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Platelet Reactivity After Clopidogrel Treatment Assessed With Point-of-Care Analysis and Early Drug-Eluting Stent Thrombosis

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Objectives The aim of this prospective trial was to assess whether platelet reactivity to clopidogrel assessed with multiple electrode platelet aggregometry (MEA) correlates with the risk of early drug-eluting stent thrombosis (ST).

Background Studies using light transmission aggregometry (LTA) have shown that insufficient suppression of platelet reactivity to adenosine diphosphate (ADP) after clopidogrel treatment is associated with an increased risk of adverse cardiovascular events after percutaneous coronary intervention (PCI). However, LTA is time- and labor-intensive and inconvenient for the routine. A point-of-care assay with similar predictive power would be of great value.

Methods Between February 2007 and April 2008, a total of 1,608 consecutive patients with coronary artery disease and planned drug-eluting stent implantation were enrolled. Before PCI, all patients received 600 mg clopidogrel. Blood was obtained directly before PCI. The ADP-induced platelet aggregation was assessed in whole blood with MEA on a Multiplate analyzer (Dynabyte, Munich, Germany). The primary end point was definite ST at 30 days.

Results The upper quintile of patients according to MEA measurements (n = 323) was defined as clopidogrel low responders. Compared with normal responders (n = 1,285), low responders had a significantly higher risk of definite ST within 30 days (2.2% vs. 0.2%; odds ratio [OR]: 9.4; 95% confidence interval [CI]: 3.1 to 28.4; $p < 0.0001$). Mortality rates were 1.2% in low versus 0.4% in normal responders (OR: 3.2; 95% CI: 0.9 to 11.1; $p = 0.07$). The composite of death or ST was higher in low versus normal responders (3.1% vs. 0.6%; OR: 5.1; 95% CI: 2.2 to 11.6; $p < 0.001$).

Conclusions Low response to clopidogrel assessed with MEA is significantly associated with an increased risk of ST. Further studies are warranted to evaluate the ability of MEA to guide antiplatelet therapy in patients undergoing PCI. (J Am Coll Cardiol 2009;53:849–56) © 2009 by the American College of Cardiology Foundation

In patients undergoing percutaneous coronary intervention (PCI), dual antiplatelet treatment with aspirin and clopidogrel is the therapy of choice for preventing thrombosis of the treated vessels and subsequent ischemic events (1).

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Despite this treatment, stent thrombosis (ST), a life-threatening event with serious clinical consequences (2,3), is still feared to occur (4,5). Platelet reactivity to clopidogrel is variable (6), and prospective studies implementing different methods of platelet function testing have reported that an attenuated response to clopidogrel is associated with an increased risk of ischemic events after PCI including ST (7–13). Light transmission aggregometry (LTA) has been the most widely used technique in this setting. However, LTA measurements are weakly standardized and time- and labor-intensive. Thus, LTA is not likely to be used as a matter of routine in clinical practice. An association of high post-treatment platelet reactivity with adverse events after PCI has also been reported in smaller study populations for a cartridge-based whole-blood point-of-care assay, which measures adenosine diphosphate (ADP)–induced agglutination of platelets on fibrinogen-coated beads as an increase in light transmittance (VerifyNow P2Y12 Assay, Accumetrics, San Diego, California) (7,14) and for a whole-blood flow-cytometry based assay that measures the phosphorylation of vasodilator-stimulated phosphoprotein (VASP), which depends on the extent of P2Y12 receptor inhibition (PLT VASP/P2Y12, Biocytex, Marseille, France) (9). A new point-of-care assay,

From the Deutsches Herzzentrum and 1. Medizinische Klinik rechts der Isar, Technische Universität München, Munich, Germany. Material for platelet function analysis on the Multiplate device were provided free of charge from Dynabyte (Munich, Germany). Dr. Sibbing received speaker fees from Dynabyte. Dr. von Beckerath received speaker fees from Eli Lilly and fees for advisory board activities from Eli Lilly and Sanofi-Aventis. Dr. Kastrati received speaker fees from Eli Lilly, Sanofi-Aventis, and Bristol-Myers Squibb.

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Abbreviations and Acronyms

ADP = adenosine diphosphate
AU = aggregation units
CAD = coronary artery disease
CI = confidence interval
DES = drug-eluting stent(s)
HR = hazard ratio
LTA = light transmission aggregometry
MEA = multiple electrode platelet aggregometry
MI = myocardial infarction
NSTEMI = non–ST-segment elevation myocardial infarction
OR = odds ratio
PCI = percutaneous coronary intervention
ROC = receiver-operator characteristic
ST = stent thrombosis
STEMI = ST-segment elevation myocardial infarction
TIMI = Thrombolysis In Myocardial Infarction
VASP = vasodilator-stimulated phosphoprotein

multiple electrode platelet aggregometry (MEA), for rapid and standardized assessment of platelet function in whole blood, has been developed recently (15). The MEA implements the principle of impedance aggregometry with no need for blood centrifugation and the ability to assess platelet function in approximately 10 min. MEA assessed on a device called the Multiplate analyzer (Dynabyte, Munich, Germany) is highly capable of detecting the effect of clopidogrel treatment, and the results of MEA correlate well with LTA (16).

At present no data exist about the value of MEA for predicting ischemic events after PCI. Moreover, no prospective large-scale trial has ever investigated the association of platelet reactivity to clopidogrel with the occurrence of both ischemic and bleeding events simultaneously, and there is still limited evidence for an association of definite ST (17) with clopidogrel low responsiveness.

The goal of this prospective trial was to assess whether platelet reactivity after clopidogrel treatment and assessed with MEA correlates with the risk of definite ST and other ischemic as well as bleeding events after drug-eluting stent (DES) placement.

Materials and Methods

Patients. Between February 2007 and April 2008, consecutive patients with coronary artery disease (CAD) and planned DES implantation in the 2 participating centers (Deutsches Herzzentrum München and I. Medizinische Klinik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany) were serially enrolled in this study. Inclusion in the present study did not preclude patients from inclusion into other clinical trials. Before PCI all patients received a loading dose of 600 mg clopidogrel. Patients on clopidogrel maintenance treatment received a 600-mg loading dose as well. The dose was recommended to be given at least 2 h before catheterization. However, a loading time <2 h did not preclude study inclusion. Directly after diagnostic angiography and before PCI, all patients received an intravenous dose of 500 mg aspirin. Coronary interventions were performed according to current standard guidelines (1). Intravenous anticoagulant treatment with unfractionated heparin or bivalirudin was administered with a dosing regimen of 140 U/kg body weight for unfractionated heparin and for bivalirudin with a dosing regimen of 0.75 mg/kg body weight for bivalirudin directly before PCI, followed by an intravenous infusion of 1.75 mg/kg/h bivalirudin for the duration of the procedure. A subset of the patients received intravenous antiplatelet therapy with the glycoprotein IIb/IIIa inhibitor abciximab (0.25 mg/kg of body weight bolus, followed by a 0.125 μg/kg/min infusion for 12 h) in addition to a reduced dose of heparin. After the procedure, patients were treated with 150 mg/day clopidogrel in the hospital for 3 days and were discharged on a dual antiplatelet regimen of 75 mg/day clopidogrel and 100 mg aspirin (twice per day).

Patients were considered eligible for the study irrespective of the clinical presentation at the time point of the PCI. Therefore, patients with an acute coronary syndrome, ST-segment elevation myocardial infarction (STEMI) and non–ST-segment elevation myocardial infarction (NSTEMI) were included, as well as patients with stable CAD. Exclusion criteria were contraindications to aspirin or clopidogrel treatment and prior treatment with glycoprotein IIb/IIIa inhibitors during the 10 days before the PCI. The present study complies with the Declaration of Helsinki and was approved by the institutional ethics committee. All patients gave written informed consent before entering the study.

Blood sampling. Whole blood was obtained from the arterial sheath of all patients after diagnostic angiography, before PCI, and at least 2 min after administration of intravenous aspirin. In all cases, blood samples were obtained before administration of intravenous PCI-related antithrombotic treatment in the catheter laboratory. Blood was placed in 4.0-ml plastic tubes containing the anticoagulant lepirudin (25 μg/ml, Refludan, hirudin blood collection tubes, Dynabyte), which are recommended to be used for MEA (15). Blood samples were kept at room temperature for at least 30 min before platelet function testing.

Point-of-care platelet function testing. The ADP-induced platelet aggregation in whole blood was assessed with MEA using a new-generation impedance aggregometer called Multiplate analyzer (Dynabyte) (15,16). Details of this method have been reported previously (16). In brief, after 1:2 dilution of whole blood with 0.9% NaCl solution and stirring for 3 min in the test cuvettes at 37°C, 6.4 μmol/l ADP was added. Platelet aggregation was continuously recorded for 5 min. Impedance with MEA is transformed to arbitrary aggregation units (AU) that are plotted against time (AU·min). Aggregation measured with MEA is quantified as AU and area under the curve of arbitrary units (AU·min). All material used including ADP was obtained from the manufacturer (Dynabyte).

Study end points and definitions. Definition of low response to clopidogrel varies from study to study, and most of the studies investigating this issue have used the upper 5% to 44% of patients (18) to define a cutoff value for low response. We prospectively defined low response to clopidogrel by setting a cutoff point at the upper quintile of MEA measurements. This definition was also used for sample size calcula-

tion. The primary end point of this study was the cumulative incidence of definite ST during a 30-day follow-up period. Definite ST was defined according to the Academic Research Consortium criteria (17) as the occurrence of an acute coronary syndrome with either angiographic or pathological confirmation of thrombosis. Secondary study end points were death from any cause, the composite of death from any cause or definite ST, and Thrombolysis In Myocardial Infarction (TIMI) major bleedings (19). We also assessed the incidence of probable ST according to Academic Research Consortium criteria (17), the composite of definite or probable ST, myocardial infarction (MI), target lesion reintervention, ischemic stroke, intracranial hemorrhage, and TIMI minor bleeding (19). The diagnosis of MI was made according to TIMI criteria (19) and based on new abnormal Q-wave appearance in the electrocardiogram and/or an increase of the creatine kinase-MB value to 3 or more times the upper limit of normal. After PCI, cardiac markers were serially measured every 8 h for the first 24 h after the procedure and then daily until discharge. The diagnosis of ischemic stroke and hemorrhage required confirmation by computed tomography or magnetic resonance imaging of the head. All events were adjudicated by an event adjudication committee blinded to the platelet function status of patients and not involved in the follow-up process.

Follow-up. Patients stayed in hospital for at least 2 days after study inclusion. Patients were interviewed by telephone call after 30 days (±7 days). Those patients with cardiac symptoms were seen in the outpatient clinic for complete clinical, electrocardiographic, and laboratory check-up. Data of patients were collected and entered into a computer database by specialized personnel. All possible information from referring physicians, relatives, and hospital readmissions were entered as well. Source documentations were checked to ensure high-quality data.

Statistical methods. In a large collaborative network meta-analysis, definite ST during the first 30 days after PCI was observed in 94 (0.7%) of 12,973 patients (20). Sample size calculation for the present study was based on the assumption that the incidence of the primary end point (definite ST) was 0.5% in clopidogrel responders (quintiles 1 to 4) and 2.5% in nonresponders (upper quintile). Choosing a power of 80% and a 2-sided α value of 0.05, an overall sample size of at least 1,460 patients was required (nQuery advisor, version 7.0, Statistical Solutions, Cork, Ireland). To compensate for loss to follow-up we aimed for the inclusion of 1,600 patients.

Variables are presented as mean ± SD, counts (percentages), and median with interquartile range. Categorical variables were compared using the chi-square test. The Kolmogorov-Smirnov test was used to test for normal distribution of continuous data. Normally distributed continuous variables were compared with a 2-sided unpaired *t* test. A receiver-operator characteristic (ROC) curve analysis was calculated to determine the ability of MEA to distinguish between patients with and without definite ST in the 30 days after the procedure. The optimal cutoff value was calculated by determining the value for ADP-induced platelet aggregation in AU·min that provided the greatest sum of sensitivity and specificity. Survival analyses were generated using the Kaplan-Meier method, and the differences between groups were assessed by the log-rank test, which allowed the calculation of odds ratios (ORs) (95% confidence intervals [CIs]) associated with the 30-day incidence of the end points of interest. A Cox proportional hazards model was used to identify independent correlates of the primary end point. The occurrence of definite ST was defined as the dependent variable. Independent variables were platelet reactivity to clopidogrel and all variables shown in Table 1 with significant differences ($p < 0.05$) between normal and low responders. The hazard ratios (HRs) and the corresponding 95% CIs were calculated.

For all statistical analyses, a value of $p < 0.05$ was considered significant. Analyses were performed using the software package S-PLUS version 4.5 (Insightful Corp., Seattle, Washington).

Results

During the study period, a total of 1,608 CAD patients treated with PCI were enrolled in this trial. Multiplate measurements in the study population were not normally distributed (1-sample Kolmogorov-Smirnov test; $p < 0.001$). The median [interquartile range] value of ADP-induced platelet aggregation after administration of 600 mg clopidogrel was 225 AU·min [141 to 369]. A broad range of values was observed (range 0 to 1,409 AU·min). The cutoff value for post-treatment Multiplate measurements defining the upper quintile (20%) of patients was 416 AU·min. According to this cutoff value, 323 patients were defined as clopidogrel low responders. The remaining patients (n = 1,285) were defined as clopidogrel normal responders.

The baseline characteristics of the study population are shown in Table 1. Among low responders, the proportion of diabetic patients, active smokers, as well as NSTEMI and STEMI patients was significantly higher compared with clopidogrel responders. In addition, low responders had a significantly higher body mass index, a higher platelet count, a significantly lower ejection fraction, and a significantly shorter time from clopidogrel loading to blood sampling compared with clopidogrel responders.

Angiographic and procedural characteristics of the study population are shown in Table 2. A total of 2,809 lesions were treated in the entire study population. Variables were well balanced between the 2 groups. The large majority of patients received a DES during the index procedure (98.0% in normal responders vs. 98.2% in low responders; $p = 0.93$). Only 23 patients (1.4%) had a follow-up shorter than 1 month.

Ischemic end points. The primary end point (definite ST) within 30 days occurred in 10 (0.6%) patients. Six (60%) of the 10 stent thromboses occurred within the first week after PCI (median 4.5 [1.3 to 8.3] days). Compared with patients with a normal response (n = 1,285), low responders (n =

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Table 1 Baseline Characteristics of the Study Population

Variable	Overall (n = 1,608)	Normal Responders (n = 1,285)	Low Responders (n = 323)	p Value
Age (yrs)	67.5 ± 10.5	67.7 ± 10.4	66.7 ± 10.9	0.12
Women	374 (23.0)	287 (22.3)	87 (26.9)	0.08
Body mass index (kg/m^2)	27.5 ± 4.4	27.3 ± 4.2	28.3 ± 4.9	<0.001
Ejection fraction (%)	54.5 ± 11.3	54.9 ± 10.9	53.2 ± 12.6	0.03
Serum creatinine (mg/dl)	1.0 ± 0.5	1.0 ± 0.5	1.0 ± 0.3	0.42
Diabetes mellitus	462 (29.0)	352 (27.4)	110 (34.1)	0.02
Insulin treatment	147 (9.1)	105 (8.2)	42 (13.0)	0.007
Oral treatment	231 (14.0)	178 (13.9)	53 (16.4)	0.24
Active smokers	216 (13.0)	156 (12.1)	60 (18.6)	0.002
Arterial hypertension	1,473 (91.6)	1,182 (92.0)	291 (90.1)	0.27
Hypercholesterolemia	1,122 (70.0)	895 (70.0)	227 (70.0)	0.83
Previous MI	507 (32.0)	396 (30.8)	111 (34.4)	0.22
Previous bypass surgery	232 (14.0)	186 (14.5)	46 (14.2)	0.92
Multivessel disease	1,369 (85.0)	1,088 (85.0)	281 (87.0)	0.29
CAD presentation				<0.001
STEMI	40 (2.5)	20 (1.6)	20 (6.2)	
Stable angina	1,075 (66.9)	881 (68.6)	194 (60.1)	
Unstable angina	357 (22.2)	291 (22.6)	66 (20.4)	
NSTEMI	136 (8.5)	93 (7.2)	43 (13.3)	
Platelet count, $\times 10^3/\mu l$	218 ± 63	213 ± 62	236 ± 64	<0.001
Time from loading (h)	4.0 [2.0–15.0]	4.0 [2.0–15.5]	3.0 [2.0–7.0]	<0.001
Comedication				
Aspirin treatment	1,593 (99.1)	1,272 (99.0)	321 (99.4)	0.51
Statin treatment	1,515 (94.2)	1,206 (93.9)	309 (95.7)	0.21

Data presented are mean ± SD or n (%). Time from clopidogrel loading (h) to blood sampling is expressed as median [interquartile range].
CAD = coronary artery disease; MI = myocardial infarction; NSTEMI = non–ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction.

323) had a significantly higher risk of definite ST within 30 days (7 [2.2%] vs. 3 [0.2%]; OR: 9.4; 95% CI: 3.1 to 28.4; p < 0.0001). Characteristics of ST patients are shown in Table 3. The clopidogrel pre-treatment interval (median [interquartile range]) for patients with definite or probable ST was 5.5 [4 to 19] h versus 3.5 [2 to 19] h for patients without ST (p = 0.14). Figure 1A shows the cumulative incidence of the primary end point during the 30-day follow-up period in clopidogrel low responders versus normal responders. Table 4 shows the number of events for definite ST and combined events for definite or probable ST across quintiles of MEA measurements. Mortality was 4 (1.2%) in low versus 5 (0.4%) in normal responders (OR: 3.2; 95% CI: 0.9 to 11.1; p = 0.07). The composite of death or ST was higher in low versus normal responders (10 [3.1%] vs. 8 [0.6%]; OR: 5.1; 95% CI: 2.2 to 11.6; p <

Table 2 Angiographic and Procedural Characteristics

Variable	Overall (n = 2,809)	Normal Responders (n = 2,221)	Low Responders (n = 588)	p Value
Number of lesions per patient	1.7 ± 1.0	1.7 ± 0.9	1.8 ± 1.0	0.12
Number of stents per lesion	1.2 ± 0.5	1.2 ± 0.5	1.2 ± 0.6	0.15
Type of intervention				0.63
Stent placement	2,713 (96.6)	2,147 (96.7)	566 (96.3)	
Balloon angioplasty	96 (3.4)	74 (3.3)	22 (3.7)	
Lesion location				0.97
Left main	159 (5.7)	126 (5.7)	33 (5.6)	
Left anterior descending	1,061 (37.8)	838 (37.7)	223 (37.9)	
Left circumflex	755 (26.9)	593 (26.7)	162 (27.6)	
Right coronary artery	777 (27.7)	620 (27.9)	157 (26.7)	
Bypass graft	57 (2.0)	44 (2.0)	13 (2.2)	
Vessel size, mm	2.8 ± 0.6	2.8 ± 0.6	2.8 ± 0.5	0.90
AHA/ACC type B2/C	2,121 (75.5)	1,667 (75.1)	454 (77.2)	0.28
Chronic occlusion	193 (6.9)	148 (6.7)	45 (7.7)	0.40
Final diameter stenosis (%)	12.9 ± 9.4	12.8 ± 9.3	13.3 ± 10.1	0.27

Data presented are mean ± SD or n (%).
AHA/ACC = American Heart Association/American College of Cardiology.

Table 3 Characteristics of Patients With Definite or Probable ST

ST Patients (Gender, Age [yrs])	MEA Value (AU·min	Pre-Treatment Interval (h)	Time to ST (Days)
Definite ST			
Patient #1 (male, 54)	1,196	12	2
Patient #2 (female, 64)	1,077	5	1
Patient #3 (female, 90)	965	4	0
Patient #4 (male, 68)	819	20	1
Patient #5 (male, 62)	606	6	7
Patient #6 (female, 67)	599	22	6
Patient #7 (male, 61)	468	2	29
Patient #8 (female, 85)	271	19	10
Patient #9 (female, 48)	244	2	8
Patient #10 (female, 74)	73	8	1
Probable ST			
Patient #11 (male, 75)	988	2	2
Patient #12 (male, 65)	730	4	18
Patient #13 (male, 72)	276	4	6
Patient #14 (male, 71)	239	46	18

The pre-treatment interval in hours is the time interval from clopidogrel loading to blood sampling. The time to ST is the time from the primary coronary intervention to the occurrence of ST.

AU = aggregation units; MEA = multiple electrode platelet aggregometry; ST = stent thrombosis.

0.001). Figure 1B shows the cumulative incidence of death or ST in the 2 groups. Table 5 shows the entire clinical outcome data for low versus normal responders at 30 days. The cumulative incidence of target lesion reintervention, ischemic stroke, and the combined incidence of definite or probable ST was significantly higher in low versus normal responders, whereas no differences were observed for probable ST alone and the incidence of MI in general. For the subgroup of patients with Q-wave infarction and MI >24 h post-PCI, significantly more events were observed in low versus normal responders.

The optimal cutoff value according to ROC analysis to predict the occurrence of 30-day ST was 468 AU·min. With this cutoff value, the Multiplate assay had 70% sensitivity, 84% specificity, and an area under the ROC curve of 0.78 (95% CI: 0.60 to 0.96, $p = 0.001$).

Predictors of ST. A Cox proportional hazards model was used to identify independent predictors for 30-day ST. The model included platelet reactivity to clopidogrel treatment (AU·min), as well as possible confounding variables of patients' baseline characteristics. These variables were diabetes mellitus, active smoking, body mass index, ejection fraction, platelet count, time from clopidogrel loading to blood sampling, and CAD presentation (including STEMI, NSTEMI, stable angina, and unstable angina, see Table 1).

In a first step, platelet reactivity (AU·min) to clopidogrel was included as a continuous variable and calculations were done for an absolute increase of 100 AU·min assessed with MEA. Results of this Cox proportional hazards model showed that platelet reactivity to clopidogrel (per 100 AU·min) was an independent predictor of 30-day ST (HR: 1.38, 95% CI: 1.14 to 1.66; $p < 0.001$). The only other independent predictor of 30-day ST was platelet count (calculated for an increase of $100 \times 10^3/\mu l$ in platelet count) (HR: 2.43, 95% CI: 1.44 to 4.09; $p < 0.001$). The remaining variables included for multivariate analysis were not found to be independent predictors of 30-day ST ($p \geq 0.18$).

In a second step and similar to the primary analysis, platelet reactivity to clopidogrel was regarded as a categorical variable (low response or normal response) for the multivariate model. With this approach, low response to clopidogrel assessed with MEA was also found to be an independent predictor for the occurrence of ST (HR: 10.95, 95% CI: 2.31 to 51.99; $p = 0.003$).

Bleeding end points. The cutoff value for post-treatment MEA measurements defining the lowest quintile of patients was 124 AU·min. According to this cutoff value, 318 patients were defined as high-responders. For the lowest quintile of patients ($n = 318$), no significant increase in TIMI major bleeding was observed compared with quintiles 2 to 5 (4 [1.3%] vs. 9 [0.7%]; $p = 0.32$). In addition, no significant difference was observed for the incidence of

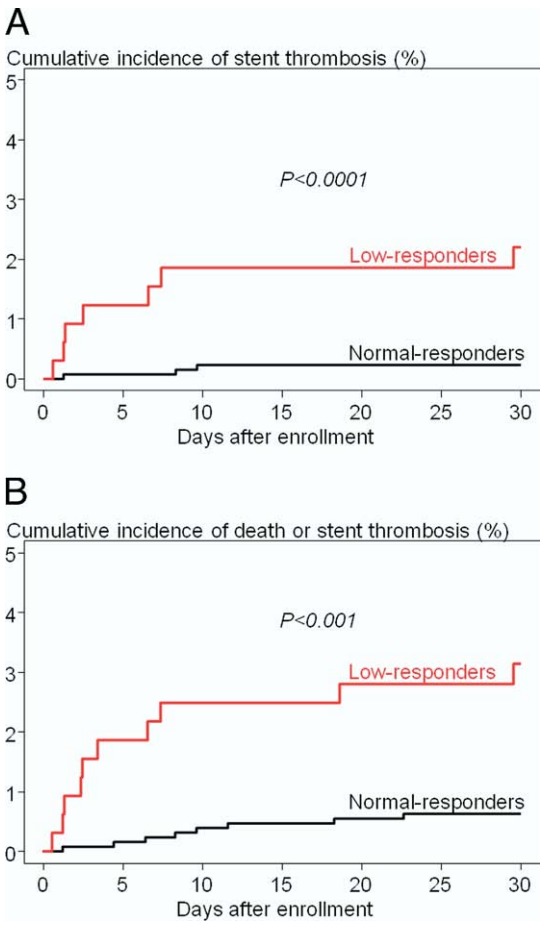

Figure 1 Kaplan-Meier Analysis

Kaplan-Meier analysis for the cumulative incidence of stent thrombosis **(A)** and for the composite of death or stent thrombosis **(B)**.

Table 4 Distribution of ST Across Quintiles

	1st Quintile	2nd Quintile	3rd Quintile	4th Quintile	5th Quintile	p Value
Definite ST	1	0	1	1	7	0.003
Combined probable/definite ST	1	0	2	2	9	0.001

Total number of events for definite ST and combined events for definite or probable ST across quintiles according to MEA measurements. The boundaries for MEA quintiles were as follows: 124, 192, 261, and 416 AU·min.
Abbreviations as in Table 3.

TIMI minor bleedings (10 [3.1%] for high responders vs. 31 [2.4%] for the remaining patients; p = 0.45). A total of 2 intracranial bleedings occurred in the study population. Albeit being above the cutoff value defining the lowest quintile of patients (124 AU·min), these 2 patients had relatively low values of ADP-induced platelet aggregation (168 AU·min, 152 AU·min).

Discussion

This is the first prospective trial investigating the impact of platelet reactivity to clopidogrel assessed with MEA on the risk of early ST and other ischemic as well as bleeding events after PCI. It is the largest study to date showing that low response to clopidogrel is associated with an increased risk of drug-eluting ST, and it is the first study so far with definite ST (17) as the primary end point. For clopidogrel low responders, an approximately 11-fold increase in the incidence of 30-day ST was observed compared with normal responders. Importantly, the study also showed that low response to clopidogrel is an independent predictor for the occurrence of ST. This finding was true regardless whether AU·min was included as a categorical or a continuous variable, whereas the latter may better reflect the biological background of platelet hyperreactivity, which is a continuum as well. Importantly, we did not observe a gradual increase of events across quintiles but a significant accumulation of stent thromboses in patients belonging to the upper quintile of MEA measurements. In fact, an MEA value close to the boundary for the upper quintile of patients (416 AU·min) was also identified by ROC analysis (468 AU·min). Using ROC analysis, we observed that MEA had a strong predictive value with an area under the curve of 0.78. Aside from ST, other ischemic events were also associated with low response to clopidogrel, which underlines the predictive value of the Multiplate analyzer for the occurrence of ischemic events after PCI.

Different methods are currently used to assess platelet function (18). Among the most commonly used methods, LTA, VerifyNow, and MEA are functional assays (in contrast to VASP) that measure ADP-induced platelet aggregation with different techniques (16,18). The LTA and VerifyNow assays are based on changes in light transmission in a liquid phase after ADP stimulation, whereas MEA is based on the principles of impedance aggregometry. The LTA and the VASP assays are time-intensive, require skilled personnel, and are therefore not suitable for clinical practice. A point-of-care approach can only be realized with the VerifyNow and MEA assays, both of which are whole-blood methods. Although a correlation exists between VerifyNow and MEA (21) and between MEA and LTA (16), it is obvious that these methods yield similar but not identical results. In this context, assessing platelet function in whole blood (MEA and VerifyNow) has some advantages over LTA using platelet-rich plasma. There is no need for centrifugation that can alter platelet function to separate platelets from other blood cells (22,23). This enables an easy and fast assessment of platelet function with the possibility to decide on treatment regimens when the patient is still in the catheter laboratory. In contrast to LTA or VerifyNow, in which aggregation occurs in a liquid phase, aggregation in MEA takes place on surfaces. This is similar to in vivo conditions, in which platelet aggregation takes place on surfaces as well, such as on ruptured plaques, at sites of vascular injury, or on stent struts. The MEA

Table 5 Clinical Outcome

Ischemic Events, n (%)	Normal Responders	Low Responders	OR (95% CI)	p Value
Definite ST	3 (0.2)	7 (2.2)	9.41 (3.11–28.44)	<0.0001
Probable ST	2 (0.2)	2 (0.6)	4.00 (0.65–24.47)	0.13
Combined probable/definite ST	5 (0.4)	9 (2.8)	7.26 (2.86–18.46)	<0.0001
Death	5 (0.4)	4 (1.2)	3.20 (0.92–11.10)	0.07
Combined death/definite ST	8 (0.6)	10 (3.1)	5.05 (2.19–11.64)	<0.001
Myocardial infarction	41 (3.2)	12 (3.7)	1.16 (0.61–2.21)	0.64
MI >24 h post-PCI	5 (0.4)	5 (1.5)	4.02 (1.28–12.63)	0.02
Q-wave MI	4 (0.3)	5 (1.5)	4.99 (1.53–16.29)	0.008
Target lesion reintervention	7 (0.5)	7 (2.2)	4.02 (1.53–10.58)	0.005
Ischemic stroke	2 (0.2)	3 (0.9)	6.01 (1.25–28.88)	0.03

CI = confidence interval; MI = myocardial infarction; OR = odds ratio; PCI = percutaneous coronary intervention; ST = stent thrombosis.

technique is a relatively new one, and data on its clinical and methodical properties are still limited. Aside from a good correlation of MEA with the golden standard of LTA (16), MEA is capable of detecting the amount of platelet inhibition achieved using different P2Y12 antagonists including clopidogrel, cangrelor, and the active metabolites of clopidogrel and prasugrel in varying doses (24,25).

Aside from the present study, no prospective study is available that shows a significant association of low response to clopidogrel and definite ST, the clinical end point with the highest interest. The RECLOSE (Low Responsiveness to Clopidogrel and Sirolimus- or Paclitaxel-Eluting Stent Thrombosis) trial (8) has shown that the cumulative incidence of definite and probable ST was higher in clopidogrel nonresponders compared with responders. However, this difference was mainly driven by the high rate of probable ST in the nonresponder group and no significant difference for the incidence of definite ST was observed, which is in contrast to the results of our study. Other prospective studies using LTA did not focus on ST specifically but defined combined primary end points including MI, stroke, cardiovascular death, or target lesion revascularization (10,12,13). Among them, the EXCELSIOR (Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate) trial (10) was the first to convincingly show that platelet aggregation above the median was associated with an increased rate of ischemic events. However, a possible association of ST and low response to clopidogrel was not reported specifically. In this context, use of combined primary end points including MI or target lesion revascularization implies the risk of assigning a significant proportion of observed events to clopidogrel low response, which in fact have other causes. Concerning the incidence of MI in our study, we only observed significant differences for infarctions >24 h post-PCI. This finding may be attributed to the fact that the etiology of immediate (<24 h) MI—representing the majority of MIs in our study—is multifactorial (e.g., occlusion of side branches, washout of myocardial enzymes in acute coronary syndromes) and probably only to a minor extent influenced by antiplatelet therapy.

Results of our study are based on the use of 1 single point-of-care assay, the Multiplate device. Recently, different studies using the VerifyNow assay have convincingly shown that high post-treatment platelet reactivity was associated with ischemic events after PCI (7,14). Price et al. (7) showed in a homogenous population (n = 380) regarding the clopidogrel pre-treatment interval and the type of stent used, that point-of-care measurements are associated with ischemic events after DES placement.

The 30-day incidence of definite ST in our study (0.6%) is consistent with a previous report (5) investigating 2,229 patients after DES implantation in which ST was observed in 0.6% of patients at 30 days and with a large meta-analysis (n = 12,973 patients) in which definite ST was reported to occur at a rate of 0.7% (20). Similar to the results of Iakovou et al. (5), we observed that the majority of stent thromboses occurred within the first week after the procedure. This emphasizes the need for early testing of platelet function; ideally before the procedure to intensify antiplatelet treatment if needed. Based on our observations, we calculated that a total number of approximately 500 platelet function tests using the Multiplate analyzer would be needed to prevent 1 ST in the low-responder group (n = 100) under the assumption that the risk of ST would be reduced by 50% (from 2.2% to 1.1%) in this group of patients when an intensified or alternative antiplatelet treatment is administered.

The predictive value of platelet function testing for bleeding events in PCI patients has never been investigated before. Here, we did not find a significant association of high response to clopidogrel treatment and the occurrence of bleeding events. A trend was observed toward more TIMI major bleeding episodes in the high-responder subgroup of patients compared with the remaining patients (1.3% vs. 0.7%, respectively) and it is perceivable that an even larger trial would find a significant relationship between clopidogrel high response assessed with MEA and bleeding risk.

Large prospective studies to determine whether individual guidance of tailored antiplatelet treatment based on the results of platelet function testing improves clinical outcome in patients after coronary stent placement are still needed (26). The GRAVITAS (Gauging Responsiveness With A VerifyNow Assay-Impact On Thrombosis And Safety) trial using the VerifyNow assay is currently on the way to address this issue in larger study populations. In the future, GRAVITAS and other prospective large-scale trials should determine whether direct guidance of tailored antiplatelet treatment based on platelet function testing could improve the clinical outcome of patients after coronary stenting.

Based on the results of our study, MEA performed on a Multiplate analyzer with all of the advantages of an easy-to-use point-of-care assay is likely to be helpful for tailoring antiplatelet treatment to the needs of individual patients treated with PCI. To address this issue specifically, a large prospective clinical trial using the Multiplate analyzer is needed, in which patients defined as low responders are randomized to intensified antiplatelet treatment using either an increased clopidogrel maintenance dose or more potent antiplatelet drugs such as prasugrel. As soon as prasugrel and the other newly developed P2Y12 inhibitors are available, assessing clopidogrel response may become mandatory to avoid unnecessary stent thromboses related to clopidogrel low responsiveness.

Study limitations. Herein we report clinical follow-up data for a time period of 30 days after the procedure. Whether assessment of platelet function with MEA is also predictive for the occurrence of long-term clinical events must to be determined separately. However, a follow-up period of 30 days has several advantages. It is the period with the highest risk of ST, and a 30-day follow-up interval attenuates the interference of drug noncompliance in the long term. In

addition it is close enough to the sole measurement time point to detect a cause–effect relationship. In daily clinical practice, the clopidogrel pre-treatment interval is variable, which obviously affects the results of platelet function testing. However, it is highly improbable that this had a relevant impact on the results of our study for 2 reasons. First, the significant association of platelet reactivity to clopidogrel and ST was maintained after adjustment for time from clopidogrel loading in the multivariate analysis. Second, patients who suffered an ST had a relatively long clopidogrel pre-treatment interval compared with patients without ST. A limitation of the study is related to the low number of events that composed the primary end point, despite the large number of patients included in this study. This reflects the increased safety associated with current PCI but also underscores the need for larger studies to corroborate present results. Results of this study are based on measurements with MEA. Although we know that MEA measurements correlate with LTA (16) and the VerifyNow assay (21), we are unable to say how the results of the present study can be extrapolated to other assays for platelet function testing.

Conclusions

A low response to clopidogrel treatment assessed with MEA is significantly associated with an increased risk of ST. Further studies are warranted to evaluate the ability of MEA to guide antiplatelet therapy in patients undergoing PCI.

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Key Words: clopidogrel ■ stent thrombosis ■ platelet aggregation ■ whole blood aggregometry.