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Prospective Evaluation of On-Clopidogrel Platelet Reactivity Over Time in Patients Treated With Percutaneous Coronary Intervention

Relationship With Gene Polymorphisms and Clinical Outcome

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Objectives This study sought to investigate the evolving pattern over time of on-clopidogrel platelet reactivity (PR) and its relationship with genotype and clinical outcomes after percutaneous coronary intervention.

Background Whether on-clopidogrel PR and role of genotype differ over time is unknown.

Methods On-clopidogrel PR before percutaneous coronary intervention, and 1 and 6 months thereafter via VerifyNow P2Y12 (Accumetrics Inc., San Diego, California), *CYP2C19*2, *17, CYP3A5*3,* and *ABCB1* polymorphisms were evaluated in 300 patients. Death, stroke, myocardial infarction, and bleedings were assessed up to 1 year.

Results On-clopidogrel PR varied significantly over time, being higher at baseline than at 1 and 6 months after. From baseline to 1 month, 83 of 300 patients varied their response status. This was mainly due to baseline poor responders becoming full responders (75 of 83). Genotype justifies roughly 18% of this trend. *CYP2C19*2* and **17* influence on PR was consistent over time, whereas that of *ABCB1* appeared of greater impact at baseline. On-clopidogrel PR at 1 month independently best predicts ischemic and bleeding events. We found a therapeutic window (86 to 238 $P2Y_{12}$ reactivity units) with a lower incidence of both ischemic and bleeding complications. A risk score was created by combining genotype (*ABCB1* and *CYP2C19*2*), baseline PR, and creatinine clearance to predict 1-month poor responsiveness and 1-year poor prognosis.

Conclusions In patients at steady state for clopidogrel undergoing percutaneous coronary intervention, PR decreases from baseline to 1 month. Genotype influences ≈18% of this trend. On-clopidogrel PR at 1 month is the strongest predictor of adverse outcomes, and this can be predicted by combining genotype to baseline phenotype and clinical variables. (J Am Coll Cardiol 2011;57:2474–83) © 2011 by the American College of Cardiology Foundation

Oral $P2Y_{12}$ inhibitors are a crucial pharmacologic tool in modern cardiovascular practice. Response to clopidogrel varies widely among patients, and those with a high residual on-clopidogrel platelet reactivity (PR) undergoing percutaneous coronary intervention (PCI) are at a greater risk for death, myocardial infarction, and stent thrombosis (1–5). Clinical, genetic, and cellular factors are involved in the clopidogrel response variability (6–11). Particularly, gene polymorphisms of proteins involved in absorption and metabolism of clopidogrel account for approximately 15% to 20% of the variation and are strongly related to poor prognosis in patients taking clopidogrel (7–10). These results have been largely provided by studies with a single phenotype assessment evaluation before or soon after PCI (1–11). Therefore, whether clopidogrel response varies throughout follow-up and whether the role of gene polymorphism differs over time is unknown.

See page 2484

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We sought: 1) to evaluate whether clopidogrel response in patients treated with PCI differs through follow-up; and 2) to assess the relationship of on-clopidogrel PR at different time points with gene polymorphisms and clinical outcome.

Methods

Patients. Individuals eligible for enrolment were patients undergoing PCI for ischemic heart disease in our center from December 2008 to May 2009 (Fig. 1). Exclusion criteria were known contraindication to dual antiplatelet therapy, history of chronic inflammatory disease, steroidal and non-steroidal anti-inflammatory drugs use, glycoprotein IIb/IIIa inhibitors administration before coronary artery angiography, significant bleeding, and/or major surgery within 4 weeks. Subjects were also excluded if they were admitted with ST-segment elevation acute coronary syndrome. Informed written consent was obtained from all patients, and the study was approved by the local ethics review board.

Study drugs and protocol. All patients were treated with aspirin (300 mg as loading dose [LD] at hospital admission, followed by 100 mg die, independently to previous or not chronic use). Clopidogrel 600 mg was given as LD at least 12 h before PCI. After intervention, clopidogrel 75 mg/day was continued for 12 months. Anticoagulant and glycoprotein IIb/IIIa inhibitors treatment was administered at the interventionalist's discretion. Of note, only 11 (3%) patients received glycoprotein IIb/IIIa inhibitors during PCI. Blood samples were drawn at baseline (just before PCI and administration of interventional therapy) and at 1 and 6 months after PCI.

Platelet function testing and clopidogrel poor response definition. To evaluate on-clopidogrel PR, we used VerifyNow (Accumetrics Inc., San Diego, California). Specific assays to test clopidogrel (VerifyNow P2Y12) are available. The results are expressed in $P2Y_{12}$ reaction units (PRU). Clopidogrel poor response was defined as a PRU value ≥235 (3).

Gene polymorphisms. Genomic deoxyribonucleic acid was extracted from whole-blood samples by Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, Wisconsin). Single nucleotide polymorphism (rs4244285, rs12248560, rs776746, and rs1045642) were genotyped by allelic discrimination assay (TaqMan Assays, Applied Biosystems, Foster City, California) on the Chromo4 Real-Time PCR System detection (Bio-Rad Laboratories, Hercules, California) using TaqMan Universal Master Mix. The amplification protocol was as follow: 50°C for 2 min, 95°C for 10 min, and 40 cycles at 95°C for 15 s, 60°C for 1 min. The data were analyzed by Opticon Monitor 3.1 software (Bio-Rad Laboratories).

Endpoints of the study. Our primary analysis compared the incidence of clopidogrel poor responders at baseline versus 1 month. Secondary assessments include: 1) incidence of clopidogrel poor responders at 1 month versus 6 months; 2) rate of death, myocardial infarction, and stroke; 3) occurrence of definite and probable stent thrombosis according to the Academic Research Consortium classification; 4) rate of bleedings according to TIMI (Thrombolysis In Myocardial Infarction) classification and BleedScore (12). Myocardial infarction is defined as the recurrence of ischemic symptoms and an elevation of creatine kinase-myocardial band ≥3× upper limit of normal. We reported all clinical events (ischemic and bleeding) after 1 month and up to 1 year of follow-up. Patients with adverse events during the first month were excluded.

Abbreviations and Acronyms

- **CI** = confidence interval
- **HR** = hazard ratio
- **LD** = loading dose
- **NSTEACS** = non–ST-segment elevation acute coronary syndrome
- **OR** = odds ratio
- **PCI** = percutaneous coronary intervention
- **PR** = platelet reactivity
- **PRU** = $P2Y_{12}$ reaction unit
- **ROC** = receiver-operator characteristic
- **TIMI** = Thrombolysis In Myocardial Infarction

Sample size and statistical analysis. We hypothesized that the number of clopidogrel poor responders would decrease by 50% after 1 month. Assuming a percentage of poor responders of 25% at baseline (1–3,5), at least 275 patients were required (alpha and beta of 0.05). Continuous data are presented as mean ± SD and were tested for normal distribution with the Kolmogorov-Smirnov test. Normally distributed values were compared by *t* test and 1-way analysis of variance; otherwise, the Mann-Whitney *U* and Kruskal-Wallis tests were used. Platelet function data obtained with VerifyNow were normally distributed. A linear mixed model was used to quantify changes of on-clopidogrel PR over time while integrating the role of baseline, genetic, and procedural characteristics. Categorical variables were summarized in terms of number and percentages and were compared by using 2-sided Fisher exact test. The exact version of McNemar test was used to compare response status at different time points. Survival curves were generated by the Kaplan-Meier method, and differences in survival between subgroups were evaluated using the log-rank test. We applied univariable and multivariable Cox proportional hazard regression models to evaluate the relation between the on-clopidogrel PR and the incidence of the composite clinical endpoint. In our multivariable model, we adjusted for a broad range of potential confounders, including clinical, angiographic, and genetic characteristics. To reduce the impact of data overfitting, we followed a stepwise modeling approach by applying a variable selection using the Akaike information criterion and a bootstrapped variance estimation of the final model. To compare the ability to discriminate between patients with and without events of baseline versus 1-month PR receiver-operator characteristic (ROC) curve analysis is performed. To obtain a model for the prediction of 1-month poor responsiveness status, the classification and regression tree method, an empirical, statistical technique based on recursive partition-

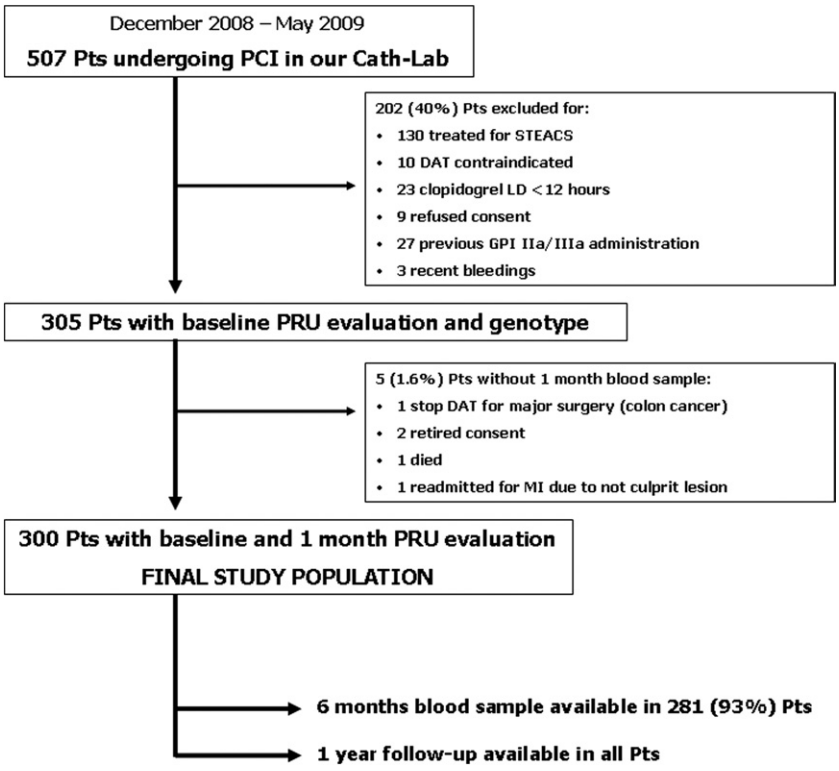

Figure 1 Study Flow Chart

The flow chart shows the progression of the study from patient enrollment to 1-year follow-up. Cath-Lab = catheterization laboratory; DAT = dual antiplatelet therapy; GPI = glycoprotein inhibitor; LD = loading dose; PCI = percutaneous coronary intervention; PRU = $P2Y_{12}$ reactivity unit; Pts = patients; STEACS = ST-segment elevation acute coronary syndrome.

ing analysis, was chosen. The classification and regression tree algorithm was used to analyze potential baseline characteristics of interest and to build up a decision tree composed of progressive binary splits that were able to predict 1-month poor response. Finally, the predictive value of this model was then assessed by determination of 1-year composite endpoint odds ratios (ORs) and 95% confidence intervals (CIs) between risk groups. A 2-sided value of $p < 0.05$ was considered significant. All analyses were performed with Statistica 8 (Statsoft Inc., Tulsa, Oklahoma), MedCalc 11.2.1 (MedCalc Software, Mariakerke, Belgium), and R-language (R Foundation, Vienna, Austria).

Results

Study population and genotype. Our study population includes 300 patients (Fig. 1). No differences were observed in baseline characteristics between various groups (Table 1). Genotype frequencies were reported in Table 1. *CYP2C19*2* and **17* were in linkage disequilibrium.

On-clopidogrel PR and clopidogrel poor responders. According to our pre-specified definition, 107 (36%), 40 (13%), and 38 (13%) patients are clopidogrel poor responders at baseline, 1 month, and 6 months, respectively ($p < 0.01$ comparing baseline vs. 1 month and baseline vs. 6 months). As shown in Figure 2, from baseline to 1 month, 83 of 300 patients changed their responsiveness status (27%, 95% CI: 23% to 33%). Fifty-five of these were admitted for non–ST-segment elevation acute coronary syndrome (NSTEACS), whereas 28 were admitted for stable disease ($p = 0.2$). This is due principally to poor responders at baseline becoming full responders after 1 month (75 of 83, 90%, 95% CI: 82% to 96%). On the contrary, the variations observed between 1 and 6 months were minimal, being limited to only 2 patients (0.7%, 95% CI: 0.1% to 2%). On-clopidogrel PR, assessed as a continuous variable, was higher at baseline (190 ± 97) than at 1 (147 ± 85, $p < 0.01$) and 6 months (146 ± 85, $p < 0.01$), whereas no significant change was observed between 1 and 6 months ($p = 0.9$). Although this PR decrease from baseline to 1 month pattern was more pronounced in patients admitted for NSTEACS (209 ± 96 vs. 159 ± 87 vs. 156 ± 89, $p < 0.01$), it was also present and significant in patients with stable disease (161 ± 91 vs. 128 ± 78 vs. 130 ± 77, $p < 0.01$).

Clinical and genetic determinants of on-clopidogrel PR. Both clinical and genetic factors displayed a significant influence on-clopidogrel PR. Age, diabetes, creatinine clearance, and admission for NSTEACS emerged as inde-

Table 1 Characteristics of the Study Population

		ABCB1, C3435T		*G681A, CYP2C19*2*		*A6986G, CYP3A5*3*		*C806T, CYP2C19*17*	
	All (n = 300)	CC (n = 69)	T carriers (n = 231)*	GG (n = 219)	A Carriers (n = 81)†	GG (n = 263)	A Carriers (n = 37)‡	CC (n = 198)	T Carriers (n = 102)§
Age, yrs	66 ± 13	65 ± 9	66 ± 14	65 ± 14	68 ± 9	66 ± 12	66 ± 18	66 ± 13	66 ± 13
Male	231 (77)	56 (81)	175 (76)	165 (75)	66 (81)	205 (78)	26 (70)	148 (75)	83 (81)
BMI, kg/m^2	27 ± 4	27 ± 3	27 ± 4	27 ± 4	28 ± 4	27 ± 4	27 ± 5	27 ± 4	28 ± 4
Diabetes	71 (24)	16 (22)	55 (24)	53 (24)	18 (22)	63 (24)	8 (22)	46 (23)	25 (24)
Hypertension	215 (72)	46 (66)	169 (73)	159 (73)	56 (69)	187 (71)	28 (76)	145 (73)	70 (69)
Hyperlipidemia	153 (51)	33 (48)	120 (51)	114 (52)	39 (48)	134 (51)	19 (51)	103 (52)	50 (49)
Current cigarette use	71 (24)	19 (27)	52 (23)	54 (25)	17 (21)	65 (24)	6 (16)	51 (25)	20 (20)
Prior MI	81 (27)	17 (27)	64 (28)	55 (25)	26 (32)	71 (27)	10 (27)	52 (26)	29 (28)
Prior PCI	47 (16)	9 (13)	38 (16)	33 (15)	14 (17)	40 (15)	7 (19)	29 (15)	18 (18)
Prior CABG	34 (11)	7 (10)	27 (12)	28 (13)	6 (8)	30 (11)	4 (11)	19 (10)	15 (14)
Admission for NSTEACS	184 (61)	39 (56)	145 (63)	136 (62)	48 (59)	160 (61)	24 (65)	123 (62)	61 (60)
Angiographic/laboratory data									
Multivessel	186 (62)	45 (65)	141 (61)	134 (61)	52 (64)	162 (62)	23 (65)	127 (64)	59 (58)
Multivessel PCI	109 (36)	27 (39)	82 (35)	82 (37)	27 (33)	95 (36)	14 (38)	70 (35)	39 (38)
Drug-eluting stent	214 (71)	49 (71)	165 (71)	164 (75)	50 (62)	186 (71)	28 (76)	139 (70)	75 (73)
LVEF, %	51 ± 11	52 ± 9	51 ± 11	52 ± 10	50 ± 12	51 ± 11	51 ± 11	50 ± 11	51 ± 10
Creatinine clearance, ml/min	82 ± 52	90 ± 63	80 ± 42	84 ± 59	78 ± 40	83 ± 55	79 ± 42	84 ± 61	79 ± 41
Medical therapy									
Time: clopidogrel to PCI, h	22 ± 8	22 ± 8	21 ± 10	21 ± 10	22 ± 9	22 ± 9	22 ± 8	21 ± 10	22 ± 8
Proton pump inhibitor	158 (53)	34 (49)	124 (54)	112 (52)	46 (56)	138 (52)	20 (54)	101 (51)	57 (55)
Aspirin at 6 months	298 (99)	68 (99)	230 (99)	218 (99)	81 (99)	261 (99)	37 (100)	197 (99)	101 (99)
Clopidogrel at 6 months	290 (97)	67 (97)	228 (99)	213 (97)	77 (95)	256 (97)	36 (97)	192 (97)	98 (96)
Aspirin at 1 year	285 (95)	66 (96)	219 (95)	208 (95)	77 (95)	250 (95)	35 (94)	188 (95)	97 (95)
Clopidogrel at 1 year	263 (88)	59 (86)	204 (88)	192 (88)	71 (87)	230 (87)	33 (89)	173 (87)	90 (88)

Values are mean ± SD or n (%). There were *74 (25%) *ABCB1* TT homozygotes; †5 (2%) *CYP2C19*2* AA homozygotes; ‡2 (1%) *CYP3A5*3* AA homozygotes; and §17 (6%) *CYP2C19*17* TT homozygotes.
BMI = body mass index; CABG = coronary artery bypass graft; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NSTEACS = non–ST-segment elevation acute coronary syndrome; PCI = percutaneous coronary intervention.

pendent predictors of on-clopidogrel PR variation overtime. Age, diabetes, and creatinine clearance had a homogenous impact over time, whereas admission for NSTEACS showed a higher influence at baseline (≈15%) as compared with 1 month (≈10%, p = 0.07) and 6 months (≈7%, p = 0.02). Regarding gene polymorphisms, *CYP2C19*2, CYP2C19*17,* and *ABCB1* justified altogether ≈18% of PR variation (6.6%, 5.2%, and 6.7%, respectively). Interestingly, the *CYP2C19*2* and **17* influence appeared constant over time, whereas that of *ABCB1* was higher at baseline (9%) and thereafter decreased gradually (6% at 1 month, p = 0.09; 5% at 6 months, p = 0.04) (Table 2).

Clinical outcomes. ISCHEMIC ADVERSE EVENTS. The composite ischemic endpoint occurred in 21 (7%) patients (6 deaths, 13 reinfarctions, 2 strokes) (Table 3). Four (1.3%) stent thromboses were observed (Table 3). At univariate analysis, multivessel PCI, admission for NSTEACS, left ventricular ejection fraction, *ABCB1* T and *CYP2C19*2* A alleles and on-clopidogrel PR (evaluated both at baseline and after 1 month) were associated to adverse events. After multivariable analysis, only admission for NSTEACS (hazard ratio [HR]: 3.5, 95% CI: 1.2 to 9.6, p = 0.04) and on-clopidogrel PR remained independent outcome predictors. On-clopidogrel PR was a stronger predictor when evaluated at 1 month (HR: 1.02, 95% CI: 1.012 to 1.026, p < 0.01, as continuous variable; HR: 28.5, 95% CI: 8 to 104, p < 0.01, as categorical variable according to the established cutoff) as compared with baseline assessment (HR: 1.01, 95% CI: 1.002 to 1.029, p = 0.04; HR: 3.1, 95% CI: 1.3 to 7.3, p = 0.02, respectively). To further display our finding graphically, survival curves were constructed that showed that poor responders at 1 month had suboptimal outcomes, whereas poor responders at baseline who subsequently became full responders at 1 month had an excellent prognosis with a remarkably low event rate (Fig. 3). Finally, at ROC analysis, the ability of on-clopidogrel PR to discriminate outcomes was significantly better when assessed at 1 month versus that recorded at baseline (differences between areas: 0.21, 95% CI: 0.05 to 0.33, p < 0.01) (Fig. 4).

BLEEDING ADVERSE EVENTS. We observed 19 (6.3%) TIMI bleeding events, 4 (1.3%) of which were major. Regarding BleedScore, 5 alarming, 21 internal, and 30 superficial bleedings occurred. At univariate analysis, age, creatinine clearance, *CYP2C19*17*, and on-clopidogrel PR (both at baseline and at 1 month) predicted composite bleeding endpoints. After multivariable analysis, age (HR: 1.03, 95% CI: 1.02 to 1.04, p = 0.04, as continuous variable), *CYP2C19*17* genotype (HR: 2.3, 95% CI: 1.03 to

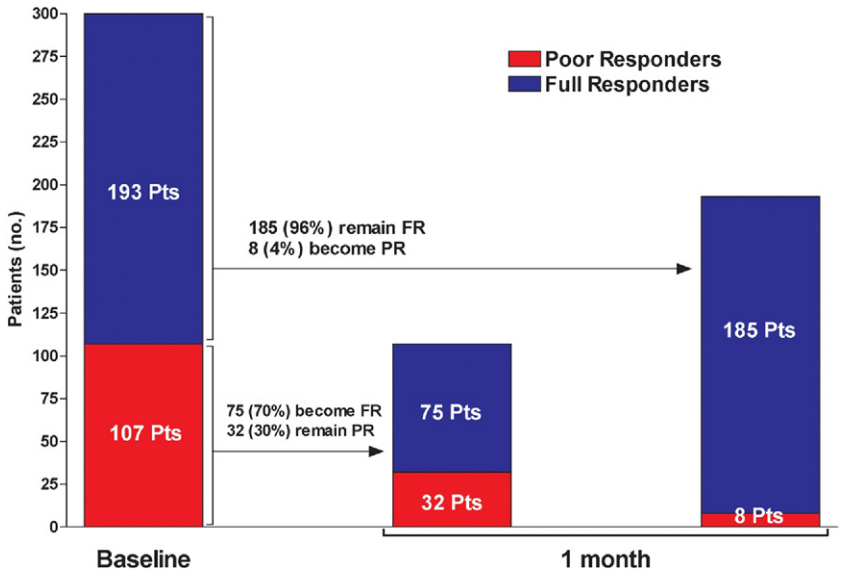

Figure 2 Responsiveness Status at Baseline and After 1 Month

Bar graph shows the responsiveness status at baseline and after 1 month. $p < 0.01$ comparing baseline versus 1-month response status; $p < 0.01$ comparing baseline versus 6-month response status; $p = 0.9$ comparing 1-month versus 6-month response status. Patients (Pts) who are poor responders (PRs) are **red** and full responders (FRs) are **blue**.

5.3, p = 0.03), and on-clopidogrel PR measured at 30 days (HR: 0.94, 95% CI: 0.93 to 0.95, p = 0.04, as continuous variable) emerged as independent predictors. As for ischemic endpoint, ROC analysis confirmed that 1-month on-clopidogrel PR values better discriminate bleeding complications (differences between areas: 0.2, 95% CI: 0.1 to 0.3, p < 0.01) (Fig. 4). Finally, in Figure 5, we showed the combined incidence of ischemic and bleeding events across groups stratified for best cutoff of 1-month on-clopidogrel PR.

Table 2 Genotype and On-Clopidogrel PR Values

	Baseline			1 Month			6 Months*		
	n (%)	PRU	p Value	n (%)	PRU	p Value	n (%)	PRU	p Value
ABCB1, C3435T									
CC	69 (23)	159 ± 97	0.03	69 (23)	125 ± 84	0.01	68 (24)	128 ± 80	0.01
CT	157 (52)	187 ± 94		157 (52)	146 ± 82		145 (52)	143 ± 81	
TT	74 (25)	227 ± 91		74 (25)	170 ± 87		68 (24)	169 ± 95	
T carriers	231 (77)	200 ± 95	<0.01	231 (77)	153 ± 84	<0.01	213 (76)	152 ± 87	<0.01
*G681A, CYP2C19*2*									
GG	219 (73)	181 ± 97	<0.01	219 (73)	133 ± 81	<0.01	204 (73)	132 ± 81	<0.01
GA	76 (25)	216 ± 92†		76 (25)	182 ± 88†		73 (26)	180 ± 85†	
AA	5 (2)	236 ± 112†		5 (2)	221 ± 105†		4 (1)	218 ± 95†	
A carriers	81 (27)	216 ± 91	<0.01	81 (27)	185 ± 90	<0.01	77 (27)	183 ± 87	<0.01
*A6986G, CYP3A5*3*									
GG	263 (87)	188 ± 95	0.5	253 (87)	148 ± 83	0.6	246 (87)	148 ± 85	0.6
GA	35 (12)	205 ± 106		34 (12)	147 ± 93		33 (12)	143 ± 87	
AA	2 (1)	226 ± 129		2 (1)	167 ± 209		2 (1)	152 ± 208	
A carriers	37 (13)	207 ± 106	0.3	37 (13)	139 ± 97	0.5	35 (12)	134 ± 93	0.4
*C806T, CYP2C19*17*									
CC	198 (66)	203 ± 92	<0.01	198 (66)	163 ± 83	<0.01	185 (66)	163 ± 81	<0.01
CT	85 (28)	171 ± 100†		85 (28)	122 ± 79†		79 (28)	119 ± 83†	
TT	17 (6)	139 ± 100†		17 (6)	88 ± 88†		17 (6)	88 ± 93†	
T carriers	102 (34)	165 ± 101	<0.01	102 (34)	117 ± 81	<0.01	96 (34)	113 ± 85	<0.01

PRU data are mean ± SD. *Blood sample available in 281 patients. †p < 0.05 versus wild type.
PR = platelet reactivity; PRU = $P2Y_{12}$ reactivity unit.

Table 3 Incidence of Adverse Events (Ischemic and Bleeding) According Genotype and Clopidogrel Responsiveness Status

	Ischemic Events				Bleeding Events				
	Death	ST	Death + MI + stroke	p Value*	Minor + Major†	p Value†	Superficial§	Internal + Alarming§	p Value‖
ABCB1, C3435T (n)									
CC (69)	0 (0)	0 (0)	1 (1.5)	0.02	7 (10.1)	0.3	8 (11.5)	8 (11.6)	0.6
T carriers (231)	6 (2.5)	4 (1.7)	20 (8.6)		12 (5.2)		22 (9.5)	18 (7.8)	
*G681A, CYP2C19*2* (n)									
GG (219)	4 (1.8)	3 (1.4)	11 (5.0)	0.03	16 (7.3)	0.4	22 (10.1)	20 (9.1)	0.4
A carriers (81)	2 (2.5)	1 (1.2)	10 (12.3)		3 (3.7)		8 (9.8)	6 (7.4)	
*A6986G, CYP3A5*3* (n)									
GG (263)	4 (1.5)	3 (1.1)	17 (6.5)	0.3	16 (6.1)	0.7	26 (9.8)	23 (8.7)	0.6
A carriers (37)	2 (5.4)	1 (2.7)	4 (10.8)		3 (8.1)		4 (10.8)	3 (8.1)	
*C806T CYP2C19*17* (n)									
CC (198)	5 (2.5)	4 (2.0)	16 (8.1)	0.1	6 (3)	0.01	18 (9.1)	10 (5)	0.01
T carriers (102)	1 (1.0)	0 (0)	5 (4.9)		13 (12.7)		12 (11.7)	16 (16)	
Baseline response (n)									
Full R (193)	3 (1.5)	2 (1.0)	7 (3.6)	<0.01	13 (6.7)	0.8	21 (10.8)	18 (9.3)	0.3
Poor R (107)	3 (2.8)	2 (1.9)	14 (13)		6 (5.6)		9 (8.4)	8 (7.5)	
1-month response (n)									
Full R (260)	1 (0.4)	1 (0.4)	4 (1.5)	<0.01	18 (7)	0.5	27 (10.4)	25 (9.6)	0.2
Poor R (40)	5 (12.5)	3 (7.5)	17 (42.5)		1 (2.5)		3 (7.5)	1 (2.5)	

Values are n (%). *The p value is for the composite endpoint. †According to TIMI classification. ‡The p value for minor + major bleedings. §According to BleedScore classification. ‖The p value for alarming (1 intracranial, 4 needing transfusion) + internal (5 melena, 5 hematuria, 1 hematemesis, 10 epistaxis) bleedings.

MI = myocardial infarction; R = responder; ST = stent thrombosis; TIMI = Thrombolysis In Myocardial Infarction.

Score risk predicting 1-month poor responsiveness status. As poor response at 1 month was identified as the strongest predictor of adverse outcomes, we elaborated a score risk, combining baseline characteristics, to predict responsiveness status at 1 month. *ABCB1* and *CYP2C19*2* gene polymorphisms (CC and GG homozygotes vs. T and/or A carriers),

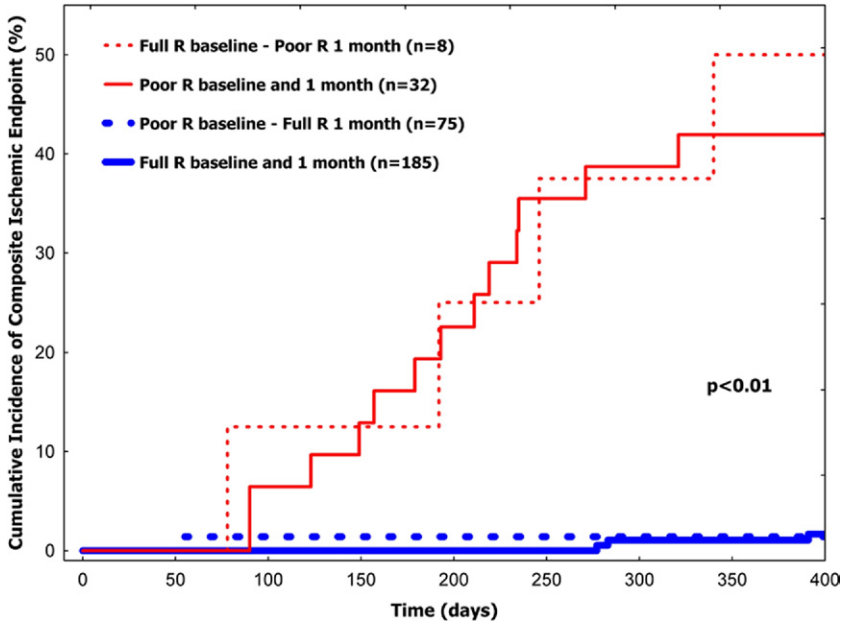

Figure 3 Cumulative Incidence of 1-Year Composite Ischemic Endpoint According to Responsiveness Status at Baseline and at 1 Month

Graph shows the cumulative incidence of 1-year composite ischemic endpoint. **Red dashed line** represents full responders (R) at baseline who became poor R at 1 month. **Solid red line** represents poor R at baseline and 1 month. **Dotted blue line** represents poor R at baseline who became full R at 1 month. **Solid blue line** represents full R at baseline and 1 month.

JACC Vol. 57, No. 25, 2011
June 21, 2011:2474–83

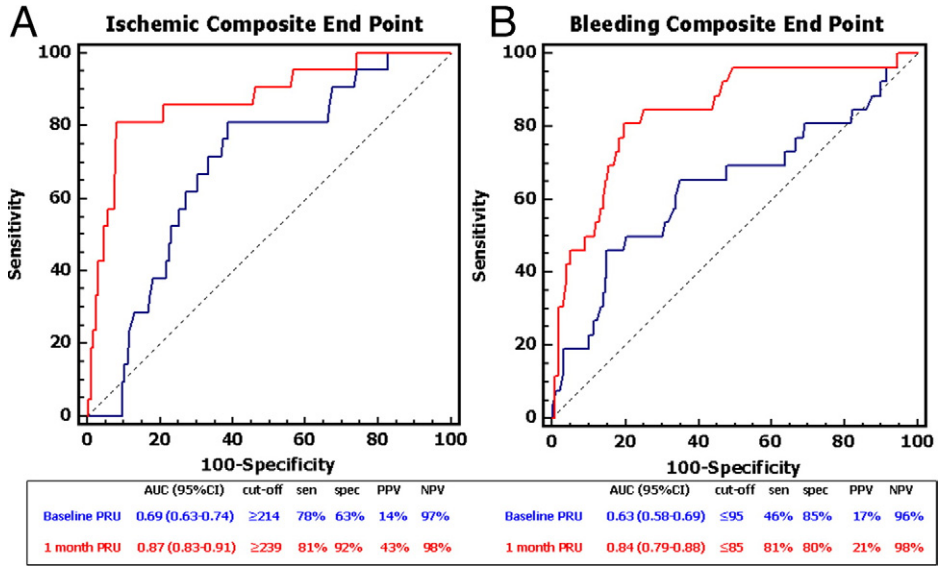

Figure 4 Comparison of ROC Curves for PRU Values at Baseline and at 1 Month

(A) Receiver-operator characteristic (ROC) analysis for ischemic composite endpoint. **(B)** ROC analysis for bleeding composite endpoint.
AUC = area under the curve; NPV = negative predictive value; PPV = positive predictive value; PRU = $P2Y_{12}$ reactivity unit; sen = sensitivity; spec = specificity.

when added to baseline on-clopidogrel PR (above vs. below 258 PRU) and creatinine clearance (above vs. below 52 ml/min) were identified as the best 1-month PR predictors (Fig. 6). Particularly, *ABCB1* and *CYP2C19*2* wild-type patients or patients with baseline PRU <258 and creatinine clearance >52 ml/min showed low PRU values at 1 month (only 6 poor responders of 196, 3%, 95% CI: 1% to 6%) (Fig. 6). On the contrary, carriers of at least 1 loss of function allele for *ABCB1* and/or *CYP2C19* plus high baseline PRU values and/or low creatinine clearance were at highest risk of 1-month high PR and as such of subsequent adverse events (Fig. 6).

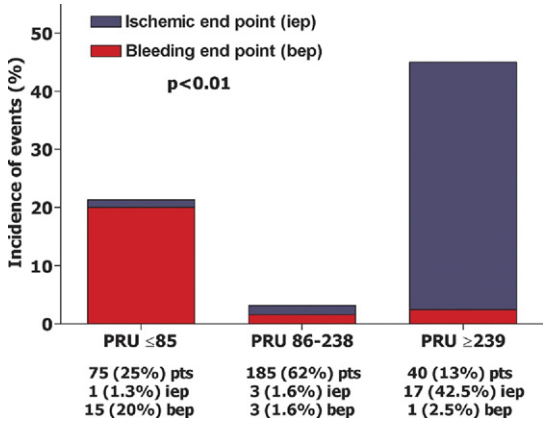

Figure 5 Adverse Events and Levels of $P2Y_{12}$ Receptor Inhibition at 1 Month

Patients are stratified into groups of enhanced responders (PRU ≤85), normal responders (PRU 86 to 238) and poor responders (PRU ≥239). BEP = bleeding endpoint; IEP = ischemic endpoint; other abbreviations as in Figure 1.

Discussion

The main findings of this prospective investigation can be summarized as follows:

1. On-clopidogrel PR showed a significant reduction from index hospitalization to 1 month. The percentage of poor responders decreased from 35% (95% CI: 30% to 41%) at baseline to 13% (95% CI: 9% to 18%) at 1 month.
2. Gene polymorphisms justified about 18% of this trend. *CYP2C19*2* and **17* influence was apparently consistent over time, whereas *ABCB1* showed a higher impact at baseline.
3. We found a "therapeutic window of PRU values" where both ischemic and bleeding adverse events are minimized. Then, on-clopidogrel PR may be used to predict both complications, particularly when assessed at 1 month after index procedure.

To the best of our knowledge, only a pilot study involving 33 stable patients has previously evaluated the pattern of on-clopidogrel PR over time showing no increase in platelet aggregation or change in the prevalence poor response over time (13). The sample size of our study was almost 10-fold greater, and we included both unstable and stable patients. Contrary to previous findings, we observed a significant decrease of on-clopidogrel PR from baseline (index hospitalization) to 1 month, without further changes up to 6

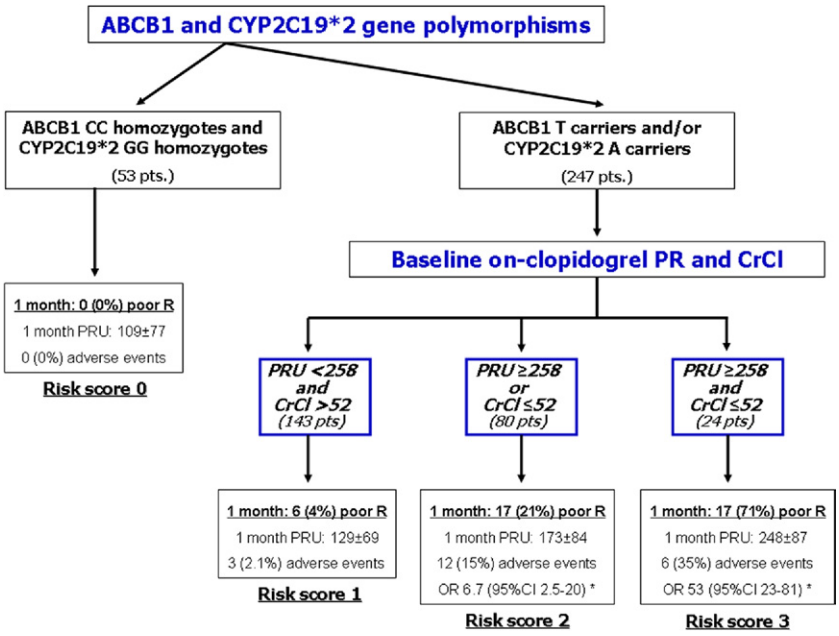

Figure 6 **Risk Score Predicting 1-Month Poor Responsiveness Status**

The score is calculated adding 1 point for the presence of each of the following variables: *ABCB1* T and/or *CYP2C*19* A carriage, baseline PR >258 PRU, and creatinine clearance (CrCl) <52 ml/min. *As compared with the risk score 1 group. Abbreviations as in Figures 1 and 3.

months. Although this pattern was more pronounced in patients admitted for NSTEACS, a consistent PR modification over time was noted also in stable patients. In our study, an LD of 600 mg of clopidogrel was systematically used. One could speculate that a higher LD (e.g., 900 mg) can induce a better early platelet inhibition, minimizing the drop from baseline to 1 month. Yet, results from previous studies are conflicting (14,15) and doses higher than 600 mg seem not to be associated with an additional significant suppression of platelet function because of limited absorption (15). Also the time between LD administration and platelet function evaluation/PCI in our study (22 ± 8 h) well reflects the daily clinical practice and it was meant to allow clopidogrel to reach a steady-state scenario.

In our study, both clinical and genetic factors influenced on-clopidogrel PR. *ABCB1* and *CYP2C19*2* and **17* accounted for approximately 18% of variability in clopidogrel platelet response. Interestingly, the reduction of on-clopidogrel PR from baseline to 1 month appeared to be homogenous across different allele variants. Moreover, for the first time, we reported that *CYP2C19*2* and **17* consistently influenced PR over time, whereas the role of *ABCB1* appeared to be reduced during follow-up. As *ABCB1* is involved in the process of clopidogrel absorption, it is reasonable to speculate that its role may be relatively more relevant in the first days after start of the treatment.

Recently, the genetic substudy of the PLATO (Platelet Inhibition and Patient Outcomes) trial (16) has reported that carriers of allele variants associated with poor clopidogrel effect showed a higher ischemic event rate mainly within the first 30 days after start of treatment. Interestingly, the prognostic impact of clopidogrel loss-of-function alleles seemed less relevant after 30 days. Our data may help to explain this finding. We found that carriers of loss-of-function alleles consistently display higher on-clopidogrel PR as compared to wild-type patients. Nevertheless, after 1 month, on-clopidogrel PR decreases significantly in wild-type patients as well as in loss-of-function allele carriers. Then, although these patients have higher on-clopidogrel PR, the number of patients with PRU values above the established cutoffs predicting adverse events is relatively small and similar to that of wild-type patients. This mechanistic observation may thus at least partially explain why the risk of adverse events as conveyed by loss-of-function alleles may be highest in the first days after the start of the treatment with clopidogrel. Alternatively, this may be partially driven by early discontinuation of clopidogrel after 30 days or by a chance finding that merits further investigation.

Similar to previous studies, we found that PR expressed as a PRU value was an independent predictor of poor prognosis. At ROC analysis, the PRU cutoff value that best discriminated ischemic events tended to be slightly inferior (214 vs. 235 to 240) to previous analyses, but this is consistent with what we previously observed in the 3T/2R (Tailoring Treatment With Tirofiban in Patients Showing Resistance to Aspirin and/or Resistance to Clopidogrel) trial (5–17), and probably due to a different study population selection. The new important information emerging from

the present analysis is the predictive role of PRU assessed at 1 month. By testing on-clopidogrel PR 1 month after index procedure, we significantly improved the ability of PR to discriminate between patients with and without adverse events via a distinct reduction of "false poor responders." Baseline PRU values are influenced by several confounding factors, particularly acute atherothrombotic events and inflammation. All these factors progressively either reduce their influence or disappear, and then the 1-month evaluation permits us to better discriminate patients with chronic and persistent high on-clopidogrel PR. Moreover, in the early phase, clinical presentation, PCI success, and complications related to procedure or hospitalization might have a stronger impact on short-term outcome than clopidogrel poor response would. Contrarily, in the later phase, all these factors are less important and the "true clopidogrel poor response" emerges as the strongest determinant of poor prognosis. This has relevant clinical implications especially in the context of current ongoing studies trying to identify a "tailored anti-platelet regimen" based on a single baseline PR assessment. Patients labeled as "poor responder" at baseline, who then became full responders after 1 month showed an excellent clinical outcome in our study, which was very close to that of patients who were full responders both at baseline and at 1-month evaluation. Thus, we may speculate that a more aggressive antiplatelet treatment may not be needed and may be even potentially harmful. On the other hand, re-evaluating PRU at 1 month after the index procedure carries several drawbacks, limiting a rapidly tailored approach, avoiding the early treatment of true poor responders, and the prevention of acute and subacute ischemic adverse events. To avoid these limitations, a stratification based on the combination of genotype and phenotype variables may be desirable. In our study population, and similar to previous studies (9), genotype information alone showed lower predictive power as compared with on-clopidogrel PR values, and it was not sufficient to discriminate the majority of patients who would be poor responders at 1 month. On the contrary, by mixing genotype and 2 simple baseline characteristics (on-clopidogrel PR and creatinine clearance), we were able to obtain a new risk score model that was able to predict the majority of poor responders after 1 month and with adverse events. Interestingly, of all clinical parameters, creatinine clearance emerged in our score. Probably, because it includes age, sex, and, in particular, renal function. Impaired renal function is frequent in patients who are elderly and diabetic, which are known factors relating to higher PR. Consistently, recent studies showed lower clopidogrel-induced antiplatelet effects and a greater prevalence of on-clopidogrel high PR in patients with chronic kidney disease (18). The proposed risk score algorithm should be regarded as the first attempt to predict high on-clopidogrel PR at 1 month from baseline variables. Therefore, future larger prospective studies are clearly in demand to evaluate the clinical utility of this or similar risk scores.

Finally, according to previous studies (8), we found that both *CYP2C19*17* polymorphism and low on-clopidogrel PR values were associated with bleeding events. As in Sibbing et al. (19), we too found a therapeutic window (between 86 and 238 PRU) with a lower incidence of both ischemic and bleeding complications. Contrarily, we used VerifyNow assay (vs. Multiplate analyzer, Verum Diagnostica, Munich, Germany), we collected bleeding events during follow-up (vs. in-hospital), and we found that blood samples collected at 1 month are better than those collected at baseline. Nevertheless, our findings are consistent and support the existence of a threshold phenomenon both for ischemic and bleeding events.

Study limitations. Our a priori sample size calculation was aimed to assess the change of PR over time after treatment with clopidogrel. Therefore, not surprisingly, there was a small number of ischemic and bleeding events noted throughout follow-up in our study. Consequently, our data and, in particular, our score to predict 1-month poor responsiveness status should be considered exploratory and new larger studies are needed to confirm them. Moreover, our study is limited by the use of only 1 test to evaluate on-clopidogrel PR, the point-of-care assay VerifyNow.

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Key Words: clinical outcome ■ clopidogrel ■ gene polymorphism ■ P2Y12 VerifyNow ■ platelet reactivity ■ poor responder.