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A Point-of-Care Platelet Function Assay and C-Reactive Protein for Prediction of Major Cardiovascular Events After Drug-Eluting Stent Implantation

Duk-Woo Park, MD,* Seung-Whan Lee, MD,* Sung-Cheol Yun, PHD,† Hae-Geun Song, MD,* Jung-Min Ahn, MD,* Jong-Young Lee, MD,* Won-Jang Kim, MD,* Soo-Jin Kang, MD,* Young-Hak Kim, MD,* Cheol Whan Lee, MD,* Seong-Wook Park, MD,* Seung-Jung Park, MD*

Seoul, Korea

Objectives This study sought to investigate clinical utility of on-site platelet function test and C-reactive protein (CRP) in patients undergoing percutaneous coronary intervention (PCI).

Background Data on long-term prognostic value of high on-treatment platelet reactivity (HTPR) on clopidogrel after PCI are limited. As a distinct biological pathway, CRP has been suggested to be associated with post-PCI atherothrombotic events.

Methods We evaluated 2,849 patients who received drug-eluting stents (DES) and had post-PCI VerifyNow P2Y12 assays (Accumetrics, San Diego, California) performed. Among them, baseline CRP measurement was available in 2,546 patients. The primary endpoint was a composite of all-cause death, nonfatal myocardial infarction, stent thrombosis, and stroke.

Results During follow-up (median, 2.2 years), the occurrence of the primary endpoint did not significantly differ among patients with and without HTPR (2.8% vs. 2.4% at 2 years; hazard ratio [HR]: 1.33, 95% confidence interval [CI]: 0.88 to 2.01; $p = 0.18$). By contrast, patients with elevated CRP levels were at significantly higher risk for the primary endpoint, as compared with those with nonelevated CRP levels (5.6% vs. 1.7% at 2 years; HR: 2.81, 95% CI:, 1.83 to 4.31; $p < 0.001$). The VerifyNow test had no incremental usefulness to classify long-term risk. However, the incorporation of CRP into a model with conventional clinical and procedural risk factors significantly improved the C-statistic for the prediction of the primary endpoint (0.729 to 0.759; $p = 0.03$).

Conclusions We failed to identify that HTPR measured by VerifyNow P2Y12 assay was significantly associated with long-term atherothrombotic risks in patients receiving DES. However, elevated CRP levels were significantly associated with worse outcomes and had incremental predictive values over conventional risk factors. (J Am Coll Cardiol 2011;58:2630–9) © 2011 by the American College of Cardiology Foundation

The clinical importance of clopidogrel has been intensified in patients undergoing percutaneous coronary intervention (PCI) with drug-eluting stents (DES), which have been associated with the ongoing propensity for late stent thrombosis, necessitating the prolonged use of dual antiplatelet therapy (1). However, a wide interindividual response to clopidogrel has been observed, and high on-treatment platelet reactivity (HTPR) is associated with adverse ischemic events after PCI (2). Multiple studies suggested a relationship between HTPR, measured by point-of-care assays, and post-PCI ischemic events (3–6). However, these studies were hampered by the small number of events, few patients, or limited duration of follow-up. It is unknown whether HTPR associated with the risks for periprocedural or mid-term events are the same as those associated with long-term risks.

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From the *Department of Cardiology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea; and the †Division of Biostatistics, Center for Medical Research and Information, University of Ulsan College of Medicine, Seoul, Korea. This study was partly supported by the Cardiovascular Research Foundation, Seoul, Korea, and by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Korea (A102065). The authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. D.-W. Park and S.-W. Lee contributed equally to this work.

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As a distinct biological pathway, the inflammatory biomarker C-reactive protein (CRP) has been postulated to be a hemostatic risk factor predicting atherothrombosis, and our previous observation suggested that a high level of CRP was associated with stent thrombosis or major cardiovascular events in patients treated with DES (7,8).

Coupled with suggested relationships of HTPR and CRP with serious clinical events, enhanced risk assessment with addition of these laboratory assays on conventional clinical and procedural risk factors would be of great clinical value if it could more accurately identify "high-risk" patients. Therefore, as a primary study objective, we tested the hypothesis that HTPR measured by the VerifyNow P2Y12 assay (Accumetrics, San Diego, California) is associated with long-term atherothrombotic events in patients receiving DES. Secondarily, on an a priori basis, we compared the incremental usefulness of onsite platelet function assay and CRP from different biological pathways to predict clinical outcome in such patients.

Abbreviations and Acronyms

CI = confidence interval
CRP = C-reactive protein
DES = drug-eluting stent
HR = hazard ratio
HTPR = high on-treatment platelet reactivity
MI = myocardial infarction
PCI = percutaneous coronary intervention
PRU = $P2Y_{12}$ reaction unit(s)
TIMI = Thrombolysis In Myocardial Infarction

Methods

Study population. Patients were eligible to be enrolled if they had undergone PCI with at least 1 DES for stable angina or ischemia, or non–ST-segment elevation acute coronary syndromes. Study patients were consecutively enrolled at the Asan Medical Center (Seoul, Korea) between March 2006 and December 2009. All patients undergoing PCI were prescribed aspirin (loading dose, 200 mg) and received optimal clopidogrel treatment (defined as a maintenance of 75 mg/day therapy for >5 days or a loading dose of 300 or 600 mg ≥12 h before PCI). After the procedure, patients were prescribed aspirin (100 to 200 mg once daily) indefinitely and clopidogrel (75 mg once daily) for at least 12 months, regardless of DES type. Treatment beyond this duration was at the discretion of the physician.

Patients with ST-segment elevation acute myocardial infarction (MI), those with periprocedural use of glycoprotein IIb/IIIa inhibitors, and those with a known platelet function disorder or thrombocytopenia (platelet count of $<80 \times 10^3/\mu l$) were excluded. Patients were also excluded if they presented with cardiogenic shock, had concomitant inflammatory conditions (such as active infection, inflammatory arthritis, or connective tissue disease) or malignancies, or had a contraindication to aspirin or clopidogrel.

This study was approved by the institutional review board of Asan Medical Center, and all patients provided written informed consent.

Laboratory measurements of platelet reactivity and CRP. Platelet function was assessed with the VerifyNow P2Y12 test 24 to 48 h post-PCI, the time at which the study patients would be expected to be near or at their steady-state level of platelet inhibition immediately before discharge. This test has been previously described in detail (9). The instrument measures platelet-induced aggregation as an increase in light transmittance and uses a proprietary algorithm to report values in $P2Y_{12}$ reaction units (PRU). The device also provides an estimated inhibition without a pre-clopidogrel sample by reporting the ratio of the results of the ADP-PGE1 and iso-TRAP channels (VerifyNow % inhibition).

Considering the biological plausibility and the reported associations with cardiovascular events, we also measured baseline CRP levels in study patients. Fasting blood samples were obtained in the morning before the procedure. CRP was assayed using a latex-enhanced high-sensitivity CRP immunoassay (COBAS INTEGRA, Roche Diagnostics, Mannheim, Germany) (8). All laboratory testing (VerifyNow P2Y12 assay and CRP values) was performed by personnel blinded to patient information and study objectives.

Endpoints, definitions, and follow-up. The primary endpoint was the first occurrence of major cardiovascular events defined as a composite of all-cause death, nonfatal MI, stent thrombosis, and stroke. The principal secondary endpoints were death from any cause; MI, stent thrombosis; stroke (from any cause); target vessel revascularization; a composite of cardiovascular death, nonfatal MI, stent thrombosis, or stroke; and bleeding, according to the Thrombolysis In Myocardial Infarction (TIMI) criteria (10).

All deaths were considered to be from cardiovascular causes unless an unequivocal noncardiovascular cause could be established. The diagnosis of acute MI was based on the universal definition of MI (11). Periprocedural or postprocedural elevations of cardiac enzymes were disregarded if ischemic signs or symptoms were absent. Stent thrombosis was assessed by the Academic Research Consortium criteria (12), with the pre-specified component of primary composite outcome being definite or probable. Stroke, as detected by the occurrence of a new neurological deficit, was confirmed by a neurologist and on imaging. Target vessel revascularization was defined as any percutaneous or surgical revascularization of the target vessel. Major and all types of (major, minor, or minimal) bleedings were assessed in accordance with TIMI criteria. All study endpoints were confirmed on the basis of source documentation from medical records and were adjudicated by an independent group of clinicians blinded to the VerifyNow P2Y12 assay and CRP values.

In this study, HTPR was defined by a PRU value >235 and/or a % inhibition <15%. This cutoff was chosen because it was similar to the cutoff (PRU and % inhibition) suggested by a prior observation of a similar ethnic population that used receiver-operator characteristic curve anal-

ysis to define the optimal cutoff of the VerifyNow P2Y12 assay for prediction HTPR on standard light transmittance aggregometry (13). The cutoff is also similar with other studies (14,15). Based on the cutoff point suggested in the literature and in our previous report (8,16), CRP levels ≥3 mg/l were considered elevated. These cutoff points of the P2Y12 assay and CRP values were pre-specified in the study protocol. In our practice, alteration of antiplatelet therapy was not done according to the results of the VerifyNow P2Y12 assay or CRP measurements.

Clinical, procedural, and outcome data were prospectively collected by independent research personnel unaware of the study aims and entered into a central database, as previously described (8). Clinical follow-up after PCI was performed via office visit or telephone contact at 1, 6, and 12 months and then every 6 months thereafter. At these visits, data pertaining to patients' clinical status, all interventions, outcome events, and adverse events were recorded. Adherence to antiplatelet medication was routinely assessed at each time of follow-up contact and also verified by pharmacy refill data. For validation of complete follow-up data, information about vital status was obtained from the National Population Registry of the Korea National Statistical Office with the use of a unique personal identification number.

Statistical methods. Continuous variables are presented as mean ± SD and were compared with the *t* test or Mann-Whitney *U* test. Categorical variables are reported as frequencies (%) and were compared with the chi-square statistic or Fisher exact test, as appropriate.

A total sample of 3,000 observations was computed to achieve 90% power at a 2-sided 0.05 significance level to detect a hazard ratio (HR) ≥2.0 with a Cox regression of the log HR on a binary covariate (high vs. normal on-treatment platelet reactivity) with a 25% or greater prevalence. The sample size was adjusted for an anticipated rate of the primary endpoint of 4.0% at a median follow-up of 2 years (17).

We used a Cox proportional hazards model to compare clinical endpoints between patients with HTPR and those without HTPR. In addition, the relationship between different degrees of platelet reactivity (PRU or % $P2Y_{12}$ inhibition by quintiles or continuous model) and outcomes were evaluated. Survival analysis for patients with and without HTPR was performed using the Kaplan-Meier method, and the differences between groups were assessed by the log-rank test.

After evaluating the relation to CRP to clinical outcomes with the use of Cox proportional hazards regression, we compared the incremental value of incorporating HTPR or elevated CRP levels into the context of conventional clinical and procedural characteristics, for prediction of primary endpoint. Estimates of the C-statistic for the Cox regression models were calculated according to the method of Pencina et al. (18). Differences in C-statistics (with 95% confidence interval [CI]) after the addition of the laboratory markers to a model with conventional clinical and procedural risk factors were obtained through the bootstrap percentile method (200 replicates) (19). All reported p values are 2-sided, and p values

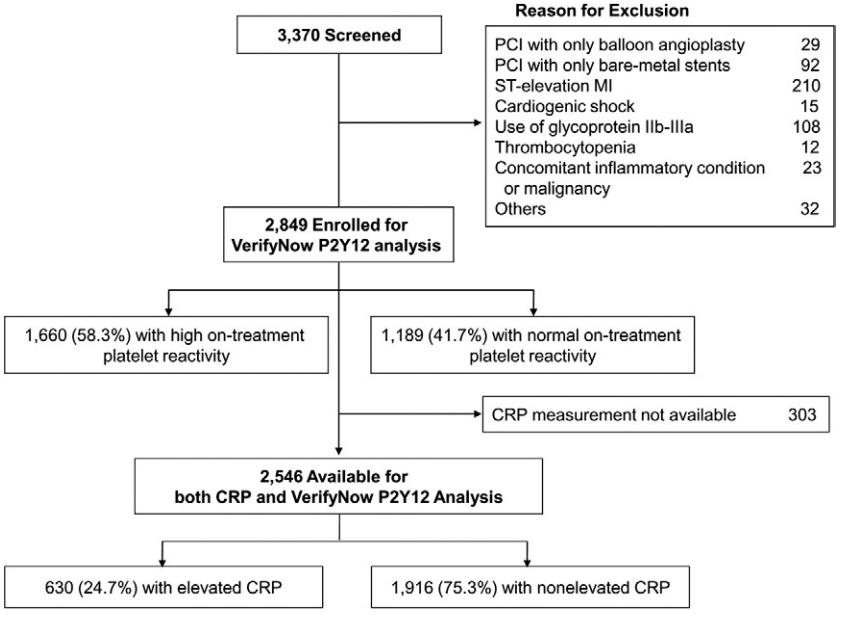

Figure 1 Study Profile

High on-clopidogrel platelet reactivity was defined by a $P2Y_{12}$ reaction unit value >235 and/or a % inhibition <15%, as measured by the VerifyNow P2Y12 assay. Elevated C-reactive protein (CRP) level was defined as value ≥3 mg/l. MI = myocardial infarction; PCI = percutaneous coronary intervention.

<0.05 were considered to indicate statistical significance. SAS software, version 9.1 (SAS Institute, Cary, North Carolina) was used for statistical analysis.

Results

Patient characteristics. During enrollment period, 3,370 patients underwent an index PCI and had VerifyNow P2Y12 assays performed 24 to 48 h after the procedure. Among them, a total of 2,849 patients met the study inclusion criteria and met none of the criteria for exclusion (Fig. 1).

Baseline characteristics of the entire population and of the patients according to the magnitude of platelet reactivity are shown in Table 1. HTPR was observed in 58.3% of the study population. Covariates of HTPR included older age, female sex, higher body mass index, and hypertension. Types of DES used were sirolimus-eluting stents (41.5% of the population), paclitaxel-eluting stents (12.5%), zotarolimus-eluting stents (19.9%), everolimus-eluting stents (15.5%), Resolute zotarolimus-eluting stents (8.0%, Medtronic, Minneapolis, Minnesota), and other DES (2.5%), and did not significantly differ among patients with or without HTPR. Status of antiplatelet therapy and cardiac-related medications are summarized in Table 2. All patients received optimal clopidogrel pre-treatment before the VerifyNow P2Y12 assay. The status of dual antiplatelet therapy at different time intervals did not differ between patients with and without HTPR.

Association of VerifyNow P2Y12 assay with clinical outcomes. The median follow-up was 2.2 years (interquartile range: 1.3 to 3.4 years). Clinical follow-up completeness is shown in Table 2. During follow-up, the primary endpoint occurred in 96 patients (2-year cumulative rate, 2.6%); a total of 72 patients died, 10 patients had nonfatal acute MI, 6 had definite or probable stent thrombosis, and 26 had a stroke. Target vessel revascularization was performed in 140 patients, and major bleeding occurred in 27 patients.

The Kaplan-Meier estimate of the event rate for the primary endpoint at 2 years was 2.8% in patients with HTPR and 2.4% in patients without HTPR (HR: 1.33; 95% CI: 0.88 to 2.01; p = 0.18) (Fig. 2, Table 3). Overall results were the same if just a PRU value >235 was used for the definition of HTPR (with this criteria, 56.0% had HTPR; HR: 1.37; 95% CI: 0.91 to 2.07; p = 0.13). Stratification by quintiles based on platelet reactivity (PRU value or % $P2Y_{12}$ inhibition) also demonstrated no significant difference in primary outcomes among quintiles (Online Fig. 1). In addition, PRU value (HR: 1.001,

Table 1 Baseline Characteristics of the Patients, According to the Magnitude of On-Treatment Platelet Reactivity

Variable	Overall Population (N = 2,849)	On-Treatment Platelet Reactivity		p Value
		High (n = 1,660)	Normal (n = 1,189)	
Clinical characteristics				
Age, yrs	61.7 ± 9.7	62.6 ± 9.5	60.4 ± 9.9	<0.001
Male	2,027 (71.1)	1,121 (67.5)	906 (76.2)	<0.001
Body mass index, kg/m^2	25.0 ± 2.9	25.1 ± 2.9	24.9 ± 2.9	0.02
Diabetes	811 (28.5)	482 (29.0)	329 (27.7)	0.43
Hypertension	1,677 (58.9)	1,013 (61.0)	664 (55.8)	0.006
Current smoking	712 (25.0)	400 (24.1)	312 (26.2)	0.19
Hypercholesterolemia	1,739 (61.0)	1,028 (61.9)	711 (59.8)	0.25
Previous myocardial infarction	167 (5.9)	90 (5.4)	77 (6.5)	0.24
Previous PCI	514 (18.0)	281 (16.9)	233 (19.6)	0.07
Previous bypass surgery	80 (2.8)	50 (3.0)	30 (2.5)	0.44
Previous stroke	141 (4.9)	90 (5.4)	51 (4.3)	0.17
Renal insufficiency	50 (1.8)	32 (1.9)	18 (1.5)	0.41
Clinical indication				0.85
Stable angina	1,586 (55.6)	921 (55.5)	664 (55.8)	
Acute coronary syndrome	1,264 (44.4)	739 (44.5)	525 (44.2)	
Ejection fraction	59.3 ± 6.8	59.2 ± 6.7	59.5 ± 7.0	0.24
Procedural characteristics				
Multivessel disease	1,469 (51.6)	845 (50.9)	624 (52.5)	0.41
Left anterior descending artery	2,095 (73.5)	1,204 (72.5)	891 (74.9)	0.15
Left main disease	307 (10.8)	159 (9.6)	148 (12.4)	0.02
ACC/AHA lesion B2 or C type lesions	2,295 (80.6)	1,332 (80.2)	963 (81.0)	0.62
Bifurcation lesion	1,009 (35.4)	601 (36.2)	408 (34.3)	0.30
No. of stents implanted	2.0 ± 1.1	2.0 ± 1.1	2.1 ± 1.2	0.32
Total stent length, mm	50.3 ± 31.5	50.0 ± 31.4	50.8 ± 31.7	0.52
Minimal stent diameter, mm	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.4	0.14

Values are mean ± SD or n (%).
ACC/AHA = American College of Cardiology/American Heart Association; PCI = percutaneous coronary intervention.

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Table 2 Status of Dual Antiplatelet Therapy and Cardiac-Related Medications and Clinical Follow-Up Completeness

Characteristic	On-Treatment Platelet Reactivity: High (n = 1,660)	On-Treatment Platelet Reactivity: Normal (n = 1,189)	p Value
Clopidogrel loading at pre-procedure			0.28
75 mg/day for >5 days	507 (30.5)	396 (33.3)	
300 mg ≥12 h before PCI	1,107 (66.7)	759 (63.8)	
600 mg ≥12 h before PCI	46 (2.8)	34 (2.9)	
Clopidogrel maintenance			
At discharge	1,658/1,660 (99.9)	1,188/1,189 (99.9)	>0.99
6 months after procedure	1,605/1,644 (97.6)	1,146/1,182 (97.0)	0.27
12 months after procedure	1,472/1,578 (93.3)	1,041/1,135 (91.7)	0.12
18 months after procedure	834/1,018 (81.9)	638/794 (80.3)	0.40
24 months after procedure	539/752 (71.7)	426/609 (70.0)	0.49
Aspirin maintenance			
At discharge	1,657/1,660 (99.8)	1,186/1,189 (99.7)	0.70
6 months after procedure	1,620/1,644 (98.5)	1,169/1,182 (98.9)	0.41
12 months after procedure	1,542/1,578 (97.7)	1,101/1,135 (97.0)	0.25
18 months after procedure	973/1,018 (95.6)	761/794 (95.8)	0.78
24 months after procedure	703/752 (93.5)	577/609 (94.7)	0.33
Cardiac-related medications at discharge			
ACE inhibitor	472 (28.4)	295 (24.8)	0.03
Beta-blockers	1,149 (69.2)	806 (67.8)	0.42
Calcium-channel blocker	1,458 (87.8)	1,048 (88.1)	0.80
Statin	1,325 (79.8)	936 (78.7)	0.48
Warfarin	12 (0.7)	6 (0.5)	0.47
Proton pump inhibitor	47 (2.8)	26 (2.2)	0.28
Clinical follow-up completeness after discharge			
1 month after procedure	1,658/1,660 (99.9)	1,185/1,189 (99.7)	0.24
6 months after procedure	1,644/1,650 (99.6)	1,182/1,184 (99.8)	0.48
12 months after procedure	1,602/1,620 (98.9)	1,154/1,162 (99.3)	0.25
18 months after procedure	1,060/1,080 (98.1)	831/845 (98.3)	0.75
24 months after procedure	801/826 (97.0)	662/682 (97.1)	0.92

Values are n (%) or n/total n (%).
ACE = angiotensin-converting enzyme; PCI = percutaneous coronary intervention.

95% CI: 0.998 to 1.003, p = 0.57) or % $P2Y_{12}$ inhibition (HR: 1.006, 95% CI: 0.997 to 1.014, p = 0.18) as a continuous variable was not associated with increasing risk for the primary endpoint. There was also no significant difference in the risk of individual and composite secondary endpoints among patients with or without HTPR.

Association of CRP levels with clinical outcomes. Baseline CRP was concomitantly measured in 2,546 patients (Fig. 1) and elevated CRP (≥3.0 mg/l) was observed in 24.7% of patients. Characteristics of the entire population and of the patients according to elevated versus non-elevated CRP levels are shown in Online Table 1.

During follow-up, there were progressive and significantly higher increases in primary endpoint in patients with elevated CRP levels than in those with non-elevated CRP levels (Fig. 3, Table 4). These results were also consistent with a broad range of clinical presentations (HR: 3.08, 95% CI: 1.73 to 5.47, p < 0.001 for stable angina vs. HR: 2.88, 95% CI: 1.50 to 5.55, p = 0.002 for acute coronary syndrome; p for interaction = 0.64). The association remained significant even after adjustment for other significant covariates or multiple potential confounders. The HRs for each component of mortality, MI, stent thrombosis, or stroke or secondary composite outcome range from 2 to 3, consistently (but not always statistically significantly) higher risk for patients with elevated CRP levels. However, there was no association between CRP levels and the risk of revascularization or bleeding.

Incremental usefulness of VerifyNow P2Y12 assay or CRP over conventional risk factors for prediction of clinical outcomes. Table 5 summarizes the improvements in model discrimination when adding HTPR or elevated CRP levels into a model with the conventional clinical and procedural risk factors for the prediction of the primary endpoint. The addition of HTPR did not significantly improve the C-statistic for predicting outcomes. However, when elevated CRP levels were incorporated into the model with conventional risk factors, the C-statistic increased significantly.

Relationship and outcomes according to VerifyNow P2Y12 assay and CRP. There was no significant correlation between the magnitude of platelet reactivity and CRP values (Online Fig. 2). Kaplan-Meier curves of the cumulative

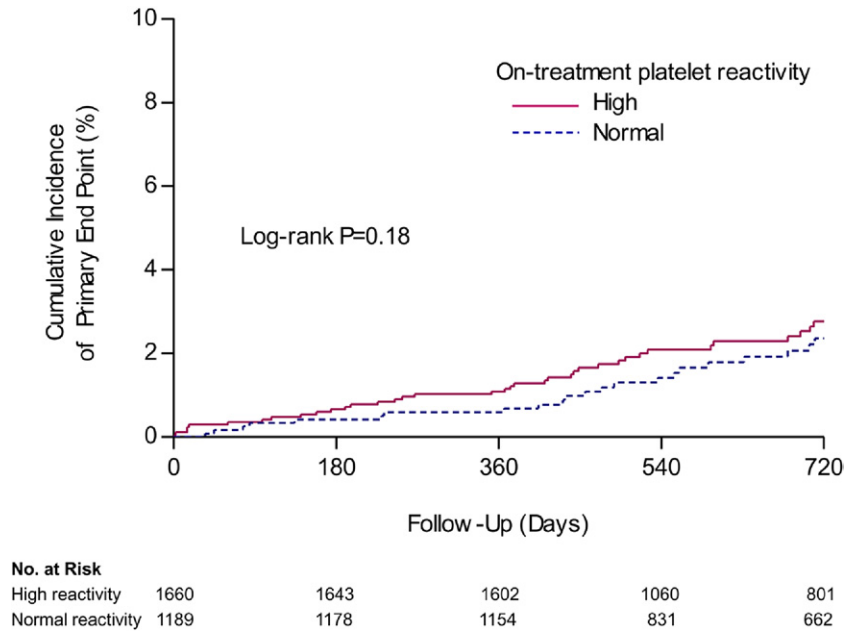

Figure 2 **Kaplan-Meier Curves of the Cumulative Probability of the Primary Endpoint According to High Versus Normal On-Treatment Platelet Reactivity**

The primary endpoint was defined as a composite of all-cause death, nonfatal myocardial infarction, stent thrombosis, and stroke. High on-clopidogrel platelet reactivity was defined by a $P2Y_{12}$ reaction unit value >235 and/or a % inhibition <15%, as measured by the VerifyNow P2Y12 assay. p values were calculated with the use of the log-rank test.

probability of the primary endpoint according to the combined status of platelet reactivity and CRP are illustrated in Figure 4. In patients with elevated CRP levels, there was a continuous separation of the events curves according to high versus normal platelet reactivity; however, these findings were not observed in patients with non-elevated CRP levels (p = 0.02 for interaction). Consequently, patients with both HTPR and elevated CRP levels were the highest-risk group for future atherothrombotic events.

Table 3 **Clinical Outcomes According to On-Treatment Platelet Reactivity**

	On-Treatment Platelet Reactivity					
	Total No. of Events*		Cumulative Event Rate at 2 Years			
Outcome	High (n = 1,660)	Normal (n = 1,189)	High (n = 1,660)	Normal (n = 1,189)	Hazard Ratio† (95% CI)	p Value
Primary endpoint						
Death, MI, stent thrombosis, or stroke	58	38	2.8	2.4	1.33 (0.88–2.01)	0.18
Secondary endpoints						
Death	40	32	2.0	2.0	1.10 (0.69–1.75)	0.71
MI	6	4	0.3	0.3	1.34 (0.37–4.83)	0.66
Stent thrombosis						
Definite	3	1	0.2	0.1	2.19 (0.23–21.05)	0.50
Definite or probable	4	2	0.2	0.2	1.45 (0.27–7.92)	0.67
Definite, probable, or possible	13	15	0.8	0.8	0.72 (0.34–1.51)	0.38
Stroke	17	9	0.9	0.7	1.54 (0.68–3.47)	0.30
Target vessel revascularization	78	62	4.3	5.1	0.94 (0.67–1.31)	0.71
Cardiovascular death, MI, stent thrombosis, or stroke	41	24	2.2	1.3	1.46 (0.88–2.43)	0.15
Bleeding, according to TIMI criteria‡						
Major	17	10	0.9	0.6	1.29 (0.59–2.83)	0.52
All type	62	36	3.3	2.6	1.31 (0.87–1.98)	0.20

Cumulative event rates are derived from Kaplan-Meier estimates. *For each endpoint, first events only are counted. †Hazard ratios are for patients with high on-treatment platelet reactivity, as compared with patients with normal on-treatment platelet reactivity. ‡Bleeding according to TIMI criteria refers to adjudicated events in accordance with previously used definitions (10).

CI = confidence interval; MI = myocardial infarction; TIMI = Thrombolysis In Myocardial Infarction.

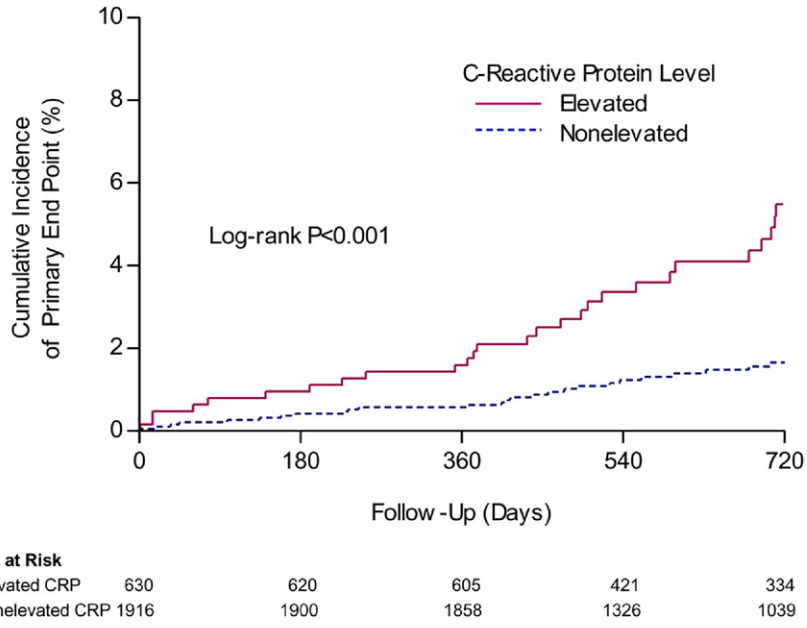

Figure 3 Kaplan-Meier Curves of the Cumulative Probability of the Primary Endpoint According to Elevated Versus Nonelevated Levels of CRP

The primary endpoint was defined as a composite of all-cause death, nonfatal myocardial infarction, stent thrombosis, and stroke. Elevated C-reactive protein (CRP) level was defined as a value ≥3 mg/l. p values were calculated with the use of the log-rank test.

Discussion

In our large cohort of patients with DES implantation, HTPR, as measured by the VerifyNow P2Y12 assay, failed to predict long-term risk of major cardiovascular events. By contrast, our data suggested that elevated CRP levels were significantly associated with atherothrombotic events and also had incremental predictive values beyond on classical risk factors.

In contrast with our findings, several studies suggested a strong association between VerifyNow P2Y12 assays and adverse events in patients with coronary stenting (3,6,14).

Table 4 Clinical Outcomes According to CRP Level

Outcome	CRP Level: Total No. of Events* Elevated (n = 630)	CRP Level: Total No. of Events* Nonelevated (n = 1,916)	CRP Level: Cumulative Event Rate at 2 Years Elevated (n = 630)	CRP Level: Cumulative Event Rate at 2 Years Nonelevated (n = 1,916)	Hazard Ratio† (95% CI)	p Value
Primary endpoint						
Death, MI, stent thrombosis, or stroke	39	45	5.6	1.7	2.81 (1.83–4.31)	<0.001
Secondary endpoints						
Death	31	34	4.6	1.3	2.94 (1.81–4.79)	<0.001
MI	4	6	0.8	0.2	2.17 (0.61–7.71)	0.23
Stent thrombosis						
Definite	2	2	0.3	0.1	3.06 (0.43–21.74)	0.26
Definite or probable	3	3	0.5	0.2	3.06 (0.62–15.14)	0.17
Definite, probable, or possible	13	13	1.9	0.5	3.18 (1.47–6.86)	0.003
Stroke	9	10	1.2	0.4	2.88 (1.17–7.10)	0.02
Target vessel revascularization	25	102	3.7	5.0	0.75 (0.48–1.16)	0.20
Cardiovascular death, MI, stent thrombosis, or stroke	27	29	4.2	0.9	3.01 (1.78–5.08)	<0.001
Bleeding, according to TIMI criteria‡						
Major	6	16	0.8	0.6	1.16 (0.46–2.97)	0.75
All	25	60	3.0	2.7	1.29 (0.81–2.06)	0.28

Cumulative event rates are derived from Kaplan-Meier estimates. *For each endpoint, first events only are counted. †Hazard ratios are for patients with elevated C-reactive protein (CRP) level, as compared with patients with non-elevated C-reactive protein level. ‡Bleeding according to TIMI criteria refer to adjudicated events in accordance with previously used definitions (10).
Abbreviations as in Table 4.

Table 5 C-Statistic for Cox Regression Models for Prediction of Primary Endpoint

Model Risk Factors and Laboratory Assay	C-Statistic	Estimated Difference (95% CI)	p Value
Conventional clinical and procedural risk factors*	0.729	Referent	Referent
Conventional clinical and procedural risk factors plus HTPR†	0.733	0.003 (−0.007 to 0.013)	0.54
Conventional clinical and procedural risk factors plus elevated CRP levels‡	0.759	0.031 (0.002 to 0.058)	0.03
Conventional clinical and procedural risk factors plus HTPR and elevated CRP levels	0.762	0.032 (0.003 to 0.062)	0.03

*Conventional clinical risk factors were age, sex, body mass index, diabetes, hypertension, hypercholesterolemia, previous myocardial infarction, previous stroke, renal insufficiency, ejection fraction, and acute coronary syndrome; conventional procedural risk factors were multivessel disease, American College of Cardiology/American Heart Association lesion B2 or C type lesions, bifurcation lesions, total number of stent, total stent length, and minimal stent diameter. †High on-clopidogrel platelet reactivity was defined by a $P2Y_{12}$ reaction units (value >235 and/or a % inhibition <15%, as measured by the VerifyNow P2Y12 assay. ‡Elevated CRP level was defined as a value ≥3 mg/l.

CI = confidence interval; CRP = C-reactive protein; HTPR = high on-treatment platelet reactivity.

However, given the small number of events, limited patient populations, and the frequent use of glycoprotein IIb/IIIa inhibitors highly interfering with the P2Y12 assay, some studies should be interpreted with caution, and the robustness of conclusions is limited (3,6). A recent large observational study suggested that the VerifyNow test was significantly associated with atherothrombotic events (14). In this study, however, HTPR cluster with traditional clinical risk factors (i.e., old age, high body mass index, diabetes, low ejection fraction) for cardiovascular events. Therefore, independent association of HTPR with clinical outcomes after adjustment for these concomitant risk factors should be evaluated to clarify whether "in vitro" platelet reactivity has direct causality for clinical events or whether it functions as just a marker.

The GRAVITAS (Gauging Responsiveness with A VerifyNow assay–Impact on Thrombosis And Safety) trial is the first large-scale clinical trial to test tailored antiplatelet therapy based on the VerifyNow P2Y12 assay in patients who received DES (15). This trial does not support high-dose clopidogrel in patients with HTPR. In addition, similar to our findings, there was no significant association of HTPR with the clinical endpoint in patients on standard clopidogrel therapy; moreover, this association could be weaker if adjustment for a higher prevalence of concomitant risk factors in the high-reactivity group were to be done. However, because of the relatively small number of events and a clinically low-risk population in both cases—the GRAVITAS trial and the current study—more large clinical studies with longer-term follow-up are mandatory to

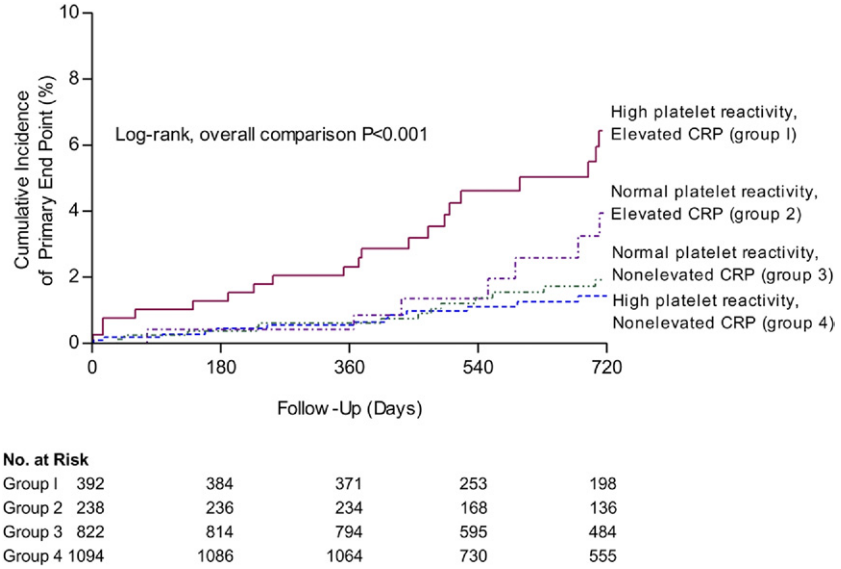

Figure 4 Kaplan-Meier Curves of the Cumulative Probability of the Primary Endpoint, According to the Status of Both On-Treatment Platelet Reactivity and CRP

Groups were represented according to decreasing incidence of the primary endpoint; group 1 indicates patients (n = 392) with high on-treatment platelet reactivity and elevated C-reactive protein (CRP) levels; group 2 indicates patients (n = 238) with normal on-treatment platelet reactivity and elevated CRP levels; group 3 indicates patients (n = 822) with normal on-treatment platelet reactivity and non-elevated CRP levels; and group 4 indicates patients (n = 1,094) with high on-treatment platelet reactivity and non-elevated CRP levels. High on-clopidogrel platelet reactivity was defined as a $P2Y_{12}$ reaction unit value >235 and/or a % inhibition <15%, as measured by the VerifyNow P2Y12 assay. Elevated CRP level was defined as a value ≥3 mg/l.

confirm whether alteration of antiplatelet therapy based on platelet function assay actually improves outcomes and to propose how these measurements may be used in the future care of patients.

In agreement with previous findings (7,8,20), the current study also demonstrated that elevated CRP was significantly associated with increased risks of major cardiovascular events in patients receiving DES. The biological plausibility in this group has been described elsewhere (8). In clinical practice, there may be substantial interest in the use of a readily available and simple marker, such as point-of-care platelet function tests or serum biomarkers, to identify high-risk patients for future cardiovascular events who could be targeted for preventive measures. Our study suggests that elevated CRP levels could more accurately identify a high risk for major cardiovascular events in patients receiving DES. However, we did not observe the long-term prognostic value of HTPR, as measured by the VerifyNow P2Y12 assay. Interestingly, on the basis of the observed differences of outcomes according to platelet reactivity in patients with elevated CRP levels, one might pose the hypothesis that elevated CRP could indicate a higher-risk population who may benefit from a selective platelet function test or tailoring antiplatelet therapy. Similarly, in the CREDO (Clopidogrel for the Reduction of Events During Observation) trial, patients with elevated CRP had increased atherothrombotic events, and the clinical benefit of clopidogrel seems greater in those with elevated CRP, but not lower CRP (21). A similar proof of concept was also documented in a large clinical trial with statin treatment (22).

In our study, the rates of the primary endpoint were lower than expected and were lower than rates reported in other studies (3–6,14), for reasons that remain unclear. This could be explained in part by differences in clinical or lesion characteristics, interventional practice, or race or ethnic group between our population of patients and those enrolled in earlier studies, as previously noted (8,23,24). By contrast, the rate of HTPR in our population was higher than rates reported in other studies (3,6,14,15), even though similar cutoff levels for HTPR were used among studies. This may be related in part to the significantly higher incidence of the Asian population having specific alleles on CYP2C19 that are associated with poor metabolization of clopidogrel, as compared with the Western population (25,26). In addition, there may be the possibility of inter-racial variability of platelet reactivity as noted in another study (27). Further studies may be needed to determine the complex relationships of genetic polymorphisms, platelet function assays, and outcomes in different ethnic populations.

Study limitations. First, our study evaluated nonrandomized, observational data. Second, given the relatively infrequent occurrence of hard endpoints, our analysis was underpowered to detect a clinically significant difference in the outcomes, and nonsignificant findings with platelet function test might have been significant with a larger cohort of patients. Therefore, our findings should be confirmed or refuted through larger clinical studies with long-term follow-up. Third, we did not perform serial measurements of the VerifyNow P2Y12 assay and CRP. Platelet reactivity or inflammatory status might be variable over time. Also, optimal platelet reactivity may vary in the early or late period after PCI. Finally, since the prognostic value of other various platelet function tests was not tested, the direct application of our findings to other studies or testing may be limited.

Conclusions

In our cohort of patients treated with DES, the VerifyNow P2Y12 assay failed to predict the risk of cardiovascular events. However, high CRP levels were significantly associated with long-term outcomes, suggesting that CRP measurement might be useful for risk stratification for these patients.

Reprint requests and correspondence: Dr. Seung-Jung Park, Department of Cardiology, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Poongnap-dong, Songpa-gu, Seoul 138-736, Korea. E-mail: sjpark@amc.seoul.kr.

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Key Words: C-reactive protein ■ drug-eluting stent(s) ■ platelet function test.

APPENDIX

For supplementary figures and a table, please see the online version of this paper.