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Level of Adenosine Diphosphate Receptor $P2Y_{12}$ Blockade During Percutaneous Coronary Intervention Predicts the Extent of Endothelial Injury, Assessed by Circulating Endothelial Cell Measurement

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Objectives We aimed to investigate whether clopidogrel-induced inhibition of platelet reactivity could reduce the level of circulating endothelial cells (CEC), reflecting the endothelial injury induced by percutaneous coronary intervention (PCI).

Background Clopidogrel loading dose before percutaneous coronary angioplasty (PCI) reduces platelet activation through a selective and irreversible blockade of the adenosine diphosphate (ADP) receptor $P2Y_{12}$. The impact of clopidogrel on endothelial cells has been scarcely studied.

Methods A total of 149 patients undergoing PCI for stable angina were enrolled. Levels of CEC were measured at baseline (H0) and 6 (H6) and 24 (H24) h after the procedure using a CD146-based immunomagnetic separation assay. The CEC delta-change (CEC at H6 − CEC at H0) was analyzed according to ADP receptor $P2Y_{12}$ blockade, assessed by a vasodilator-stimulated phosphoprotein (VASP) assay after a 600-mg loading dose of clopidogrel.

Results The PCI induced a significant rise in CEC levels 6 h after the procedure. The CEC peak value was significantly higher in patients with high on-treatment platelet reactivity (VASP index ≥50%: 59.6 ± 27.5 cells/ml) as compared with good responders (VASP index <50%: 27 ± 22 cells/ml; $p = 0.04$). The endothelial injury, assessed by CEC delta-change between H6 and H0, was significantly higher in the high on-treatment platelet reactivity group compared with the good responders group (52.6 ± 25.6 vs. 18.6 ± 23.5, respectively; $p < 0.001$) and correlated with the VASP index ($r = 0.59$; $p < 0.001$). In multivariate analysis, VASP group, the number of diseased vessels, and the number of implanted stents independently predicted the endothelial injury ($p < 0.001$).

Conclusions Optimal ADP receptor $P2Y_{12}$ blockade reduces the endothelial injury during PCI. This protective effect of clopidogrel on endothelial cells could add to the clinical benefit associated with this drug. (J Am Coll Cardiol 2010; 56:1024–31) © 2010 by the American College of Cardiology Foundation

Platelet interactions with the endothelium participate in the initiation and progression of the atherosclerotic plaque (1,2) and its complications. In vivo platelet activation not only induces thrombus formation but also triggers an inflammatory response responsible for vascular injury and involving cytokines and chemokines such as chemokine ligand-5 (3), macrophage inflammatory protein-1 (4), interleukin-1 beta (5), and soluble CD40 ligand (6–8).

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Clopidogrel is a key antiplatelet agent that prevents thrombotic events in patients with atherothrombosis and particularly in those undergoing percutaneous coronary intervention (PCI) (9). Clopidogrel mainly acts by inducing a selective and irreversible blockade of the adenosine disphosphate (ADP) receptor $P2Y_{12}$, thus inhibiting platelet aggregation (10). Its effect on ADP receptor $P2Y_{12}$ can be selectively and specifically assessed by measuring the ratio of phosphorylated and dephosphorylated vasodilator-stimulated phosphoprotein (VASP). Determination of the VASP ratio allows the division of patients into good responders (GR), who have a low risk of recurrent thrombotic event, and patients with high on-treatment platelet reactivity (HTPR), who have a high risk of an event following PCI (11–16). Recent studies have reported that in addition to its antiplatelet effects, clopidogrel therapy reduces the inflammatory response and improves endothelial function in patients with coronary artery disease (CAD) (17–19). Moreover, using $P2Y_{12}^{(-/-)}$ deficient mice, Evans et al. (20) demonstrated a role for platelet ADP receptor $P2Y_{12}$ in the vessel wall response to arterial injury. These findings are of critical interest because the endothelium plays a central role in the maintenance of vascular health by modulating vasomotor tone, thrombotic, inflammatory, and cellular proliferation pathways, thus affecting the short- and long-term biology of vessel walls. According to the response to injury theory (21), mechanical damage or chronic exposure to cardiovascular risk factors alters the regulatory functions and integrity of the endothelium. As a result, endothelial cells detached from injured vessels (22) constitute a hallmark of these deleterious processes. Circulating endothelial cells (CEC) are mature endothelial cells that represent a noninvasive and specific marker reflecting vascular damage, remodeling, and dysfunction (23–25). Thanks to a consensus definition of CEC and a standardized protocol for identifying these cells (26), there is good agreement among laboratories with regard to CEC counts. These cells are present at very low levels in healthy subjects, whereas elevated levels have been reported in various pathologic situations. Circulating endothelial cells have both a diagnostic and prognostic significance in CAD (27–29). Previous studies, including some from our group, have reported that PCI leads to a significant increase in CEC, reflecting the endothelial injury induced by the procedure (22,30–33). However, the possibility that clopidogrel therapy could impact CEC levels has never been investigated before. We therefore postulated that ADP receptor $P2Y_{12}$ blockade induced by clopidogrel loading dose (LD) could have a protective effect on endothelium assessed by decreased CEC levels.

The aim of the present study was to monitor early changes in CEC number in patients undergoing PCI identified as GR for clopidogrel compared with HTPR patients (13).

Methods

A prospective, multicenter study was performed. A total of 149 patients undergoing PCI with bare-metal stent implantation for stable angina or silent ischemia were enrolled after informed consent was obtained. The protocol was approved by the local ethics committee of Marseille, Sud Mediterranee, and was in accordance with the declaration of Helsinki.

Exclusion criteria were acute coronary syndrome, drug-eluting stent implantation, lesion located in a saphenous vein graft, contraindication to clopidogrel therapy, bleeding diathesis, platelet count <100,000/l, New York Heart Association functional class III or IV, acute or chronic inflammation, neoplasia, or other concomitant illness known to interact with CEC count.

PCI. All patients had PCI with bare-metal stent implantation through the radial route using a 6-F sheath. The day before the procedure they received an oral LD of aspirin (250 mg) and clopidogrel (600 mg). During the procedure, a bolus of 50 IU/kg heparin was administrated and then adjusted to ensure an activated clotting time between 200 and 250 s. Glycoprotein IIb/IIIa inhibitors were not used. The maintenance dose of clopidogrel and aspirin were 75 and 160 mg, respectively, daily for at least 1 month.

VASP assay. The VASP phosphorylation analysis was performed within 24 h of blood collection by an experienced investigator using a platelet VASP kit (Diagnostica Stago, Asnières, France) as previously described (11,13–16). Blood samples were collected 12 ± 2 h after the clopidogrel LD and before PCI in all patients. Briefly, blood samples were incubated in vitro with ADP and/or prostaglandin E1 (PGE1) before fixation. Each sample was indirectly immunolabeled by incubation with a 16C2 monoclonal antibody followed by staining with a goat anti-mouse fluorescein isothiocyanate polyclonal reagent (Stago, Asnières, France). Flow cytometric analysis was performed using an EpicsXL 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 using EpicsXL software. Mean fluorescence intensity (MFI) corresponding to each experimental condition (ADP or ADP + PGE1) was determined to establish a ratio directly correlated with VASP phosphorylation state. The ratio, $100 \times ([MFI^{PGE1} - MFI^{ADP + PGE1}]/MFI^{PGE1})$, is expressed in this study as a VASP index corresponding to a ratio of the VASP phosphorylation of activated platelets versus at-rest platelets and

Abbreviations and Acronyms

ADP = adenosine disphosphate
BMI = body mass index
CAD = coronary artery disease
CEC = circulating endothelial cell(s)
GR = good responders
HTPR = high on-treatment platelet reactivity
LD = loading dose
MFI = mean fluorescence intensity
PCI = percutaneous coronary intervention
VASP = vasodilator-stimulated phosphoprotein

is expressed as a percentage of platelet reactivity. The intra-assay coefficient of variation was <5%, and the inter-assay coefficient of variation was <8%. This test provides a standardized, reproducible, and specific measure of ADP receptor $P2Y_{12}$ blockade activity. Based on previous studies linking the VASP index to a clopidogrel LD and clinical outcome, we divided the patient population into 2 groups using a cut-off value of 50% of post-treatment platelet reactivity: GR with a VASP index <50% and patients with HTPR with a VASP index ≥50% (11,13–16).

Enumeration of CEC. Four milliliters of blood were collected into EDTA by venipuncture of the antecubital vein, immediately before (H0) and 6 (H6) and 24 (H24) h after PCI. As previously reported, these sample times allow monitoring of the peak level of CEC increase reflecting the endothelial damage induced by PCI (32). The CEC were counted according to a standardized protocol (26). Briefly, the first 2 ml of drawn blood was discarded to avoid contamination by mural endothelial cells dislodged by the venipuncture. The CEC were counted by an operator unaware of the patients' clinical features. Immunoseparation of CEC from blood was performed at 4°C with magnetic beads (Dynabeads M-450, Invitrogen, Carlsbad, California) coated with S-Endo1 (Biocytex, Marseille, France), a monoclonal antibody directed against the endothelial antigen CD146. To avoid nonspecific binding of leukocytes to CD146-coated beads, the cell suspensions were flushed vigorously through a pipette tip during the washing steps and then suspended in acridine orange (3 μg/ml in phosphate-buffered saline, Sigma-Aldrich, Saint-Quentin Fallavier, France) before being counted under a fluorescence microscope. The CEC were identified according to the following consensus morphologic and immunologic criteria: rosetted cells with sizes of 20 to 50 μm and bearing more than 10 beads or bearing fewer than 10 beads but with well-preserved and recognizable morphology (clear nucleus in a well-delineated cytoplasm) and *Ulex europeaus* lectin-1 binding. The number of CEC was expressed as cells/ml of blood.

Statistical analysis. The CEC delta-change (CEC at H6 − CEC at H0) was calculated to measure the endothelial injury induced by PCI independently from CEC baseline level (32). Comparisons between both groups were performed using Student *t* tests for quantitative variables and chi-square or Fisher exact tests for frequencies. The Student *t* test for paired samples was used to compare CEC means before and 6 h after PCI in each group. Associations between continuous variables were analyzed using Pearson correlation tests. Multivariate analyses using multiple linear regressions were performed to determine variables potentially related to CEC delta-change. Variables relevant to the models were selected based on a threshold p value ≤0.2 in univariate analysis (hypertension, diabetes, family history of CAD, VASP group [GR and HTPR], number of diseased vessels, and number of stents) and/or their clinical interest (age, sex, body mass index [BMI], statin therapy, and aspirin). The final model expressed the standardized beta coefficient, which represents the change in SD units in the dependent variable resulting from a change of 1 SD in the different independent variables. All of the tests were 2-sided. Statistical significance was defined as $p < 0.05$. Statistical analysis was performed using the SPSS version 15.0 software package (SPSS, Inc., Chicago, Illinois).

Results

Baseline characteristics of GR and HTPR patients. The final sample included 149 patients, corresponding to 61 GR patients and 88 patients with HTPR. Patient characteristics are displayed in Table 1. The GR group included 61 (41%) patients, whereas the HTPR group comprised 88 (59%) patients. The mean VASP index was 23.7 ± 12.5% in the GR group and 65.1 ± 9.7% in the HTPR group. The 2 groups of patients were similar in age and sex ratio. Regarding cardiovascular risk factors, the prevalence of diabetes and BMI were significantly higher in the HTPR group compared with the GR group (31.8% vs. 8.2%, p = 0.001 and 27.3 ± 4.9 kg/m^2 vs. 25.4 ± 4.3 kg/m^2, p = 0.02, respectively), whereas prevalence of smoking, hypertension, dyslipidemia, and family history of CAD was similar in both groups. No significant difference was found among medications on admission (including clopidogrel chronic ther-

Table 1 Comparison of Baseline Characteristics of GR and HTPR Groups

	GR (n = 61)	HTPR (n = 88)	p Value
Age (yrs)	64.4 ± 12.5	64.8 ± 10.9	0.82
Women	18 (29.5)	18 (20.5)	0.25
Cardiovascular risk factors			
BMI (kg/m^2)	25.4 ± 4.3	27.3 ± 4.9	0.02
Smoker	17 (27.9)	26 (29.5)	0.86
Hypertension	32 (52.5)	51 (58.0)	0.62
Diabetes	5 (8.2)	28 (31.8)	0.001
Dyslipidemia	32 (52.5)	59 (67.0)	0.09
Family history of CAD	19 (31.1)	18 (20.5)	0.18
Medications			
Statins	37 (60.7)	56 (63.6)	0.73
Aspirin	23 (37.7)	48 (54.5)	0.05
Clopidogrel	32 (52.5)	42 (47.7)	0.62
Beta-blocker	33 (54.1)	41 (46.6)	0.41
Intervention			
No. of diseased vessels	1.8 ± 0.8	1.7 ± 0.8	0.37
No. of stents	1.9 ± 1.0	1.6 ± 0.9	0.09
Stent diameter (mm)	3.1 ± 0.4	3.1 ± 0.4	0.55
Stent length (mm)	21.9 ± 11.1	23 ± 13	0.58
Biology			
Hemoglobin (g/dl)	13.3 ± 1.2	13.5 ± 1.3	0.30
White blood count (g/l)	7.1 ± 2.2	9.0 ± 12.6	0.25
Platelets (×10^3/mm^3)	215 ± 46	228 ± 67	0.21
Creatinine (μmol/l)	83.4 ± 31.1	79.9 ± 26.4	0.49
Fibrinogen (g/l)	3.4 ± 0.9	3.5 ± 0.9	0.54

Values are mean ± SD or n (%).
BMI = body mass index; CAD = coronary artery disease; GR = good responders; HTPR = high on-treatment platelet reactivity.

apy) and baseline laboratory data between the 2 groups. Angiographic and interventional data were also similar in the 2 groups, including the number of diseased vessels.

CEC increase induced by PCI is reduced in GR patients and correlated to the level of ADP receptor $P2Y_{12}$ blockade. The endothelial injury induced by PCI was monitored by CEC counts determined at H0, H6, and H24. The kinetics of CEC released from the vessel wall is displayed in Figure 1. No significant difference in pre-procedural CEC counts was observed between GR and HTPR patients (8.3 ± 8.8 cells/ml vs. 6.8 ± 8 cells/ml, respectively; $p = 0.3$). Consistent with the inclusion criteria limited to patients with stable CAD, these baseline values were low and within expected normal range (31). In both groups of patients, PCI induced a significant rise in CEC levels detectable 6 h after the procedure compared with pre-procedural values (GR and HTPR groups: 26.9 ± 22.5 cells/ml vs. 8.3 ± 8.8 cells/ml, $p < 0.0001$ and 59.6 ± 27.5 cells/ml vs. 6.8 ± 8 cells/ml, p 0.0001, respectively). Interestingly, the CEC peak value was significantly higher in the HTPR group compared with the GR group (59.6 ± 27.5 cells/ml vs. 27 ± 22 cells/ml, $p < 0.001$). Twenty-four hours after the procedure, although CEC levels had significantly decreased compared with peak values in both groups, a significant difference was still observed compared with baseline (16.3 ± 18.3 cells/ml, $p = 0.003$ and 20.6 ± 13.1 cells/ml, $p < 0.001$, respectively, for GR and HTPR) (Fig. 1). The extent of endothelial injury was assessed by the difference in CEC count between H6 and H0, which defined CEC delta-change. This CEC delta-change evaluates the impact of the procedure independently from baseline CEC level (29). Interestingly, the endothelial injury assessed by CEC delta-change was significantly higher in HTPR compared with GR patients (52.6 ± 25.6 vs. 18.6 ± 23.5, respectively; $p < 0.001$) (Fig. 2). In addition, a significant correlation between the VASP index and CEC delta-change ($r = 0.59$; $p < 0.001$) was observed in the study population, indicating that patients with the higher ADP receptor $P2Y_{12}$ blockade had the lower endothelial injury (Fig. 3).

ADP receptor $P2Y_{12}$ blockade is an independent predictor of CEC increase during PCI. A linear regression analysis was used to determine predictors of endothelial injury following PCI. The univariate analysis showed that the VASP group (GR and HTPR), family history of CAD, and the number of stents were significantly associated with the endothelial injury (Table 2). However, after adjustment for

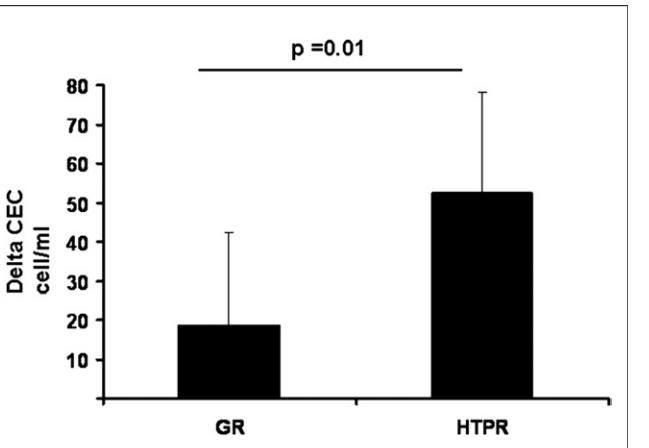

Figure 2 Comparison of CEC Delta-Change Between GR and HTPR Patients

The CEC delta-change is the CEC value 6 h after PCI − CEC value at baseline. Results are means ± SD cells/ml. Abbreviations as in Figure 1.

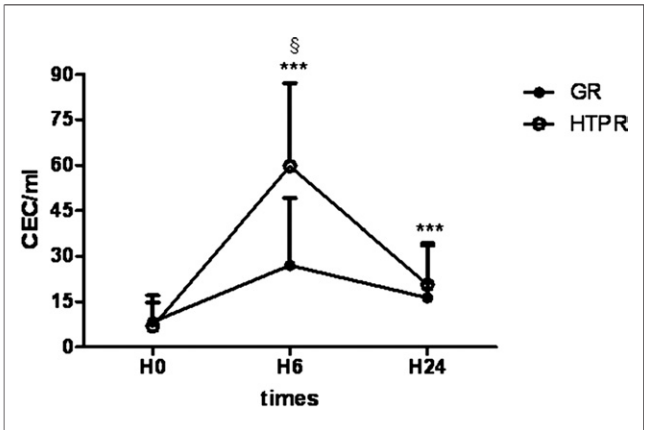

Figure 1 CEC Levels in GR and HTPR Patients

The circulating endothelial cells (CEC) levels were determined using a CD146-based immunomagnetic separation assay in good responders (GR) (VASP index <50%) and high on-treatment platelet reactivity (HTPR) patients (vasodilator-stimulated phosphoprotein [VASP] index ≥50%) before and 6 and 24 h after percutaneous coronary intervention. Results are mean ± SD cells/ml. ***$p <$ 0.001 compared with baseline value between groups. §$p < 0.001$ between GR and HTPR patients.

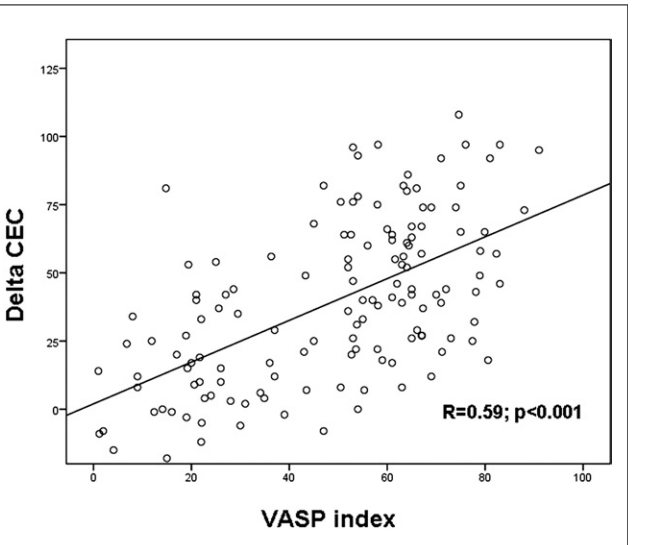

Figure 3 Correlation Between CEC Delta-Change and VASP Index

Abbreviations as in Figure 1.

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Table 2 Univariate and Multivariate Analysis for Predictors of CEC Delta-Change

	Univariate Analysis		Multivariate Analysis	
	CEC H6 − H0, Mean ± SD or R*	p Value	Beta†	p Value
Age (yrs)	0.07	0.42	0.009	0.907
Sex				
Women	37.9 ± 29.9	0.76	0.068	0.384
Men	39.8 ± 29.8			
Cardiovascular risk factors				
BMI (kg/m^2)	0.08	0.38	−0.016	0.849
Smoker				
Yes	40.5 ± 29.5	0.79		
No	38.9 ± 30.0			
Hypertension				
Yes	43.2 ± 30.8	0.10	0.114	0.171
No	34.8 ± 28.0			
Diabetes				
Yes	48.2 ± 33.0	0.06	0.023	0.782
No	36.7 ± 28.3			
Dyslipidemia				
Yes	39.8 ± 27.7	0.82		
No	38.7 ± 32.8			
Family history of CAD				
Yes	28.6 ± 28.7	0.02	−0.065	0.423
No	42.9 ± 29.4			
Medication				
Statins				
Yes	37.7 ± 29.5	0.41	−0.139	0.146
No	42.1 ± 30.3			
Aspirin				
Yes	40.8 ± 29.3	0.58	0.021	0.825
No	38.0 ± 30.3			
Clopidogrel				
Yes	39.1 ± 29.8	0.92		
No	39.6 ± 29.9			
Beta-blocker				
Yes	38.8 ± 28.6	0.82		
No	40.0 ± 31.1			
Intervention				
No. of diseased vessels	0.12	0.18	0.219	0.009
No. of stents	−0.17	0.05	−0.169	0.046
Stent diameter (mm)	0.10	0.28		
Stent length (mm)	−0.03	0.73		
Biology				
Hemoglobin (g/dl)	0.05	0.59		
White blood count (g/l)	0.05	0.55		
Platelets (×10^3/mm^3)	0.03	0.79		
Creatinine (μmol/l)	−0.05	0.61		
Fibrinogen (g/l)	0.11	0.21		
VASP group				
GR (VASP <50%)	18.9 ± 23.5	<0.001	0.514	<0.001
HTPR (VASP ≥50%)	52.6 ± 25.6			

*R = Spearman correlation coefficient. †Beta = standardized beta coefficient (beta represents the change of the SD in quality of life score resulting from a change of 1 SD in the independent variable).

CEC = circulating endothelial cells; VASP = vasodilator-stimulated phosphoprotein; other abbreviations as in Table 1.

age, sex, BMI, hypertension, diabetes mellitus, family history of CAD, statin therapy, number of disease vessels, and number of stents implanted, the multivariate approach identified 3 independent predictors of endothelial injury assessed by CEC delta-change: VASP group ($p < 0.001$), number of diseased vessels ($p = 0.009$), and number of stents implanted ($p = 0.046$) (Table 2). These 3 parameters were similarly linked to CEC peak values (H6) (data not shown). Altogether, these data indicated that disease severity, mechanical trauma, and ADP receptor $P2Y_{12}$ blockade are independent predictors of the endothelial injury in clopidogrel-treated patients undergoing PCI.

Discussion

To our knowledge, this is the first prospective study demonstrating a protective effect of clopidogrel on the endothelium during PCI. Indeed, a correlation was found between the level of ADP receptor $P2Y_{12}$ blockade, as measured by the VASP index, and the endothelial injury, assessed by CEC peak values and delta-change. In multivariate analysis, VASP group, number of diseased vessels, and number of implanted stents independently predicted CEC delta-change, indicating that ADP receptor $P2Y_{12}$ blockade was an independent determinant of the endothelial injury.

Clopidogrel is widely used in patients with CAD to reduce the risk of thrombotic events. Interestingly, a large interindividual variability in response to the drug has been observed in patients with CAD (14,34). Of importance, several investigators have since reported a strong relationship between the response to clopidogrel and occurrence of post-PCI thrombotic events (11–16,35,36). The VASP index is the most specific platelet assay to evaluate $P2Y_{12}$ ADP receptor blockade and is not influenced by other antiplatelet agents or anticoagulants (37). Therefore, the assessment of ADP receptor $P2Y_{12}$ blockade, which represents the biologic effect of the drug, is also of clinical significance. In fact, the 50% cut-off value of the VASP index used in the present study was shown to have a very high negative predictive value for major adverse cardiovascular events (11–16). Using the same threshold of platelet reactivity, we found that optimal clopidogrel responsiveness is associated with reduced CEC elevation following PCI.

Increased levels of CEC have been identified across a broad spectrum of cardiovascular diseases and have been linked to the clinical severity, as well as the clinical outcome, of CAD (24). Several studies have highlighted the interest in the early evaluation of endothelial damage following PCI (30–32). We have previously reported that CEC elevation is detectable as early as the end of the procedure, whereas peak values are observed 6 h after the end of the intervention (32). These kinetics indicate that CEC increase not only reflects the direct mechanical injury related to balloon inflation and stent deployment but also more widespread deleterious responses related to inflammation or ischemia participating in delayed endothelial detachment (38,39). In

addition, we have previously observed CEC released from ruptured plaques in acute coronary syndrome, and their prognosis values have been documented (27–29). Given the key role of endothelial denudation in atherothrombosis, these data indicate that reduced endothelial vulnerability evidenced by CEC levels may participate in the clinical benefit of clopidogrel over recurrent thrombotic events. In addition, these findings may be of particular interest in patients undergoing drug-eluting stent implantation because of the relationship between endothelial damage and drug-eluting stent thrombosis (40).

Comparison of baseline characteristics of the 2 subgroups of patients indicated that they differed according to BMI and the number of patients with diabetes mellitus. These observations are consistent with previous reports of an association between these clinical factors and reduced responsiveness to antiplatelet agents (31–42). In addition, diabetes has already been associated with high numbers of CEC (43). However, the possibility that these risk factors impact the difference observed in CEC level post-PCI was ruled out by multivariate analysis. Determination of factors influencing CEC delta-change evidenced the role of clopidogrel responsiveness. ADP receptor $P2Y_{12}$ blockade was shown to independently predict CEC delta-change or peak level, demonstrating a protective effect of clopidogrel-dependent inhibition of platelet reactivity on endothelial damage during PCI. The mechanisms underlying the protection of the endothelium by clopidogrel remain to be fully elucidated. In line with the hypothesis of a beneficial reduction of endothelium-platelet interactions, short-term clopidogrel treatment in patients undergoing PCI has been shown to limit agonist-induced platelet P-selectin and CD40L expression and subsequent release of inflammatory mediators like tumor necrosis factor known to participate in CEC release (44,45). Similarly, the link between inhibition of platelet function and reduction of various inflammatory markers has been recently demonstrated during long-term clopidogrel treatment (46). In addition, improvement of endothelial function in patients with CAD under chronic clopidogrel therapy has been shown to occur concomitantly with a reduction of the oxidative stress marker 8-iso-PGF2alpha and inflammatory markers such as high-sensitivity C-reactive protein and sCD40L (19).

Beside platelet-dependent mechanisms, findings from animal models and in vitro studies have identified platelet-independent effects of thienopyridines on the vasculature. These effects included a desensitization of aortic rings to vasoconstrictors after intravenous administration of clopidogrel (47) and a dose-dependent increment of nitric oxide production in ECV304 cells incubated with clopidogrel (48). Finally, the recently described expression of ADP receptor $P2Y_{12}$ by human coronary endothelial cells and HUVEC (49) allows us to hypothesize that clopidogrel could also impact the endothelium by direct interaction with an endothelial receptor. More insight into the targets of ADP receptor $P2Y_{12}$ blockade was evidenced using a model of arterial injury in bone marrow–transplanted chimeric mice, which showed that reduced vessel wall response to injury in $P2Y_{12}$-deficient mice was mainly dependent on platelet ADP receptor $P2Y_{12}$ blockade (20).

Study limitations. Limitations of the present study include the lack of outcome data and the slightly higher rate of patients considered to have HTPR compared with that in previous studies.

In the present study, we observed that, in addition to the level of ADP receptor $P2Y_{12}$ blockade, the number of diseased vessels and number of implanted stents were also independently associated with the endothelial injury. These data indicate that, besides platelet reactivity, disease severity also determines endothelial vulnerability to PCI and is consistent with increasing endothelial dysfunction in advanced CAD (50). Moreover, the CEC increase is also dependent on the number of implanted stents, in line with the critical role of mechanical trauma in endothelial detachment (51). Altogether, these data suggest that CEC increase may be a reliable marker integrating the impact of procedural and clinical risk factors together with platelet reactivity on endothelial vulnerability during PCI, with potential prognostic significance.

Conclusions

The present study demonstrated that the level of ADP receptor $P2Y_{12}$ blockade induced by clopidogrel reduces the endothelial injury after PCI. Because disruption of endothelial integrity promotes thrombosis and restenosis, which represent the main limitations of PCI, the findings suggest that optimized ADP receptor $P2Y_{12}$ blockade may reduce vascular events, not only by preventing acute thrombosis but also by exerting protective endothelial effects.

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Key Words: ADP receptor $P2Y_{12}$ blockade ■ circulating endothelial cells ■ clopidogrel ■ endothelial injury.