CLINICAL RESEARCH

Impact of P2Y$_{12}$ Inhibition by Clopidogrel on Cardiovascular Mortality in Unselected Patients Treated by Percutaneous Coronary Angioplasty

A Prospective Registry

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Objectives The aim of this study was to determine whether low platelet response to the P2Y$_{12}$ receptor antagonist clopidogrel as assessed by Vasodilator-stimulated phosphoprotein flow cytometry test (VASP- FCT) predicts cardiovascular events in a high-risk population undergoing percutaneous coronary intervention (PCI).

Background Impaired platelet responsiveness to clopidogrel is thought to be a determinant of cardiovascular events after PCI. The platelet VASP-FCT is a new assay specific to the P2Y$_{12}$ adenosine diphosphate receptor-pathway. In this test, platelet activation is expressed as platelet reactivity index (PRI).

Methods Four-hundred sixty-one unselected patients undergoing urgent (n = 346) or planned (n = 115) PCI were prospectively enrolled. Patients were classified as low-response (LR) and response (R) to clopidogrel, depending on their PRI. Optimal PRI cutoff was determined by receiver-operator characteristic curve analysis to 61% (LR: PRI ≥61% and R: PRI <61%). Follow-up was obtained at a mean of 9 ± 2 months in 453 patients (98.3%).

Results At follow-up, total cardiac mortality rates and possible and total stent thrombosis were higher in LR patients. Multivariate analysis identified creatinine clearance (hazard ratio [HR]: 0.95; 95% confidence interval [CI]: 0.93 to 0.98, p < 0.001), drug-eluting stent (HR: 5.73; 95% CI: 1.40 to 23.43, p = 0.015), C-reactive protein (HR: 1.01; 95% CI: 1.001 to 1.019, p = 0.024), and LR to clopidogrel (HR: 4.00; 95% CI: 1.08 to 14.80, p = 0.037) as independent predictors of cardiac death. The deleterious impact of LR to clopidogrel on cardiovascular death was significantly higher in patients implanted with drug-eluting stent.

Conclusions In patients undergoing PCI, LR to clopidogrel assessed by VASP-FCT is an independent predictor of cardiovascular death at the PRI cutoff value of ≥61%. The LR clinical impact seems to be dependent on the type of stent implanted. (J Am Coll Cardiol Intv 2010;3:648–56) © 2010 by the American College of Cardiology Foundation

Combined pharmacological inhibition of the P2Y$_{12}$ receptor by thienopyridines and of the cyclooxygenase pathway by aspirin is currently considered the reference antiplatelet strategy to prevent thrombotic complication of percutaneous coronary intervention (PCI) with stent (1,2). Clopidogrel elicits a dose-dependent inhibition of adenosine diphosphate (ADP)-induced aggregation by specific and irreversible blockade of the P2Y$_{12}$ receptor (2). However, a

wide interindividual variability in platelet response to clopidogrel has been reported (3). The mechanisms for suboptimal response to clopidogrel are not fully elucidated, although genetic, metabolic, cellular, and clinical factors have been proposed as important determinants (1,4–6). Although recent data have suggested that residual platelet aggregability during clopidogrel treatment could portend worse cardiovascular outcome in patients treated by PCI (7,8), to date no definitive evidence based on hard end points has been published. Moreover, although a "low-response" (LR) to clopidogrel might be overcome by dose titration (9,10), the appropriate test to evaluate the platelet effects of clopidogrel and the optimal threshold of responsiveness that should be reached remain debatable (11).

Several studies showed that measurement of vasodilator-stimulated phosphoprotein (VASP) phosphorylation might be employed for monitoring the effects of thienopyridines on platelets (10,12–15). Compared with platelet aggregometry, the VASP assay affords several advantages: 1) specificity of the P2Y$_{12}$ signaling; 2) no need for a pretreatment measurement; 3) lack of interference with aspirin or glycoprotein IIb/IIIa antagonists; 4) standardization of the procedure; 5) measurement on whole blood; and 6) stability of the measurement up to 48 h (16).

The relationship between VASP phosphorylation and the occurrence of clinical cardiovascular events has been evaluated in several studies (12–15). Low response to clopidogrel was not associated with mortality but with a higher number of major clinical events (15,17). However these studies included a relatively low risk population and used different cutoff values to define LR to clopidogrel. In fact, the optimal platelet reactivity index (PRI) cutoff for the prediction of cardiovascular events remains undetermined. In the present study, we examined whether LR to clopidogrel assessed by VASP phosphorylation is an indicator of major cardiovascular events after PCI.

Methods

Patients and design of the prospective registry. Consecutive patients undergoing PCI between September 2007 and December 2008 in our institution for acute coronary syndrome (ACS) or stable coronary artery disease were in-

cluded. The study was performed in accordance with the Declaration of Helsinki. Informed written consent was obtained from all patients. The study protocol was approved by the institutional ethics committee "Comité de Protection des Personnes EST IV, Strasbourg, France."

Blood samples. Whole blood samples were drawn from venous punctures at least 6 h after a loading dose (300 or 600 mg) of clopidogrel. Blood was immediately collected into a vacutainer tube containing 0.129 mol/l sodium citrate (BD Vacutainer, Becton Dickinson, Sparks, Maryland) and sent to the hemostasis laboratory (EFS-Alsace, Strasbourg, France).

Analysis of VASP phosphorylation by flow cytometry. The VASP phosphorylation was assessed with standardized flow cytometric assay (Platelet VASP; Diagnostica Stago [Biocytex], Asnières, France) (3). Briefly, a citrated blood sample was incubated with prostaglandin E1 (PGE1) or with PGE1 and ADP for 10 min and fixed with paraformaldehyde, after which the platelets were permeabilized with nonionic detergent. The cells were labeled with a primary monoclonal antibody against serine 239-phosphorylated VASP (16C2), followed by a secondary fluorescein isothiocyanate-conjugated polyclonal goat antimouse antibody. Analyses were performed on a Becton Dickinson FACS Calibur flow cytometer as previously described (3). A PRI was calculated from the median fluorescence intensity (MFI) of samples incubated with PGE1 and ADP according to the formula: $PRI\ VASP = ([MFI[PGE1] - MFI[PGE1 + ADP]/MFI[PGE1]) \times 100$. The PRI, expressed as a percentage, is the difference in VASP fluorescence intensity between resting (+PGE1) and activated (+ADP) platelets (3).

Clinical end points. Cardiovascular death was defined as any death with demonstrable cardiovascular cause or any death that was not clearly attributable to a noncardiovascular cause. The diagnosis of ST-segment elevation myocardial infarction (STEMI) was based on the evidence of a new or presumably new ST-segment elevation in 2 consecutive leads and an increase in biochemical markers of myocardial necrosis. The diagnosis of non–ST-segment elevation myocardial infarction (NSTEMI) was defined as the occurrence of ischemic symptoms, ST-segment depression, and/or T-wave

Abbreviations and Acronyms

ACS = acute coronary syndrome

ADP = adenosine diphosphate

BMS = bare-metal stent(s)

DES = drug-eluting stent(s)

LR = low response/responders (to clopidogrel)

MACE = major adverse cardiovascular events

MFI = median fluorescence intensity

NSTEMI = non–ST-segment elevation myocardial infarction

PCI = percutaneous coronary intervention

PGE1 = prostaglandin E1

PRI = platelet reactivity index

R = response/responders (to clopidogrel)

STEMI = ST-segment elevation myocardial infarction

VASP = vasodilator-stimulated phosphoprotein

abnormalities and an increase of biochemical markers of myocardial necrosis. Post-PCI troponin elevation was not considered as recurrent myocardial infarction. Stent thrombosis was defined according to the Academic Research Consortium criteria as: definite = ACS and angiographic or pathologic evidence of stent thrombosis; probable = unexplained death within 30 days or target-vessel infarction without angiographic information; and possible = unexplained death after 30 days of stent placement. Major bleeding was defined as intracranial bleeding, life-threatening bleeding, or any decrease in hemoglobin requiring transfusion.

Follow-up information was achieved by a written questionnaire sent to cardiologist and referring physician completed by phone.

Statistical analysis. Continuous variables are expressed as median (interquartile range); categorical variables are expressed as frequencies and percentages. Student *t* tests have been used to compare continuous variables between 2 groups. If necessary (i.e., for non-Gaussian distribution), nonparametric Mann-Whitney *U* test has been used. Chi-square test or Fisher exact tests have been used for comparison of categorical variables. Continuous variables were analyzed for normal distribution with the Shapiro-Wilk test. Time to event was defined as the time from PCI to the date of event, with patients censored at death, loss to follow-up, or end of the study (June 30, 2009).

Kaplan-Meier analyses were used to construct survival plots of time to death after PCI and compared with log-rank test. Multivariate analysis of survival rates has been done with Cox models. Variables with p < 0.10 in univariate analysis were entered into a stepwise ascending multivariate analysis. The results of the Cox regression are presented as hazard ratio, 95% confidence intervals (CIs), and p values.

The optimal cutoff value for VASP was determined by using a receiver-operator characteristic curve analysis based on the maximal value of the Youden index. A p value < 0.05 was considered statistically significant. Statistical analysis was performed with SPSS version 13.0 software (SPSS, Inc., Chicago, Illinois).

Results

Baseline characteristics and biological response to clopidogrel assessed by VASP. Four-hundred sixty-one consecutive patients treated by planned (24.9%) or urgent PCI (75.1%) were enrolled. Indication for PCI was STEMI in 151 patients (32.8%), NSTEMI in 154 patients (33.4%), unstable angina in 41 patients (8.9%) and stable angina or silent ischemia in 115 patients (24.9%). The median value of PRI (%) in the cohort was 55 (25th, 75th percentile: 37.78, 70). Patients were assigned in 2 separate groups on the basis of the PRI cutoff value of 61%, which was determined by receiver-
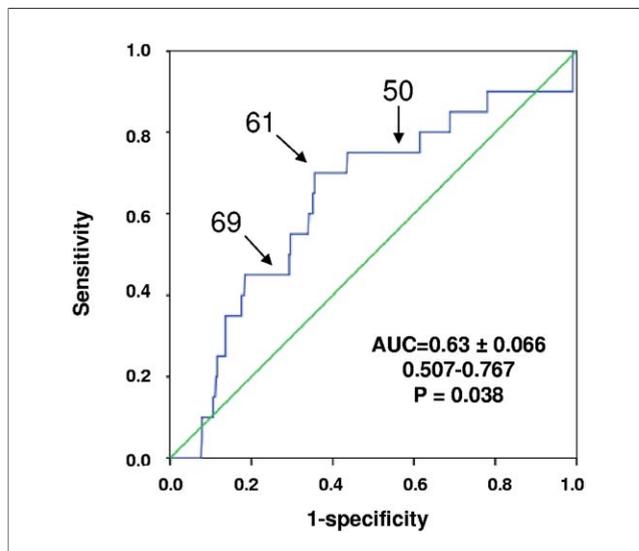
Figure 1. ROC Curve for the Prediction by PRI% of Cardiac Death

The area under the receiver-operator characteristic (ROC) curve SEM and the 95% confidence interval are indicated. AUC = area under the curve; PRI = platelet reactivity index.

operator characteristic curve analysis (Fig. 1). According to this cutoff, the median value of VASP in the responders (R) group (PRI <61%) was 41.37 (26.8, 51.8) and 73.15 (66.70, 79.08) in LR group (PRI ≥61%). Two-hundred and seventy-seven (60.1%) patients had a PRI below 61% and were considered as R, and 184 patients (39.9%) had a PRI equal to or above 61% and were considered as LR. Baseline demographic, clinical, biological, and angiographic characteristics of the 2 groups are described in Tables 1–3. At follow-up (9 ± 2 months), the rate of dual antiplatelet therapy was equivalent between groups (LR: 85%; R: 87%; p = NS).

Determinants of impaired platelet response to clopidogrel. The loading dose of clopidogrel was 300 mg in 349 patients (75.7) and ≥600 mg in 106 patients (23%). Six patients were taking long-lasting clopidogrel therapy and did not receive any loading dose. The loading dose was similar between R and LR groups. The delay that elapsed between clopidogrel loading dose and VASP measurement was below 12 h in 68 patients (14.7%), between 12 and 24 h in 174 patients (37.7%), 24 and 48 h in 70 patients (15.2%), 48 and 72 h in 61 patients (13.2%), and over 72 h in 88 patients (19.1%). The delay between clopidogrel loading dose and VASP assay was similar in LR and R; the mean delay was 24 h in both groups.

Of note, LR patients were more frequently obese (52.2% vs. 40.7%, p = 0.016) and presented more often with type 2 diabetes mellitus (44.6% vs. 32.1%, p = 0.008). No correlation between smoking and PRI value could be shown.

Impact of P2Y$_{12}$ inhibition by clopidogrel on cardiovascular outcome. Clinical outcomes were available in 453 patients (98.3%), at a mean follow-up of 9 ± 2 months (range 6 to

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Table 1. Baseline Demographic and Clinical Characteristics

Variable	Total Cohort (N = 461)	Low Responders (n = 184)	Responders (n = 277)	p Value
Age (yrs)	65.40 (55.8–75.40)	63.95 (54.62–73.28)	66.25 (57.52–75.90)	0.16
Risk factors/past medical history				
Current smoking	228 (49.5)	83 (45.1)	145 (52.3)	0.13
Hypertension	253 (54.9)	104 (56.5)	149 (53.8)	0.57
Obesity (BMI >30 kg/m^2)	206 (45.3)	95 (52.2)	111 (40.7)	0.016
Hyperlipidemia	241 (52.3)	100 (54.3)	141 (50.9)	0.51
Diabetes mellitus type 2	171 (37.1)	82 (44.6)	89 (32.1)	0.008
Prior STEMI	81 (17.6)	31 (16.8)	50 (18.1)	0.80
Prior NSTEMI	27 (5.9)	14 (7.6)	13 (4.7)	0.23
Prior CABG	40 (8.7)	14 (7.6)	26 (9.4)	0.61
Prior PCI	130 (28.2)	53 (28.8)	77 (27.8)	0.83
Prior stroke	20 (4.3)	7 (3.8)	13 (4.7)	0.82
Peripheral vascular disease	32 (6.9)	15 (8.2)	17 (6.1)	0.46
Chronic renal insufficiency	36 (7.8)	18 (9.8)	18 (6.5)	0.22
Treatment				
Aspirin	461 (100)	184 (100)	277 (100)	1
Clopidogrel	461 (100)	184 (100)	277 (100)	1
ACE inhibitors	448 (98.5)	178 (97.8)	270 (98.9)	0.45
Beta-blockers	442 (96.7)	175 (95.6)	267 (97.4)	0.30
Statin	450 (98.5)	180 (98.4)	270 (98.5)	1
Oral anticoagulants	29 (6.4)	15 (8.3)	14 (5.1)	0.24
GP IIb/IIIa antagonists	109 (27.6)	58 (36.9)	51 (21.4)	0.001
PPI	314 (83.7)	121 (80.1)	193 (86.2)	0.15
Calcium inhibitors	22 (5.1)	9 (5.2)	13 (5)	1

Values are n (range) or n (%).

ACE = angiotensin-converting enzyme; BMI = body mass index; CABG = coronary artery bypass graft surgery; GP = glycoprotein; NSTEMI = non–ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; PPI = proton pomp inhibitor; STEMI = ST-segment elevation myocardial infarction.

14 months). Follow-up was missing in 8 patients: 6 LR and 2 R patients.

Death occurred in 26 patients (5.7%), cardiovascular death occurred in 20 patients (4.4%), STEMI occurred in 8 patients (1.8%), NSTEMI occurred in 23 patients (5.1%), and total stent thrombosis occurred in 24 patients (5.3%). Forty-two patients (9.3%) required target lesion revascularization. Overall, total major adverse cardiovascular events (MACE) rate was 15.7% (71 of 453). At follow-up major

bleeding occurred in 4 patients: 3 in R patients, and 1 in LR patients (p = NS). The distribution of major adverse events in LR and R groups is shown in Table 4. Although no differences in definite or probable stent thrombosis could be shown between groups, total (definite + probable + possible) and possible stent thrombosis were more frequent in LR patients. Likewise, death was more frequent in LR patients.

The Kaplan-Meier curves representing cardiovascular mortality dependent of PRI cutoff of 61% and 50% are shown, respectively, in Figures 2 and 3. Survival was significantly higher in patients with PRI below 61%. However by setting the cutoff at 50%, PRI was unable to discriminate a higher risk group.

By univariate Cox analysis (Table 5), Killip class III to IV, drug-eluting stent (DES), renal dysfunction, and LR to clopidogrel, defined as a PRI above or equal to 61% were associated with total death. Killip class III to IV, DES, renal dysfunction, C-reactive protein, and LR to clopidogrel defined as a PRI above or equal to 61%, were significantly related to cardiac death. Three-vessel disease, DES implantation, and unstable angina were associated with MACE.

Table 2. Angiographic Characteristics

	Low Responders (n = 184)	Responders (n = 277)	p Value
LAD	124 (67.4)	192 (69.3)	0.70
CX	76 (41.3)	99 (35.7)	0.24
RCA	104 (56.5)	157 (56.7)	0.99
Left main coronary artery	17 (9.2)	21 (7.6)	0.60
Three-vessel disease	66 (35.9)	73 (26.4)	0.038
Drug-eluting stent	81 (46.3)	128 (48.1)	0.77

Values are n (%).

CX = circumflex artery; LAD = left anterior descending artery; RCA = right coronary artery.

Table 3. Biological Characteristics

	Low Responders (n = 184)	Responders (n = 277)	p Value
Glycemia (g/l)	1.30 (1.06–1.70)	1.13 (0.97–1.53)	0.004
HbA1C (%)	6.3 (5.8–7.5)	6.2 (5.60–7.30)	0.150
Creatinine (μmol/l)	92 (74.7–114.25)	85 (70.57–105.55)	0.034
Tn admission (μg/l)	0.41 (0.14–1.81)	0.21 (0.08–0.78)	0.008
Tn H6 (μg/l)	2.89 (0.20–25.71)	0.70 (0.14–5.67)	0.008
Tn peak (μg/l)	12.18 (1.41–49.06)	5.24 (0.43–25.03)	0.018
CPK (U/l)	209 (89–505)	135 (80.5–308)	0.028
BNP (ng/l)	103 (47.3–266.50)	142 (52–328)	0.197
CRP (mg/l)	7 (4–24.30)	5.65 (4–16.63)	0.057
WBC ($\times10^9$/l)	9.28 (7.21–11.30)	8.58 (7.10–11.28)	0.341
Hb (g/dl)	13.5 (12.2–14.65)	13.4 (12–14.60)	0.374
Platelets ($\times10^9$/l)	244 (188–293)	240.5 (195.3–285)	0.944
Total cholesterol (g/l)	1.77 (1.49–2.15)	1.67 (1.37–2.07)	0.171
LDLc (g/l)	1.10 (0.76–1.44)	1.03 (0.76–1.34)	0.534
HDLc (g/l)	0.40 (0.30–0.40)	0.40 (0.30–0.50)	0.162
TG (g/l)	1.27 (0.92–1.85)	1.16 (0.79–1.64)	0.045
PRI (%)	73.15 (66.70–79.08)	41.37 (26.8–51.8)	0.0001

Values are n (range).

BNP = brain natriuretic peptide; CPK = creatinine phosphokinase; CRP = C-reactive protein; Hb = Hemoglobin; HDLc = high-density lipoprotein cholesterol; LDLc = low-density lipoprotein cholesterol; PRI = platelet reactivity index; TG = triglycerides; Tn = troponin; Tn H6 = troponin 6 h after admission; WBC = white blood cells.

As expected, patients implanted with at least 1 DES were more frequently diabetic, underwent more frequently planned PCI, were more frequently treated for LAD lesion, and received longer stents (data not shown).

Multivariate Cox regression analysis identified LR to clopidogrel (PRI ≥61%), DES, renal dysfunction, and C-reactive protein as independent predictors of cardiac death (Table 6). Cardiac death was higher in LR patients treated with DES in comparison with R patients treated with DES and bare-metal stent (BMS)-treated patients regardless of platelet response (LR or R) (Fig. 4).

Table 4. MACE at Follow-Up

|	Total Cohort (n = 453)	Low Responders (n = 178)	Responders (n = 275)	p Value
Total death	26 (5.7)	17 (9.6)	9 (3.3)	0.005
Cardiac death	20 (4.4)	14 (7.9)	6 (2.2)	0.004
STEMI	8 (1.8)	3 (1.7)	5 (1.8)	1.00
NSTEMI	23 (5.1)	11 (6.2)	12 (4.4)	0.39
TLR	42 (9.3)	18 (10.1)	24 (8.7)	0.62
Definite stent thrombosis	9 (2)	5 (2.8)	4 (1.5)	0.49
Probable stent thrombosis	8 (1.8)	5 (2.8)	3 (1.1)	0.27
Possible stent thrombosis	7 (1.5)	6 (3.3)	1 (0.4)	0.017
All stent thrombosis	24 (5.3)	15 (8.3)	9 (3.3)	0.018
MACE	71 (15.7)	35 (19.7)	22 (13.1)	0.06

Values are n (%). The follow-up was available in 453 of 461 patients.

MACE = major adverse cardiovascular events; TLR = target lesion revascularization; other abbreviations as in Table 1.
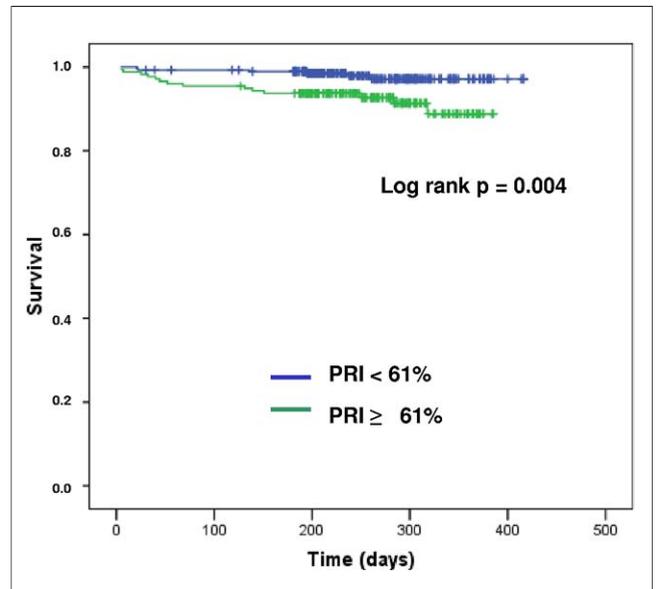
Figure 2. Kaplan-Meier Analysis for the Probability of Cardiac Survival According to the PRI 61% Threshold

Blue line = patients with platelet reactivity index (PRI) <61%; **green line** = patients with PRI ≥61%. Abbreviations as in Figure 1.

Discussion

The data from this prospective registry suggest that LR to clopidogrel identified by VASP phosphorylation analysis in a high-risk population is indicative of increased cardiovascular mortality after PCI. Our results underline the rele-
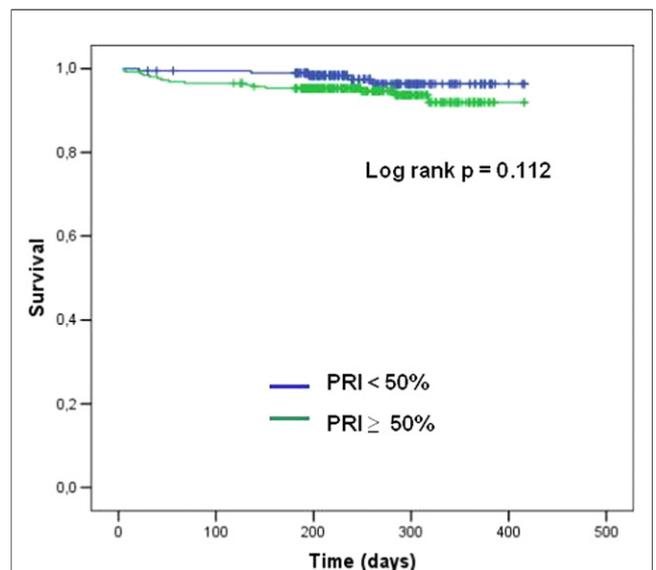
Figure 3. Kaplan-Meier Analysis for the Probability of Cardiac Survival According to the PRI 50% Threshold

Blue line = patients with platelet reactivity index (PRI) <50%; **green line** = patients with PRI ≥50%. Abbreviations as in Figure 1.

Table 5. Univariate Analysis for Prediction of Cardiac Death, Total Death, and MACE

Variable	Cardiac Death		Total Death		MACE	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Age	1.02 (0.98–1.06)	0.29	1.02 (0.98–1.05)	0.30	1.01 (0.99–1.03)	0.38
Sex	1.97 (0.81–4.83)	0.14	1.78 (0.78–4.06)	0.17	0.98 (0.57–1.67)	0.94
Diabetes mellitus	2.16 (0.89–5.24)	0.09	1.83 (0.82–4.10)	0.14	1.38 (0.86–2.21)	0.19
Obesity	1.25 (0.52–3.0)	0.62	1.27 (0.57–2.83)	0.56	1.02 (0.64–1.63)	0.94
Hypertension	0.81 (0.34–1.94)	0.63	0.59 (0.26–1.32)	0.20	0.92 (0.58–1.47)	0.74
Dyslipidemia	0.48 (0.19–1.91)	0.11	0.63 (0.28–1.43)	0.27	0.65 (0.40–1.04)	0.07
Smoking	0.86 (0.35–2.07)	0.73	0.75 (0.33–1.69)	0.49	1.36 (0.85–2.17)	0.20
History of stroke	1.47 (0.20–11.03)	0.71	1.18 (1.16–8.77)	0.87	1.90 (0.69–5.22)	0.22
History of STEMI	1.16 (0.39–3.46)	0.79	0.94 (0.32–2.74)	0.90	0.77 (0.40–1.52)	0.46
History of NSTEMI	2.82 (0.83–9.62)	0.098	2.22 (0.66–7.47)	0.20	1.41 (0.61–3.27)	0.42
STEMI	1.41 (0.58–3.45)	0.45	1.49 (0.66–3.35)	0.34	1.37 (0.85–2.21)	0.19
NSTEMI	1.35 (0.55–3.30)	0.51	1.46 (0.65–3.29)	0.36	0.87 (0.52–1.45)	0.59
Unstable angina	1.83 (0.54–6.27)	0.33	1.54 (0.46–5.21)	0.48	1.99 (1.02–3.91)	0.045
Stable angina	0.03 (0.00–2.11)	0.11	0.03 (0.001–1.44)	0.08	0.43 (0.22–0.84)	0.014
Killip III to IV	9.21 (3.67–23.14)	0.0001	9.16 (3.88–26.63)	0.0001	3.06 (1.56–5.97)	0.001
Three-vessel disease	1.24 (0.50–3.12)	0.64	0.97 (0.40–2.35)	0.95	1.66 (1.03–2.66)	0.037
LAD	1.80 (0.60–5.38)	0.29	1.38 (0.55–3.47)	0.50	1.23 (0.73–0.73)	0.44
DES	4.81 (1.59–14.61)	0.006	3.16 (1.27–7.86)	0.01	1.65 (1.02–2.68)	0.043
Stent length >30 mm	1.65 (0.22–12.33)	0.63	1.33 (0.18–9.86)	0.78	1.94 (0.70–5.33)	0.20
Creatinine clearance (ml/min)	0.96 (0.94–0.98)	<0.001	0.97 (0.95–0.98)	<0.001	0.99 (0.98–1.00)	0.31
CRP	1.008 (1.0–1.025)	0.033	1.007 (1.001–1.015)	0.059	1.004 (0.10–1.01)	0.12
PRI ≥61%	3.65 (1.40–9.50)	0.008	2.71 (1.18–6.20)	0.02	1.54 (0.97–2.46)	0.07
PRI ≥50%	2.22 (0.80–6.12)	0.12	1.86 (0.77–4.51)	0.17	1.36 (0.83–2.22)	0.22
PRI ≥69%	2.42 (1.00–5.85)	0.049	1.85 (0.80–4.24)	0.15	1.14 (0.67–1.92)	0.64

CI = confidence interval; DES = drug-eluting stent(s); HR = hazard ratio; other abbreviations as in Tables 1 to 4.
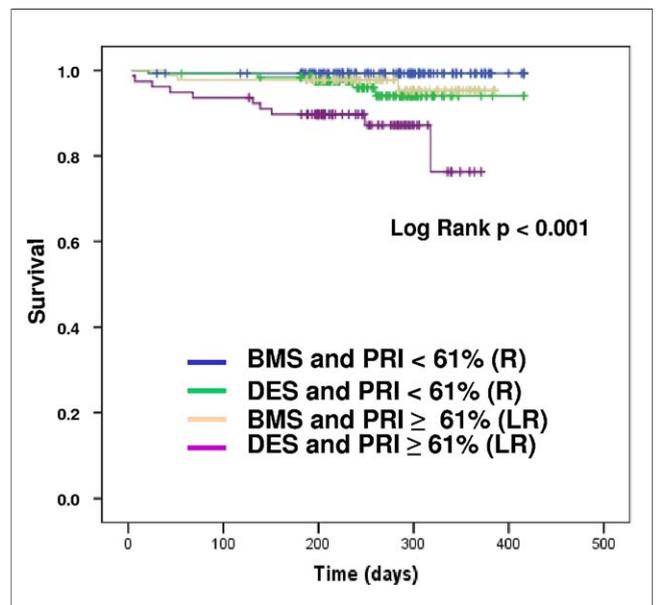
vance of the 61% threshold for PRI to identify patients at higher risk of cardiovascular mortality and total stent thrombosis. The deleterious impact of LR to clopidogrel on cardiovascular outcome seems higher in patients treated with DES. Finally, LR to clopidogrel seems to be associated with diabetes mellitus and obesity.

Monitoring of the P2Y$_{12}$ inhibition by clopidogrel: value of VASP assay. Clopidogrel effect depends on the generation of active metabolites to achieve a degree of blockade of the P2Y$_{12}$ receptor that substantially blunts the extent of P2Y$_{12}$-mediated amplification of platelet stimulation. The hepatic conversion of clopidogrel in active metabolites involves the enzymatic activities of several P450 cyto-

Table 6. Multivariate Analysis for Cardiac Death Prediction

Variable	Cardiac Death	p Value
DES	5.73 (1.40–23.43)	0.015
Creatinine clearance	0.95 (0.93–0.98)	<0.001
CRP	1.01 (1.001–1.019)	0.024
PRI ≥61%	4.00 (1.08–14.80)	0.037

Values are hazard ratio (95% confidence interval).
Abbreviations as in Tables 3 and 5.

Log Rank p < 0.001

BMS and PRI < 61% (R)
DES and PRI < 61% (R)
BMS and PRI ≥ 61% (LR)
DES and PRI ≥ 61% (LR)

Figure 4. Kaplan-Meier Analysis of Cardiac Survival With Respect to Platelet Responsiveness to Clopidogrel and Stent Type

BMS = bare-metal stent(s); DES = drug-eluting stent(s); PRI = platelet reactivity index.

chromes (4,6). Among them, the CYP2C19 has an important role. Indeed, the carriage of at least 1 copy of the CYP2C19*2 allele was recently associated with decreased concentrations of active metabolite (18) and was shown to greatly increase the risk of adverse thrombotic events in young survivors of acute myocardial infarction (4). Although the concept of "genotyping" seems somewhat appealing, it is difficult to translate it into clinical practice, because the results of such assessment would be unlikely to be available during the early high thrombotic risk period after ACS. In addition, the possible impact of the redundancy described among the cytochromes deserves further clarification, and the impact of CYP2C19 on the metabolism of more potent thienopyridines such as prasugrel might be limited (18). Another approach is to directly measure the active metabolite concentrations in the plasma, but this remains technically challenging. Consequently, a more suitable approach to assess biological response to clopidogrel is to provide a pharmacodynamic assessment of P2Y$_{12}$ pathway blockade (16). Platelet aggregometry presents several limitations, including but not limited to: 1) wide interindividual variability of the response to ADP and subsequent large overlap between clopidogrel-treated and -untreated patients; 2) lack of specificity for the P2Y$_{12}$ pathway; 3) the need for a large sample volume; 4) the complexity of the pre-analytic phase; and 5) the wide variability of the results among laboratories (7,8,16). For these reasons, the VASP phosphorylation assay is considered the gold standard to monitor thienopyridines therapy.

Impact of P2Y$_{12}$ inhibition by clopidogrel on cardiovascular outcome. Several studies have recently highlighted the deleterious impact of LR to clopidogrel on stent thrombosis (13,14), post-stent ischemic events (7), and peri-procedural infarction (8). These studies have been performed in different subgroups of patients undergoing PCI, including patients with STEMI or NSTEMI and patients undergoing elective PCI. In the present work, total death and cardiovascular death occurred in 5.7% and 4.4% of patients, respectively. These mortality rates were higher than those reported in the study by Bonello et al. (17) (2.7% and 2.1%, respectively). However, it should be emphasized that the latter study had enrolled lower-risk patients (34% stable angina, 40% silent ischemia, and 26% low-risk NSTEMI). By comparison, our study population was mainly ACS patients (32.8% STEMI, 33.4% NSTEMI). The rates of cardiovascular events reported in the present work are in line with those of a recent meta-analysis (19). Moreover, the mortality rate among patients with LR to clopidogrel is in accordance with a recent publication by Marcucci et al. (20). The impact of the use of different methodologies in the evaluation of clopidogrel effect on platelet function deserves further investigation. In a recent study, Paniccia et al. (21) reported a strong correlation between platelet aggregometry and the VerifyNow cartridge but only weak correlation

between VASP-related PRI value and values obtained by the VerifyNow assay. These 2 tests do not measure the same thing: light transmission aggregometry as well as the automated VerifyNow device measure the global aggregatory response of platelets stimulated by ADP. The latter integrates numerous factors, including receptor activation, intracellular signaling, integrin activation, and platelet secretion. In contrast, the VASP phosphorylation assay only measures the P2Y$_{12}$ responsiveness to ADP on a very proximal signaling event (16).

VASP threshold for the prediction of cardiovascular events. In our study, the analysis of receiver-operator characteristic curve for the prediction of cardiac mortality with the VASP assay determined an optimal PRI cutoff of 61%. Briefly, P2Y$_{12}$ inhibition by clopidogrel follows a Gaussian distribution; the "biological" threshold was defined in our lab as the mean PRI value minus 2 SD in patients untreated by clopidogrel. According to this biological definition, almost 30% of patients receiving clopidogrel standard regimen (300 mg/75 mg) treatment were undistinguishable from untreated patients (3). The optimal PRI threshold is still a matter of debate, with regard to the "biological threshold (PRI: 69%)" (3) in comparison with the "clinical efficacy threshold" (14). Barragan et al. (14) first reported, in a retrospective study, that a PRI value higher than 50% might be indicative of subacute stent thrombosis. In a pragmatic and empiric approach, the threshold of "clinical" efficacy was therefore set at 50%. The clinical relevance of this cutoff was later confirmed in a prospective study according to a very high negative predictive value for MACE prediction after PCI (17). Moreover, Blindt et al. (15) observed that a PRI value above 48% was the only independent predictor of stent thrombosis in high-risk PCI. In rats, Schumacher et al. (22) showed that a 50% PRI value corresponded to a nearly 90% P2Y$_{12}$ receptor blockage. However, in the present work, the 50% cutoff was not predictive of total or cardiac mortality after PCI, whereas the threshold of 69% was slightly associated with cardiovascular mortality. At follow-up, the 61% cutoff was indicative of both total and cardiac mortality as well as possible and total stent thrombosis. Overall, our study validates for the first time the usefulness of the VASP assay after PCI to predict hard end points.

Impact of LR to clopidogrel in patients treated with DES on cardiovascular outcome. In our registry, the implantation of DES was associated with an increased risk of total death and cardiac death at 9-month follow-up. Interestingly, the deleterious impact of LR to clopidogrel on cardiovascular outcome seems higher in patients treated with DES. This result should be taken with great caution, given the inherent limitation of registry data, the limited size of the cohort, and the possible contribution of unmeasured confounding factors. Of note, increased mortality with DES as compared with BMS has already been reported in very large nationwide registries (23,24). Our result is also consistent with a

recent report by Trenk et al. (5). In this study, LR to clopidogrel was particularly deleterious in patients treated with DES (5). One might hypothesize that the behavior of patients with LR to clopidogrel is comparable to the behavior of untreated patients. In the large Duke registry, Eisenstein et al. (25) observed that patients with DES under clopidogrel treatment at 12 months have a lower rate of death at 24 months. This relationship between long-term clopidogrel treatment and long-term death was not observed among patient with BMS (25). Likewise, the cessation of clopidogrel treatment in patients with DES is associated with increased death and MACE at 6-, 12-, and even 24-month follow-up (26). These findings support the view that the delayed endothelium healing after DES requires a more robust and sustained platelet inhibition than after BMS placement (5). Altogether, these data strongly suggest that the evaluation of platelet responsiveness to clopidogrel could be useful for the management of patients treated with DES.

Impact of diabetes mellitus and obesity on P2Y$_{12}$ pathway blockade. We observed a significantly higher proportion of diabetic and obese patients among the LR group. Low responsiveness to antiplatelet drugs has already been reported in diabetic persons (27), and the mechanism for this remains poorly understood. Possible hypotheses include the following: 1) nonenzymatic glycation of platelet membrane proteins that might alter receptor functions and/or signaling pathways; 2) overexpression of platelet integrins such as glycoprotein IIb/IIIa; 3) increased thromboxane A$_2$ synthesis; 4) decreased nitric oxide and antioxidant production; 5) disturbed calcium homeostasis; 6) impairment of platelet inhibition by insulin, through P2Y$_{12}$ pathway, due to insulin resistance; and 7) increased fibrinogen levels (27,28). Angiolillo et al. (29) showed that patients with diabetes exhibit higher platelet reactivity and reduced sensitivity to clopidogrel. Moreover, high platelet reactivity in diabetic patients taking dual antiplatelet therapy was associated with a higher risk of long-term adverse cardiovascular events. In agreement with their finding, we found a higher proportion of LR among diabetic patients. Some authors have suggested that low platelet response to thienopyridines observed in diabetic patients could mainly result in lower levels of circulating active metabolites, whereas ex vivo, the extent of platelet inhibition by thienopyridines was shown to be comparable in type 2 diabetes mellitus and nondiabetic patients (30).

In agreement with previous works, our study confirms that obesity is associated with lower platelet response to clopidogrel (8,28,31). The relationship between obesity and LR to clopidogrel is complex and might be related to insufficient dosage of drug or adipose tissue-induced inflammation. Our data are also in line with the observation in the TRITON–TIMI 38 study (TRial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibi-

tioN with Prasugrel–Thrombolysis In Myocardial Infarction 38), in which low body mass index was associated with more bleeding complications. Relative overdosing of antithrombotic medications together with higher platelet inhibition activation observed in lean patients were proposed to explain this adverse outcome (32).

Study limitations. Single time point laboratory assessment represents a common limitation to most studies assessing the prognostic implication of platelet function. In addition, the timing of the VASP analysis was not fully homogeneous. The VASP testing was not compared with other techniques of platelet function. The potential impact of higher doses of clopidogrel has not been investigated in LR patients. We could not exclude that a disproportionate noncompliance to clopidogrel in the LR group could have impaired the cardiovascular outcome. The adjudication of cardiovascular events was not realized by an independent committee. However, this adjudication was realized by 2 physicians not aware of the PRI values. Given the relatively low number of cardiovascular deaths (20), the interpretation of the multivariate analysis including 4 variables should be interpreted with caution. As with similar evaluation of registry data, there are inherent limitations mainly with known or unknown confounding factors.

Conclusions

The results of the present prospective registry suggest that low responsiveness to clopidogrel, assessed by flow cytometric VASP phosphorylation defined as a PRI ≥61%, is an independent predictor of all-cause mortality, cardiovascular death, and possible and total stent thrombosis after PCI. The impact of LR to clopidogrel was particularly deleterious in patients treated with DES.

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