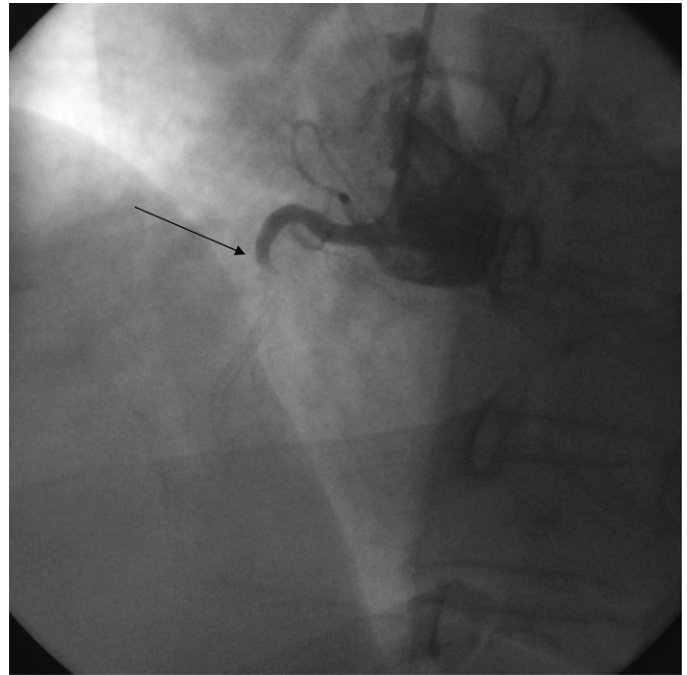


Figure 3. Re-occlusion of RCA at proximal portion of stent.

A thrombelastograph (TEG) Platelet Mapping assay (Haemoscope Corp., Niles, IL) using arachidonic acid (AA; 1 mmol/L) and ADP (2 μ mol/L) was used immediately before the start of a repeat PCI to determine the effect of aspirin and clopidogrel, respectively. The repeat catheterization showed a totally occluded stent in the

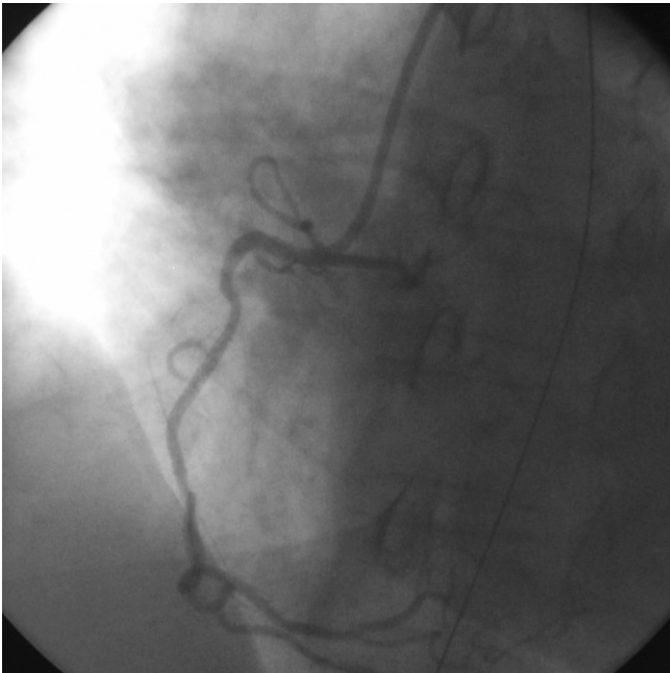


Figure 2. Patent RCA after stent (first).

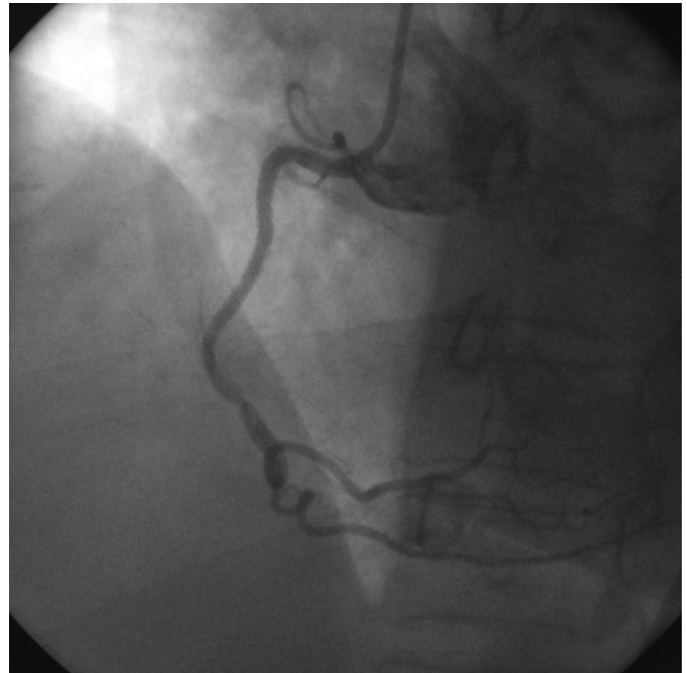


Figure 4. Patent RCA after stent (second).

TEG® Analysis Results

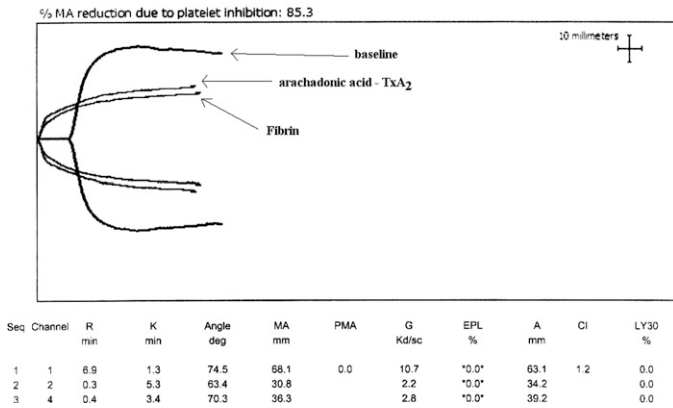


Figure 5. Eighty-five percent reduction in MA with arachidonic acid assay. Calculation of percent inhibition. y-axis, strength or stiffness (shear modulus) of the developed clot, measured in millimeters; x-axis, time; 10 mm, 10 minutes; MA, maximum amplitude (on y-axis) of tracing, which is a direct function of the maximum dynamic properties of fibrin and platelet bonding through the GPIIb/IIIa receptor and represents the ultimate strength of the fibrin clot; % inhibition of ADP receptor, $100 - \{100 \times [(MA \text{ of ADP}) - (MA \text{ of fibrin})] \times [(MA \text{ of baseline}) - (MA \text{ of fibrin})]^{-1}\}$; % inhibition of TxA2 receptor, $100 - \{100 \times [(MA \text{ of AA}) - (MA \text{ of fibrin})] \times [(MA \text{ of baseline}) - (MA \text{ of fibrin})]^{-1}\}$.

right coronary artery at the proximal portion of the stent (Figure 3). A bolus of 5000 units unfractionated heparin was given. Pre-dilation and re-stenting with a 3.0 × 15-mm Vision (Guidant Corp.) stent returned flow to TIMI-III (Figure 4). Immediately after the intervention, the patient was treated with 300 mg clopidogrel, and abciximab was administered as a .25-mg intravenous bolus and .125-µg/kg/min infusion to reduce the risk of acute recurrent stent thrombosis.

The Platelet Mapping assay used immediately before the start of the repeat PCI showed 85% inhibition of the thromboxane A₂ pathway (Figure 5) and 0% inhibition of

TEG® Analysis Results

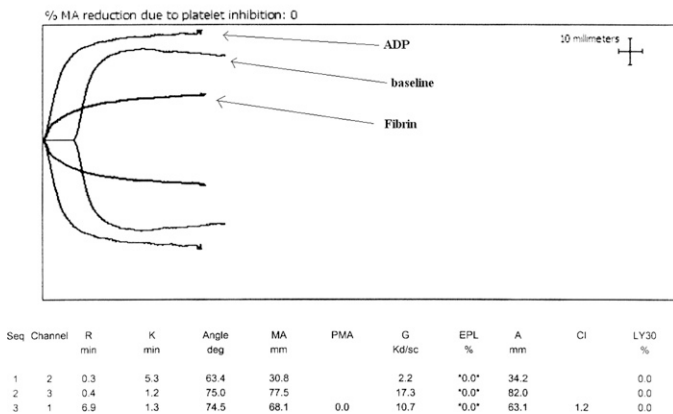


Figure 6. Lack of MA reduction with ADP assay.

TEG® Analysis Results

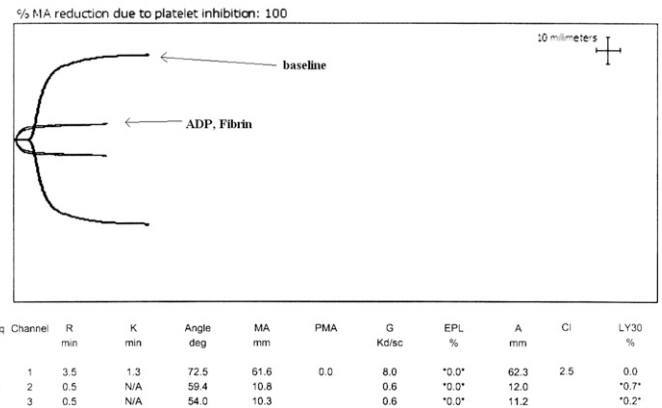


Figure 7. Complete inhibition of ADP receptor with ADP assay.

the ADP receptor (Figure 6). The absence of ADP inhibition, as shown by the Platelet Mapping assay, prompted a change in the ADP antagonist from clopidogrel to ticlopidine. The following day, the patient was re-tested for ADP inhibition. The assay showed 100% inhibition of the ADP receptor (Figure 7). Initially, the improvement in ADP inhibition was attributed to ticlopidine. It was subsequently learned that the patient had received abciximab after the repeat catheterization.

The slight anomaly of Figure 6 is likely a result of human error in laboratory technique. The ADP maximum amplitude measured greater than the baseline maximum amplitude. This was probably because of inadequate mixing of kaolin and blood in the baseline sample. The large disparity between the ADP and fibrin sample maximum amplitude (MA) showed a very significant lack of ADP inhibition. The calculated %MA reduction most likely would remain at 0% if the baseline sample achieved full activation.

The patient's remaining hospitalization was uneventful, and she was discharged to home on ticlopidine and warfarin. A Persantine Cardiolute stress test was performed at 18 months after PCI for an evaluation of angina. The stress test showed anteroapical ischemia; therefore, she underwent an elective cardiac catheterization. The angiographic data from the catheterization showed an ejection fraction of 50–55%, a focal 75% stenosis of the distal LAD, and 30% restenosis in the mid-segment of the RCA stent. The residual stenosis of the RCA stent was approximately the same diameter as the distal RCA and angiographically deemed not critical enough for PCI. In view of her coronary anatomy, medical therapy was recommended for angina relief. Further PCI or coronary artery bypass graft (CABG) will be considered if symptoms persist. She has not experienced any further cardiac events to date.

DISCUSSION

PCIs are performed more than one million times per year in the United States and two million times per year worldwide. The FDA reports that 15–30% of patients treated with a conventional uncoated stent will occlude their stent within 1 year. Use of drug eluting stents (DESs), available in the United States since 2003, can help to reduce the recurrence of stenosis (8.9% coated vs. 36.3% uncoated), repeat cardiac procedures (4.2% vs. 16.8%), and combined occurrence of repeat angioplasty, bypass surgery, heart attacks, and death (8.8% vs. 21%) (8). Multiple large prospective randomized studies have also shown the ability of anti-platelet therapy to improve patient outcomes in the setting of ACS and PCI (1,2,9). However, a subset of patients remains that will re-stenose after treatment with the newest drug eluting stents and most current anti-platelet regimens. A growing body of evidence suggests the beneficial effects of anti-platelet drugs are reduced or negated if they are unable to effectively inhibit the target receptor (5–7).

The TEG Platelet Mapping assay is a moderately complex point-of-care platelet test. It differs from conventional platelet aggregation assays in several ways: (i) the assay uses whole blood and not platelet-rich plasma; (ii) platelet activity is not measured as a percentage of light transmission through aggregated platelets but measured as a ratio of clot strength, MA, based on maximal platelet activation, complete inhibition of platelet activation, and targeted (ADP, TxA2) receptor activation (Figure 5). Maximal platelet activity is achieved by adding a 1-mL sample of whole blood to a vial containing kaolin. After gentle inversion, 360 μ L of this blood is transferred into the TEG cup of the analyzer. The large amount of thrombin generated binds to platelet protease activating receptors (PARs) and ultimately results in full activation of the platelet. A sample with complete inhibition of platelet activation is achieved by heparinizing 360 μ L of whole blood. Heparin in the sample binds to antithrombin (AT). The AT–heparin complex in turn binds to thrombin, resulting in its inactivation. The conversion of fibrinogen to fibrin is achieved by the addition of 10 μ L of reagent containing reptilase and factor XIIIa (Reagent P1; Haemoscope Corp.). Reptilase cleaves fibrinogen to fibrin and factor XIIIa provides fibrin cross-linking in the absence of thrombin, yet neither induces platelet activation. The resulting clot is composed entirely of fibrin, with no platelet contribution. Targeted receptor activation is also achieved using a 360- μ L heparinized whole blood sample containing 10 μ L of reagent P1 (Haemoscope Corp.). Platelet activation is achieved by the addition of 10 μ L of reagent containing either AA or ADP (reagent P2 and P3; Haemoscope Corp.). The result is a whole blood clot with platelet contribution derived through a specified group of platelet receptors.

Many platelet receptors use integrin activation (inside-out signaling) of GP IIb/IIIa. On activation, the GP IIb/IIIa

receptor binds fibrinogen, leading to platelet aggregation. GP IIb/IIIa is the only platelet receptor capable of binding fibrinogen. A platelet receptor assay dependent on the final pathway of fibrinogen–GP IIb/IIIa would require this pathway to be intact. The abciximab our patient was administered completely inhibited all GP IIb/IIIa activity as shown by Figure 7. It is possible that ticlopidine-induced ADP inhibition was occurring in Figure 7; however, the evidence of any such effect was masked by abciximab. Abciximab inhibits ADP-mediated expression of the GP IIb/IIIa receptor. This inhibition of GP IIb/IIIa obscures any quantification of ADP inhibition when using the TEG Platelet Mapping assay.

In our patient, we faced recent stent thrombosis and 0% inhibition of the ADP receptor. The ADP antagonist was changed to ticlopidine because the relatively low risk of neutropenia and thrombotic thrombocytopenia purpura (TTP) associated with ticlopidine was outweighed by the risk of further MACE. Although the decision was made to change the ADP inhibitor in our patient, other therapeutic options exist. Clopidogrel is a concentration dependent inhibitor of the platelet ADP receptor P2Y₁₂ but not of ADP receptors P2X₁ or P2Y₁. Increasing the dose of clopidogrel could also increase the concentration of the active metabolite responsible for P2Y₁₂ blockade. Because clopidogrel is dependent on CYP3A4 for metabolism, limiting compounds competitively binding with CYP3A4 could increase the amount of clopidogrel transformed to its active metabolite. Current data are conflicted on the impact of CYP3A4-dependent statins when used in conjunction with clopidogrel. Ex vivo testing has shown clopidogrel metabolism to be inhibited by >90% when present at equal molar concentrations with atorvastatin (10). However, an in vivo study examining the impact of CYP3A4-dependent statins when administered with clopidogrel showed no significant difference in clinical benefit (11). Conversely, administering compounds that increase CYP3A4 activity, such as the herbal remedy St. John's wort, can potentially amplify the inhibitory effect of clopidogrel on the ADP receptor (10). Our patient was maintained on atorvastatin 80 mg daily throughout her hospitalization.

Our use of a TEG Platelet Mapping assay diagnosed clopidogrel resistance in the setting of acute stent thrombosis. We were unable to show the efficacy of ticlopidine initially because of abciximab and subsequently because repeat Platelet Mapping was not ordered after the effect of abciximab had subsided. No studies currently exist examining the use of ticlopidine in the setting of clopidogrel resistance. Further study is needed to definitively define the use of ticlopidine in these circumstances.

REFERENCES

1. Yusuf S, Zhao F, Mehta SR, et al. 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.

2. Steinhubl S, Berger PB, Mann JT III, et al. CREDO Investigators. Clopidogrel for the reduction of events during observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA*. 2002;288:2411–20.
3. Burns T, Moss A, Hilleman D. Antiplatelet drug resistance: not ready for prime time. *Pharmacotherapy*. 2005;25:1621–8.
4. Michaelson A. Platelet function testing in cardiovascular diseases. *Circulation*. 2004;110:489–93.
5. Eikelboom JW, Hirsh J, Weitz JI, et al. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular event. *Circulation*. 2002;105:1650–5.
6. Matekzky S, Shenkman 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.
7. Steinhubl S, Talley JD, 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 GOLD (AU-Assessing Ultegra) multi-center study. *Circulation*. 2001;103:2572–8.
8. US Food and Drug Administration. FDA news “FDA Approves Drug-Eluting Stent for Clogged Heart Arteries” 24 April 2003. Available at: <http://www.fda.gov/bbs/topics/NEWS/2003/NEW00896.html>. Accessed December 2005.
9. The Lancet CAPRIE Steering Committee. A randomized, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet*. 1996;348:11329–39.
10. Clark TA, Waskell LA. The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atorvastatin. *Drug Metab Dispos*. 2003;31:53–9.
11. Mukherjee D, Line-Rogers E, Fang J, Munir K, Eagle KA. Lack of clopidogrel-CYP3A4 statin interaction in patients with acute coronary syndrome. *Heart*. 2005;91:23–6.