Comparison of Platelet Function Tests in Predicting Clinical Outcome in Patients Undergoing Coronary Stent Implantation

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Context High on-treatment platelet reactivity is associated with atherothrombotic events following coronary stent implantation.

Objective To evaluate the capability of multiple platelet function tests to predict clinical outcome.

Design, Setting, and Patients Prospective, observational, single-center cohort study of 1069 consecutive patients taking clopidogrel undergoing elective coronary stent implantation between December 2005 and December 2007. On-treatment platelet reactivity was measured in parallel by light transmittance aggregometry, VerifyNow P2Y12 and Plateletworks assays, and the IMPACT-R and the platelet function analysis system (PFA-100) (with the Dade PFA collagen/adenosine diphosphate [ADP] cartridge and Innovance PFA P2Y). Cut-off values for high on-treatment platelet reactivity were established by receiver operating characteristic curve analysis.

Main Outcome Measurement The primary end point was defined as a composite of all-cause death, nonfatal acute myocardial infarction, stent thrombosis, and ischemic stroke. The primary safety end point included TIMI (Thrombolysis In Myocardial Infarction) criteria major and minor bleeding.

Results At 1-year follow-up, the primary end point occurred more frequently in patients with high on-treatment platelet reactivity when assessed by light transmittance aggregometry (11.7%; 95% confidence interval [CI], 8.9%-15.0% vs 6.0%; 95% CI, 4.2%-8.2%; $P<.001$), VerifyNow (13.3%; 95% CI, 10.2%-17.0% vs 5.7%; 95% CI, 4.1%-7.8%; $P<.001$) and Plateletworks (12.6%; 95% CI, 8.8%-17.2% vs 6.1%; 95% CI, 3.8%-9.2%; $P=.005$), which also had modest ability to discriminate between patients having and not having a primary event: light transmittance aggregometry (area under the curve [AUC], 0.63; 95% CI, 0.58-0.68), VerifyNow (AUC, 0.62; 95% CI, 0.57-0.67), and Plateletworks (AUC, 0.61; 95% CI, 0.53-0.69). The IMPACT-R, Dade PFA collagen/ADP, and Innovance PFA P2Y were unable to discriminate between patients with and without primary end point at 1-year follow-up (all AUCs included 0.50 in the CI). None of the tests identified patients at risk for bleeding.

Conclusions Of the platelet function tests assessed, only light transmittance aggregometry, VerifyNow, and Plateletworks were significantly associated with the primary end point. However, the predictive accuracy of these tests was only modest. None of the tests provided accurate prognostic information to identify low-risk patients at higher risk of bleeding following stent implantation.

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DUAL ANTIPLATELET THERAPY with aspirin and clopidogrel reduces atherothrombotic complications in patients undergoing percutaneous coronary intervention (PCI) with stenting.[1,2] However, the individual response to dual antiplatelet therapy is not uniform, and consistent findings across multiple investigations support the association between a lower degree of platelet inhibition, a high on-treatment platelet reactivity, and the occurrence of atherothrombotic events.[3-10]

The major drawbacks of these previous investigations are the relatively small sample size of the studied populations and the fact that on-treatment platelet reactivity was evaluated by only 1 platelet function test per study. There is currently no consensus regarding the most appropriate method to quantify the magnitude of on-treatment platelet reactivity. Therefore, the aim of The Popular Study (Do Platelet Function Assays Predict Clinical Outcomes in Clopidogrel-Pretreated Patients Undergoing Elective PCI) was to evaluate the ability of multiple platelet function tests

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in predicting atherothrombotic events, including stent thrombosis, in clopidogrel-pretreated patients undergoing PCI with stent implantation.

METHODS

Study Population

Consecutive patients with established coronary artery disease scheduled for elective PCI with stent implantation were included in this study. All patients received optimal clopidogrel treatment (defined as a maintenance of 75 mg/d therapy for >5 days or a loading dose of 300 mg ≥24 hours before PCI or 600 mg ≥4 hours before PCI) and aspirin (80-100 mg/d ≥10 days) unless they were receiving long-term anticoagulation with warfarins. According to our institutional practice, all patients (after receiving drug-eluting and bare-metal stenting) were treated with clopidogrel for at least 1 year since 2003. Clopidogrel and aspirin maintenance doses were 75 mg and 80 to 100 mg daily, respectively. Higher maintenance doses were not used. Adherence to antiplatelet medication was routinely assessed by outpatient visits at 6 weeks, 3 months, and 1 year and also verified by pharmacy refill data.

All interventions were performed according to current guidelines[11] and the choice of stent type and periprocedural use of glycoprotein IIb/IIIa inhibitors was left to the operator's discretion, but the latter were always administered after blood collection. Patients using concomitant medication known to affect platelet function other than aspirin (ie, nonsteroidal anti-inflammatory agents, dipyramidole, upstream glycoprotein IIb/IIIa inhibitors) and patients with a known platelet function disorder or a whole blood platelet count of less than $150 \times 10^3/\mu L$ were excluded. Written informed consent was obtained before PCI. All data were prospectively collected and entered into a central database. Clinical follow-up was obtained by additional telephone contact to all patients at 30 days and 12 months and verified using source documents from medical records from the referring hospitals.

The study was conducted according to the principles of the Declaration of Helsinki and the laws and regulations applicable in the Netherlands. The local institutional review board (Verenigde Commissies Mensgebonden Onderzoek) approved the study.

Follow-up and End Points

The primary end point of Popular was defined as a composite of all-cause death, nonfatal myocardial infarction (the occurrence of ischemic symptoms and a spontaneous troponin T value [ie, not periprocedural or postprocedural] or creatine kinase myocardial >the upper limit of normal), stent thrombosis (according to Academic Research Consortium criteria[12]), and ischemic stroke (focal loss of neurologic function caused by an ischemic event). The primary safety end point was defined as major or minor bleeding according to the modified Thrombolysis In Myocardial Infarction (TIMI) Study Group criteria.[13]

Exploratory end points included elective target vessel revascularization (revascularization of the vessel treated at the time of inclusion in the study), elective nontarget vessel revascularization (revascularization of a vessel different from the one treated at the time of enrollment), and hospitalization for ischemia (hospitalization with ischemic symptoms, ie, evidence for ischemia on electrocardiogram but without elevated cardiac markers).

An independent committee with blinding for platelet function data adjudicated all end points through review of medical record source documents.

Blood Sampling

Before heparinization, whole blood samples were drawn from the femoral or radial artery sheath into 3.2% citrate tubes for light transmittance aggregometry and for testing using IMPACT-R (Matis Medical Inc, Beersel, Belgium). Testing with VerifyNow P2Y12 (Accumetrics, San Diego, California) was performed using Greiner tubes, according to the manufacturer's test protocol. For the platelet function analysis system (PFA-100; Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany), 3.8% buffered citrated blood was used according to the manufacturer's test protocol. Blood samples for whole blood count were drawn into tubes containing K3-EDTA and tubes containing dphenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK [50 µmol/L]) to perform testing with the Plateletworks (Helena Laboratories, Beaumont, Texas).

Platelet Function Measurements

The magnitude of on-treatment platelet reactivity was quantified using the platelet function tests in parallel: light transmittance aggregometry with adenosine diphosphate (ADP) 5 and 20 µmol/L as the agonist, the VerifyNow P2Y12 assay, the Plateletworks assay using ADP tubes, the IMPACT-R assay (with and without ADP prestimulation), and the Dade PFA collagen/ADP test cartridge (PFA-100 system). Halfway through the study, the final prototype of the novel Innovance PFA P2Y PFA-100 system became available for performance evaluation. Except for the Innovance PFA P2Y, which is still under development at time of this publication, all platelet function tests were commercially available at the start of the study. All platelet function measurements were performed within 2 hours after blood collection. A detailed description of the platelet function tests is summarized in supplementary material (eAppendix, http://www.jama.com).[14-18]

Statistical Analysis

Sample size calculation was based on the ISAR-REACT I trial,[19] which included a cohort with similar selection criteria and the same treatment strategy. Therefore, we assumed an incidence of the primary end point of 6%. The study was designed on the basis of the superiority principle to have 80% power to observe an incidence of the primary end point in patients exhibiting high on-treatment platelet reactivity of 10% and 4% in patients without high on-treatment platelet reactivity. On this basis, 380 patients were needed in each group. To compensate for loss to follow-up, we aimed for a population of 800 as measured with each test.

Table 1. Baseline Characteristics of the Total Population

Characteristic	No. With Characteristic/ No. in Population (%)[a]
Clinical parameters	
Age, mean (SD), y	64 (10.6)
Male sex	802/1069 (75.0)
Hypertension[b]	823/1069 (76.9)
Hypercholesterolemia[c]	858/1069 (80.3)
Diabetes mellitus[d]	199/1069 (18.6)
Family history[e]	646/1069 (60.4)
Current smoking	119/1069 (11.1)
Left ventricular ejection fraction <45%	165/1069 (15.4)
Renal insufficiency[f]	86/1069 (8.0)
Prior myocardial infarction	583/1069 (54.5)
Medication	
Aspirin	955/1068 (89.4)
Loading dose clopidogrel	548/1068 (51.3)
Proton pump inhibitor	297/1068 (27.8)
Warfarins	108/1068 (10.1)
Laboratory parameters, mean (SD)	
Platelet count, $\times 10^3/\mu L$	271.7 (81.6)
White blood cell count, /μL	7600 (2300)
Hemoglobin, g/dL	13.9 (3.4)
Procedural parameters	
No. of stents implanted	1669
Minimal stent diameter, mean (SD), mm	3.1 (0.8)
Total stent length, mean (SD), mm	28.1 (16.8)
Bifurcation lesion	33/1069 (3.1)
Drug-eluting stent	675/1063 (63.5)
Left anterior descending artery	515 (48.2)

SI conversions: to convert creatinine to μmol/L, multiply by 88.4; low-density lipoprotein cholesterol to mmol/L, multiply by 0.0259.
[a] Reported as No./total No. (%) unless otherwise noted.
[b] Systolic blood pressure greater than 140 mm Hg or diastolic blood pressure greater than 90 mm Hg.
[c] A fasting low-density lipoprotein cholesterol at least 131 mg/dL or being on statin therapy at the time of inclusion.
[d] According to World Health Organization criteria.
[e] One or more first-degree relatives have developed coronary artery disease younger than the age of 55 years (men) or 65 years (women).
[f] Creatinine level greater than 1.36 mg/dL.

Continuous variables are presented as mean plus or minus SD. Categorical data are reported as frequencies (percentages). Categorical variables were compared using the χ^2 test. Normally distributed continuous variables were compared with a 2-sided unpaired *t* test. Since the PFA-100 system confines detection of a closure time to a 300-second window, and because the majority of patients receiving adequate antiplatelet therapy exhibit nonclosure according to Innovance PFA P2Y, the results of the PFA-100 system are depicted as a Kaplan-Meier time-to-aperture-closure plot and a log-rank test was used.

To evaluate a platelet function assay's ability to distinguish between patients with and without primary end point at 1-year follow-up, a receiver operating characteristic (ROC) curve analysis was calculated for each test. The optimal cut-off level was calculated by determining the smallest distance between the ROC curve and the upper left corner of the graph. Patients above the optimal cut-off level were considered to exhibit high on-treatment platelet reactivity. Survival analysis for patients with and without high on-treatment platelet reactivity, according to the ROC of the specific test, was performed using the Kaplan-Meier method and the differences between groups were assessed by the log-rank test. The measure of effect was the odds ratio (OR) and estimated from a logistic regression analysis. A second ROC curve analysis was performed based on the 1-year primary safety end point combining TIMI major and minor bleeding.

Logistic regression modeling was used to identify independent correlates of the primary end point and to adjust for potential confounders (classic cardiovascular risk factors, renal failure, left ventricular ejection fraction <45%, total stent length, number of lesions treated, amount of stents implanted, bifurcation lesions, comedication [including use of clopidogrel loading dose, warfarins, proton pump inhibitors, calcium channel blockers, statins, or glycoprotein IIb/IIIa inhibitors], laboratory parameters [hemoglobin, platelet count, and mean platelet volume], left anterior descending coronary artery, or graft-stenting). All univariate variables with a *P* value <.10 were included in multivariate analysis. Whether a variable had additional contribution to a logistic regression model without that variable was tested with the likelihood ratio test. The Hosmer-Lemeshow goodness-of-fit test was performed to assess the adequacy of the model. All statistical analyses were performed with R (version 2.9, http://www.r-project.org) and a 2-tailed *P* value <.05 was considered significant.

RESULTS

In total, 1328 consecutive patients were invited to participate in the study with 21 (1.6%) refusing to participate. Another 238 patients were initially included in the study but since no stent was implanted, they were also excluded (eg, patients underwent only balloon angioplasty or a fractional flow reserve measurement demonstrating nonischemic coronary disease), resulting in a population of 1069 consecutive patients. Owing to irregularities in platelet assay supply, particularly in the supply of the Plateletworks as well as technical failure in a minority of platelet function tests, not all platelet function assays were performed in every patient. As a consequence, light transmittance aggregometry was performed in 1049 patients with 5 μmol/L ADP and in 1051 patients with 20 μmol/L ADP; the VerifyNow P2Y12 cartridge was used in 1052 patients; the Plateletworks assay in 606 patients; and the IMPACT-R in 910 patients without prestimulation and in 905 with ADP prestimulation. The PFA COL/ADP was performed in 812 patients and Innovance PFA P2Y in 588 patients.

Baseline characteristics of the cohort are depicted in TABLE 1. Baseline characteristics of the subpopulations according to the tests performed are summarized in eTable 1 (http://www.jama.com), demonstrating that the subpopulations tested were well balanced (except for white blood cell counts, *P* = .04; all *P* values were >.85). All patients received optimal clopidogrel pretreatment, 50.6% received a maintenance dose of 75 mg daily therapy for more than 5 days, 41.6% received a loading dose of 300 mg at least 24 hours before PCI, and 8.3% received a loading dose of 600 mg at least 4 hours before PCI. There were 995 patients (89.4%) who received 80 to 100 mg aspirin daily for more than 10 days.

Clinical outcome at 12 months was available for 1067 (99.8%) patients. Adherence for clopidogrel was 95.2% after 6 months and 82.1% after 1 year. During 1-year follow-up, a total of 18 patients died (1.7%), 64 patients had nonfatal acute myocardial infarction (6.0%), 13 presented with definite stent thrombosis (1.2%), and 14 patients experienced nonfatal ischemic stroke (1.3%). Three possible stent thromboses occurred (0.3%) and no probable stent thromboses were found. A total of 55 patients (5.1%) presented with bleeding: 33 TIMI-major (3.1%) and 24 TIMI-minor bleeding (2.2%).

ROC Curve Analysis

Receiver operating characteristic curve analysis demonstrated that light transmittance aggregometry (both 5 µmol/L ADP and 20 µmol/L), the VerifyNow P2Y12 cartridge, and the Plateletworks assay were able to distinguish between patients with and without ischemic events at 1-year follow-up. Conversely, neither the IMPACT-R with and without ADP prestimulation nor the PFA collagen/ADP or Innovance PFA P2Y were able to discriminate between patients with and without postprocedural events. TABLE 2 displays the area under the curve (AUC) and optimal cut-off value for every test. eFigure 1 (http://www.jama.com) depicts the optimal cut-off values per test and the percentages of patients exhibiting high on-treatment platelet reactivity according to the test. Baseline characteristics for every test, for patients with and without high on-treatment platelet reactivity, are depicted in eTable 2, showing significant differences between the 2 groups.

Logistic regression modeling was used to identify independent predictors for the primary end point. The model included on-treatment platelet reactivity according to the various tests as a categorical variable (patients with vs without high on-treatment platelet reactivity using the cut-off defined with the ROC analysis) and multiple potential confounders. Independent predictors of 1-year primary end point were age (calculated for an increase of 10 years [OR, 1.22; 95% confidence interval {CI}, 0.97-1.51; $P=.08$]), hypertension (OR, 2.50; 95% CI, 1.30-4.82; $P=.006$), hypercholesterolemia (OR, 0.57; 95% CI, 0.33-0.98; $P=.04$), left ventricular ejection fraction of less than 45% (OR, 1.83; 95% CI, 1.07-3.11; $P=.06$), and a prior coronary artery bypass grafting (OR, 1.91; 95% CI, 0.96-3.81; $P=.06$). Procedural factors independently predicting the primary end point were total stent length (OR, 0.97; 95% CI, 0.94-1.00; $P=.05$), number of lesions treated (OR, 1.92; 95% CI, 1.10-3.39; $P=.02$), number of stents implanted (OR, 2.4; 95% CI, 1.38-4.30; $P=.002$), left anterior descending artery stenting (OR, 1.79; 95% CI, 1.11-2.88; $P=.017$) or graft stenting (OR, 2.88; 95% CI, 1.00-8.32; $P=.049$), stenting a bifurcation lesion (OR, 5.43; 95% CI, 1.91-15.45; $P=.002$), and a clopidogrel loading dose (OR, 1.73; 95% CI, 2.73-1.09; $P=.02$). The remaining variables included for multivariate analysis were not found to be independent correlates of the primary end point ($P>.10$) and were not included in the model.

The addition of high on-treatment platelet reactivity to this statistical model revealed that high on-treatment platelet reactivity as measured with light transmittance aggregometry (both 5 µmol/L ADP and 20 µmol/L), the VerifyNow-P2Y12 cartridge, and the Plateletworks assay significantly improved the AUC. Likewise, the likelihood ratio test demonstrated that high on-treatment platelet reactivity, according to these tests, provided an additional contribution to the model

Table 2. Area Under the Receiver Operating Characteristic Curve for Prediction of Composite Outcome

	Light Transmittance Aggregometry				IMPACT-R			
Values by Test	5 µmol/L	20 µmol/L	VerifyNow P2Y12	Plateletworks	Spontaneous	ADP Stimulated	PFA-100 Collagen/ADP	Innovance PFA P2Y
AUC, % (95% CI)	0.63 (0.58-0.68)	0.62 (0.56-0.67)	0.62 (0.57-0.67)	0.61 (0.53-0.69)	0.56 (0.50-0.62)	0.53 (0.48-0.59)	0.50 (0.46-0.55)	0.56 (0.48-0.63)
Sensitivity, % (95% CI)	60.2 (49.8-69.8)	54.6 (44.2-64.5)	60.4 (50.2-69.9)	63.0 (49.6-74.6)	56.4 (45.4-66.9)	44.0 (33.3-55.3)	62.9 (51.2-73.2)	60.9 (46.5-73.6)
Specificity, % (95% CI)	59.1 (56.0-62.2)	63.9 (60.8-66.8)	63.1 (60.0-66.1)	58.5 (54.4-62.6)	52.5 (49.1-55.9)	53.5 (50.1-56.9)	44.3 (40.8-47.9)	29.0 (25.3-32.9)
Optimal cut-off, %[a]	42.9	64.5	236[a]	80.5	8.4[b]	3.0[b]	116[b]	299[b]
NPV, %	94	93.8	94.3	93.9	90	91.2	92.5	89.7
PPV, %	11.7	12	13.3	12.6	7.2	7.7	5.3	4.8
	Values for Backward Regression Models[c]							
AUC, %	0.73	0.73	0.74	0.77	0.72	0.72	0.72	0.72
P value for addition to model[d]	.004	.001	<.001	.001	.2	.83	.15	.27

Abbreviations: ADP, adenosine diphosphate; AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
[a] Calculated by determining smallest distance between receiver operating characteristic curve and upper left corner of the graph.
[b] Cut-off % units are P2Y12 reaction units for VerifyNow, % surface coverage for IMPACT-R spontaneous and IMPACT-R ADP stimulated tests, and closure time in seconds for the PFA-100 system.
[c] AUC of different backward regression models for the prediction of the primary end point at 1-year follow-up.
[d] Backward regression values are for the prediction of the primary end point at 1-year follow-up. Likelihood ratio test for additional value of high on-treatment platelet reactivity (increase in AUC) as measured with multiple platelet function tests. AUC and optimal cut-off values for each test. Model 1: classic cardiovascular risk factors: age, hypertension, hypercholesterolemia, left ventricular ejection fraction less than 45%, and prior coronary artery bypass grafting (AUC, 0.66). Model 2: model 1 plus procedural risk factors: total stent length, number of lesions treated, number of stents implanted, left anterior descending stenting, graft-stenting, bifurcation lesion, and clopidogrel loading dose vs maintenance dose (AUC, 0.72; *P* value for addition, .001). Model 3: model 2 plus high on-treatment platelet reactivity.

(Table 2). The goodness-of-fit test demonstrated that the predicting model was adequate (all *P* values >.10). On the contrary, the AUC did not improve when high on-treatment platelet reactivity as measured with IMPACT-R (with and without ADP prestimulation) or the PFA test cartridges (PFA COL/ADP and Innovance PFA P2Y) were added to the model.

Table 3. Clinical Outcome Based on Testing With Light Transmittance Aggregometry, VerifyNow P2Y12, and Plateletworks

Platelet Function Test	On-Treatment Platelet Reactivity, No. (%)		OR (95% CI)	*P* Value
	Normal	High		
Light transmittance aggregometry, 5 µmol/L ADP	<42.9% Aggregation (n = 604)	≥42.9% Aggregation (n = 445)		
Death combined[a]	36 (6.0)	52 (11.7)	2.09 (1.34-3.25)	<.001
Death	6 (1.0)	11 (2.5)	2.53 (0.93-6.88)	.06
MI	24 (4.0)	37 (8.3)	2.19 (1.29-3.72)	.003
Stent thrombosis	6 (1.0)	7 (1.6)	1.59 (0.53-4.77)	.40
Stroke	7 (1.2)	6 (1.3)	1.17 (0.39-3.49)	.78
Target vessel revascularization	18 (3.0)	7 (1.6)	0.52 (0.22-1.26)	.14
Nontarget vessel revascularization	21 (3.5)	8 (1.8)	0.51 (0.22-1.16)	.10
Rehospitalization	16 (2.6)	11 (2.5)	0.93 (0.43-2.03)	.87
Light transmittance aggregometry, 20 µmol/L ADP	<64.5% Aggregation (n = 659)	≥64.5% Aggregation (n = 392)		
Death combined[a]	41 (6.2)	47 (12.0)	2.05 (1.32-3.19)	.001
Death	11 (1.7)	6 (1.5)	0.92 (0.34-2.50)	.86
MI	24 (3.6)	37 (9.4)	2.76 (1.62-4.68)	.0001
Stent thrombosis	4 (0.6)	9 (2.3)	3.85 (1.18-12.58)	.017
Stroke	8 (1.2)	5 (1.3)	1.05 (0.34-3.24)	.93
Target vessel revascularization	21 (3.2)	4 (1.0)	0.31 (0.11-0.92)	.03
Nontarget vessel revascularization	23 (3.5)	6 (1.5)	0.43 (0.17-1.07)	.06
Rehospitalization	21 (3.2)	6 (1.5)	0.47 (0.19-1.18)	.10
VerifyNow P2Y12	<236[b] (n = 646)	≥236[b] (n = 406)		
Death combined[a]	37 (5.7)	54 (13.3)	2.53 (1.63-3.91)	<.001
Death	9 (1.4)	9 (2.2)	1.60 (0.63-4.08)	.32
MI	23 (3.6)	40 (9.9)	2.96 (1.74-5.02)	<.001
Stent thrombosis	5 (0.8)	8 (2.0)	2.58 (0.84-7.93)	.09
Stroke	6 (0.9)	7 (1.7)	1.87 (0.62-5.61)	.26
Target vessel revascularization	16 (2.5)	9 (2.2)	0.89 (0.39-2.04)	.79
Nontarget vessel revascularization	20 (3.1)	9 (2.2)	0.71 (0.32-1.57)	.40
Rehospitalization	18 (2.8)	8 (2.0)	0.70 (0.30-1.63)	.41
Plateletworks	<80.5 Aggregation (n = 344)	≥80.5 Aggregation (n = 262)		
Death combined[a]	21 (6.1)	33 (12.6)	2.22 (1.25-3.93)	.005
Death	9 (2.6)	4 (1.5)	0.58 (0.18-1.89)	.36
MI	10 (2.9)	25 (9.5)	3.52 (1.66-7.47)	<.001
Stent thrombosis	3 (0.9)	6 (2.3)	2.66 (0.66-10.75)	.15
Stroke	3 (0.9)	4 (1.5)	1.76 (0.39-7.94)	.45
Target vessel revascularization	12 (3.5)	5 (1.9)	0.54 (0.19-1.55)	.24
Nontarget vessel revascularization	11 (3.2)	7 (2.7)	0.83 (0.32-2.17)	.71
Rehospitalization	10 (2.9)	7 (2.7)	0.92 (0.34-2.44)	.86

Abbreviations: ADP, adenosine diphosphate; CI, confidence interval; MI, myocardial infarction, OR, odds ratio.
[a] Includes all-cause death, as well as nonfatal MI, stent thrombosis, and stroke.
[b] Units are P2Y12 reaction units.

Relationship Between High On-Treatment Platelet Reactivity and Clinical Outcome

At 1-year follow-up, the primary end point occurred more frequently in patients with high on-treatment platelet reactivity compared with patients without high on-treatment platelet reactivity, when platelet function was evaluated with light transmittance aggregometry (11.7% [95% CI, 8.9%-15.0%] vs 6.0% [95% CI, 4.2%-8.2%]; *P*<.001 using 5 µmol/L ADP and 12.0% vs 6.2%; *P*=.001 using 20 µmol/L ADP, respectively), the VerifyNow P2Y12 assay (13.3% [95% CI, 10.2%-17.0%] vs 5.7% [95% CI, 4.1%-7.8%]; *P*<.001), and the Plateletworks assay (12.6% [95% CI, 8.8%-17.2%] vs 6.1% [95% CI, 3.8%-9.2%]; *P*=.005). One-year follow-up for patients with and without high on-treatment platelet reactivity according to each platelet function test is depicted in TABLE 3 and TABLE 4.

The survival rate free from the primary end point was significantly lower in patients with high on-treatment platelet reactivity when measured with light transmittance aggregometry 5 µmol/L ADP and 20 µmol/L ADP, VerifyNow, Plateletworks, and Innovance PFA P2Y as compared with patients without high on-treatment platelet reactivity, whereas no significant relation was detected when platelet function was assessed by the IMPACT-R (both with and without prestimulation) or by the PFA collagen/ADP (FIGURE 1).

The occurrence of the primary end point was also compared when groups were divided in quintiles according to on-treatment platelet reactivity (FIGURE 2). Patients in the higher quintiles according to the light transmittance aggregometry 5 µmol/L ADP and 20 µmol/L ADP and the VerifyNow P2Y12 assay were at significantly higher risk for the primary end point. In contrast, no significant difference in the occurrence of the primary end point was

observed between quintiles as measured with the IMPACT-R tests and Plateletworks. Since the PFA-100 system confines detection of a closure time to a 300-second window, the results of both PFA cartridges are depicted as time-to-aperture-closure Kaplan-Meier curves. Closure times as measured by the PFA COL/ADP were not significantly different between patients with and without a primary end point.

Relationship Between Platelet Reactivity and Bleeding

A second ROC analysis demonstrated that none of the performed tests were able to discriminate between patients with and without bleeding (all AUCs included 0.50 in the CI). Stratification by quintiles based on on-treatment platelet reactivity demonstrated no significant difference in the occurrence of bleeding between the quintiles (eFigure 2). In addition, no significant increase in bleeding was observed in the lowest quintile of patients compared with quintiles 2 to 5. A third ROC analysis further demonstrated that the platelet function tests were not able to predict postdischarge (>48 hours) minor or major bleedings (all AUCs included 0.50 in the CI).

COMMENT

High on-treatment platelet reactivity, when assessed by light transmittance aggregometry (both 5 µmol/L and 20 µmol/L ADP), VerifyNow P2Y12 assay, and Plateletworks, was significantly associated with atherothrombotic events. In contrast, the shear stress-based tests IMPACT-R (with and without ADP prestimulation) and the Dade PFA-100 system (the collagen/ADP and Innovance PFA P2Y) did not show an association with outcome.

The criterion standard light transmittance aggregometry has been the most widely used technique and has clearly demonstrated the relationship between high on-treatment platelet reactivity and subsequent atherothrombotic events.[4-6] Popular found an optimal diagnostic cut-off level discriminating patients with atherothrombotic events from those without, similar to that found by Gurbel et al.[4] However, light transmittance aggregometry is not suitable for routine use in clinical practice due to the poor reproducibility, the long sample processing time, and the need for specialized technicians. Therefore, several new, more easy-to-use platelet function tests have been intro-

Table 4. Clinical Outcome Based on Testing With IMPACT-R, IMPACT-R ADP, PFA 100 Collagen/ADP, and Innovance PFA P2Y

Platelet Function Test	On-Treatment Platelet Reactivity, No. (%)		OR (95% CI)	*P* Value
	Normal	High		
IMPACT-R	<8.4 (n = 481)[a]	≥8.4 (n = 429)[a]		
Death combined[b]	36 (7.5)	42 (9.8)	1.34 (0.84-2.14)	.21
Death	5 (1.0)	11 (2.6)	2.51 (0.86-7.27)	.08
MI	28 (5.8)	25 (5.8)	1.00 (0.57-1.75)	.99
Stent thrombosis	5 (1.0)	6 (1.4)	1.35 (0.41-4.46)	.62
Stroke	4 (0.8)	7 (1.6)	1.98 (0.58-6.8)	.27
Target vessel revascularization	15 (3.1)	6 (1.4)	0.44 (0.17-1.15)	.08
Nontarget vessel revascularization	15 (3.1)	9 (2.1)	0.67 (0.29-1.54)	.33
Rehospitalization	12 (2.5)	12 (2.8)	1.12 (0.5-2.53)	.78
IMPACT-R ADP	<3.0 (n = 419)[a]	≥3.0 (n = 486)[a]		
Death combined[b]	33 (7.9)	32 (8.6)	1.11 (0.69-1.78)	.68
Death	7 (1.7)	8 (1.6)	0.99 (0.35-2.74)	.98
MI	21 (5.0)	30 (6.2)	1.25 (0.70-2.21)	.45
Stent thrombosis	5 (1.2)	5 (1.0)	0.86 (0.25-2.99)	.81
Stroke	6 (1.4)	5 (1.0)	0.72 (0.22-2.36)	.58
Target vessel revascularization	7 (1.7)	14 (2.9)	1.75 (0.70-4.37)	.23
Nontarget vessel revascularization	8 (1.9)	16 (3.3)	1.75 (0.74-4.13)	.20
Rehospitalization	12 (2.9)	12 (2.5)	0.86 (0.38-1.93)	.71
PFA 100 collagen/ADP	<116 (n = 452)[a]	≥116 (n = 360)[a]		
Death combined[b]	43 (9.5)	27 (7.5)	0.77 (0.47–1.28)	.31
Death	11 (2.4)	4 (1.1)	0.45 (0.14-1.43)	.16
MI	20 (6.6)	30 (5.6)	0.83 (0.46-1.49)	.52
Stent thrombosis	6 (1.3)	3 (0.8)	0.63 (1.60-2.50)	.50
Stroke	4 (0.9)	4 (1.1)	1.27 (0.31-5.00)	.75
Target vessel revascularization	13 (2.9)	7 (1.9)	0.67 (0.26-1.69)	.39
Nontarget vessel revascularization	12 (2.7)	11 (3.1)	1.15 (0.51-2.63)	.73
Rehospitalization	10 (2.2)	6 (1.7)	0.75 (0.27-2.08)	.58
Innovance PFA P2Y	<299 (n = 413)	≥299 (n = 175)		
Death combined[b]	28 (6.8)	18 (10.3)	1.59 (0.85-2.94)	.15
Death	4 (1.0)	6 (3.4)	3.57 (1.01-12.5)	.03
MI	20 (4.8)	11 (6.3)	1.32 (0.62-2.77)	.47
Stent thrombosis	4 (1.0)	1 (0.6)	0.59 (0.07-5.26)	.63
Stroke	5 (1.2)	1 (0.6)	0.47 (0.05-4.00)	.48
Target vessel revascularization	15 (3.6)	2 (1.1)	0.31 (0.07-1.35)	.10
Nontarget vessel revascularization	13 (3.1)	2 (1.1)	0.36 (0.08-1.59)	.16
Rehospitalization	11 (2.7)	2 (1.1)	0.42 (0.09-1.92)	.25

Abbreviations: CI, confidence interval; MI, myocardial infarction, OR, odds ratio.
[a] Units are % surface coverage for IMPACT-R spontaneous and IMPACT-R ADP stimulated tests and closure time in seconds for the PFA 100 system.
[b] Includes all-cause death, as well as nonfatal MI, stent thrombosis, and stroke.

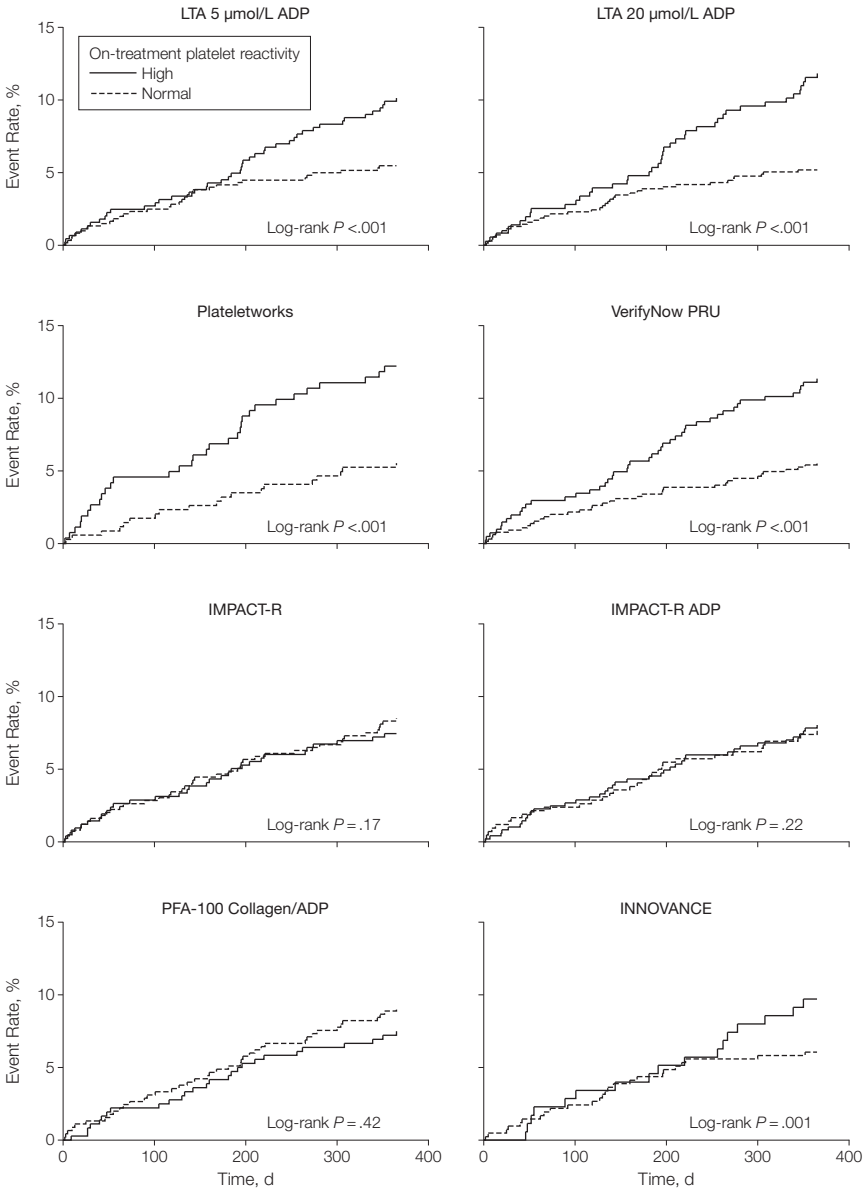
duced. Our study revealed that the VerifyNow P2Y12 cartridge is capable of identifying patients who are at risk for atherothrombotic events after undergoing PCI. Our optimal diagnostic cut-off value of 236 P2Y12 reaction units is consistent with that reported in previous reports.[7,8,20] We demonstrated a relation between the Plateletworks ADP assay and clinical outcome and established an optimal cut-off value. Plateletworks ADP had the largest increase in predictive value of all evaluated tests. However, rapid performance (within 10 minutes after blood withdrawal) of this assay is required since the ADP-induced platelet aggregates disaggregate after this time point, resulting in an unreliable test result as described in eAppendix (http://www.jama.com).[16] Therefore, the use of the Plateletworks in routine clinical practice might be limited.

We reported performance data of the prototype Innovance PFA P2Y, which in its final design became available halfway through the inclusion period. Although the sample size has insufficient statistical power, the survival analysis demonstrated a lower incidence of the primary end point in patients without high on-treatment platelet reactivity.

In light of the Popular data, should high on-treatment platelet reactivity be used as a prognostic marker in clinical practice? Despite growing evidence that high on-treatment platelet reactivity is associated with adverse clinical outcome, platelet function testing is not widely implemented in clinical practice due to a lack of consensus on the optimal method and on the optimal cut-off values of the different tests to identify patients at higher risk. Popular provides additional evidence, including optimal cut-off values, that 3 tests might be used (light transmittance aggregometry, VerifyNow, and Plateletworks). However, other risk factors such as diabetes mellitus and poor left ventricular function have also been demonstrated to predict atherothrombotic events poststent implantation.[21-24] Furthermore, these same risk factors have been shown to be associated with high on-treatment platelet reactivity[25,26] and thus, high on-treatment platelet reactivity is probably a composite of several of these risk factors as well as the response to antiplatelet therapy.

In Popular, high on-treatment platelet reactivity added to the overall risk model. The modest contribution of high on-treatment platelet reactivity might be attributed to its relatively low-risk population, excluding higher-risk patients (ie, ST-elevation myocardial infarction). The greater importance of high on-treatment platelet reactivity in patients at higher risk has been demonstrated by Marcucci et al and Sibbing and et al.[7,9]

Figure 1. Kaplan-Meier Analysis



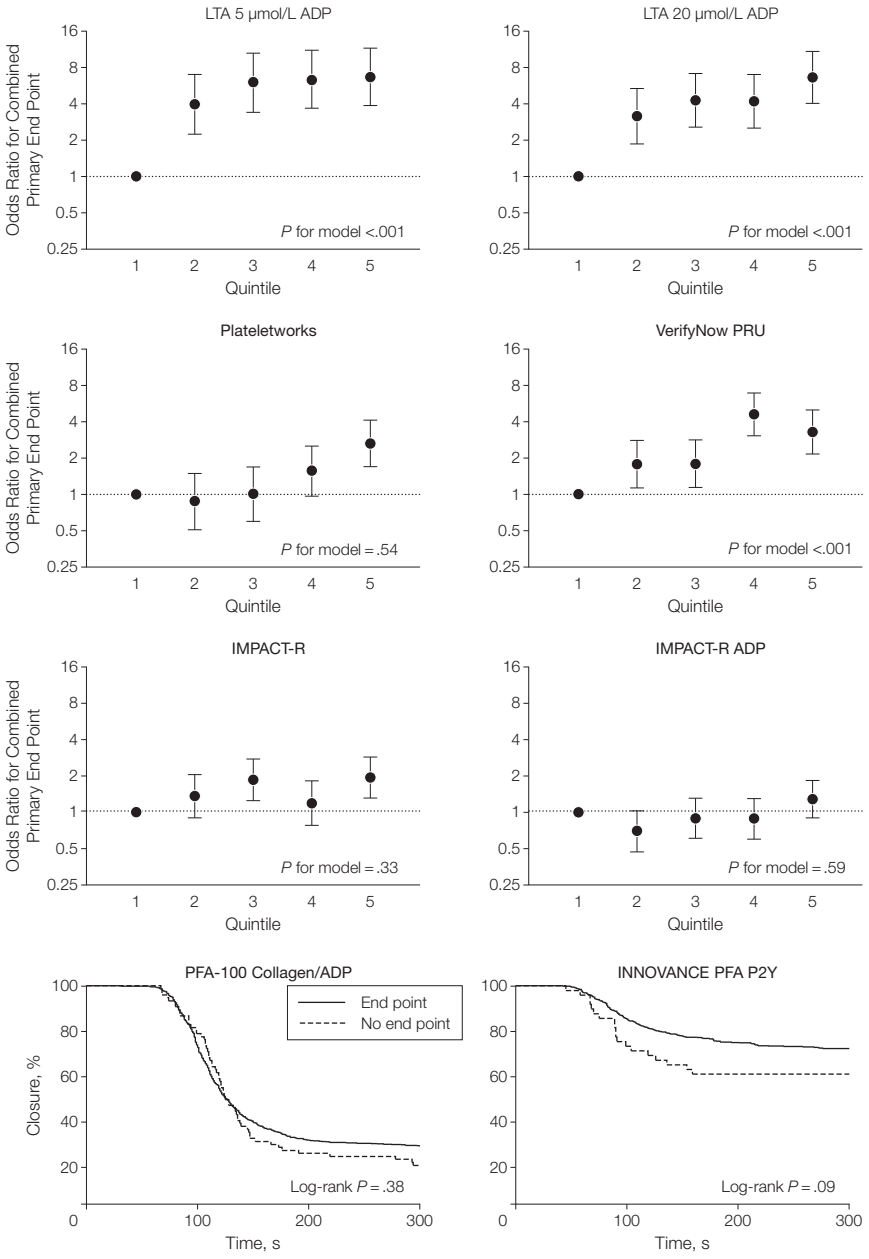
Kaplan-Meier analysis is for the event rate of the combined primary end point in patients with and without high on-treatment platelet reactivity as measured by multiple platelet function tests. LTA indicates light transmittance aggregometry; ADP, adenosine diphosphate.

Despite numerous data on the association between high on-treatment platelet reactivity and adverse outcome, there are only preliminary data concerning the benefit of tailoring therapy based on the results of platelet function testing.[27] Therefore, the correct treatment, if any, of high on-treatment platelet reactivity remains unknown pending the completion of currently ongoing clinical trials: the GRAVITAS (NCT00645918), the DANTE (NCT00774475), the ARCTIC (NCT00827411), as well as the TRIGGER-PCI (NCT00910299), which may reveal whether individualized antiplatelet treatment based on platelet function testing improves outcome. Until then, clinical practice should not be guided by (point-of-care) platelet function testing.

Some issues merit careful consideration. First, the sample size of Innovance PFA P2Y was too small to have sufficient statistical power to detect the relationship between high on-treatment platelet reactivity and clinical outcome. Second, not all currently available platelet function tests were included with additional tests including the Multiplate (Dynabyte Informationssysteme GmbH, Munich, Germany), the thromboelastograph, and the flowcytometric vasodilator–stimulated phosphoprotein (VASP) analysis. However, at the start of our inclusion, the Multiplate and the platelet assay for the thromboelastograph were not available. Furthermore, the published results with the VASP assay were mainly preliminary and did not provide a solid base for choosing VASP as one of the platelet function tests. Third, patients received 3 different, but adequate, clopidogrel dosing strategies. Previous studies have demonstrated differences in the effect on platelet reactivity of these 3 dosing regimens. However, these 3 regimens are current clinical practices and our study therefore reflects the clinical relevance of monitoring platelet function in daily practice.

In conclusion, of the platelet function tests assessed, only light transmittance aggregometry, VerifyNow, and Plateletworks were significantly associated with the primary end point. However, the predictability of these 3 tests was only modest. None of the tests provided accurate prognostic informa-

Figure 2. Odds Ratios for the Primary End Point



Odds ratios are for the combined primary end point by quintiles of on-treatment platelet reactivity according to multiple platelet function assays. Error bars indicate 95% confidence intervals. Cumulative Kaplan-Meier time-to-aperture-closure plot in patients with and without the combined primary end point according to the PFA-100 system and INNOVANCE PFA. Quintiles for light transmittance aggregometry 5 µmol/L ADP (1, ≤27.1%; 2, >27.1%-36.6%; 3, >36.6%-44.1%; 4, >44.1%-52.1%; 5, >52.1%-78.6%), light transmittance aggregometry 20 µmol/L ADP (1, ≤45.4%; 2, >45.4%-56.0%; 3, >56.0%-63.9%; 4, >63.9%-70.7%; 5, >70.7%-96.6%), Plateletworks (1, ≤36.4%; 2, >36.4%-66.5%; 3, >66.5%-82.5%; 4, >82.5%-93.8%; 5, >93.8%-100%), reaction units using the VerifyNow P2Y12 test (1, ≤146; 2, >146-198; 3, >198-235; 4, >235-276; 5, >276-413), % surface coverage using the IMPACT-R test (1, ≤3.9%; 2, >3.9%-6.7%; 3, >6.7%-9.6%; 4, >9.6%-13.2%; 5, >13.2%-30.7%), and the IMPACT-R ADP (1, 20.5%->5.4%; 2, 5.4%->3.2%; 3, 3.2%->2.2%; 4, 2.2%->1.3%; 5, ≤1.3%).

tion to identify patients at higher risk of bleeding. Thus, Popular does not support the use of platelet function testing to guide clinical practice in a low-risk population of patients undergoing elective PCI.

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Analysis and interpretation of data: Breet, van Werkum, Bouman, Kelder, Ruven, Bal, Deneer, van der Heyden, Rensing, Suttorp, Hackeng, ten Berg.
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Critical revision of the manuscript for important intellectual content: Breet, van Werkum, Bouman, Kelder, Ruven, Bal, Deneer, Harmsze, van der Heyden, Rensing, Suttorp, Hackeng, ten Berg.
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Obtained funding: van Werkum, Hackeng, ten Berg.
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Study supervision: van Werkum, Ruven, Bal, Deneer, van der Heyden, Rensing, Suttorp, Hackeng, ten Berg.
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Online-Only Material: eAppendix, eTables, and eFigures are available at http://www.jama.com.
Additional Contributions: Innovance PFA P2Y is under development at the time of this publication and not available for sale.
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