CLINICAL RESEARCH　　　　　　　　　　　　　　　　**Interventional Cardiology**

Increased Risk in Patients With High Platelet Aggregation Receiving Chronic Clopidogrel Therapy Undergoing Percutaneous Coronary Intervention

Is the Current Antiplatelet Therapy Adequate?

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Objectives	We sought to determine whether patients receiving chronic clopidogrel therapy undergoing nonemergent stenting who display high on-treatment preprocedural platelet aggregation measured by standard light transmittance aggregometry and thrombelastography (TEG) will be at increased risk for poststenting ischemic events.
Background	Patients exhibiting heightened platelet reactivity to adenosine diphosphate (ADP) might be at increased risk for recurrent ischemic events after coronary stenting.
Methods	A total of 100 consecutive patients receiving chronic antiplatelet therapy consisting of aspirin (325 mg qd) and clopidogrel (75 mg qd) were studied before undergoing nonemergent stenting. Patients were followed for 1 year after coronary stenting for the occurrence of death, myocardial infarction, stent thrombosis, stroke, or ischemia requiring a hospital stay.
Results	All patients were aspirin responsive. Patients with ischemic events (23 of 100, 23%) within 1 year had greater on-treatment prestent ADP-induced platelet aggregation than patients without ischemic events by aggregometry and TEG ($p < 0.001$ for both measurements). Of patients with an ischemic event, 70% and 87% displayed high on-treatment platelet reactivity at baseline by aggregometry and TEG, respectively. High on-treatment platelet reactivity as measured by aggregometry and TEG were the only variables significantly related to ischemic events ($p < 0.001$ for both assays). The administration of eptifibatide reduced periprocedural elevation in platelet reactivity, with no significant differences in bleeding events.
Conclusions	Patients receiving chronic clopidogrel therapy undergoing nonemergent percutaneous coronary intervention who exhibit high on-treatment ADP-induced platelet aggregation are at increased risk for postprocedural ischemic events. These findings might have implications for the alteration in clopidogrel maintenance dose and use of glycoprotein IIb/IIIa inhibitors in selected patients.　(J Am Coll Cardiol 2007;49:657–66) © 2007 by the American College of Cardiology Foundation

Platelet aggregation is a central process in the development of ischemic complications after percutaneous coronary intervention (PCI) (1–4). Dual antiplatelet therapy with aspirin and clopidogrel are the gold standard to attenuate platelet function during PCI. However, despite the superior protection documented in clinical trials with dual antiplate-

let therapy, it has been demonstrated that nearly 20% of patients undergoing PCI will experience recurrent ischemic or thrombotic events (5). It has been repeatedly shown that a significant percentage of patients display no demonstrable antiplatelet effect by ex vivo measurements after a 300-mg clopidogrel loading dose (6,7). It has been hypothesized that patients exhibiting clopidogrel nonresponsiveness and heightened platelet reactivity to adenosine diphosphate (ADP) are inadequately protected and might be at increased risk for recurrent events (6,8).

　A relationship between clopidogrel nonresponsiveness and post-PCI ischemic events in clopidogrel-naïve patients has been recently suggested (5,9–11). Clinical studies indicated that postprocedural heightened platelet reactivity is associated with increased myocardial necrosis and inflammation marker

release (3,12). Furthermore, increased post-treatment platelet reactivity has been associated with recurrent ischemic events and stent thrombosis (4,5,11). These studies emphasize the potentially sizeable risks associated with inadequate platelet inhibition from current antiplatelet therapy.

Among patients receiving maintenance dose clopidogrel therapy, a certain percentage display heightened platelet reactivity to ADP (4,6,13). Despite this evidence, there is no established recommendation for clopidogrel loading in patients receiving chronic clopidogrel therapy undergoing PCI. Furthermore, there are no sufficient data correlating heightened platelet reactivity with adverse cardiovascular events in these patients. The goal of the present study was to quantify this relationship. We hypothesized that patients receiving chronic clopidogrel therapy undergoing non-emergent PCI who display high preprocedural platelet reactivity, as measured by standard light transmittance aggregometry (LTA) and thrombelastography (TEG), are at increased risk for post-PCI ischemic events.

Methods

This study was approved by the investigational review board. One hundred consecutive patients receiving clopidogrel therapy (75 mg qd) for ≥1 month before undergoing non-emergent coronary stenting were enrolled after giving informed consent. A clopidogrel loading dose was not administered. All patients had received at least 81 mg aspirin for 7 days before the procedure. Patients were >18 years old. The exclusion criteria were: a history of bleeding diathesis, acute myocardial infarction within 48 h, elevated cardiac markers (above upper limits normal for the respective assay), cerebrovascular event within 3 months, illicit drug or alcohol abuse, prothrombin time >1.5 times control, platelet count <100,000/mm^3, hematocrit <30%, creatinine >4.0 mg/dl, and glycoprotein (GP) IIb/IIIa use before the procedure.

On the day of the procedure and daily thereafter, 325 mg aspirin was administered. Eptifibatide was administered at the discretion of the treating physician with the ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial) study protocol as a double bolus (180 μg/kg) followed by an infusion (2 μg/kg/min) for 18 to 24 h after procedure. Unfractionated heparin was administered according to the ESPRIT dosing regimen (60 U/kg) as a bolus to all patients in the catheterization laboratory immediately before stenting (14). Clopidogrel was prescribed in all patients for at least 6 months after procedure.
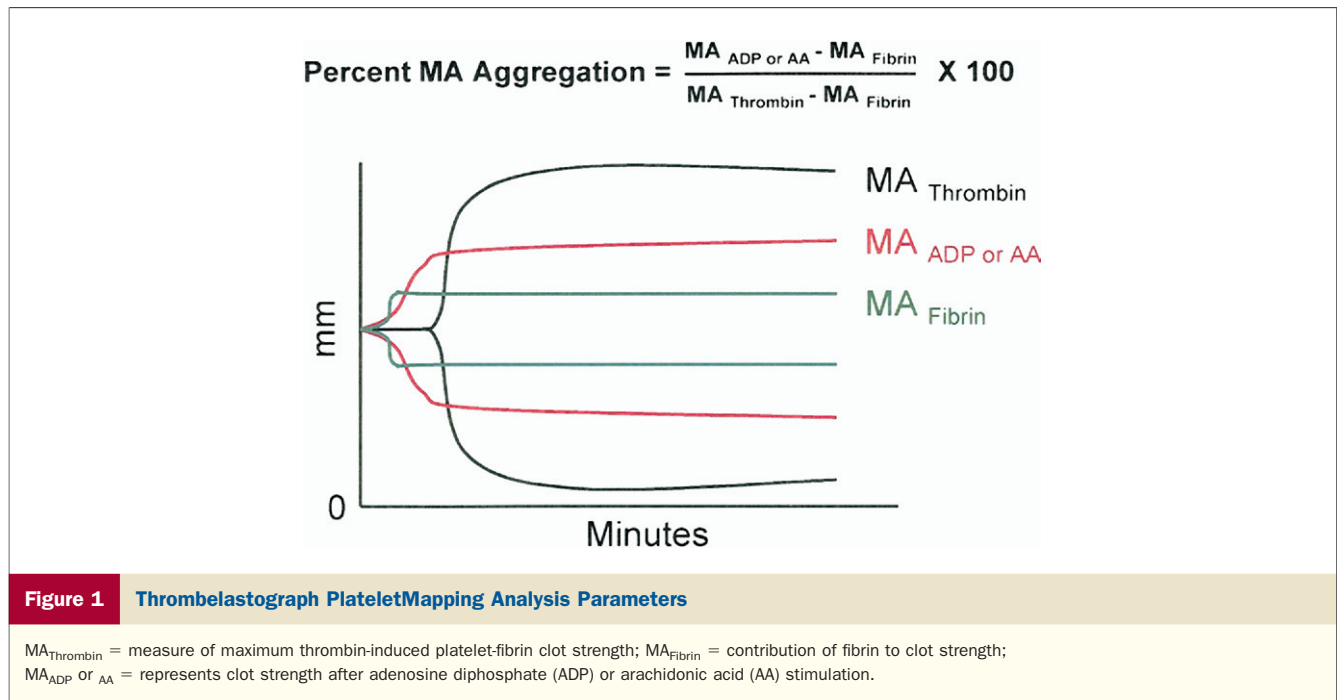
Blood sampling. Baseline blood samples were obtained from patients in a fasting state in the catheterization laboratory through the indwelling femoral vessel sheath. Samples were transferred to separate vacutainer blood collecting tubes (Becton-Dickinson, Franklin Lakes, New Jersey) containing 3.8% trisodium citrate or lithium heparin. After discarding the first 2 to 3 ml of free flowing blood, the vacutainer tubes were filled to capacity and gently inverted 3 to 5 times to ensure complete mixing of the anticoagulant. Baseline samples were obtained before coronary intervention and at 3 h and 18 to 24 h after stenting. In patients treated with eptifibatide, the 18 to 24 h blood draw was performed within 1 h after completion of the eptifibatide infusion.

Platelet aggregation. The blood-citrate tubes were centrifuged at 120 g for 5 min to recover platelet rich plasma (PRP) and further centrifuged at 850 g for 10 min to recover platelet poor plasma (PPP). The PRP and PPP were stored at room temperature to be used within 2 h. Platelet aggregation was assessed as described previously (3). Briefly, platelets were stimulated with 5 μmol/l ADP and 1 mmol/l arachidonic acid (AA). Aggregation was assessed with a Chronolog Lumi-Aggregometer (Model 490-4D) with the Aggrolink software package (Chronolog, Havertown, Pennsylvania). Aggregation was expressed as the maximum percent change in light transmittance from baseline, with PPP as a reference.

Thrombelastograph Hemostasis Analyzer With Platelet-Mapping. The Food and Drug Administration-approved Thrombelastograph Hemostasis Analyzer With Platelet-Mapping assay (Haemoscope Corp., Niles, Illinois) relies on the measurement of thrombin-induced clot strength to enable a quantitative analysis of platelet function (15,16). The assay uses heparin as an anticoagulant to eliminate thrombin activity in the sample. Reptilase and factor XIIIa (activator F) are used to generate a cross-linked fibrin clot to isolate the fibrin contribution to clot strength (17). The contribution of P2Y$_{12}$ receptor or cyclooxygenase pathways to the clot formation can be measured by the addition of the appropriate agonist, ADP or AA.

Blood was analyzed according to the manufacturer's instructions. One milliliter heparinized blood was transferred to a vial containing kaolin and mixed by inversion. Five hundred microliters of the activated blood were then transferred to a vial containing heparinase and mixed to neutralize heparin. The neutralized blood (360 μl) was immediately added to a heparinase-coated cup and assayed in the TEG analyzer to measure the thrombin-induced clot strength (MA$_{thrombin}$). Heparinized blood (340 μl) was added to a noncoated cup containing reptilase and activator F to generate a whole blood crosslinked clot in the absence of thrombin generation or platelet stimulation (MA$_{fibrin}$). A third sample (340 μl) of heparinized blood was added to a nonheparinase-coated cup in the presence of the activator F and ADP (2 μmol) or AA (1 mmol/l) to generate a whole blood-crosslinked clot with platelet activation (MA$_{ADP}$ or MA$_{AA}$). Platelet aggregation in response to ADP or AA is calculated with computerized software on the basis of the

$$\text{Percent MA Aggregation} = \frac{MA_{ADP \text{ or } AA} - MA_{Fibrin}}{MA_{Thrombin} - MA_{Fibrin}} \times 100$$

Figure 1   Thrombelastograph PlateletMapping Analysis Parameters

$MA_{Thrombin}$ = measure of maximum thrombin-induced platelet-fibrin clot strength; MA_{Fibrin} = contribution of fibrin to clot strength; $MA_{ADP \text{ or } AA}$ = represents clot strength after adenosine diphosphate (ADP) or arachidonic acid (AA) stimulation.

formula: %Aggregation = $[(MA_{ADP \text{ or } AA} - MA_{fibrin})/(MA_{thrombin} - MA_{fibrin})] \times 100$ (Fig. 1).

Definitions and clinical outcomes. HIGH ON-TREATMENT PLATELET REACTIVITY. The cut points for high on-treatment platelet reactivity (HPR) were defined as ≥50% ADP-induced platelet aggregation after stimulation with 5-μmol ADP at baseline as measured by LTA or ≥70% ADP-induced aggregation with 2-μmol ADP at baseline as measured by TEG (17). All patients below these cut points were defined as exhibiting normal on-treatment platelet reactivity (NPR). All tables are representative of 5 μmol/l ADP-induced LTA aggregation values.

ASPIRIN RESISTANCE. Aspirin resistance was defined as >20% platelet aggregation after stimulation by 1-mmol/l AA as measured by LTA or more than 50% platelet aggregation after stimulation by 1-mmol/l AA as measured by TEG (18).

CLINICAL OUTCOMES. Patients were contacted by telephone at 1, 6, and 12 months to identify the occurrence of adverse events. Patient records including electronic source documents were obtained and reviewed by 2 physicians blinded to the study who adjudicated events. Ischemic events were defined as: death secondary to any cardiovascular cause, stroke, myocardial infarction (in-hospital and after discharge), ischemia requiring a hospital stay, and target vessel revascularization (TVR), nontarget vessel revascularization (NTVR), or medical management. Myocardial infarction was defined as the occurrence of ischemic symptoms and a troponin-I value > upper limits of normal (>1.5 ng/ml). Unstable angina was defined as the occurrence of ischemic symptoms requiring a hospital stay. Bleeding was quantified according to the Thrombolysis In Myocardial

Infarction (TIMI) criteria (19). In brief, minor bleeding was defined as clinically overt bleeding accompanied by a fall in hemoglobin of 3.0 to 5.0 g/dl or a fall in hematocrit of 9% to <15%. Major bleeding occurred when the hemoglobin decreased >5 g/dl or the hematocrit decreased ≥15%.

Statistical analysis. Categorical variables are expressed as n (%), and continuous variables are expressed as mean ± SD with p < 0.05 considered statistically significant. The Fischer Exact test and Mann-Whitney Rank Sum test were used for comparison of categorical and continuous variables between platelet reactivity groups. Multivariate Cox regression analysis was used to evaluate the significance of the following variables concerning ischemic events within 1 year of follow-up: HPR (measured by TEG and LTA), age, history of diabetes, history of hypertension, smoking (current), bare-metal stent (BMS) versus drug-eluting stent (DES), and patients presenting post-acute coronary syndrome. Pearson product moment correlation was used to measure the correlation between LTA and TEG assays. All statistical calculations for the aforementioned analyses were performed with SigmaStat software (Point Richmond, California). A receiver operator curve (ROC) analysis was used to determine the ability of LTA and TEG to predict ischemic events. This analysis was performed with MedCalc software (Mariakerke, Belgium).

Determination of sample size. It has been demonstrated that approximately 20% of patients with a history of prior coronary revascularization undergoing repeat PCI will experience a recurrent ischemic event within 1 year of repeat coronary intervention (20). We have previously demonstrated that patients in the upper quartile of platelet reactivity are at approximately 3 times greater risk for repeat ischemic events (17). Therefore, we hypothesized that 30% and 10% of patients with HPR and NPR in the current study will experience a

recurrent ischemic event, respectively. With the sample size calculation from SigmaStat software, it is estimated that the sample size required for 95% power with the alpha of 0.05 is approximately 100 patients.

Results

Patients. One hundred patients receiving chronic clopidogrel (≥1 month) and aspirin therapy were enrolled. Among these patients, 12 were admitted with myocardial infarction (enrolled ≥48 h after infarction) and 13 had unstable angina. The remainder of the patients had stable angina, and the procedures were nonemergent. A total of 30 patients received treatment with GP IIb/IIIa inhibitors at

the discretion of the physician. Serial platelet assays were completed for all subjects.

Demographics. Patient demographics and procedural characteristics are shown in Tables 1 and 2, respectively. Briefly, cardiovascular risk factors and multivessel interventions using drug-eluting stents were common. No significant differences in age, gender, ethnicity, body mass index, baseline medications or hematological data existed between reactivity groups. Patients with HPR exhibited a higher prevalence of hypertension, diabetes, and use of calcium-channel blockers.

Platelet aggregation. All patients were aspirin responsive. Mean preprocedural ADP-induced platelet aggregation was

Table 1	Patient Demographics			
	Total Group (n = 100)	HPR Group (n = 22)	NPR Group (n = 78)	HPR vs. NPR p Value
Age (yrs)	66 ± 11	67 ± 10	66 ± 11	NS
Gender and ethnicity, n (%)				
Caucasian male	60 (60)	13 (59)	47 (60)	NS
Caucasian female	17 (17)	4 (18)	13 (17)	NS
African-American male	12 (12)	1 (5)	11 (14)	NS
African-American female	11 (11)	4 (18)	7 (9)	NS
BMI (kg/m^2)	30 ± 6	31 ± 7	30 ± 6	NS
Systolic BP (mm Hg)	145 ± 22	154 ± 19	143 ± 23	0.04
Diastolic BP (mm Hg)	76 ± 16	77 ± 15	76 ± 17	NS
Subject presentation, n (%)				
Elective	75 (75)	18 (82)	57 (73)	NS
Unstable angina	13 (13)	2 (9)	11 (14)	NS
Myocardial infarction	12 (12)	2 (9)	10 (13)	NS
Risk factors/medical history, n (%)				
Smoking	56 (56)	14 (64)	42 (54)	NS
Family history of CAD	53 (53)	13 (59)	40 (51)	NS
Hypertension	74 (74)	19 (86)	55 (71)	0.07
Hyperlipidemia	83 (83)	19 (86)	64 (82)	NS
Diabetes	44 (44)	14 (64)	30 (38)	0.01
Renal disease	12 (12)	3 (14)	9 (12)	NS
Peripheral vascular disease	13 (13)	3 (14)	10 (13)	NS
Prior myocardial infarction	40 (40)	6 (27)	34 (44)	NS
Prior CABG	31 (31)	9 (41)	22 (28)	NS
Prior PTCA	49 (49)	13 (64)	36 (46)	0.07
Prior CVA	15 (15)	3 (14)	12 (15)	NS
Baseline medications, n (%)				
Beta-blockers	84 (84)	18 (82)	66 (85)	NS
ACE inhibitors	71 (71)	14 (64)	57 (73)	NS
Calcium-channel blockers	26 (26)	9 (41)	17 (22)	0.04
Lipid-lowering agents				
3A4 pathway metabolized	74 (74)	18 (82)	56 (72)	NS
Non-3A4 pathway metabolized	14 (14)	1 (5)	13 (17)	NS
Laboratory data				
WBC (× 1,000/mm^3)	7.4 ± 2.2	7.4 ± 2.7	7.4 ± 1.9	NS
Platelets (× 1,000/mm^3)	233 ± 75	236 ± 86	231 ± 71	NS
Hemoglobin (g/dl)	13.2 ± 2	13.1 ± 2	13.3 ± 2	NS
Hematocrit (%)	40 ± 5	39.5 ± 5	40.0 ± 5	NS
Creatinine (g/dl)	1.1 ± 0.4	1.0 ± 0.3	1.1 ± 0.4	NS

ACE = angiotensin-converting enzyme; BMI = body mass index; BP = blood pressure; CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; CVA = cerebrovascular accident; HPR = high on-treatment platelet reactivity; NPR = normal on-treatment platelet reactivity; NS = not significant; PTCA = percutaneous transluminal coronary angioplasty; WBC = white blood cells.

Table 2	Procedural Characteristics			
	Total Group (n = 100)	HPR Group (n = 22)	NPR Group (n = 78)	HPR vs. NPR p Value
Length of procedure (min)	62 ± 28	62 ± 34	62 ± 26	NS
Ejection fraction (%)	50 ± 11	50 ± 12	50 ± 10	NS
Number of vessels treated	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	NS
Number of lesions treated	1.4 ± 0.6	1.6 ± 0.7	1.3 ± 0.6	NS
Lesion morphology				
De novo, n (%)	89 (89)	19 (86)	70 (90)	NS
Bifurcation lesions	5 (5)	1 (5)	4 (5)	NS
Calcified lesions	17 (17)	2 (9)	16 (20)	NS
Thrombus present	4 (4)	0 (0)	4 (5)	NS
Total occlusion	10 (10)	3 (14)	7 (9)	NS
Dissection	1 (1)	0 (0)	1 (1)	NS
Lesion location, n (%)				
LAD	38 (38)	7 (32)	31 (40)	NS
CX	32 (32)	10 (45)	22 (28)	NS
RCA	34 (34)	9 (41)	25 (32)	NS
SVG	11 (11)	2 (9)	9 (12)	NS
Stent types, n (%)				
Drug-eluting	75 (75)	17 (77)	58 (75)	NS
Bare-metal	24 (24)	5 (23)	19 (24)	NS
PTCA only	1 (1)	0 (0)	1 (1)	NS
Reference vessel diameter (mm)	3.0 ± 0.5	3.0 ± 0.4	3.0 ± 0.5	NS
Total lesion length (mm)	22.1 ± 16	26.0 ± 24	21.0 ± 13	NS
Prestenosis (%)	88 ± 5	89 ± 6	88 ± 5	NS
Poststenosis (%)	4 ± 2	5 ± 3	4 ± 2	NS
Procedural success, n (%)	97 (97)	21 (95)	76 (97)	NS

CX = circumflex artery; LAD = left anterior descending artery; RCA = right coronary artery; SVG = saphenous vein graft; other abbreviations as in Table 1.

38 ± 16% as measured by LTA and 55 ± 21% as measured by TEG (Table 3). Baseline on-treatment aggregation was significantly higher in patients with ischemic events than in patients without events as measured by LTA (50 ± 13% vs. 31 ± 13% respectively, p < 0.0001) (Fig. 2A) and TEG (76 ± 13% vs. 49 ± 13% respectively, p < 0.0001) (Fig. 2B). A strong correlation was shown between these assays (p < 0.0001, r = 0.82) (Fig. 3).

Ischemic events. One-year follow-up data were completed for all patients. A total of 26 ischemic events occurred in 23 patients (23%) within 1 year of discharge (Table 4). There were no strokes or in-hospital deaths. One patient in the NPR group (presenting with unstable angina) and 2 patients

in the HPR group (1 presenting with unstable angina and 1 undergoing non-emergent PCI) suffered periprocedural myocardial infarctions, and 2 patients in the HPR group developed subacute stent thrombosis within 1 week of revascularization. Of the 23 patients (30%), 7 suffered ischemic events after completion of their prescribed clopidogrel treatment. The mean time from discharge to initial event occurrence was 147 ± 106 days (Fig. 4).

Relation of HPR to events. Of patients with an ischemic event, 70% displayed HPR at baseline, whereas only 8% of patients without an ischemic event displayed HPR at baseline as measured by LTA (Fig. 2A). Similarly, the TEG assay demonstrated that 87% of patients with an ischemic event displayed baseline HPR, whereas only 17% of patients without an ischemic event displayed baseline HPR (Fig. 2B). This resulted in positive predictive values of 73% and 67% and negative predictive values of 91% and 94% for the LTA and TEG, respectively, demonstrating 87% test efficiency with the LTA and 85% test efficiency with the TEG. By analyzing the area under a combined receiver-operating characteristic (ROC) curve, LTA and TEG demonstrated the ability to distinguish between ischemic and nonischemic groups (area = 0.862, p = 0.0001 for LTA; area = 0.881, p = 0.0001 for TEG) (Fig. 5). Within the total group, 16 patients exhibited concordant HPR as measured by both LTA

Table 3	Mean Platelet Aggregation and Percentage of Patients With HPR		
Platelet Assay	Baseline	95% CI	HPR (% of Patients)
LTA-5 μmol/l ADP (%)	38 +/− 16	(34–40)	22
TEG-MA$_{ADP}$ (%)	55 +/− 21	(50–59)	30
			Aspirin Resistance (% of Patients)
LTA-1 mmol/l AA (%)	2 ± 2	(0–4)	0
TEG-MA$_{AA}$ (%)	2 ± 2	(0–4)	0

AA = arachidonic acid; ADP = adenosine diphosphate; CI = confidence interval; HPR = high on-treatment platelet reactivity; LTA = light transmittance aggregometry; NPR = normal on-treatment platelet reactivity; TEG = thrombelastography.
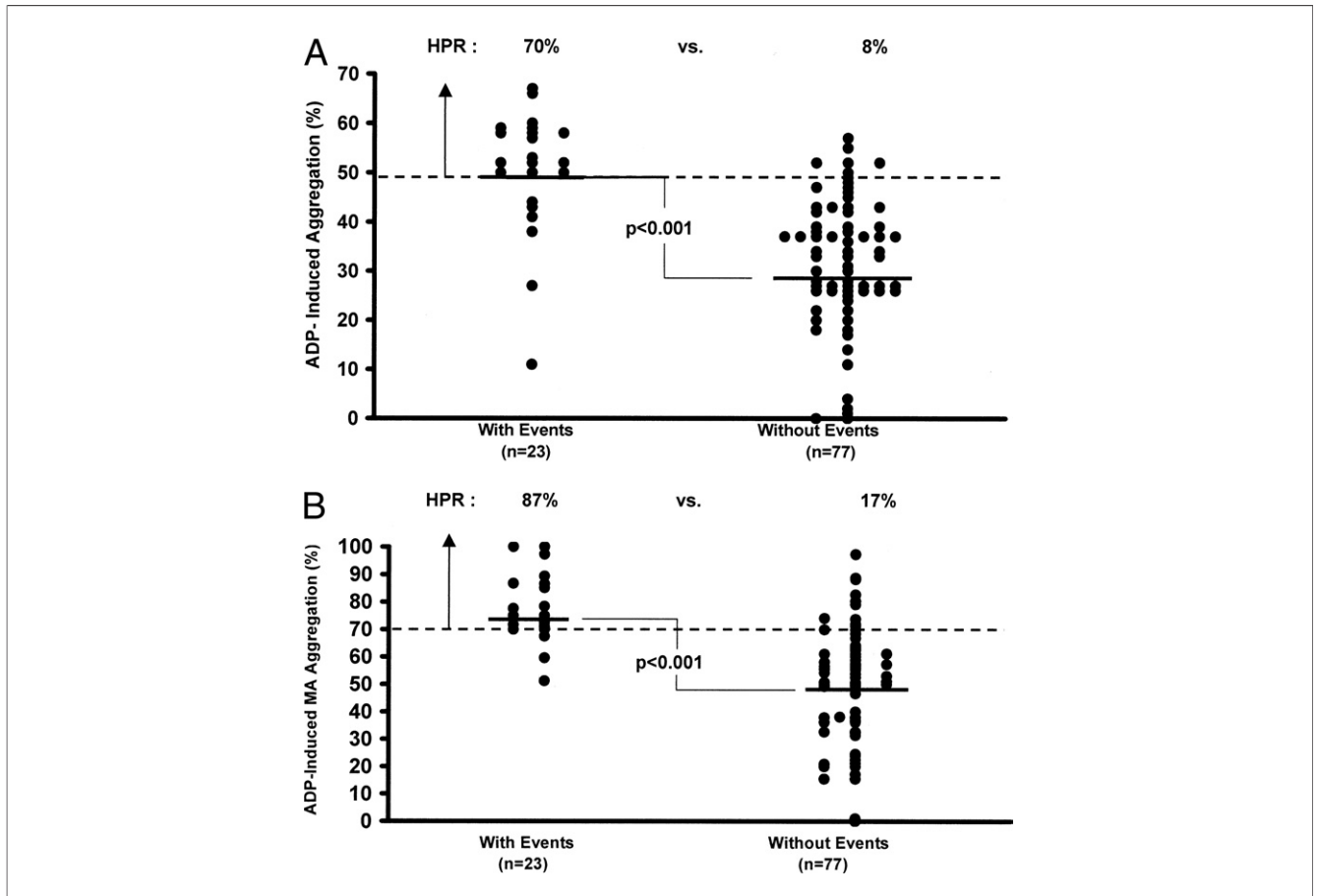
Figure 2   **Relation of HPR to Ischemic Events**

(A) Graph demonstrating percentage of patients with and without ischemic events displaying high on-treatment platelet reactivity (HPR) as measured by 5 μmol/l adenosine diphosphate (ADP)-induced light transmittance aggregometry. **Dashed line** indicates cut point for HPR. **(B)** Graph demonstrating percentage of patients with and without ischemic events displaying HPR as measured by 2-μmol/l ADP-induced thrombelastography. **Dashed line** indicates cut point for HPR.
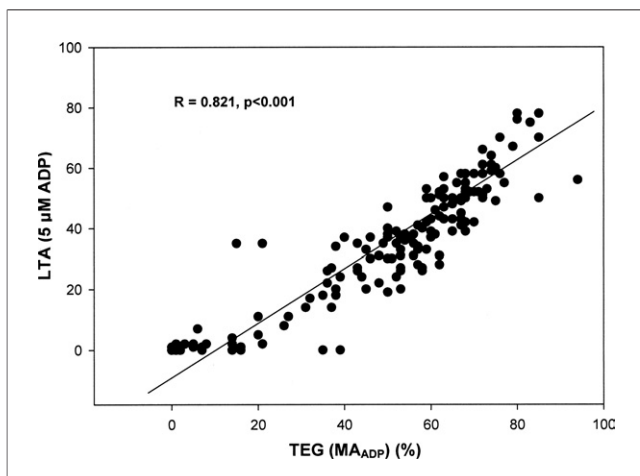

Figure 3   **Linear Regression Model Representing the Correlation Between LTA and TEG**

ADP = adenosine diphosphate; LTA = light transmittance aggregometry;
TEG = thrombelastograph.

and TEG, 21 patients demonstrated discordant HPR by either LTA or TEG, and the remaining 63 patients displayed NPR as measured by both assays. Eighty-eight percent of patients with concordant HPR, 29% of patients with discordant HPR, and 5% of patients with NPR suffered ischemic events. Despite the higher prevalence of hypertension, diabetes, and use of calcium channel blockers in patients with HPR, analysis with multivariate Cox regression demonstrated no prognostic significance between these risk factors and ischemic events. High on-treatment platelet reactivity as measured by LTA and TEG were the only variables significantly related to ischemic events ($p < 0.001$) (Table 5).

Effect of eptifibatide treatment on platelet reactivity and ischemic events. In patients not treated with eptifibatide, there was an 18% relative increase in mean platelet aggregation by LTA from baseline to 3 h after procedure ($39 \pm 17\%$ to $48 \pm 14\%$, $p = 0.0004$) followed by a return to preprocedural aggregation values by 18 to 24 h. However, an increase was not observed with the TEG assay ($56 \pm 21\%$ to 55 ± 24, $p = 0.793$). In patients

Table 4	Initial Adverse Events					
	Total Group (n = 100)		HPR Group (n = 22)		NPR Group (n = 78)	
	Day 0–30	Day 31–365	Day 0–30	Day 31–365	Day 0–30	Day 31–365
Ischemic events (n)						
Death	0	0	0	0	0	0
Myocardial infarction	4	1	3	1	1	0
Target vessel revascularization	0	8	0	6	0	2
Stroke	0	0	0	0	0	0
Nontarget vessel revascularization	0	3	0	2	0	1
Rehospitalization for ischemia	2	5	2	2	0	3
Total patients with ischemic events, n (%)	6 (6)	17 (17)	5 (23)	11 (50)	1 (1)	6 (8)
Bleeding events (n)						
Major bleeding	1	0	1	0	0	0
Minor bleeding	2	0	1	0	1	0
Total patients with bleeding events, n (%)	3 (3)	0 (0)	2 (10)	0 (0)	1 (1)	0 (0)

HPR = high on-treatment platelet reactivity; NPR = normal on-treatment platelet reactivity.

treated with eptifibatide, a 97% relative reduction in mean platelet aggregation was observed from baseline to 3 h as measured by LTA, compared with a 90% relative reduction observed with TEG. By 18 to 24 h, patients treated with eptifibatide had significant platelet inhibition compared with baseline (Figs. 6A and 6B). Within 1 month after procedure 6 patients suffered a recurrent ischemic event, 4 receiving clopidogrel alone and 2 receiving clopidogrel and eptifibatide. Within 1 year after procedure, recurrent ischemic events occurred in 80% of patients with HPR receiving clopidogrel alone versus 57% of patients with HPR receiving clopidogrel and eptifibatide. In the NPR group, 11% of patients receiving clopidogrel alone and 4% of patients receiving both clopidogrel and eptifibatide suffered recurrent ischemic events (Table 6). Although not statistically significant, this resulted in a 15% relative risk reduction in ischemic events 1 month after procedure and a 35% relative risk reduction in ischemic events 1 year after procedure (p = 0.56 and p = 0.325, respectively). Bleeding events occurred in 1 patient receiving clopidogrel alone (minor)

Mean Time to Initial Event: 147+/-106 days

Figure 4   Cumulative Event Occurrence Over 1 Year

Graph demonstrating cumulative event occurrence over 365 days.

and in 2 patients receiving clopidogrel and eptifibatide (1 major and 1 minor).

Discussion

The current study demonstrates that patients receiving chronic clopidogrel therapy undergoing nonemergent PCI who exhibit high on-treatment preprocedural ADP-induced platelet aggregation as measured by LTA (≥50% ADP-induced aggregation) or TEG (≥70% ADP-induced aggregation) are at increased risk for recurrent ischemic events. Of 23 patients suffering recurrent ischemic events, 16 patients displayed HPR as measured by LTA, whereas 20 patients displayed HPR as measured by TEG. Moreover, mean platelet aggregation was significantly higher in patients suffering recurrent ischemic events than in patients without recurrent events. Of further interest was the observation that patients not treated with eptifibatide displayed an 18% increase in mean platelet aggregation from baseline to 3 h after procedure as measured by LTA. These findings suggest that selected patients exhibiting preprocedural platelet inhibition during chronic clopidogrel therapy might nevertheless remain at risk for periprocedural thrombotic events due to heightened platelet reactivity to ADP that occurs after stenting.

A possible explanation for the absence of an increase in platelet reactivity to ADP when studied by TEG involves the use of whole blood as compared with platelet-rich plasma. Heptinstall et al. (21) have demonstrated the important effects that leukocytes and erythrocytes have on adenine nucleotide metabolism that modify the overall response of platelets to ADP.

Our results are concordant with several previous studies that have demonstrated the relationship between insufficient platelet inhibition, postcoronary stent thrombosis, and ischemic cardiovascular events (4,5,10,11). The recent CREST study (Clopidogrel Effect on Platelet Reactivity in Patients With Stent Thrombosis) demonstrated significantly higher
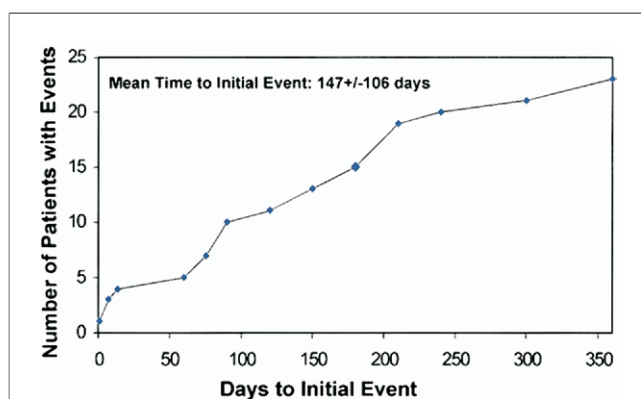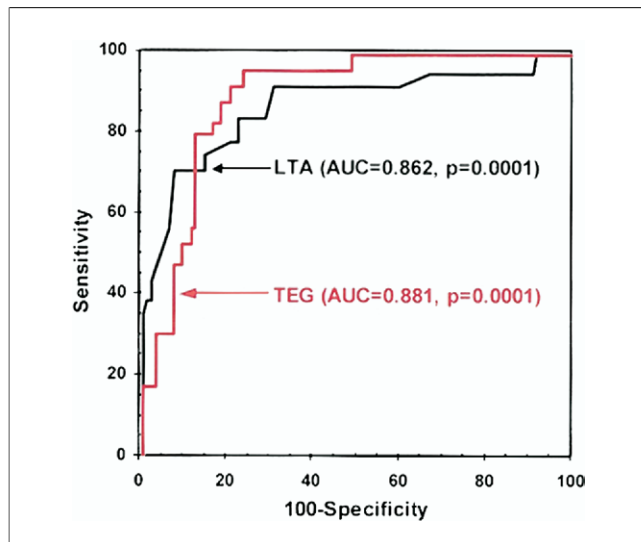
Figure 5   Combined Receiver-Operator Curve for LTA and TEG

Combined receiver-operating characteristic curve for 5-μmol/l ADP-induced LTA and 2-μmol/l ADP-induced TEG at baseline. An area of 0.862 and an area of 0.881 were observed below the curves of LTA and TEG, respectively, with p = 0.0001 for both areas. AUC = area under the curve; other abbreviations as in Figure 3.

5 μmol/l and 20 μmol/l LTA ADP-induced platelet aggregation in patients with stent thrombosis as compared with patients without stent thrombosis (4), and the PRE-PARE POST-STENTING (Platelet Reactivity in Patients and Recurrent Events Post-Stenting) study reported increased ADP-induced aggregation measured by TEG in patients suffering ischemic events compared with patients without events (5). Furthermore, Matetzky et al. (10) reported that patients in the highest quartile of ADP-induced aggregation who were stented for acute ST-segment elevation myocardial infarction had a 40% likelihood for a recurrent cardiovascular event within 6 months. A similar study by Barragan et al. (11) showed high P2Y$_{12}$ receptor reactivity as measured by vasodilator-stimulated phosphoprotein (VASP) phosphorylation levels in patients with stent thrombosis. Each of these studies supports the notion that inadequate platelet inhibition, the occurrence of stent thrombosis, and recurrent ischemic events are significantly related.

This study is the first to correlate the occurrence of ischemic events with platelet reactivity to ADP in patients receiving chronic clopidogrel therapy undergoing non-emergent PCI. There is currently no existing standard of treatment for clopidogrel loading in these patients. The possibility of further inhibiting platelet reactivity in patients receiving chronic clopidogrel therapy with an additional 600-mg loading dose was demonstrated by Kastrati et al. (22), suggesting routine reloading with clopidogrel in all patients undergoing PCI regardless of ongoing clopidogrel treatment. In addition, Kastrati (22) has also suggested increasing the current clopidogrel maintenance dose to 150 mg qd to further inhibit ADP-induced aggregation. Moreover, our current study demonstrated that the administration of eptifibatide reduced periprocedural elevation in platelet reactivity measured by LTA. Because further platelet inhibition is attainable in patients receiving chronic clopidogrel therapy, and a reduction in platelet reactivity is associated with a decrease in ischemic complications, an additional loading dose of clopidogrel (300 mg or 600 mg), the use of a GP IIb/IIIa inhibitor or an increase in maintenance dose therapy to 150 mg qd are all therapeutic considerations for patients receiving chronic therapy undergoing non-emergent PCI.

Thrombelastography was implemented in the current study to assess its reliability as a monitoring tool for analyzing the response to antiplatelet therapy. The TEG, combined with the PlateletMapping assay, was able to identify a subtherapeutic response that highly correlated with the more labor intensive method of LTA. Eighty-seven percent of patients encountering recurrent ischemic events exhibited HPR that was identified by TEG. These findings are concordant with the results of the PREPARE POST-STENTING study (5). Therefore, this assay might affect the delivery of individualized antiplatelet therapy.

Table 5	**Prognostic Significance of Selected Variables for Ischemic Events According to Multivariate Cox Regression Analysis**			
	LTA		**TEG**	
	p Value	Odds Ratio (95% CI)	p Value	Odds Ratio (95% CI)
HPR (LTA)	<0.001	34.6 (8.3–144.2)	—	—
HPR (TEG)	—	—	<0.001	26.8 (6.7–107.5)
Age (yrs)	0.407	1.0 (0.9–1.0)	0.835	1.0 (0.9–1.0)
Presentation*	0.713	1.36 (0.2–7.1)	0.264	0.4 (0.6–2.1)
Diabetes	0.810	0.6 (0.2–3.1)	0.561	0.6 (0.2–2.6)
Hypertension	0.134	4.9 (0.6–38.8)	0.405	2.2 (0.3–13.7)
Smoking (current)	0.978	1.0 (0.1–6.7)	0.906	1.1 (0.2–7.6)
BMS	0.725	0.8 (0.2–3.2)	0.313	0.5 (0.1–2.0)

*>48 h after ACS.

ACS = acute coronary syndrome; BMS = bare-metal stents; CI = confidence interval; HPR = high on-treatment platelet reactivity; LTA = light transmittance aggregometry; TEG = thrombelastography.
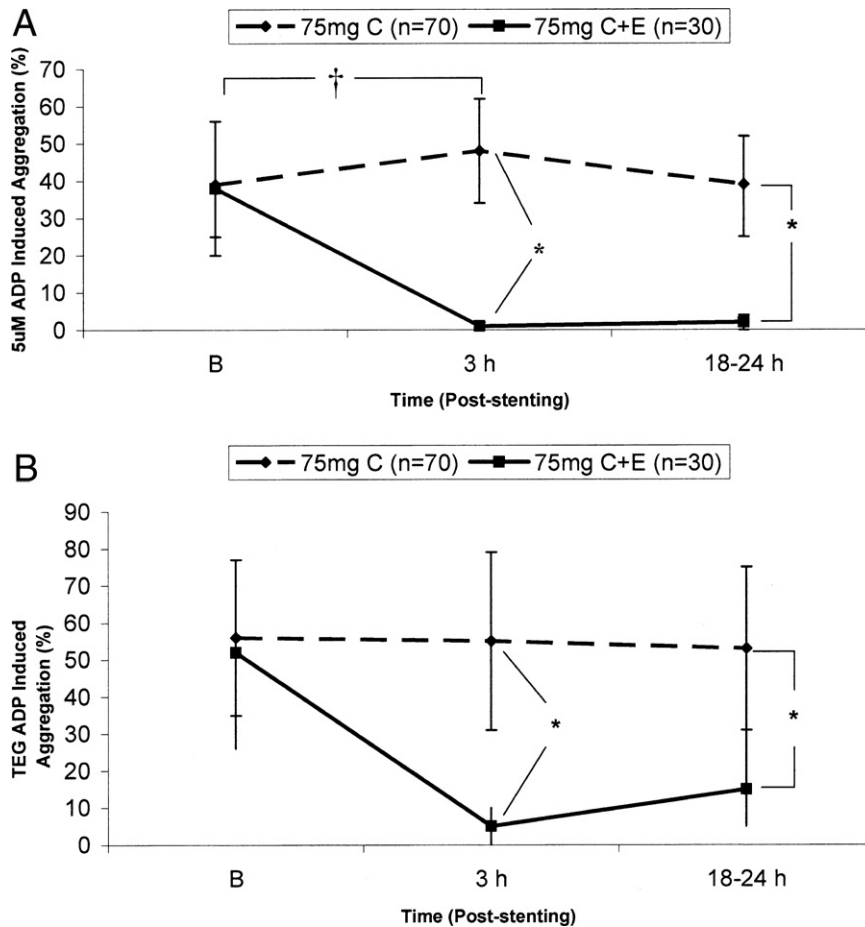
Figure 6   **A Comparison of Platelet Reactivity With and Without Eptifibatide**

(A) Graph demonstrating mean 5 μmol/l ADP-induced platelet aggregation with and without eptifibatide measured by LTA at baseline, 3 h and 18 to 24 h after percutaneous coronary intervention (PCI). *p < 0.0001 for the change in platelet aggregation in patients receiving eptifibatide versus no eptifibatide. †p = 0.0004 for platelet aggregation 3 h after PCI in patients receiving eptifibatide versus baseline. **(B)** Graph demonstrating mean 2 μmol/l ADP-induced platelet aggregation with and without eptifibatide measured by TEG at baseline, 3 h and 18 to 24 h after PCI. *p < 0.0001 for change in platelet aggregation in patients receiving eptifibatide versus no eptifibatide. Abbreviations as in Figure 3.

Study limitations. Although the study strongly suggests that HPR is associated with increased ischemic events after PCI, the results overall are limited by a small sample size. Therefore, larger randomized clinical trials in this subset of patients are necessary to confirm our findings. A second limitation of the study is that patients were not given an additional loading dose of clopidogrel. The clinical benefit of reloading patients already treated with chronic clopidogrel therapy with a further 300- or 600-mg dose remains

unclear. Moreover, inherent differences within our study population by virtue of their need for repeat PCI could have impacted the prevalence of high platelet reactivity. In addition, flow cytometric platelet surface P-selectin and GP IIb/IIIa expression were not measured for additional indicators of platelet reactivity to ADP. Finally, medication was not provided and distributed by our study. Therefore, clopidogrel noncompliance might have contributed to pre-PCI high platelet reactivity and could have affected long-term outcomes, because no additional loading dose was administered. However, preprocedural compliance to clopidogrel and aspirin was verified by nursing staff, and postprocedural compliance was assured through repetitive telephone contacts and outpatient source documentation.

In conclusion, patients receiving chronic clopidogrel therapy undergoing nonemergent PCI, who exhibit high on-treatment ADP-induced platelet aggregation by LTA or

Table 6   **Relation Between Platelet Reactivity, GP IIb/IIIa Use, and Ischemic Events**

	HPR (n = 22)	NPR (n = 78)
With GP IIb/IIIa Inhibitor, n (%)	4/7 (57)	1/23 (4)
Without GP IIb/IIIa Inhibitor, n (%)	12/15 (80)	6/55 (11)

GP = glycoprotein; HPR = high on-treatment platelet reactivity; n = number of patients with an ischemic event/number of patients in each quadrant; NPR = normal on-treatment platelet reactivity; % = percentage of patients in each quadrant with an ischemic event.

the TEG point-of-service device, are at increased risk for postprocedural ischemic events. Our data support a mechanistic link between the platelet physiologic response and clinical outcomes. The results of this study have potential implications for clinicians who do not administer additional clopidogrel (before or after PCI) to patients receiving chronic clopidogrel therapy. Platelet function testing might improve patient outcomes by assessing the risk for recurrent ischemic events and adjusting subsequent antiplatelet treatment. These treatments might include reloading with a dose of 300 mg or 600 mg of clopidogrel, additional antiplatelet treatment with a GP IIb/IIIa inhibitor, or an increased clopidogrel maintenance dose. Future prospective studies are required to determine the efficacy of these regimens in patients with HPR.

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REFERENCES

1. Tantry US, Bliden KP, Gurbel PA. Resistance to antiplatelet drugs: current status and future research. Expert Opin Pharmacother 2005; 6:2027–45.
2. Gurbel PA, Bliden KP, Hayes KM, Tantry U. Platelet activation in myocardial ischemic syndromes. Expert Rev Cardiovasc Ther 2004;2: 535–45.
3. Gurbel PA, Bliden KP, Zaman KA, Yoho JA, Hayes KM, Tantry US. Clopidogrel loading with eptifibatide to arrest the reactivity of platelets: results of the Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets (CLEARPLATELETS) study. Circulation 2005;111:1153–9.
4. Gurbel PA, Bliden KP, Samara W, et al. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. J Am Coll Cardiol 2005;46:1827–32.
5. Gurbel PA, Bliden 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.
6. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003;107:2908–13.
7. Gurbel PA, Bliden KP, Hayes KM, Yoho JA, Herzog WR, Tantry US. The relation of dosing to clopidogrel responsiveness and the incidence of high post-treatment platelet aggregation in patients undergoing coronary stenting. J Am Coll Cardiol 2005;45:1392–6.
8. Tantry US, Bliden KP, Gurbel PA. What is the best measure of thrombotic risks- pretreatment platelet aggregation, clopidogrel responsiveness, or posttreatment platelet aggregation? Catheter Cardiovasc Interv 2005;66:597.
9. Patti G, Colonna G, Pasceri V, Pepe LL, Montinaro A, Di Sciascio G. Randomized trial of high loading dose of clopidogrel for reduction of periprocedural myocardial infarction in patients undergoing coronary intervention. Circulation 2005;111:2099–106.
10. Matetzky 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.
11. Barragan P, Bouvier JL, Roquebert PO, et al. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. Catheter Cardiovasc Interv 2003;59:295–302.
12. Gurbel PA, Bliden KP, Tantry US. Effect of clopidogrel with and without eptifibatide on tumor necrosis factor-alpha and C-reactive protein release after elective stenting: results from the CLEAR PLATELETS-1b study. J Am Coll Cardiol 2006;48:2186–91.
13. Gurbel PA, Bliden KP. Durability of platelet inhibition by clopidogrel. Am J Cardiol 2003;91:1123–5.
14. O'shea JC, Madan M, Cantor WJ, et al. Design and methodology of the ESPRIT trial: evaluating a novel dosing regimen of eptifibatide in percutaneous coronary intervention. Am Heart J 2000;140:834–9.
15. Rivard GE, Brummel-Ziedins KE, Mann KG, Fan L, Hofer A, Cohen E. Evaluation of the profile of thrombin generation during the process of whole blood clotting as assessed by thromboelastography. J Thromb Haemost 2005;3:2039–43.
16. Kawasaki J, Katori N, Kodaka M, Miyao H, Tanaka K. Electron microscopic evaluations of clot morphology during thromboelastography. Anesth Analg 2004;99:1440–4.
17. Bliden KP, Tantry U, Zaman K, et al. High platelet reactivity is a risk factor for post-discharge ischemic complications following elective coronary stenting. J Am Coll Cardiol 2005;47:45B.
18. Tantry US, Bliden KP, Gurbel PA. Overestimation of platelet aspirin resistance detection by thrombelastograph platelet mapping and validation by conventional aggregometry using arachidonic acid stimulation. J Am Coll Cardiol 2005;46:1705–9.
19. Antman EM, Cohen M, Bernink PJ, et al. The TIMI risk score for unstable angina/non-ST-elevation MI: a method for prognostication and therapeutic decision making. JAMA 2000;284:835–42.
20. Singh M, Williams BA, Gersh BJ, et al. Geographical differences in the rates of angiographic restenosis and ischemia-driven target vessel revascularization after percutaneous coronary interventions: results from the Prevention of Restenosis With Tranilast and its Outcomes (PRESTO) Trial. J Am Coll Cardiol 2006;47:34–9.
21. Heptinstall S, Johnson A, Glenn JR, White AE. Adenine nucleotide metabolism in human blood—important roles for leukocytes and erythrocytes. J Thromb Haemost 2005;10:2331–9.
22. Kastrati A, Beckerath N, Joost A, Pogatsa-Murray G, Gorchakova O, Schomig A. Loading with 600 mg clopidogrel in patients with coronary artery disease with and without chronic clopidogrel therapy. Circulation 2004;110:1916–9.