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High Platelet Reactivity on Clopidogrel Therapy Correlates With Increased Coronary Atherosclerosis and Calcification

A Volumetric Intravascular Ultrasound Study

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OBJECTIVES This study sought to evaluate the relationship between platelet reactivity and atherosclerotic burden in patients undergoing percutaneous coronary intervention (PCI) with pre-intervention volumetric intravascular ultrasound (IVUS) imaging.

BACKGROUND Atherosclerosis progresses by the pathologic sequence of subclinical plaque rupture, thrombosis, and healing. In this setting, increased platelet reactivity may lead to more extensive arterial thrombosis at the time of plaque rupture, leading to a more rapid progression of the disease. Alternatively, abnormal vessel wall biology with advanced atherosclerosis is known to enhance platelet reactivity. Therefore, it is possible that by either mechanism, increased platelet reactivity may be associated with greater atherosclerotic burden.

METHODS This study included patients who underwent PCI with pre-intervention IVUS imaging and platelet reactivity functional assay ($P2Y_{12}$ reaction units) performed >16 h after PCI, after the stabilization of clopidogrel therapy (administered before PCI). Platelet reactivity >230 $P2Y_{12}$ reaction units defined high on-treatment platelet reactivity (HPR).

RESULTS Among 335 patients (mean age 65.0 years, 71% men), there were 109 patients with HPR (32.5%) and 226 without HPR (67.5%), with HPR being associated with diabetes and chronic renal insufficiency. By IVUS analysis, patients with HPR had significantly greater target lesion calcium lengths, calcium arcs, and calcium indexes. Furthermore, patients with HPR tended to have longer lesions and greater volumetric dimensions, indicating higher plaque volume, larger total vessel volume, and also greater luminal volume, despite similar plaque burden. By multivariate analysis controlling for baseline clinical variables, HPR was the single consistent predictor of all IVUS parameters examined, including plaque volume, calcium length, and calcium arc.

CONCLUSIONS Increased platelet reactivity on clopidogrel treatment, defined as >230 $P2Y_{12}$ reaction units, is associated with greater coronary artery atherosclerotic disease burden and plaque calcification. (J Am Coll Cardiol Img 2012;5:540–9) © 2012 by the American College of Cardiology Foundation

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Enhanced platelet reactivity plays a pivotal role in arterial ischemic events, acute coronary syndromes, and complications of percutaneous coronary intervention (PCI) (1). In addition, the complex interactions among platelets, inflammatory cells, vascular cells, and chemokines also play a major role in atherosclerotic plaque and neointimal formation (2,3). Putative mechanisms whereby platelets may promote atherosclerosis include: 1) releasing chemokines and their precursors, which trigger the atherogenic recruitment of vascular cells or modulate processes such as angiogenesis or lipoprotein metabolism; 2) inducing chemokine secretion by endothelial and other vascular cells; and 3) binding and presenting vascular cell–derived chemokines to trigger arrest of circulating mononuclear cells (2,4–8). Huo et al. (9) showed that the injection of activated platelets exacerbated atherosclerotic lesion formation, a process involving platelet surface receptors that facilitate mononuclear cell recruitment. The deposition of the most abundant platelet chemokine, platelet factor-4, has been correlated with lesion severity and symptomatic atherosclerosis, suggesting that persistent platelet activation may contribute to the evolution of vascular lesions and supporting the rationale for long-term antiplatelet therapy in patients at risk for atherosclerosis (10). These observations not only extend the current view of platelets as being responsible for adhesion to the endothelium and propagation of endovascular thrombosis but also suggest that activated platelets play an important role in promoting the atherosclerotic process itself, in particular the stages relating to acute coronary syndromes. Furthermore, it is also well described that alterations in the vascular wall or situations inducing high shear stress may lead to secondary platelet activation (11,12). Therefore, as either a primary cause or a secondary consequence, there is a strong rationale to expect that platelet activation may be associated with atherosclerotic plaque burden.

Antiplatelet therapy is a cornerstone of cardiovascular disease management and secondary prevention (13). Significant reductions in ischemic complications in a wide range of patients with coronary artery disease have been demonstrated in major randomized controlled trials by the use of dual-antiplatelet therapy with a thienopyridine plus aspirin (14,15). However, clopidogrel nonresponsiveness (characterized as high on-treatment platelet reactivity [HPR]) (16) has been recognized to correlate with adverse events after acute coronary syndromes and PCI (17–24). Because most patients with known coronary artery disease are receiving long-term antiplatelet therapy, assessment of platelet reactivity and ascertainment of HPR status are relevant to clinical practice and patient outcomes. Importantly, a recent meta-analysis demonstrated that HPR is associated with long-term cardiovascular events after PCI, including death, myocardial infarction, and stent thrombosis (24). Although it is widely assumed that this is due to increased thrombotic events, a preliminary study recently reported that HPR may be associated with increased coronary artery atherosclerotic burden as assessed by cine angiography (25). In the present study, we sought to extend these findings and determine if HPR, as an indicator of high residual platelet reactivity in patients receiving clopidogrel, correlates with more extensive atherosclerotic disease as determined by volumetric intravascular ultrasound (IVUS) imaging, the gold-standard imaging modality for the assessment of atherosclerotic burden and calcification.

ABBREVIATIONS AND ACRONYMS

CSA = cross-sectional area

EEM = external elastic membrane

HPR = high on-treatment platelet reactivity

IVUS = intravascular ultrasound

PCI = percutaneous coronary intervention

METHODS

Patient population, PCI, and IVUS image acquisition. We analyzed 335 consecutive patients who underwent PCI with pre-intervention IVUS imaging and who had platelet function testing performed on the day after PCI. Only a single culprit target lesion and associated target vessel per patient were included in this study. IVUS of other vessels was not clinically indicated and was not performed. Key enrollment criteria were as follows: guideline-appropriate requirement for PCI, typically based on severe disease, positive stress test results, or presentation with unstable coronary syndromes; age >18 years; and signed informed consent. Exclusion criteria were as follows: presentation with ST-segment elevation myocardial infarction; serum creatinine ≥2.0 mg/dl;

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prior heart transplantation; active autoimmune disease; illicit drug use; human immunodeficiency virus infection; prior malignancy with mediastinal irradiation, bone marrow transplantation, or high-dose chemotherapy; and adult congenital heart disease.

PCI procedures were performed according to current standard guidelines, and the type of stent implanted and the use of pharmacological agents were at the discretion of the operator. The decision to perform IVUS was also at the discretion of the operator and was made entirely independently of this study, with >80% of all IVUS images acquired before this study was conceived. If the patient had no prior exposure to clopidogrel (n = 132), a dose of 600 mg was administered no later than 2 h before PCI; patients already treated with clopidogrel before hospitalization received a loading dose of 300 mg before PCI (n = 203). After PCI, all patients received aspirin, clopidogrel, and a statin. For the purposes of this analysis, patients with anemia (hemoglobin <10.5 g/dl), those with thrombocytopenia (platelet count <125,000/ml), and those receiving glycoprotein IIb/IIIa inhibitors during or after PCI were excluded because of the possibility of interference with platelet assay measurements.

Platelet reactivity testing. The VerifyNow point-of-care assay (Accumetrics, San Diego, California) was used to measure platelet reactivity. This test has been previously described in detail (26); it is a turbidimetry-based optical detection device that measures platelet-induced aggregation in a system containing fibrinogen-coated beads. The instrument measures changes in light transmission and thus the rate of aggregation in whole blood. In the cartridge used for this assay, there is a channel in which inhibition of the $P2Y_{12}$ receptor is measured; this channel contains adenosine diphosphate as a platelet agonist and prostaglandin E_1 as a suppressor of intracellular free calcium levels, to reduce the nonspecific contribution of adenosine diphosphate binding to $P2Y_{12}$ receptors. Venous blood samples anticoagulated with sodium citrate 0.109 mol/l (ratio 9:1) were tested from each patient 16 to 24 h after PCI (as part of the clinically determined morning

Table 1. Baseline Patient Characteristics in the Overall Study Population and in the Groups With and Without HPR

	Overall Group (n = 335)	HPR (n = 109)	No HPR (n = 226)	p Value
Age (yrs)	65.0 (58.0–73.0)	66.0 (58.4–74.0)	65.0 (57.8–72.0)	0.28
Men	71.0 (238/335)	61.5 (67/109)	75.7 (171/226)	0.01
Body surface area (m^2)	1.93 (1.79–2.13)	1.95 (1.80–2.16)	1.93 (1.79–2.12)	0.70
Platelet count (×1,000/μl)	196 (164–232)	189 (158–220)	198 (167–236)	0.037
Prior MI	21.5 (72/335)	18.3 (20/109)	23.0 (52/226)	0.40
Stroke/TIA	6.6 (22/335)	3.7 (4/109)	8.0 (18/226)	0.16
Congestive heart failure*	7.5 (25/335)	8.3 (9/109)	7.1 (16/226)	0.67
Hypertension	77.9 (261/335)	79.8 (87/109)	77.0 (174/226)	0.67
Hyperlipidemia	80.5 (269/334)	81.5 (88/108)	80.1 (181/226)	0.88
Diabetes mellitus†	31.6 (106/335)	45.9 (50/109)	24.8 (56/226)	0.0002
Chronic renal insufficiency‡	9.3 (31/335)	14.7 (16/109)	6.6 (15/226)	0.026
Current smoking	9.9 (33/335)	8.3 (9/109)	10.6 (24/226)	0.56
Additional medication use				
Beta-blockers	66.9 (220/329)	67.3 (72/107)	66.7 (148/222)	1.00
Statins	70.9 (234/330)	66.7 (72/108)	73.0 (162/222)	0.25
ACE inhibitors	34.8 (115/330)	32.4 (35/108)	36.0 (80/222)	0.54
Anatomical location of target lesion				
Left main	2.1 (7/333)	3.7 (4/108)	1.3 (3/225)	0.22
LAD	44.4 (148/333)	47.2 (51/108)	43.1 (97/225)	0.48
LAD branch (diagonal)	3.6 (12/333)	3.7 (4/108)	3.6 (8/225)	1.00
LCx	14.1 (47/333)	9.3 (10/108)	16.4 (37/225)	0.093
LCx branch (ramus intermedius, OM, LPL, LPDA)	8.1 (27/333)	9.3 (10/108)	7.6 (17/225)	0.67
RCA	24.6 (82/333)	23.1 (25/108)	25.3 (57/225)	0.69
RCA branch (AV continuation, RPDA, RPL)	3.0 (10/333)	3.7 (4/108)	2.7 (6/225)	0.73

Values are median (interquartile range) or % (n/N). *New York Heart Association functional class III or IV. †Requiring medical therapy. ‡Glomerular filtration rate <60 ml/min/1.72 m^2.

ACE = angiotensin-converting enzyme; AV = atrioventricular; HPR = high on-treatment platelet reactivity; LAD = left anterior descending coronary artery, LCx = left circumflex coronary artery; LPDA = left posterior descending artery; LPL = left posterolateral; MI = myocardial infarction; OM = obtuse marginal; RCA = right coronary artery; RPDA = right posterior descending artery; RPL = right posterolateral; TIA = transient ischemic attack.

blood tests) but before the next dose of clopidogrel. Results are expressed as $P2Y_{12}$ reaction units. Platelet reactivity > 230 $P2Y_{12}$ reaction units was used to define patients with HPR clopidogrel resistance (27).

IVUS image analysis. Target lesion IVUS imaging studies were performed after intracoronary administration of 200 μg nitroglycerin using a commercially available IVUS system (Atlantis SR Pro, 40-MHz catheter, Boston Scientific Corporation, Natick, Massachusetts; Eagle Eye, 20-MHz catheter, or Revolution 45-MHz catheter, Volcano Corporation, Rancho Cordova, California) and before any coronary intervention. The IVUS catheter was advanced distal to the stenosis, and imaging was performed with retrograde pullback to the aorto-ostial junction at an automatic pullback speed of 0.5 mm/s. All analyses were performed offline without knowledge of the platelet assay results using planimetry software (Indec Systems Inc., Mountain View, California). The minimal luminal cross-sectional area (CSA) site was the image slice with the smallest luminal CSA. The reference sites were the most normal appearing cross-sections within 5 mm proximal and distal to the stenosis, but before any side branch, and were used to calculate a mean reference. For each patient, the lesion with the smallest luminal CSA was chosen for analysis. The lesion itself was defined as the segment between the proximal and distal reference sites whose length (in millimeters) was calculated using the pullback duration and pullback speed. Quantitative analysis included measurement of the external elastic membrane (EEM) and luminal CSA every 1 mm within the length of the lesion. Plaque and media CSA was calculated as EEM minus luminal CSA. Once a complete set of CSA measurements were obtained, EEM, plaque plus media, and luminal volumes were calculated using Simpson's rule. Plaque burden was calculated as plaque and media divided by EEM volume or CSA. A remodeling index was calculated as the lesion divided by the mean reference EEM CSA. Calcium was identified as an echo signal brighter than the adventitia with acoustic shadowing. The maximal arc of calcium (in degrees) within the lesion was measured with the electronic protractor centered on the lumen. Calcium length (in millimeters) within the lesion was measured as the length of the lesion in which there was IVUS-detectable calcium. Calcium index was calculated as: total calcium length/lesion length × maximal calcium arc/360°.

Clinical patient follow-up. All patients had follow-up completed out to 12 months or to fatal events. Follow-up data were collected from patients using scripted telephone interviews and simultaneous electronic medical record review. If telephone contact was not established, a mailed questionnaire was used. Major adverse cardiovascular events (death, myocardial infarction, or target lesion revascularization) were adjudicated by an independent committee. The Social Security Death Index was used to obtain the vital status of patients. These methods are the standard operation of the Columbia University Medical Center PCI outcomes database registry, which includes data collection and follow-up under institutional review board approval.

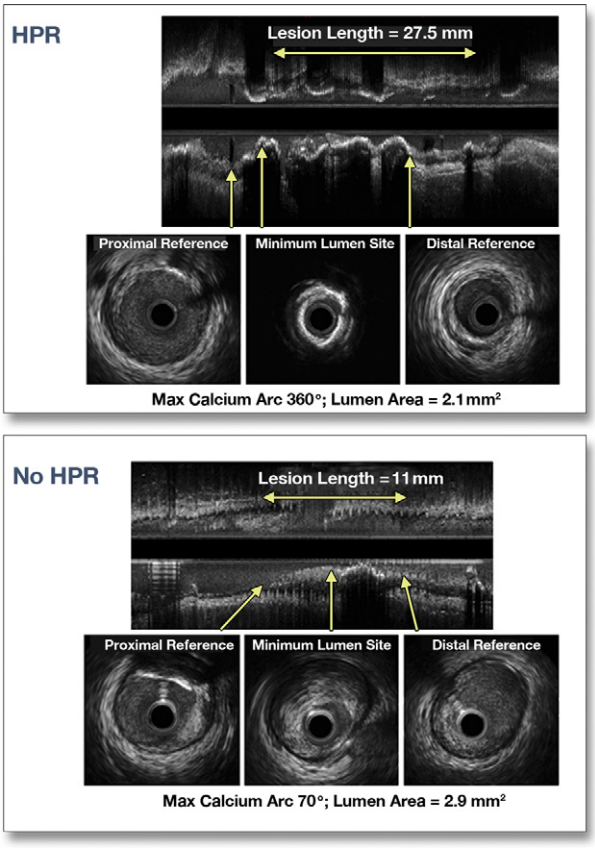

Figure 1. Illustrative Volumetric Intravascular Ultrasound Images From Patients With and Without HPR

(Top) An example of a patient with high on-treatment platelet reactivity (HPR). This long lesion (27.5 mm from proximal to distal reference site) contains diffuse calcification (note the acoustic shadows beyond regions of calcification), with a maximal calcium arc of 360° at the minimum luminal area (MLA) site. **(Bottom)** A patient without HPR. Although not intended to be representative, the lesion length was shorter (11 mm from proximal to distal reference site) with noncalcified plaque at the MLA site; the maximal arc of calcium (70°) was located at the proximal reference. Both lesions had luminal areas at the lesion site of <3.0 mm², considered to be highly hemodynamically significant.

Statistical analyses. Continuous variables are expressed as mean ± SD or as median (interquartile range) as indicated and were compared using Student *t* tests or Wilcoxon rank sum tests if applicable. Discrete variables and clinical outcomes are expressed as frequencies and percents and were compared using chi-square tests, unless the observations in any cell were <5, in which case Fisher exact tests were used. All statistical tests were 2-sided, with a significance level of <0.05. Multivariate linear regression analysis was used to assess the relationship between HPR and IVUS measurements while controlling for the following baseline factors: age, sex, diabetes, and chronic renal failure. A stepwise selection algorithm with entry and stay criteria of 0.1 and 0.1 was used to identify multivariate predictors. Adjusted mean values for IVUS measurements were calculated using least squares mean estimation from a linear regression model. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Among the 335 patients analyzed, there were 109 with HPR (32.5%) and 226 without HPR (67.5%). Patient baseline characteristics are presented in Table 1; HPR was associated with diabetes and chronic renal insufficiency.

Volumetric IVUS analysis was performed in all 335 patients (Fig. 1, Table 2). There was no difference in proximal or distal reference segments in terms of plaque area or plaque burden. Plaque burden at the minimal luminal site was similar between the 2 groups. The HPR group had significantly greater calcification lengths, calcification arcs, and calcium indexes. In addition, patients with HPR tended to have longer lesions and greater volumetric dimensions despite a similar plaque burden. Similar results were obtained when only patients receiving clopidogrel at the time of enrollment were evaluated (Table 3).

Clinical events were rare (Table 4), and there was no significant difference between patients with and those without HPR. However, the present study was not designed or powered to detect differences in clinical outcomes.

After exploratory analyses to identify univariate predictors, multivariate analysis was performed against each of the IVUS-derived variables using the following candidate predictors: HPR, age, sex, diabetes, and chronic renal insufficiency. Table 5 indicates that HPR was the single consistent predictor of all IVUS parameters examined. Male sex

Table 2. Intravascular Ultrasound Measurements in the Overall Study Population and by Groups According to Platelet Reactivity Status

	Overall Group (n = 335)	HPR (n = 109)	No HPR (n = 226)	p Value
Reference site*				
EEM CSA (mm^2)	12.0 (9.3–15.2)	12.2 (9.8–15.0)	11.6 (9.1–15.4)	0.355
Luminal CSA (mm^2)	6.5 (5.1–7.9)	6.7 (5.1–8.0)	6.3 (5.1–7.9)	0.535
Plaque burden	0.45 (0.38–0.52)	0.45 (0.39–0.53)	0.45 (0.37–0.52)	0.751
Minimum luminal area site				
EEM CSA (mm^2)	10.7 (8.5–13.6)	11.0 (8.5–14.0)	10.6 (8.4–13.5)	0.310
Luminal CSA (mm^2)	3.0 (2.3–3.8)	3.1 (2.4–4.0)	3.0 (2.2–3.7)	0.229
Plaque and media CSA (mm^2)	7.5 (5.5–10.0)	7.9 (5.6–10.0)	7.2 (5.4–10.0)	0.434
Plaque burden	0.71 (0.64–0.77)	0.70 (0.64–0.77)	0.71 (0.65–0.77)	0.720
Volumetric data				
EEM volume (mm^3)	100.9 (58.6–174.4)	111.6 (66.6–177.5)	89.4 (57.4–169.0)	0.0526
Luminal volume (mm^3)	38.5 (25.0–61.7)	44.4 (26.3–69.5)	36.3 (22.6–58.8)	0.0244
Plaque and media volume (mm^3)	58.6 (33.1–108.7)	71.2 (36.7–111.2)	53.9 (32.9–103.6)	0.0726
Plaque burden	0.61 (0.55–0.66)	0.61 (0.56–0.66)	0.61 (0.55–0.66)	0.869
Lesion length (mm)	8.1 (5.5–13.1)	8.8 (6.3–14.0)	7.6 (5.2–12.8)	0.0710
Calcium length (mm)	4.7 (1.7–8.8)	5.9 (2.6–10.5)	4.3 (1.4–7.4)	0.0096
Maximal calcium arc (°)	110 (50–190)	130 (70–260)	90 (40–170)	0.0149
Calcium index	0.17 (0.04–0.40)	0.25 (0.07–0.45)	0.15 (0.03–0.38)	0.0250
Remodeling index	0.90 (0.79–1.02)	0.92 (0.79–1.03)	0.90 (0.79–1.02)	0.834

Values are median (interquartile range). *Calculated as the average of proximal and distal reference sites.
CSA = cross-sectional area; EEM = external elastic membrane; HPR = high on-treatment platelet reactivity.

Table 3. Intravascular Ultrasound Measurements in Patients Already Receiving Clopidogrel at Enrollment by Groups According to Platelet Reactivity Status

	Overall Group (n = 203)	HPR (n = 58)	No HPR (n = 145)	p Value
Reference site*				
EEM CSA (mm^2)	11.6 (9.3–15.0)	11.9 (10.2–14.5)	11.4 (9.1–15.4)	0.461
Luminal CSA (mm^2)	6.3 (5.0–7.6)	6.4 (5.3–7.6)	6.3 (5.0–7.6)	0.659
Plaque burden	0.46 (0.38–0.53)	0.47 (0.39–0.53)	0.46 (0.38–0.52)	0.592
Minimum luminal area site				
EEM CSA (mm^2)	10.7 (8.7–13.9)	10.6 (8.5–13.9)	10.7 (8.8–13.9)	0.829
Luminal CSA (mm^2)	3.0 (2.3–3.7)	3.0 (2.5–3.8)	3.0 (2.2–3.7)	0.485
Plaque and media CSA (mm^2)	7.5 (5.5–10.3)	7.7 (5.5–9.9)	7.5 (5.5–10.3)	0.714
Plaque burden	0.71 (0.64–0.77)	0.72 (0.63–0.77)	0.71 (0.65–0.78)	0.867
Volumetric data				
EEM volume (mm^3)	92.3 (55.8–174.4)	106.4 (67.8–200.3)	86.8 (51.8–161.2)	0.0962
Luminal volume (mm^3)	37.4 (22.6–59.5)	42.2 (26.3–69.5)	34.7 (20.5–53.8)	0.0526
Plaque and media volume (mm^3)	56.0 (32.9–110.6)	67.4 (37.4–127.5)	51.3 (30.9–102.9)	0.111
Plaque burden	0.62 (0.55–0.66)	0.61 (0.56–0.67)	0.62 (0.55–0.66)	0.822
Lesion length (mm)	7.6 (5.3–13.0)	8.6 (6.4–14.5)	7.3 (4.9–12.5)	0.0652
Calcium length (mm)	4.4 (1.3–8.7)	7.0 (2.9–11.0)	3.8 (1.1–6.9)	0.0022
Maximal calcium arc (°)	100 (40–180)	125 (70–270)	90 (33–165)	0.0055
Calcium index	0.15 (0.03–0.42)	0.28 (0.07–0.53)	0.11 (0.02–0.40)	0.0116
Remodeling index	0.91 (0.79–1.03)	0.90 (0.78–1.04)	0.91 (0.80–1.02)	0.954

Values are median (interquartile range). *Calculated as the average of proximal and distal reference sites.
Abbreviations as in Table 2.

also correlated with EEM, luminal, and volumetric plaque burden.

Table 6 depicts the IVUS parameters in the 2 groups after adjustment for age, sex, diabetes, and chronic renal failure. Figure 2 indicates that the trends observed in the univariate analyses between the 2 study groups with respect to the IVUS parameters were significantly strengthened after adjustment for clinical variables.

DISCUSSION

Our data demonstrate that platelet reactivity, as assessed by HPR measurement, correlates with diabetes and chronic renal insufficiency as well as with increased calcification and atheroma burden as assessed by IVUS in patients with coronary artery disease requiring PCI. Compared with patients without HPR, those with HPR exhibited increased lesion lengths and plaque volumes despite also having larger luminal volumes. Our analysis also confirmed other previously reported observations, including the fact that men have more plaque than women (28). Importantly, ours is not the first study to suggest that HPR is related to the extent of coronary artery atherosclerotic disease. However, the single prior study that we are aware of that suggested this relationship did not incorporate a sensitive and objective imaging modality to determine atherosclerotic burden but rather relied entirely on cine angiography with visual estimation of disease severity (25). Therefore, our IVUS-based findings add significantly to this former study, extending our understanding of this relationship and demonstrating that HPR is independently associated with sensitive measures of coronary atheroma burden and calcification.

Altered vessel wall characteristics may lead to HPR. Our findings of increased calcification and plaque burden in patients with HPR raise important biologic questions. Foremost, as a potential mechanistic explanation for our findings, intrinsic changes in the biology of the vessel wall may arise

Table 4. Clinical Outcomes in the Overall Study Population and in the Groups With and Without HPR

	Overall Group (n = 335)	HPR (n = 109)	No HPR (n = 226)	p Value
MACEs	18.2 (61/335)	22.9 (25/109)	15.9 (36/226)	0.132
Myocardial infarction	0.9 (3/335)	0.9 (1/109)	0.9 (2/226)	1.000
TLR	17.9 (60/335)	22.9 (25/109)	15.5 (35/226)	0.128
Death	0.6 (2/335)	0.0 (0/109)	0.9 (2/226)	1.000

Values are % (n/N). No patient underwent coronary artery bypass graft surgery.
HPR = high on-treatment platelet reactivity; MACE = major adverse cardiovascular event (death, myocardial infarction, or TLR); TLR = target lesion revascularization.

Table 5. Multivariate Analysis Results for IVUS Variables and Respective Predictors

IVUS Variable and Respective Independent Predictor	Coefficient	Standard Error	p Value
Lesion length/mm			
HPR	1.58	0.83	0.0585
Maximal calcium/°			
HPR	32.4	12.5	0.0101
Calcium length/mm			
HPR	1.76	0.70	0.0118
EEM volume/mm^3			
HPR	23.2	10.5	0.0284
Male	31.6	10.9	0.0039
Luminal volume/mm^3			
HPR	7.9	3.3	0.0161
Male	7.4	3.4	0.0281
Plaque and media volume/mm^3			
HPR	15.3	7.5	0.0432
Male	24.2	7.8	0.0021

Candidate predictors included HPR, age, sex, diabetes, and chronic renal failure.
IVUS = intravascular ultrasound; other abbreviations as in Table 2.

because of diabetes, chronic renal insufficiency, or plaque development (Tables 1, 5, and 6), which lead to functional platelet abnormalities and increased platelet reactivity (29). In support of this, both diabetes and chronic renal insufficiency play a significant role in arterial calcification and were preliminarily reported to be associated with HPR in another study (30). Furthermore, alterations in arterial shear forces due to developing atherosclerotic plaque may activate platelets (11), with additional human in vivo data corroborating the notion that primary vascular disease processes lead to platelet activation (12). Therefore, there is biologic plausibility to suggest that our findings may be due to atherosclerotic disease secondarily activating platelets and leading to HPR.

Table 6. Mean Values for Intravascular Ultrasound Variables in Relation to Platelet Reactivity After Adjustment for Age, Sex, Diabetes, and Chronic Renal Failure

	HPR (n = 109)	No HPR (n = 226)	p Value
Lesion length (mm)	11.0 ± 0.7	9.4 ± 0.5	0.0585
Maximal calcium arc (°)	152.8 ± 10.3	120.4 ± 7.2	0.0101
Calcium length (mm)	7.2 ± 0.6	5.4 ± 0.4	0.0118
EEM volume (mm^3)	133.6 ± 8.7	110.4 ± 6.6	0.0284
Luminal volume (mm^3)	49.2 ± 2.7	41.3 ± 2.0	0.0161
Plaque and media volume (mm^3)	84.4 ± 6.2	69.0 ± 4.7	0.0432

Values are mean ± SD.
Abbreviations as in Table 2.

Increased platelet reactivity may promote atherosclerosis. As an alternative explanation, HPR may be a marker of intrinsic platelet characteristics or functionality that promote plaque formation and calcification. Importantly, silent plaque rupture with arterial thrombosis is a form of vascular wound healing that leads to atherosclerotic progression (31). Therefore, it is possible that increased platelet reactivity may potentiate arterial thrombosis at the time of plaque rupture, thereby driving inflammation and atherosclerotic progression. Moreover, in addition to facilitating arterial thrombosis, several studies have shown a key role for platelets in mediating various nonthrombotic, inflammatory pathways of plaque progression (32–36). On the endothelial layer, platelet-derived chemokines, in conjunction with increased expression of adhesion molecules, promote the recruitment of circulating monocytes that may migrate across the endothelial lining and augment plaque progression. Furthermore, activated circulating platelets may induce monocyte differentiation (32) or form platelet-monocyte complexes that increase the adhesive and migratory capacities of monocytes and promote the additional recruitment of inflammatory cells (37,38). Collectively, there are multiple possible mechanisms whereby patients with high platelet reactivity may develop more advanced atherosclerotic and calcific arterial disease. Importantly, although we measured platelet reactivity and ascertained HPR in patients receiving clopidogrel, it is critical to emphasize that high baseline platelet reactivity (in patients receiving no antiplatelet therapy) is very strongly correlated with subsequent platelet reactivity after the initiation of clopidogrel therapy (39,40). Therefore, HPR is a reliable measure for determining those patients with high intrinsic platelet reactivity during the preceding years of plaque development, when they are not receiving clopidogrel.

Vascular calcification and platelet reactivity. It has been established that calcification of the coronary arteries accurately identifies coronary atherosclerosis (41). However, the relationship between vascular calcification and atherosclerosis is complex; Burke et al. (31,42) identified that vascular calcification is least in eroded plaques, greatest in acute and healed plaque ruptures, and intermediate in stable plaques. Furthermore, at the cellular level, multiple cell types are potentially implicated in the process of vascular ossification (43,44). Again, increased vascular calcification may be either a cause or an effect of HPR. Thus, more extensive, calcific plaque may lead to

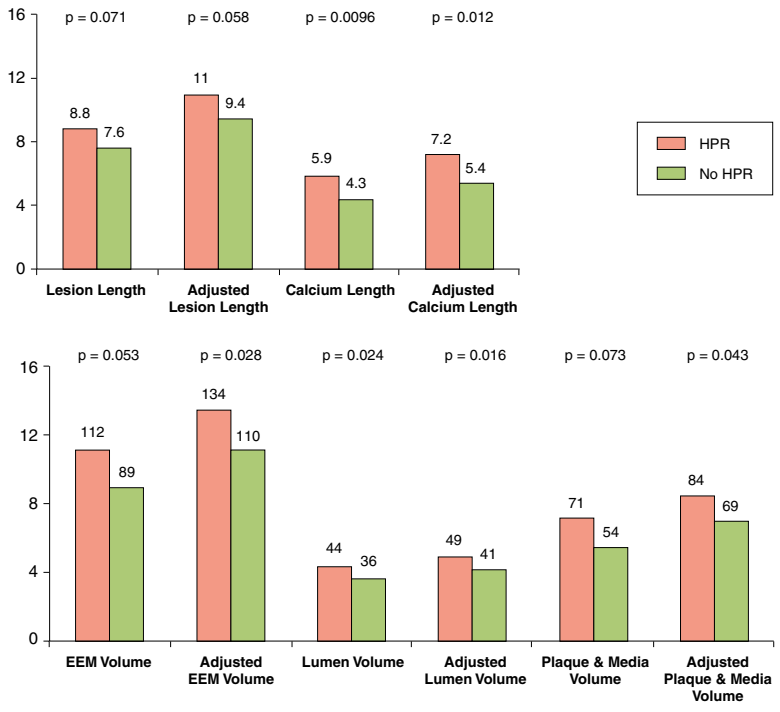

Figure 2. Relationship Between Intravascular Ultrasound–Derived Arterial Wall Measurements and Platelet Reactivity

Data represent mean values per group. Adjustments were performed for age, sex, diabetes, and chronic renal failure. EEM = external elastic membrane; HPR = high on-treatment platelet reactivity.

platelet activation, or increased platelet reactivity may augment the progression of atherosclerosis and arterial calcification.

Study limitations. This was a single-center study. Only patients with available pre-intervention IVUS images were included in the study. Platelet reactivity was assessed at only a single time point, at a relatively late stage in the development of atherosclerotic disease. Furthermore, it was recently shown that platelet reactivity changes during the first weeks after the initiation of clopidogrel therapy (27,45). Therefore, in patients commencing this therapy, the measurement of platelet reactivity soon after PCI may not precisely reflect long-term HPR status. Only the culprit lesion and vessel were analyzed, and we did not assess more global measures of atherosclerotic disease. Our study demonstrates only correlative associations, and the possibility that platelet reactivity is causatively related to atherosclerotic burden and calcification, or vice versa, remains speculative. Although we attempted to account for potential confounding (Tables 5 and 6), we are unable to completely exclude the possibility of residual confounding in our results.

CONCLUSIONS

In a large group of patients undergoing PCI with volumetric IVUS, we have identified that relative resistance to the antiplatelet effects of clopidogrel as defined by HPR status is associated with increases in both atherosclerotic burden and plaque calcification. Further studies are now required to precisely define the mechanisms whereby platelets that are relatively resistant to the effects of clopidogrel are associated with this increase in atherosclerotic disease and if this may be a contributing factor in adverse clinical events. The higher resolution provided by optical coherence tomography compared with IVUS may enable the further identification of atheromatous characteristics that are associated with high platelet reactivity. These findings have important implications for our understanding of the pathobiology and treatment of atherosclerotic disease.

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Key Words: atherosclerosis ■ clopidogrel ■ plaque progression ■ platelet reactivity ■ platelets.