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Adjusted Clopidogrel Loading Doses According to Vasodilator-Stimulated Phosphoprotein Phosphorylation Index Decrease Rate of Major Adverse Cardiovascular Events in Patients With Clopidogrel Resistance

A Multicenter Randomized Prospective Study

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Objectives This study evaluates the clinical impact of adjusting the loading dose of clopidogrel according to vasodilator-stimulated phosphoprotein (VASP) index in patients with clopidogrel resistance undergoing percutaneous coronary intervention (PCI).

Background Clopidogrel resistance plays a key role in ischemic recurrence after PCI. In vitro tests of clopidogrel resistance can accurately predict major adverse cardiac events after PCI.

Methods In this prospective, randomized, multicenter study, clopidogrel resistance was defined as a VASP index of more than 50% after a 600-mg loading dose. Patients with clopidogrel resistance undergoing coronary stenting were randomized to a control group or to the VASP-guided group, in which patients received additional bolus clopidogrel to decrease the VASP index below 50%.

Results A total of 162 patients were included. The control (n = 84) and VASP-guided groups (n = 78) had similar demographic, clinical, and biological characteristics. In the VASP-guided group, dose adjustment was efficient in 67 patients (86%) and VASP index was significantly decreased (from 69.3 ± 10 to 37.6 ± 13.8; $p < 0.001$). Eight major adverse cardiac events (5%) were recorded during the 1-month follow-up, with a significantly lower rate in the VASP-guided group compared with the control group (0% vs. 10%; p = 0.007). There was no difference in the rate of major and minor bleeding (5% vs. 4%; p = 1).

Conclusions This is the first study to suggest that adjusting the clopidogrel loading dose according to platelet monitoring using the VASP index is safe and may significantly improve the clinical outcome after PCI in patients with clopidogrel resistance despite a first 600-mg loading dose. (J Am Coll Cardiol 2008;51:1404–11) © 2008 by the American College of Cardiology Foundation

Cardiovascular diseases are the most common cause of mortality and morbidity in Western countries. Percutaneous coronary intervention (PCI) has become the most frequently used form of coronary revascularization. Since the mid-1990s, stent deployment has been the gold standard to reduce the rate of acute closure (1) and in-stent restenosis (2,3). Addition of the P_2Y_{12} adenosine diphosphate (ADP) receptor blocker, clopidogrel, to aspirin in patients undergoing PCI has dramatically reduced the rate of major adverse cardiac events (MACE) (4,5). However, not all patients benefit to the same extent from these improvements

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in antiplatelet therapy, and some continue to suffer ischemic recurrences, including stent thrombosis, which are associated with significant mortality and morbidity (5,6). The pathophysiology of recurrent ischemic events is multifactorial, although numerous reports have demonstrated that clopidogrel resistance is a major precipitating factor (7–9).

In vitro tests of platelet reactivity, such as ADP-induced platelet aggregometry and vasodilator-stimulated phosphoprotein (VASP) phosphorylation analysis, accurately detect biological clopidogrel resistance, which is associated with worse outcome after PCI (7–9). However, there is no consensus on the definition of biological clopidogrel resistance, and its prevalence remains unclear. In a previous prospective study in patients undergoing PCI, we observed that a cutoff value of 50% in the VASP index was predictive of MACE at 6 months. Using this threshold value, the test demonstrated a sensitivity of 100% in predicting MACE of clinical interest (10). Cuisset et al. (11) reported in a recent prospective, randomized, single-center study that increasing the clopidogrel loading dose from 300 to 600 mg is likely to improve clinical outcome after coronary angioplasty. However, despite the 600-mg loading dose, some patients remained clopidogrel-resistant and the rate of MACE observed at 1 month, although decreased, was still 5%.

Several reports have highlighted the large interindividual response to clopidogrel, regardless of the test used to assess platelet reactivity, and the optimal loading and maintenance doses are still being debated (12,13). We hypothesized that the loading dose may need to be individually adjusted according to the patient's biological response to clopidogrel to decrease the rate of MACE after stenting. We conducted this study to evaluate the clinical benefit of adjusting the clopidogrel loading dose according to platelet reactivity, as assessed by the VASP index, to the rate of MACE in patients undergoing stenting who presented clopidogrel resistance despite a first 600-mg loading dose.

Methods

This was a multicenter, prospective, controlled, randomized study. The study protocol was in accordance with the Declaration of Helsinki and was approved by the local ethics committees of our institutions. All patients gave written informed consent before inclusion.

All patients undergoing PCI for refractory angina pectoris under optimal medical therapy, silent ischemia on thallium scintigraphy, or non–ST-segment elevation myocardial infarction (NSTEMI) were eligible for inclusion. Exclusion criteria were persistent ST-segment elevation myocardial infarction, a failed PCI, New York Heart Association functional class III or IV, sudden death, contraindications to antiplatelet therapy, platelet count <100 g/l, history of bleeding diathesis, and concurrent severe illness with expected survival of <1 month.

The VASP index was measured 24 h after the first 600-mg bolus of clopidogrel. All patients with a VASP index above 50% were prospectively included in the study and were randomly assigned to the control group or to the VASP-guided group.

In the control group, PCI was carried out without an additional bolus of clopidogrel. In the VASP-guided group, the overall dose of clopidogrel was adjusted individually, before PCI, to obtain a VASP index below 50%. Briefly, after the initial dose up to 3 additional boluses of 600 mg may be given in 24 h increments, and the VASP index was assessed 12 h after administration until a VASP index below 50% was obtained (Fig. 1). If these additional boluses were unable to decrease the VASP index below 50%, PCI was performed without further loading-dose adjustment.

Angioplasty procedure. The PCI was carried out according to international guidelines, using a standard technique, through the femoral route (14). Systematic stent implantation was achieved in all patients. Either a drug-eluting or bare-metal stent could be used according to French Society of Cardiology guidelines. The sheath was removed immediately at the end of the procedure in all cases. Routine care before and after the procedure was undertaken for all

Abbreviations and Acronyms

ADP = adenosine diphosphate
MACE = major adverse cardiac events
MFI = mean fluorescence intensity
NSTEMI = non–ST-segment elevation myocardial infarction
PCI = percutaneous coronary intervention
PGE1 = prostaglandin E1
TIMI = Thrombolysis In Myocardial Infarction
VASP = vasodilator-stimulated phosphoprotein

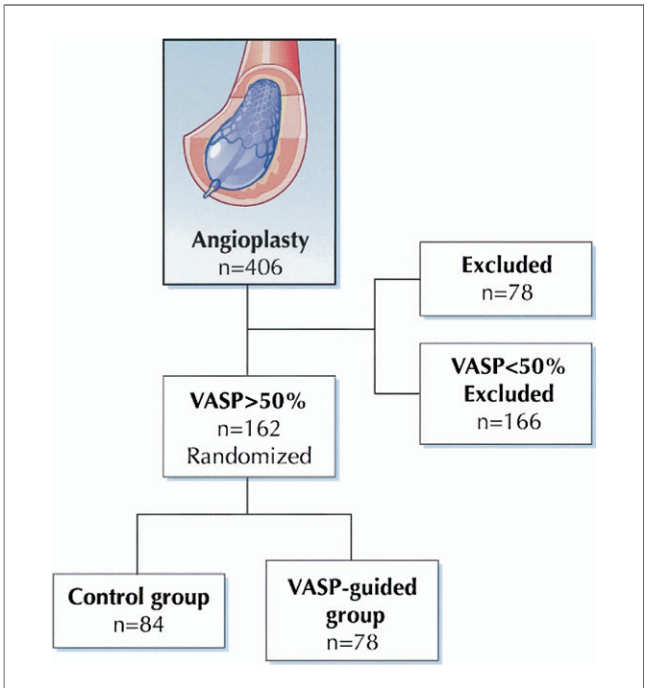

Figure 1 Study Design

VASP = vasodilator-stimulated phosphoprotein.

patients, including pre-treatment clopidogrel (600-mg initial bolus) 12 h before the procedure followed by 75 mg daily for at least 1 month. In addition, all patients received aspirin, 160 mg daily, for at least 1 month, with a dose administered 12 h before stenting.

For patients with NSTEMI, anticoagulation with unfractionated heparin was begun in the intensive care unit before PCI. Glycoprotein IIb/IIIa inhibitors were administered at the discretion of the interventional cardiologist and were used according to guidelines (15).

The interventional cardiologist and the treating physician were not aware of the VASP index results at the time of PCI.

Blood samples. Blood samples for VASP phosphorylation analysis were drawn by atraumatic venipuncture of the antecubital vein. The initial blood drawn was discarded to avoid measuring platelet activation induced by needle puncture; blood was collected into a Vacutainer (BD, Franklin Lakes, New Jersey) containing 3.8% trisodium citrate and filled to capacity. The Vacutainer was inverted 3 to 5 times for gentle mixing and sent immediately to the hemostasis laboratory.

VASP phosphorylation analysis. The VASP phosphorylation analysis was performed within 24 h of blood collection by an experienced investigator using Platelet VASP kits (Diagnostica Stago, Asnières, France) according to the manufacturer's instructions (7,10). Briefly, blood samples were incubated in vitro with ADP and/or prostaglandin E1 (PGE1) before fixation. Each sample was indirectly immunolabeled by incubation with 16C2 monoclonal antibody followed by staining with a goat antimouse fluorescein isothiocyanate polyclonal reagent (BioCytex, Marseille, France). Flow cytometric analysis was performed using a Coulter EPICS XL cytometer (Beckman Coulter Inc., Fullerton, California). Platelet population was identified on its forward and side scatter distributions and 3,000 platelet events were gated and analyzed for mean fluorescence intensity (MFI) using EPICS XL software. The MFI corresponding to each experimental condition (ADP, ADP + PGE1) was determined to establish a ratio directly correlated with the VASP phosphorylation state. The ratio, $100 \times [(MFI^{PGE1} - MFI^{ADP+PGE1})/MFI^{PGE1}]$, is expressed in this study as a VASP phosphorylation index (VASP index) corresponding to a ratio of the VASP phosphorylation of activated platelets versus resting platelets and expressed as a percentage of platelet reactivity. The intra-assay coefficient of variation was <5%, and the inter-assay coefficient of variation was <8%. The normal platelet reactivity in healthy subjects is above 70% (16).

End points. End points were recorded by an investigator who was not aware of the treatment status and clinical characteristics of patients. The primary end point was the rate of MACE (defined in the next section). Secondary end points, recorded to assess safety, were major and minor bleeding. Major bleeding was defined as intracranial bleeding or clinically-overt bleeding associated with a decrease in hemoglobin of 5 g/dl, according to the Thrombolysis In Myocardial Infarction (TIMI) criteria (17). Minor bleeding was also defined according to TIMI criteria (17).

MACE. Clinical follow-up was performed 1 month after PCI. Events recorded included cardiovascular death, angiographically-confirmed stent thrombosis, recurrent acute coronary syndrome as defined by the American College of Cardiology/American Heart Association guidelines (14), and recurrent revascularization by either coronary angioplasty or bypass surgery.

We defined stent thrombosis using the Academic Research Consortium classification (18) as an angiographic confirmation of stent thrombosis associated with at least 1 of the following signs present within 48 h: new onset of ischemic symptoms at rest, recent changes suggestive of acute ischemia on resting electrocardiogram, or typical rise and fall in cardiac biomarkers. Stent thrombosis was further characterized as acute (0 to 24 h) or subacute (>1 day and ≤30 days).

Drug therapy compliance was assessed. The treating physician and the investigators who evaluated the clinical end points were blinded to the results of platelet testing and to group assignment.

Power calculation. We postulated that the average difference in the rate of MACE between the 2 groups would be 15% (0% vs. 15%) (10). Therefore, for 90% power and an alpha risk of 5%, we estimated that 76 patients should be included in each group. We estimated that 5% of patients would be lost to follow-up, so the target number of included patients was 160.

Statistical analysis. Statistical analysis was performed using SPSS 15 software (SPSS Inc., Chicago, Illinois). Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as frequency and percentage. Comparison between categorical variables was performed using the chi-square test or Fisher exact test when frequencies were below 5. Analysis of variance was used to compare characteristics of quantitative variables. Kaplan-Meier curves were used to assess MACE-free survival.

Results

Patients. From March to July 2007, 406 consecutive patients admitted for PCI to 4 participating cardiology centers in Marseille were prospectively screened for inclusion. A total of 244 patients were not included: 78 met exclusion criteria and 166 (48%) had a VASP index below 50% after the first 600-mg bolus of clopidogrel. Therefore, 162 patients undergoing coronary angioplasty with stent implantation fulfilled the inclusion criteria. Eighty-four patients were randomized to the control group. Seventy-eight patients were randomized to the VASP-guided group and received up to 3 additional loading doses in an attempt to obtain a VASP index below 50% (Fig. 1).

Table 1 Baseline Characteristics

	Control Group (n = 84)	VASP-Guided Group (n = 78)	p Value
Gender, female/male	17/67	19/59	0.5
Age, yrs*	66.6 ± 11.1	66.3 ± 10.1	0.9
BMI, kg/m^2*	27.2 ± 5.1	27.6 ± 5.1	0.6
Previous myocardial infarction, n (%)	20 (24)	22 (28)	0.5
History of CABG, n (%)	10 (12)	6 (8)	0.4
Cardiovascular risk factors, n (%)			
Present smoking	35 (42)	27 (35)	0.4
Dyslipidemia	45 (54)	41 (53)	0.9
Diabetes mellitus	36 (43)	31 (40)	0.7
Hypertension	51 (61)	47 (60)	1
Family history of CAD	14 (17)	21 (27)	0.1
Treatment on admission			
Beta-blocker	32 (38)	29 (37)	0.9
Aspirin	43 (51)	44 (56)	0.5
Statin	41 (49)	41 (53)	0.6
PCI indication, n (%)			
Silent ischemia	18 (21)	14 (18)	0.6
Stable angina	27 (32)	27 (35)	0.7
NSTEMI	40 (48)	35 (45)	0.7
Angiography and intervention			
LVEF, %*	59.4 ± 12.1	58.7 ± 13.2	0.7
Number of diseased vessels*	2.1 ± 1.1	2.4 ± 1.3	0.1
Number of treated vessels*	1.4 ± 0.6	1.5 ± 0.7	0.4
Number of stents per patient*	2 ± 1	2.2 ± 1.2	0.3
Number of DES per patient*	0.96 ± 1.1	0.97 ± 1.1	0.9
Stent length per patient, mm*	29 ± 15	33 ± 18	0.1
GP IIb/IIIa inhibitors, n (%)	17 (20)	13 (17)	0.6
Biology			
Time between first LD and VASP measurement, h*	24.5 ± 13.1	23.6 ± 13	0.7
VASP after first loading dose, %*	67.8 ± 10.5	69.3 ± 10	0.4
VASP after sensitization, %*	—	37.6 ± 13.8†	—
Hemoglobin, g/dl*	13.8 ± 5.4	13.4 ± 1.5	0.5
Leucocytes, g/l*	7.8 ± 2.3	7.5 ± 2.5	0.5
Platelets, 10^3 g/l*	240 ± 68	230 ± 76	0.4
Fibrinogen, g/l*	3.8 ± 1	3.9 ± 1	0.5
Creatinine, μmol/l*	93 ± 43	98 ± 37	0.4

*Mean ± SD. †$p < 0.0001$ between VASP after first loading dose and VASP after sensitization.

BMI = body mass index; CABG = coronary artery bypass graft; CAD = coronary artery disease; DES = drug-eluting stents; GP = glycoprotein; LD = loading dose; LVEF = left ventricular ejection fraction; MI = myocardial infarction; NSTEMI = non–ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; SD = standard deviation; VASP = vasodilator-stimulated phosphoprotein.

Baseline characteristics are summarized in Table 1. Demographic data and body mass index were similar in the 2 groups. The prevalence of cardiovascular risk factors was similar, including diabetes and smoking ($p = 0.7$ and 0.4, respectively). The PCI indications were balanced among the 3 inclusion criteria and were similar between the 2 randomized groups. The rate of patients undergoing PCI for NSTEMI was similar (48% vs. 45%; $p = 0.7$). The 2 groups did not differ in left ventricular ejection fraction ($p = 0.7$). The PCI data were also similar including the number of drug-eluting stents per patient and stent length ($p = 0.9$ and 0.1, respectively). Consistent with the study protocol, there is a significant difference in the time between the first clopidogrel loading dose and performance of PCI (26 ± 3 h vs. 65 ± 24 h; $p < 0.0001$).

Platelet parameters. The mean time between the first loading dose of clopidogrel and blood sampling was similar in the 2 groups (24.5 ± 13.1 h vs. 23.6 ± 13 h; $p = 0.7$) (Table 1). The VASP index after the first loading dose of clopidogrel was also similar (67.8 ± 10.5 vs. 69.3 ± 10; $p = 0.7$).

Figure 2 summarizes the effect of each additional clopidogrel loading dose on the VASP index in patients randomized to the VASP-guided group. After the second bolus of clopidogrel, 38 patients had a VASP index below 50% and the mean VASP index was significantly decreased from baseline (50.5 ± 17.3 vs. 69.3 ± 10; $p < 0.001$). Forty patients required a third loading dose of clopidogrel. Following this, 26 patients (65%) were still resistant and required a fourth loading dose to achieve the target VASP

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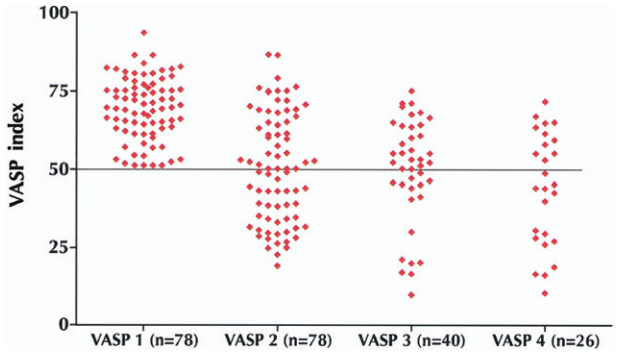

Figure 2 Effects of Each Additional Bolus of Clopidogrel on the VASP Index in the VASP-Guided Group

VASP 1 is the VASP index after the initial loading dose as given to patients in both groups; VASP 2 is that after the second loading dose, and so on. Abbreviation as in Figure 1.

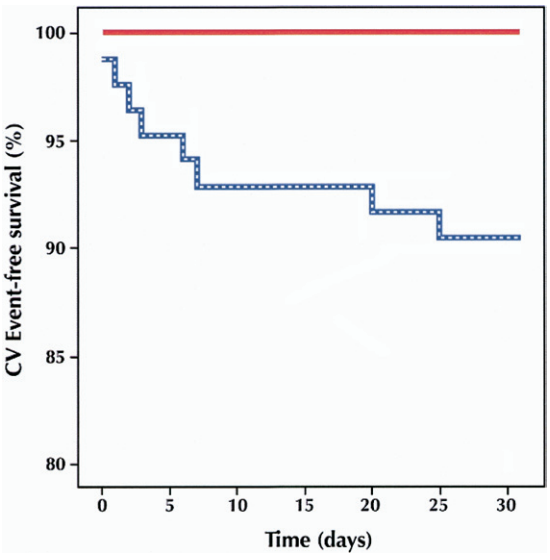

Figure 3 Kaplan-Meier Analysis for 30-Day MACE

Kaplan-Meier analysis for 30-day major adverse cardiovascular (CV) events (MACE) according to group. Log-rank = 7.75, $p < 0.005$. **Blue** = control group; **red** = VASP-guided group. Abbreviation as in Figure 1.

index. Additional loading doses were unable to decrease the VASP index below 50% in 11 patients (14% of patients randomized to the VASP-guided group). The mean VASP index after sensitization in the VASP-guided group was 37.6 ± 13.8% ($p < 0.0001$, vs. after first loading dose).

Clinical outcomes. One-month follow-up was completed in all patients. Eight (5%) MACE occurred during follow-up (Table 2), all in the control group (10%) (Fig. 3), resulting in a statistically significant difference between the groups ($p = 0.007$). The distribution of cardiovascular events is summarized in Table 2.

Hemorrhagic complications and side effects of clopidogrel. There was 1 major hemorrhagic complication in each group: an intracranial bleed requiring surgery in the control group and a femoral hematoma requiring transfusions in the VASP-guided group (Table 3). The overall rate of bleeding was similar in the 2 groups (5% vs. 4%, $p = 1$). No other side effect of clopidogrel was reported.

Discussion

The data from this multicenter prospective study suggest that adjusting the clopidogrel loading dose according to the VASP index among patients with clopidogrel resistance who were undergoing coronary stenting may significantly improve clinical outcome. To our knowledge, this is the first randomized study to demonstrate the clinical benefit of prospective platelet monitoring of clopidogrel efficiency in patients undergoing stenting.

In the present study, we used the VASP index to monitor platelet reactivity. The original VASP assay described by Schwarz et al. (16) was modified by Barragan et al. (7) to track VASP phosphorylation using a flow cytometric assay to establish a ratio directly correlated with VASP phosphorylation state. This VASP index, expressed as a mean percentage of platelet reactivity, is inversely correlated with clopidogrel efficiency. In their retrospective study, Barragan et al. (7) observed a strong correlation between subacute stent thrombosis and a VASP index higher than 50%. These results were later confirmed in a prospective study, and the 50% threshold demonstrated a very high negative predictive value for MACE after PCI (11). Moreover, Blindt et al. (19), in a prospective study, observed that a VASP index above 48% was the only independent predictor of stent thrombosis in high-risk PCI. In a recent experimental study to track the antithrombotic effects of clopidogrel in rats, Schumacher et al.

Table 2 Primary End Points: MACE During 1-Month Follow-Up

End Point	Control Group	VASP-Guided Group
Cardiovascular death, n (%)	2 (2)	0
Acute stent thrombosis, n (%)	1 (1)	0
Subacute stent thrombosis, n (%)	3 (4)	0
Recurrent acute coronary syndrome, n (%)	2 (2)	0
All MACE, n (%)	8 (10)*	0

*p = 0.007.
MACE = major adverse cardiac events; other abbreviation as in Table 1.

Table 3 Secondary End Points: Bleeding During 1-Month Follow-Up

End Point	Control Group	VASP-Guided Group
Major bleeding, n (%)	1	1
Minor bleeding, n (%)	3 (4)	2 (3)
All, n (%)	4 (5)	3 (4)

Abbreviation as in Table 1.

(20) demonstrated that a 50% VASP index corresponded to a nearly 90% P_2Y_{12} receptor blockage.

Current common clinical practice consists of pretreatment with a 300-mg loading dose of clopidogrel at least 6 h before the procedure in patients undergoing PCI for various clinical indications (15). In 1 study (11), the prevalence of clopidogrel resistance after the standard 300-mg loading dose was 75% using the VASP index, but a 600-mg loading dose was associated with a faster and greater antiplatelet effect, reducing the rate of patients with resistance (21–25). In the present study, the 600-mg loading dose significantly decreased the rate of patients with resistance to 52%. Two recent studies also suggest a better clinical outcome in the setting of coronary stenting with a 600-mg loading dose compared with a 300-mg dose (9,26). Nevertheless, the rate of MACE at 1 month was still 5% despite the increased loading dose (9).

Considering the difficulty in identifying patients resistant to clopidogrel, we hypothesized that the clopidogrel loading dose should be individually adjusted according to a platelet function assay. Because of the very high sensitivity and the reproducibility of the 50% threshold, the VASP index allows simple platelet monitoring. Gurbel et al. (27) demonstrated that the VASP index is strongly correlated with ADP-induced platelet aggregometry, which is considered the gold standard.

The ADP-induced platelet aggregometry definition of "no response" is not consensual and corresponds for Gurbel et al. (21) to a <10% decrease in absolute change in aggregation. This definition is based on platelet responsiveness and not on post-treatment platelet reactivity, which has been advocated by recent studies (9,11) on the topic and was used in this study. Moreover, in contrast to the VASP index, the predictive value of this biologically-derived threshold is unknown. Because of these different definitions of resistance, it is possible that some of the patients classified as "low responders" when using ADP-induced platelet aggregometry may be classified as "nonresponders" when using the VASP index. The data in the present study concur in that the rate of nonresponders is higher than previously reported with a 600-mg loading dose using ADP-induced platelet aggregometry (21). This could also be related to the high proportion of patients with NSTEMI, a condition that is associated with an increased prevalence of clopidogrel nonresponders (28). Furthermore, as demonstrated by Morel et al. (29), ADP-induced platelet aggregometry is sensitive to glycoprotein IIb/IIIa or aspirin administration and therefore may not identify all nonresponders.

Interestingly, most events in this study were angiographically-proven stent thrombosis, a condition that is consistent with insufficient platelet inhibition by clopidogrel. The high frequency of this complication as well as the high overall dose of clopidogrel required in the VASP-guided group are probably linked to the selection of patients according to resistance to the 600-mg clopidogrel loading dose and to the very high negative predictive value of the VASP index (11). Moreover, most patients had PCI for NSTEMI, which has been associated with increased platelet activity and greater prevalence of clopidogrel resistance (29).

In this study, 2 MACE were recorded after the first 10 days of follow-up in the control group. Although not statistically significant, this difference in the rate of MACE under chronic clopidogrel therapy may have several explanations. In particular, it is possible that loading dose adjustment may provide a lasting sensitization to clopidogrel. Moreover, reducing platelet reactivity during PCI and the first days after stent implantation may prevent thrombus formation, avoiding later events. However, in this study, the VASP index was not measured under chronic clopidogrel therapy, and we cannot ascertain whether adjusting the clopidogrel loading dose allows for sustained sensitization, thus preventing delayed events. The play of chance could also be involved in this difference in event rates more than 10 days after dose adjustment.

Among patients with resistance to a 600-mg loading dose of clopidogrel in the VASP-guided group, 14% remained resistant despite an additional 1,800 mg of clopidogrel. The prognosis in this subset of patients and their management should be addressed in future studies. Campo et al. (30) demonstrated that ticlopidine could be an alternative treatment because crossover resistance appears infrequently. New ADP antagonists, such as prasugrel, may also be useful in such patients.

Although the expected rate of events in this small subset of patients was 15%, none were recorded. This may be related to the play of chance and to the multifactorial nature of MACE. Furthermore, the sensitivity of the test may not be as high as expected.

In the present study, we observed a clinical benefit from adjusting the clopidogrel loading dose according to post-treatment platelet reactivity among patients with resistance despite a 600-mg loading dose. Moreover, this was well tolerated and did not increase the rate of bleeding complications or other side effects of the drug. Our data validate adjusting the clopidogrel loading dose according to the VASP index, before PCI, in daily clinical practice to improve the clinical outcome in coronary stenting. This approach is associated in this study with an increased length of hospital stay. However, given the observed safety of the VASP-guided loading dose of clopidogrel, dose adjustment could be realized before hospitalization for PCI.

Study limitations. The sample size of the present study is relatively small and therefore does not allow for definitive conclusions. Further large multicenter studies are needed to confirm our results. Delaying PCI until dose adjustment was performed may have induced a bias associated with time-dependent effects of other antianginal therapy. To prevent bias in future trials, inclusion of a placebo-controlled group would correct for the time imbalance between the groups. The VASP phosphorylation analysis is standardized, reproducible, and accurate, but it is expensive and technically demanding, which limits its widespread use

in clinical practice. The cost-effectiveness ratio of this approach should be evaluated in future trials.

Conclusions

This is the first study suggesting that adjusting clopidogrel loading dose according to platelet reactivity measured by VASP index is safe and may significantly improve clinical outcome after PCI in patients with clopidogrel resistance. Moreover, the very high sensitivity of the VASP index at a cutoff value of 50% may be considered of clinical interest in avoiding subacute stent thrombosis and may justify routine monitoring for clopidogrel resistance in high-risk population subsets.

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