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Elevated Plasma Fibrinogen Rather Than Residual Platelet Reactivity After Clopidogrel Pre-Treatment Is Associated With an Increased Ischemic Risk During Elective Percutaneous Coronary Intervention

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Objectives This study was undertaken to determine the roles of serum fibrinogen and residual platelet reactivity after clopidogrel pre-treatment on ischemic events after elective percutaneous coronary intervention (PCI).

Background Both elevated serum fibrinogen and high platelet reactivity with thienopyridines are associated with ischemic cardiovascular events. Elevated fibrinogen also contributes to high on-clopidogrel platelet reactivity. It is unknown whether fibrinogen and residual platelet reactivity are associated with adverse cardiovascular events through independent or interactive effects.

Methods A total of 189 patients undergoing elective PCI with clopidogrel pre-treatment (75 mg daily for ≥7 days or a 600-mg bolus ≥12 h before recruitment) were prospectively enrolled. Baseline fibrinogen and platelet function using the VerifyNow P2Y12 assay (Accumetrics, San Diego, California) were obtained. Markers of ischemic myocardial injury were measured every 8 h after PCI.

Results Incidence of troponin-defined periprocedural myocardial infarction (PPMI) (troponin I/T >3× upper limit of normal) was 13.9% and associated with elevated fibrinogen (363.1 ± 131.0 mg/dl vs. 309.1 ± 99.6 mg/dl; p = 0.017), higher age (68.2 ± 10.1 years vs. 63.0 ± 11.8 years; p = 0.040), and elevated platelet count. Fibrinogen level and age remained independently associated with PPMI following multiple variable and interaction testing. The incidence of creatine kinase-myocardial band (CK-MB)–defined PPMI (CK-MB >3× upper limit of normal) was 5.8% and associated with elevated fibrinogen (403.4 ± 128.0 mg/dl vs. 313.5 ± 104.6 mg/dl; p = 0.007). Platelet reactivity measurements were not associated with PPMI by either definition. Fibrinogen ≥345 mg/dl was significantly associated with both CK-MB–defined (p = 0.026) and troponin I/T–defined PPMI (p = 0.036). Fibrinogen effects were most prominent in the absence of systemic inflammation (C-reactive protein ≤0.5 mg/dl).

Conclusions Elevated fibrinogen is independently associated with the risk of ischemic myocardial injury following elective PCI with clopidogrel pre-treatment regardless of platelet reactivity as measured by the VerifyNow assay. (J Am Coll Cardiol 2013;61:23–34) © 2013 by the American College of Cardiology Foundation

Antiplatelet therapy with aspirin and a thienopyridine reduces major adverse cardiac events (MACE) following percutaneous coronary intervention (PCI) (1,2). Platelet function tests, including traditional light transmission aggregometry (LTA) and contemporary point-of-care assays, have identified variable patient response to thienopyridine agents, particularly clopidogrel (3). Furthermore, in a number of studies, lower platelet inhibition at a single time point during thienopyridine therapy (also called "higher on-treatment platelet reactivity") has been associated with a higher risk for adverse thrombotic and ischemic cardiovascular events following PCI (4,5). However, platelet function testing does not reliably predict ischemic outcomes and supports the notion that high residual platelet reactivity is not the only factor contributing to these events (4).

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Abbreviations and Acronyms

ADP = adenosine diphosphate
AUC = area under the curve
CI = confidence interval
CK-MB = creatine kinase-myocardial band
CRP = C-reactive protein
LTA = light transmittance aggregometry
MACE = major adverse cardiac events
OR = odds ratio
PCI = percutaneous coronary intervention
PI = platelet inhibition
PPMI = periprocedural myocardial infarction
PRU = P2Y12 reactivity unit
ROC = receiver-operating characteristic
ULN = upper limit of normal

Multiple prior studies have identified serum fibrinogen as a risk factor for both short- and long-term adverse cardiovascular events (6–15). As an acute phase reactant involved in the final common pathway of the coagulation cascade and essential component of platelet crosslinking in thrombus formation, fibrinogen possesses a clear biological mechanism for its adverse cardiovascular effects (16). A relationship between serum fibrinogen and on-treatment platelet reactivity has also been demonstrated (6,17), making it unclear whether these risk factors are associated with adverse ischemic events through independent or interactive effects. This study was undertaken to clarify the roles of fibrinogen and on-clopidogrel residual platelet reactivity on short-term ischemic cardiac outcomes following PCI.

Methods

Patient selection. The study protocol was approved by the Institutional Review Board of the University of California, San Diego, and written informed consent was obtained from study participants. Patients with established coronary artery disease undergoing elective PCI requiring treatment with clopidogrel were prospectively recruited and enrolled. Subjects included those undergoing PCI for stable angina or unstable angina, or elective PCI with objective evidence of myocardial ischemia. All subjects were required to have negative biomarkers of ischemic cardiac injury prior to enrollment. All subjects received either daily clopidogrel 75 mg for a minimum of 7 days or a 600-mg clopidogrel bolus loading dose at least 12 h before enrollment. Exclusion criteria included age younger than 18 years, anemia (hemoglobin $<$10 g/dl), thrombocytopenia (platelet count $<$100,000 cells/μl), diagnosis of inherited platelet disorders, serum triglycerides $>$400 mg/dl, chronic liver disease, intravenous glycoprotein IIb/IIIa inhibitor administration (within previous 30 days), and positive markers of myocardial injury or acute myocardial infarction (within the previous 72 h). Subjects were not excluded for diagnosis of systemic inflammatory diseases, such as rheumatoid arthritis or systemic lupus erythematosus, or renal failure.

Measurements. Baseline demographic information and laboratory data including complete blood count, complete lipid panel, serum markers of inflammation (C-reactive protein [CRP] and fibrinogen), and cardiac markers of myocardial ischemia (creatine kinase-myocardial band [CK-MB], troponin I and/or troponin T) were obtained in all subjects. Standard laboratory tests were performed by the clinical laboratory at the University of California, San Diego Medical Center. Fibrinogen was measured using the Beckman Coulter ACL-TOP analyzer (Beckman Coulter Life Sciences, Indianapolis, Indiana) with Instrumentation Laboratory reagent (Instrumentation Laboratory, Lexington, Massachusetts). Cardiac markers of ischemic injury were measured every 8 h following PCI until hospital discharge or up to 24 h to assess degree of myocardial injury. Platelet function testing was performed using the bedside rapid platelet function VerifyNow P2Y12 assay (Accumetrics, San Diego, California). VerifyNow P2Y12 assay design has been previously described (18). Briefly, the VerifyNow system utilizes cartridge-based assays and optical turbidometry to rapidly assess platelet function. The P2Y12 assay features platelet P2Y12 receptor-specific activation using adenosine diphosphate (ADP) and prostaglandin E1 agonists to activate platelets following thienopyridine use. On-treatment platelet reactivity is reported as "result" P2Y12 reactivity units (PRU). High-concentration thrombin receptor-activating protein maximally activates platelets to determine a pre-treatment "base" PRU. Platelet inhibition (PI) or percent change in platelet reactivity from baseline is calculated as: $(1 - [\text{result PRU/base PRU}]) \times 100\%$.

Sample collection. Whole blood samples were obtained from the radial or femoral artery via the catheter introducer sheath immediately prior to PCI or from a vein in the antecubital fossa using standard phlebotomy techniques. Blood was transferred into standard tubes for serologic assays and Bio-One Vacuette tubes (Greiner Bio-One North America, Monroe, North Carolina) containing 3.2% sodium citrate for platelet function testing.

Statistical analysis. The study was conducted using a nested case-control design. Sensitive troponin I/T–defined periprocedural myocardial infarction (PPMI) was used as the primary study endpoint, and CK-MB–defined PPMI was used as the secondary study endpoint. Separate analyses using both endpoints were planned. Sample size calculation was performed based on a previous study showing a relationship between elevated serum fibrinogen at presentation and 48-h MACE (acute myocardial infarction and/or cardiac death) in patients hospitalized for acute coronary syndrome (7). In order to detect a 51 mg/dl mean difference in fibrinogen level between cases and controls, an alpha of 0.05, and power of 80%, an estimated 25 subjects were needed in each group. Enrollment was continued until 25 troponin I/T–defined PPMI events occurred.

Study data were collected and analyzed using SPSS software (version 18.0, SPSS, Chicago, Illinois). Categorical variables are reported as percentages, whereas continuous variables are reported as mean ± SD. Normal distribution of continuous variables was confirmed using Kolmogorov-Smirnov testing. The primary PPMI endpoint was prospec-

tively defined as either troponin I or troponin T level >3× the upper limit of normal (ULN) (troponin I reference range 0 to 0.5 ng/ml; troponin T reference range 0 to 0.03 ng/ml) (19), whereas the secondary PPMI endpoint was defined as CK-MB level >3× ULN (CK-MB reference range 0 to 4.8 ng/ml) (19). Definitions of high on-clopidogrel platelet reactivity used in this study included PRU ≥230 (20) and PRU ≥208 (21) based on results of the GRAVITAS (Gauging Responsiveness with A VerifyNow assay–Impact on Thrombosis And Safety) trial. Relationships between continuous variables and outcomes were determined using independent-samples 2-tailed *t*-tests. Relationships between categorical variables and outcomes were determined using chi-square tests. Variables with significant univariate relationships ($p < 0.05$) were entered together into a multiple variable logistic regression model for analysis of each outcome. Specific testing between serum fibrinogen, measures of platelet reactivity (PRU or PI), and markers of systemic inflammation (CRP, platelet count, white blood cell count) within a multiple variable model was prospectively planned to evaluate for interdependent effects. Significant variables after multiple variable testing ($p < 0.05$) were tested for interactions if necessary. To determine a cutoff point for serum fibrinogen level predicting PPMI, a receiver-operating characteristic (ROC) curve was plotted for each outcome definition. The fibrinogen cutoff level producing the maximum sum of sensitivity and specificity was determined, and a 2-tailed p value reported.

Results

Baseline subject characteristics. A total of 189 subjects (age 63.8 ± 11.6 years, 74.1% male) were enrolled in this study (Table 1). A high prevalence of cardiovascular risk factors and prior coronary revascularization was observed (PCI 63.0%, coronary artery bypass grafting 18.0%). Baseline fibrinogen was 318.7 ± 107.8 mg/dl, whereas only 26.3% of subjects had elevated CRP >0.5 mg/dl. Mean on-treatment PRU was 211.6 ± 91.5 (45% with PRU ≥230), and PI was 35.9 ± 24.9%. There was no angiographic evidence of an adverse event in any study subject following PCI. A ROC curve was plotted to demonstrate the relationship between PI and high on-clopidogrel platelet reactivity defined as PRU ≥230 (area under the curve [AUC]: 0.950; $p < 0.001$) (Fig. 1). PI level of 30.9% was determined to produce the greatest sum of sensitivity and specificity in predicting PRU ≥230 (86.5% sensitivity, 90.6% specificity; $p < 0.001$).

Troponin-defined PPMI. Post-procedural troponin I or troponin T was obtained in 95.2% of subjects (n = 180) with troponin I/T–defined PPMI occurring in 13.9% of subjects (Table 2). Subjects with troponin I/T–defined PPMI had higher fibrinogen levels (363.1 ± 131.0 mg/dl vs. 309.1 ± 99.6 mg/dl; p = 0.017), were older (68.2 ± 10.1 years vs. 63.0 ± 11.8 years; p = 0.040), and had higher platelet counts (243.6 ± 64.1 cells × $10^3/\mu l$ vs. 218.4 ± 55.5 cells × $10^3/\mu l$; p = 0.041) compared with those without a troponin I/T–defined PPMI. There was a trend towards a greater number of stents used per procedure among the PPMI group (2.08 ± 0.91 vs. 1.68 ± 1.02; p = 0.065), whereas prevalence of elevated CRP >0.5 mg/dl (24.0% vs. 25.7%; p = 0.86), on-treatment PRU (226.0 ± 81.5 vs. 209.8 ± 92.1; p = 0.41), and PI (31.1 ± 20.3% vs. 36.2 ± 25.5%; p = 0.34) were similar between those with

Table 1 Baseline Subject Population Characteristics

Clinical characteristics	
Age, yrs	63.8 ± 11.6
Male	74.1%
BMI, kg/m^2	28.4 ± 5.5
Past PCI	63.0%
Past CABG	18.0%
Past MI	29.7%
Hypertension	90.5%
Hyperlipidemia	89.4%
Diabetes mellitus	46.6%
Family history of CVD	29.9%
Smoking history	16.1%
Current PCI indication	
Stable angina	56.1%
Unstable accelerating angina	23.8%
Other symptoms	10.6%
Positive stress test	9.5%
Medications	
Aspirin	97.4%
Statin	83.5%
Beta-blocker	74.1%
ACE inhibitor/ARB	61.9%
Nitrates	30.0%
Calcium channel blocker	24.3%
Clopidogrel (75 mg daily ≥7 days)	84.7%
Percutaneous coronary intervention	
Drug-eluting stent	86.6%
Bare-metal stent	9.1%
PTCA	32.1%
Total stents used	325
Stents used per procedure	1.7 ± 1.0
Total vessels treated	226
Vessels treated per procedure	1.2 ± 0.5
Type C lesion treated	53.4%
Bifurcation lesion treated	17.1%
Vessel treated	
Left main	4.9%
LAD	37.6%
Circumflex	30.1%
RCA	21.7%
Bivalirudin	85.9%
Unfractionated heparin	11.9%
Glycoprotein IIb/IIIa inhibitor	8.2%

Values are mean ± SD or %.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; CVD = cardiovascular disease; LAD = left anterior descending coronary artery; MI = myocardial infarction; PCI = percutaneous coronary intervention; PTCA = percutaneous transluminal coronary angioplasty; RCA = right coronary artery.

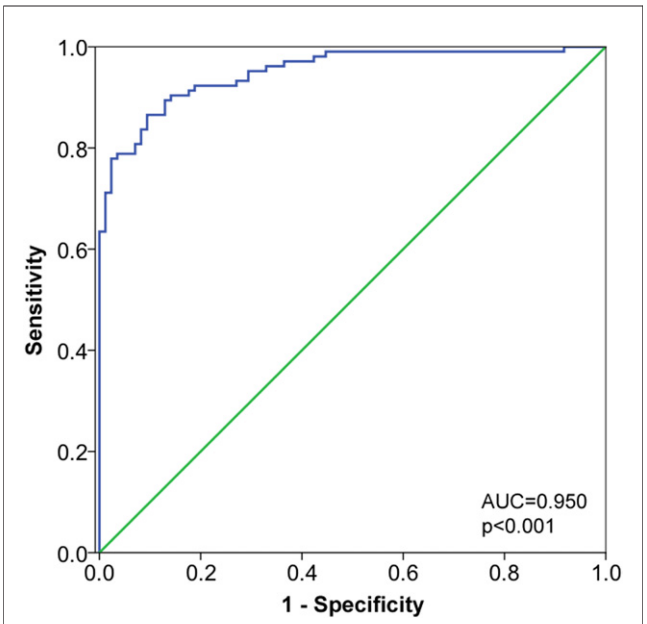

Figure 1 ROC Curve Relating Level of Platelet Inhibition With High On-Clopidogrel Platelet Reactivity

Platelet inhibition level <30% produced maximum sensitivity and specificity in predicting high on-clopidogrel platelet reactivity defined as VerifyNow platelet reactivity units (PRU) ≥230 (area under the curve [AUC] = 0.950; 86.5% sensitivity, 90.6% specificity; p < 0.001). ROC = receiver-operating characteristic.

and without a troponin I/T–defined PPMI. The prevalence of high on-clopidogrel platelet reactivity defined as PRU ≥230 (48.0% vs. 45.2%; p = 0.79), PRU ≥208 (52.0% vs. 51.6%; p = 0.97), or PI <30% (60.0% vs. 45.2%; p = 0.17) was also similar between those with and without a troponin I/T–defined PPMI.

Fibrinogen level, age, and platelet count were entered together into a multiple variable regression model and both fibrinogen (p = 0.031) and age (p = 0.035) remained independently associated with troponin I/T–defined PPMI (Table 3). Further testing did not show an interaction between fibrinogen level and age. After on-treatment PRU and PI were separately included into multiple variable regression models with significant univariate factors (fibrinogen level, age, and platelet count), only serum fibrinogen level and age remained significantly associated with troponin I/T–defined PPMI. The aforementioned multiple variable regression testing was repeated while including other markers of systemic inflammation (CRP and white blood cell count) (Table 3). When controlling for these inflammatory markers, serum fibrinogen remained significantly associated with troponin I/T–defined PPMI, whereas age and platelet function measures did not.

CK-MB–defined PPMI. CK-MB measurements were obtained in all subjects. The incidence of CK-MB–defined PPMI was 5.8% (Table 4). There was no difference in baseline characteristics, medical history, medication use, or lipid profile between subject groups with or without a PPMI. Among procedural characteristics suggesting higher PCI complexity, including treatment of type C or bifurcation lesions, only the number of stents used per procedure (2.36 ± 0.92 vs. 1.68 ± 1.02; p=0.027) was associated with occurrence of PPMI. On platelet function testing, baseline PRU (312 ± 32.7 vs. 328.2 ± 57.8; p = 0.38), on-treatment PRU (224.7 ± 96.4 vs. 210.8 ± 91.4; p = 0.62), and PI (30.2 ± 20.0% vs. 36.1 ± 25.3%; p = 0.45) were not different between the 2 groups. The prevalence of high on-clopidogrel platelet reactivity defined as PRU ≥230 (45.5% vs. 44.9%; p = 1.00), PRU ≥208 (45.5% vs. 51.7%; p = 0.69), or PI <30% (63.6% vs. 45.5%; p = 0.24) was also similar between those with and without a CK-MB–defined PPMI. However, subjects with PPMI had higher fibrinogen levels (403.4 ± 128.0 mg/dl vs. 313.5 ± 104.6 mg/dl; p = 0.007) without increase in CRP (CRP >0.5 mg/dl; 36.4% vs. 25.7%; p = 0.48). Serum fibrinogen level alone remained significantly associated with CK-MB–defined PPMI after significant univariate factors were included together with either on-treatment PRU or PI within multiple variable models. Furthermore, this relationship remained significant when other markers of systemic inflammation (CRP, platelet count, and white blood cell count) were included in the models.

ROC curve relating fibrinogen to PPMI. A ROC curve showed a fibrinogen cutoff level of 345 mg/dl to have optimal combined sensitivity and specificity for CK-MB–defined PPMI (AUC: 0.700, p = 0.026; 72.7% sensitivity, 64% specificity, positive predictive value 11.1%, negative predictive value 97.4%) (Fig. 2A). The ROC curve analysis did not yield a significant fibrinogen cutoff value to predict troponin I/T–defined PPMI (AUC: 0.590; p = 0.15). However, those with a serum fibrinogen level ≥345 mg/dl had higher occurrence of PPMI by both troponin I/T >3× ULN (20.9% vs. 9.7%; p = 0.036) and CK-MB >3× ULN (11.1% vs. 2.6%; p = 0.023) (Fig. 3). Compared with dichotomous markers of high on-treatment platelet reactivity, only elevated fibrinogen level ≥345 mg/dl was associated with increased risk of PPMI defined by either troponin I/T (odds ratio [OR]: 2.5; 95% confidence interval [CI]: 1.04 to 5.77) (Fig. 4) or CK-MB (OR: 4.75; 95% CI: 1.21 to 18.5) (Fig. 5) definition.

Fibrinogen and C-reactive protein. A secondary analysis was performed to further evaluate the relationship between fibrinogen level, systemic inflammation, and the risk of PPMI. In the absence of systemic inflammation (CRP ≤0.5 mg/dl, 73.7% of subjects), there was a higher incidence of PPMI among those with fibrinogen ≥345 mg/dl (troponin I/T–defined: 27.8% vs. 9.4%, p = 0.007; CK-MB–defined: 16.2% vs. 1.0%, p = 0.002) (Fig. 3). A ROC curve analysis in subjects without elevated CRP also showed a fibrinogen level ≥345 mg/dl had greatest sensitivity and specificity for predicting a CK-MB–defined PPMI (AUC: 0.821, p = 0.004; sensitivity 85.7%, specificity 76.1%, positive predictive value 16.2%, and negative predictive value 99.0%) (Fig. 2B).

An interaction variable including elevated fibrinogen ≥345 mg/dl and CRP ≤0.5 mg/dl was entered together

Table 2 Subject Characteristics Stratified by Occurrence of a Troponin I/T (>3× ULN) Defined PPMI

	No Troponin I/T–Defined PPMI (n = 155)	Troponin I/T–Defined PPMI (n = 25)	p Value
Age, yrs	63.0 ± 11.8	68.2 ± 10.1	0.040
Male	72.3	84.0	0.215
BMI, kg/m^2	28.5 ± 5.6	27.1 ± 4.3	0.221
Cardiovascular history			
Past PCI	63.2	64.0	0.941
Past CABG	18.1	16.0	1.00
Past MI	29.6	33.3	0.711
Hypertension	89.0	96.0	0.475
Hyperlipidemia	90.3	80.0	0.163
Diabetes mellitus	44.5	60.0	0.150
Family history of CVD	30.7	32.0	0.898
Smoking history	15.8	20.0	0.568
Current PCI indication			0.949
Stable angina	56.1	52.0	
Unstable accelerating angina	23.2	28.0	
Other symptoms	11.0	12.0	
Positive stress test	9.7	8.0	
Medications			
Aspirin	97.4	96.0	0.531
Statin	83.8	76.0	0.392
Beta-blocker	76.1	64.0	0.197
ACE inhibitor/ARB	61.3	56.0	0.615
Nitrates	28.4	28.0	0.968
Calcium channel blocker	24.5	20.0	0.623
Clopidogrel (75 mg daily ≥7 days)	85.2	80.0	0.552
Laboratory values			
HDL, mg/dl	39.6 ± 13.3	39.1 ± 12.9	0.864
LDL, mg/dl	78.2 ± 31.8	84.1 ± 38.0	0.417
Triglycerides, mg/dl	118.5 ± 73.2	137.6 ± 71.9	0.237
WBC, cells × 10^3/μl	7.1 ± 2.2	8.1 ± 1.6	0.150
Hemoglobin, g/dl	12.9 ± 1.6	13.3 ± 1.7	0.320
Platelet count, cells × 10^3/μl	218.4 ± 55.5	243.6 ± 64.1	0.041
CRP >0.5 mg/dl	25.7	24.0	0.860
Fibrinogen, mg/dl	309.1 ± 99.6	363.1 ± 131.0	0.017
Percutaneous coronary intervention			
Drug-eluting stent	85.1	91.7	0.536
Bare-metal stent	9.1	12.5	0.706
PTCA	28.6	33.3	0.633
Stents used per procedure	1.68 ± 1.02	2.08 ± 0.91	0.065
Vessels treated per procedure	1.23 ± 0.48	1.35 ± 0.57	0.304
Type C lesion treated	53.5	56.0	0.819
Bifurcation lesion treated	16.3	23.8	0.368
Bivalirudin	85.3	88.0	1.00
Unfractionated heparin	11.9	12.0	1.00
Glycoprotein IIb/IIIa inhibitor	8.1	12.0	0.456
Platelet function testing			
Base PRU	362.7 ± 58.1	324.1 ± 60.7	0.837
Result PRU	209.8 ± 92.1	226.0 ± 84.5	0.411
Platelet inhibition	36.2 ± 25.5	31.1 ± 20.3	0.341
Result PRU ≥230	45.2	48.0	0.791
Result PRU ≥208	51.6	52.0	0.971

Values are mean ± SD or %.

CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; PPMI = periprocedural myocardial infarction; PRU = P2Y12 reactivity units; WBC = white blood cell; other abbreviations as in Table 1.

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Table 3 Details of Multiple Variable Regression Analyses of Factors Associated With Troponin I/T–Defined PPMI

	Analysis Including Listed Factors (p Value)	Analysis Including Listed Factors, CRP, and WBC Count (p Value)*
Significant univariate factors alone		
Fibrinogen level	0.031	0.020
Age	0.035	0.071
Platelet count	0.107	0.376
Significant univariate factors including interaction variable		
Fibrinogen level	0.040	0.018
Age	0.068	0.114
Fibrinogen and age	0.549	0.638
Significant univariate factors including PRU		
Fibrinogen level	0.036	0.022
Age	0.033	0.069
Platelet count	0.122	0.419
PRU	0.634	0.599
Significant univariate factors including PI		
Fibrinogen level	0.043	0.024
Age	0.031	0.062
Platelet count	0.107	0.388
PI	0.544	0.403

Analyses were performed by including all listed factors together within the regression model as well as in combination with markers of systemic inflammation (CRP >0.5 mg/dl and WBC count). Fibrinogen level remained significantly associated with troponin I/T–defined PPMI after testing with other significant univariate factors, interaction testing, and controlling for markers of platelet function as well as systemic inflammation. *CRP >0.5mg/dl and WBC count did not achieve statistically significant p-values <0.05 in any of these analyses.

PI = platelet inhibition; other abbreviations as in Table 2.

with fibrinogen and CRP level into multiple variable regression models to predict the occurrence of PPMI. This interaction term remained predictive of CK-MB–defined PPMI (OR: 11.6; 95% CI: 1.13 to 119) (whereas fibrinogen or CRP level alone did not), but was not predictive of troponin I/T–defined PPMI when either including or excluding age and platelet count covariates. An interaction variable between lower fibrinogen <345 mg/dl and CRP ≤0.5 mg/dl was also entered together with fibrinogen and CRP level into a multiple variable regression model to predict the absence of a CK-MB–defined PPMI, and was found to be an independent negative predictor of CK-MB–defined PPMI (OR: 0.086; 95% CI: 0.008 to 0.882) (whereas fibrinogen or CRP level alone was not). In the overall cohort, subjects with fibrinogen ≥345 mg/dl in the absence of systemic inflammation (CRP ≤0.5 mg/dl) had the highest incidence of CK-MB–defined PPMI (Fig. 6), whereas those with fibrinogen <345 mg/dl in the absence of systemic inflammation had the lowest incidence of CK-MB–defined PPMI (Fig. 7) compared with other variables known to affect the risk of PPMI.

Discussion

This study was undertaken to determine the roles of serum fibrinogen and residual platelet reactivity after clopidogrel pre-treatment on ischemic events after elective PCI. The key findings of this study are: 1) elevated serum fibrinogen (≥345 mg/dl) is independently associated with periprocedural myocardial infarction; 2) fibrinogen level is a significant positive and negative predictor of PPMI in the absence of baseline systemic inflammation (CRP ≤0.5 mg/dl); 3) no association between on-clopidogrel residual platelet reactivity and PPMI is observed; and 4) a PRU >230 as measured with the VerifyNow assay corresponds to a platelet inhibition of <30.9%.

Using universal definitions of PPMI according to troponin I/T and CK-MB enzyme levels, elevated serum fibrinogen level was associated with higher rate of PPMI regardless of on-clopidogrel platelet reactivity using the point-of-care VerifyNow P2Y12 assay. By ROC curve analysis, fibrinogen level ≥345 mg/dl significantly predicted PPMI and identified individuals undergoing elective PCI with nearly a 5-fold increased odds of experiencing a CK-MB–defined PPMI. A secondary analysis demonstrated that a fibrinogen level of 345 mg/dl was an even stronger positive and negative predictor of PPMI among those without baseline systemic inflammation. No relationships between measures of platelet reactivity using the VerifyNow P2Y12 assay and PPMI were detected in this study, but the significance of these negative findings may be limited due to inadequate study power. Measurement of sensitive periprocedural ischemic markers of myocardial injury (CK-MB and troponin I/T) within a controlled environment, during which medication noncompliance was not an issue, add to the reliability of these findings. Furthermore, patients with acute myocardial infarction were excluded to minimize confounding variables, and no patients had an angiographic etiology for a PPMI. These findings are highly pertinent from both a mechanistic and clinical standpoint.

Mechanistically, both serum fibrinogen and activated platelets are key components of arterial thrombus formation (22). Circulating platelets are activated by soluble platelet agonists (such as ADP, epinephrine, thrombin, and collagen) causing conformational shifts of glycoprotein IIb/IIIa receptors and externalization of these receptors to the platelet cell surface. Activated receptors then bind to circulating fibrinogen and fibrin molecules, and facilitate platelet crosslinking, thrombosis, and clot formation. Platelet activation also facilitates release of platelet alpha-granules, containing additional fibrinogen and other coagulation factors, further promoting localized thrombus formation (22,23). Due to the interplay between fibrinogen and ADP-mediated platelet activation during thrombus formation, uncertainty exists regarding the independent effects of these factors on adverse thrombotic and ischemic cardiac events. However, the results of the current study suggest that an elevated fibrinogen level ≥345 mg/dl is related to significant platelet cross-linking and thrombus formation independent of residual P2Y12 receptor-mediated platelet

Table 4 Subject Characteristics Stratified by Occurrence of a CK-MB (>3× ULN) Defined PPMI

	No CK-MB–Defined PPMI (n = 178)	CK-MB–Defined PPMI (n = 11)	p Value
Age, yrs	63.7 ± 11.7	65.7 ± 10.1	0.568
Male	73.6	81.8	0.731
BMI, kg/m^2	28.5 ± 5.5	27.2 ± 4.6	0.457
Cardiovascular history			
Past PCI	62.9	63.6	1.00
Past CABG	18.5	9.1	0.692
Past MI	29.3	36.4	0.735
Hypertension	89.9	100	0.604
Hyperlipidemia	90.4	72.7	0.096
Diabetes mellitus	46.1	54.5	0.584
Family history of CVD	30.1	27.3	1.00
Smoking history	16.0	18.2	0.692
Current PCI indication			0.798
Stable angina	56.7	45.5	
Unstable accelerating angina	23.0	36.4	
Other symptoms	10.7	9.1	
Positive stress test	9.6	9.1	
Medications			
Aspirin	97.2	100	1.00
Statin	84.2	72.7	0.394
Beta-blocker	74.7	63.6	0.480
ACE inhibitor/ARB	62.4	54.5	0.751
Nitrates	29.2	36.4	0.735
Calcium channel blocker	24.2	27.3	0.731
Clopidogrel (75 mg daily ≥7 days)	84.3	90.9	1.00
Laboratory values			
HDL, mg/dl	39.8 ± 13.4	41.3 ± 13.2	0.721
LDL, mg/dl	78.0 ± 31.0	90.5 ± 51.8	0.219
Triglycerides, mg/dl	120.1 ± 73.3	131.9 ± 44.4	0.597
WBC, cells × 10^3/μl	7.1 ± 2.0	8.3 ± 1.8	0.051
Hemoglobin, g/dl	12.9 ± 1.6	13.9 ± 2.0	0.056
Platelet count, cells × 10^3/μl	220.2 ± 56.2	237.5 ± 63.1	0.328
CRP >0.5 mg/dl	25.7	36.4	0.484
Fibrinogen, mg/dl	313.5 ± 104.6	403.4 ± 128.0	0.007
Percutaneous coronary intervention			
Drug-eluting stent	85.8	100	0.365
Bare-metal stent	9.1	9.1	1.00
PTCA	31.3	45.5	0.334
Stents used per procedure	1.68 ± 0.99	2.36 ± 0.92	0.027
Vessels treated per procedure	1.24 ± 0.49	1.10 ± 0.32	0.202
Type C lesion treated	52.8	63.6	0.485
Bifurcation lesion treated	16.9	20.0	0.681
Bivalirudin	86.1	81.8	0.656
Unfractionated heparin	11.5	18.2	0.623
Glycoprotein IIb/IIIa inhibitor	7.6	18.2	0.224
Platelet function testing			
Base PRU	328.2 ± 57.8	312.5 ± 32.7	0.383
Result PRU	210.8 ± 91.4	224.7 ± 96.4	0.624
Platelet inhibition, %	36.1 ± 25.3	30.2 ± 20.0	0.446
Result PRU ≥230	44.9	45.5	1.00
Result PRU ≥208	51.7	45.5	0.688

Values are mean ± SD or %.

CK-MB = creatine kinase-myocardial band; other abbreviations as in Tables 1 and 2.

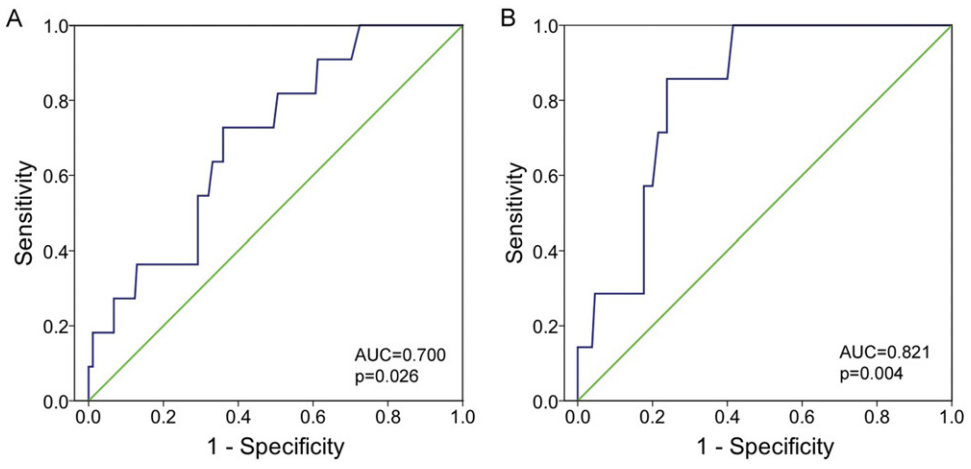

Figure 2 ROC Curve Relating Fibrinogen Level With and Without Systemic Inflammation to PPMI

(A) ROC curve relating fibrinogen level to PPMI. Fibrinogen level ≥345 mg/dl produced the greatest sensitivity and specificity in predicting a CK-MB (>3× ULN) defined PPMI (AUC: 0.700; 72.7% sensitivity; 64% specificity; 11.1% positive predictive value; 97.4% negative predictive value; p = 0.026). **(B)** ROC curve relating fibrinogen level without systemic inflammation to PPMI. Fibrinogen level ≥345 mg/dl in the absence of systemic inflammation (CRP ≤0.5 mg/dl) produced the greatest sensitivity and specificity in predicting a CK-MB (>3× ULN) defined PPMI (AUC: 0.821; sensitivity 85.7%; specificity 76.1%; positive predictive value 16.2%; and negative predictive value 99.0%; p = 0.004). AUC = area under the curve; CK-MB = creatine kinase-myocardial band; PPMI = periprocedural myocardial infarction; ULN = upper limit of normal.

activity during clopidogrel therapy, and in the absence of general inflammation.

Clinically, both elevated serum fibrinogen and high on-treatment platelet reactivity signify increased risk of adverse ischemic cardiovascular events. Large epidemiological studies have shown fibrinogen to be an independent long-term risk factor for primary MACE, including death, nonfatal myocardial infarction, and stroke (12–15). Other smaller studies with shorter follow-up periods have also shown fibrinogen to be an independent risk factor for repeat MACE in those with existing coronary artery disease (6–11,24). Similarly, the GRAVITAS trial and a number of smaller studies have shown that higher platelet reactivity with clopidogrel therapy is independently associated with an increased risk of adverse thrombotic and ischemic cardiovascular events following PCI (20,21). However, the positive predictive value of platelet reactivity measures for such events is low, and there is limited consensus on the definition of suboptimal platelet inhibition (4,5,20,21,25).

Our group (17,26) and others (6,27) have previously shown an association between elevated fibrinogen level and lower platelet reactivity during antiplatelet therapy. Using the VerifyNow IIb/IIIa assay, we have shown an association between fibrinogen level ≥375 mg/dl and inadequate inhibition of platelet aggregation during PCI despite single- or double-bolus administration of the potent glycoprotein IIb/IIIa inhibitor eptifibatide (26). In another study, using the VerifyNow P2Y12 assay, we demonstrated that fibrinogen level ≥375 mg/dl was associated with higher residual platelet reactivity following clopidogrel pre-treatment before PCI (17). Althoff et al. (6) used multiple electrode aggregometry (Multiplate analyzer, Dynabyte, Munich, Germany) and identified both serum fibrinogen and recent myocardial infarction as independent risk factors for subsequent elevated platelet reactivity during dual-antiplatelet therapy with aspirin and clopidogrel. Bernlochner et al. (27) used the Multiplate analyzer to demonstrate a relationship between a fibrinogen level ≥350 mg/dl and higher platelet aggregation following PCI and dual antiplatelet treatment using aspirin and clopidogrel. Recognizing the association between increased fibrinogen and platelet reactivity, the current study was designed to prospectively evaluate the roles of baseline serum fibrinogen and platelet reactivity following dual antiplatelet therapy (aspirin and clopidogrel) in predicting a short-term, adverse ischemic cardiac event following elective PCI.

In this study, a fibrinogen level ≥345 mg/dl had the greatest sensitivity and specificity after ROC curve analysis for predicting CK-MB–defined PPMI, and this level also remained significantly associated with troponin I/T–defined PPMI. Other groups, including Arnau Vives et al. (7), Toss et al. (8), and Kaski et al. (24), studied higher-risk individuals with non–ST-segment elevation acute coronary syndromes and reported fibrinogen cutoff levels of 375 mg/dl (upper tertile/ROC curve), 400 mg/dl (upper tertile), and 447 mg/dl (ROC curve) to be predictive of repeat occurrence of MACE. The ROC curve analyses from studies by Toss et al. (8), Kaski et al. (24), and the current study show similar results, with AUC ranges of 0.70 to 0.73 (sensitivity 60% to 73%, specificity 64% to 70%) and fibrinogen cutoffs possessing low positive predictive values (6.2% to 14.9%) and high

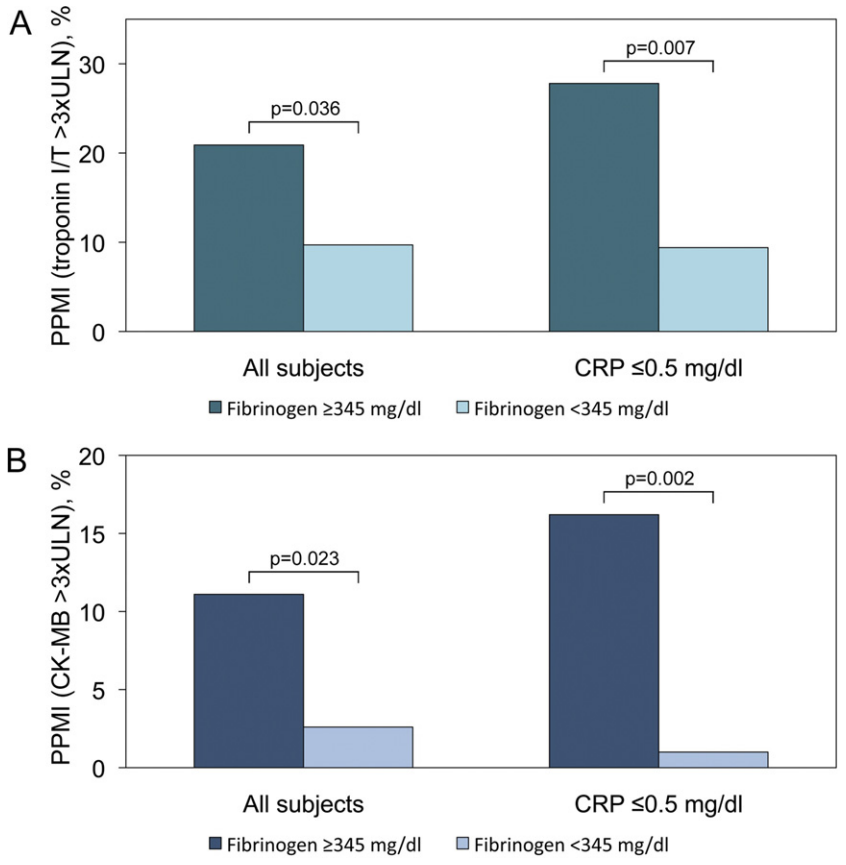

Figure 3 **Fibrinogen Level and PPMI in Elective PCI**

The incidence of both troponin I/T (>3× ULN) defined PPMI (20.9% vs. 9.7%; p = 0.036) **(A)** and CK-MB (>3× ULN) defined PPMI (11.1% vs. 2.6%; p = 0.023) **(B)** was greater in those with elevated fibrinogen level ≥345 mg/dl. In subjects with elevated fibrinogen (≥345 mg/dl) without systemic inflammation (CRP ≤0.5 mg/dl), an even higher incidence of PPMI was observed (troponin I/T–defined 27.8% vs. 9.2%; p = 0.007 **[A]** and CK-MB–defined 16.2% vs. 1.0%; p = 0.002 **[B]**). CRP = C-reactive protein; PCI = percutaneous coronary intervention; other abbreviations as in Figure 1.

negative predictive values (94.4% to 98.8%). The variation observed between our cutoff value of 345 mg/dl and the others is likely attributed to differences in study populations (lower-risk elective PCI subjects in our study) and outcomes measured. The overall predictive ability of these cutoffs suggests that depressed fibrinogen level accurately signifies a lower risk of secondary thrombotic/ischemic cardiac events, whereas elevated fibrino-

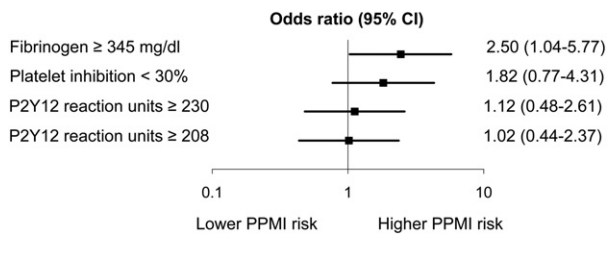

Figure 4 **Risk of PPMI (Troponin I/T >3× ULN) With Elevated Fibrinogen and High On-Clopidogrel Platelet Reactivity**

Elevated fibrinogen level (≥345 mg/dl) is associated with an increased risk of a troponin I/T–defined PPMI (odds ratio: 2.5; 95% confidence interval (CI): 1.04 to 5.77), whereas no such risk is observed with on-clopidogrel treatment platelet reactivity. PI = platelet inhibition; other abbreviations as in Figure 2.

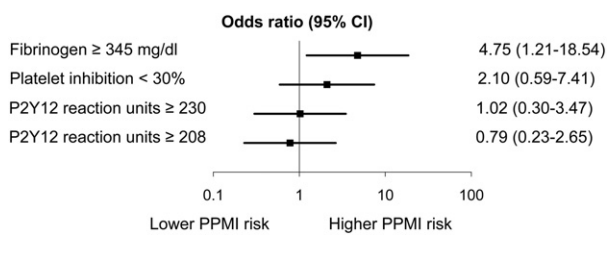

Figure 5 **Risk of PPMI (CK-MB >3× ULN) With Elevated Fibrinogen and High On-Clopidogrel Platelet Reactivity**

Elevated fibrinogen level (≥345 mg/dl) is associated with a much greater risk of a PPMI (odds ratio: 4.75; 95% CI: 1.21 to 18.5), whereas no increased risk is observed with higher on-clopidogrel platelet reactivity. Abbreviations as in Figures 2, 3, and 4.

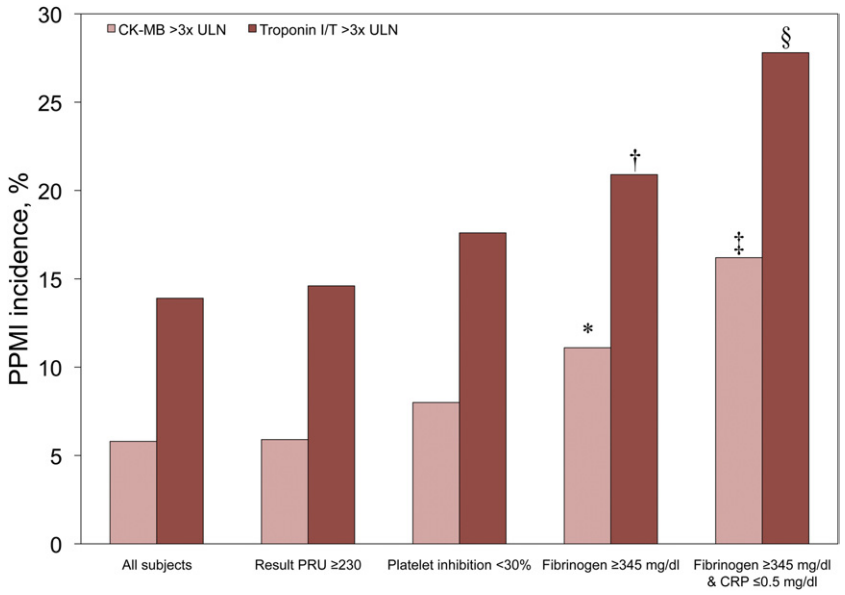

Figure 6 **Incidence of PPMI in the Presence of Factors Associated With an Increased Risk of Ischemic Myocardial Injury During PCI**

Subjects with baseline fibrinogen level ≥345 mg/dl had a higher incidence of both CK-MB–defined PPMI (*controlled for platelet reactivity and inflammatory markers) and troponin I/T–defined PPMI (†controlled for age, platelet count, platelet reactivity, and inflammatory markers) supported by multiple variable testing. Subjects with an elevated baseline fibrinogen level in the absence of systemic inflammation (CRP ≤0.5 mg/dl) had an even higher incidence of CK-MB–defined PPMI based on multiple variable interaction testing (‡controlled for fibrinogen and CRP), but higher incidence of troponin I/T–defined PPMI was only supported by univariate analysis (§). *†‡Multiple variable regression p value <0.05; §univariate p value <0.05. Abbreviations as in Figures 2 and 3.

gen level alone does not reliably predict such events. Of note, we demonstrated an incrementally increased AUC (0.821) and predictive values (sensitivity 85.7%, specificity 76.1%, positive predictive value 16.2%, and negative predictive value 99.0%) after performing ROC curve analysis among subjects with CRP ≤0.5 mg/dl, suggesting an improved positive and negative predictor role for fibrinogen in the absence of systemic inflammation.

Platelet function testing after clopidogrel pre-treatment is similarly limited in its ability to positively predict secondary ischemic cardiac events. The current study did not show an association between measures of platelet reactivity and PPMI, though it may have been inadequately powered to evaluate these relationships. This finding is inconsistent with the results of several smaller studies that used the VerifyNow P2Y12 assay to predict adverse thrombotic cardiac outcomes following PCI for both acute coronary syndromes (28,29) and elective indications (30,31). The ROC curve analyses used in each of these studies resulted in PRU cutoffs of 235, 236, and 240 to predict adverse cardiac events. These analyses as a group produced ranges of AUC (0.62 to 0.71), sensitivity (60% to 81%), specificity (53% to 70%), positive predictive value (12% to 81%), and negative predictive value (91% to 99%) that indicate, similar to serum fibrinogen concentration, lower VerifyNow PRU predicts superior clinical outcomes, but higher PRU alone does not reliably predict adverse ischemic events.

In the current study, we also report a strong association between VerifyNow PRU ≥230 and PI <30.9%. This finding can be helpful in interpreting the results of studies identifying clinically significant VerifyNow PRU cutoffs and those using traditional LTA to identify clinically significant levels of PI. A number of previous studies have used LTA with a 10 μmol/l ADP agonist and ROC curve analyses to identify PI of 30% as a significant cutoff, below which individuals are at greater risk of adverse cardiac events (32–35). In addition, the current study shows that although 45% of subjects have high on-clopidogrel platelet reactivity defined as PRU ≥230, a slightly larger percentage (53%) of subjects attained PI <30%, and a small portion of these individuals would be considered as appropriate clopidogrel responders using a PRU-based definition alone. Most importantly, it might not be appropriate to define cutoff values that are based on a single assay or level of platelet reactivity, but consideration should be given to reporting outcomes in future studies based on a range of inhibition of platelet reactivity as it is more generalizable and better understood by the majority of clinicians.

Study limitations. The current study was not designed to re-examine the relationship between fibrinogen and platelet reactivity, but rather to assess for interactions between these variables in predicting a short-term adverse cardiac event. The results of these analyses showed a relationship between fibrinogen and PPMI independent of VerifyNow P2Y12 platelet reactivity measures. A relationship between platelet reactivity and PPMI was not found in this study, but conclusions drawn from this result may be limited due to

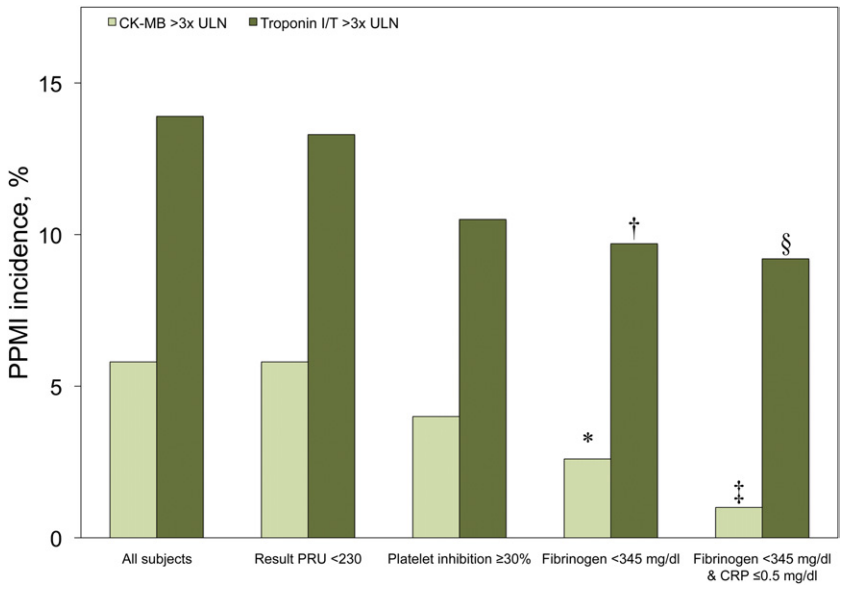

Figure 7 **Incidence of PPMI in the Absence of Factors Associated With an Increased Risk of Ischemic Myocardial Injury During PCI**

Subjects with baseline fibrinogen level <345 mg/dl had lower incidence of both CK-MB–defined PPMI (*controlled for platelet reactivity and inflammatory markers) and troponin I/T–defined PPMI (†controlled for age, platelet count, platelet reactivity, and inflammatory markers) supported by multiple variable testing. Subjects with baseline fibrinogen level <345 mg/dl and absence of systemic inflammation (CRP ≤ 0.5 mg/dl) had a lower occurrence of CK-MB–defined PPMI based on multiple variable interaction testing (‡controlled for fibrinogen and CRP), but lower incidence of troponin I/T–defined PPMI was only supported by univariate analysis (§). *†‡Multiple variable regression p value <0.05; §univariate p value <0.05. Abbreviations as in Figures 2 and 3.

inadequate study power. Results may also be limited to the VerifyNow P2Y12 assay methodology, and other tests of platelet reactivity may yield different results. The findings of this study do not provide insight into whether the relationship between high platelet reactivity and ischemic cardiovascular events demonstrated in previous studies is a direct one or mediated through the effect of serum fibrinogen. Future studies evaluating platelet reactivity and adverse cardiovascular events should include simultaneously measured serum fibrinogen to clarify this relationship. Furthermore, though relationships between fibrinogen, low CRP, and PPMI were observed in a secondary analysis, limited conclusions should be drawn regarding lack of similar relationships in those with higher CRP. The significance of the reported fibrinogen cutoff may vary when related to different populations or outcome measurements.

Conclusions

In elective PCI patients, elevated serum fibrinogen is independently associated with PPMI regardless of on-treatment platelet reactivity or platelet inhibition using the VerifyNow P2Y12 assay. Fibrinogen level ≥345 mg/dl signifies an increased risk of a PPMI, especially in the absence of systemic inflammation (CRP ≤0.5 mg/dl), whereas no relationship between high platelet reactivity and PPMI is present in this elective PCI study population. Future studies relating platelet reactivity and adverse cardiac events should measure baseline fibrinogen to clarify whether such a relationship is a direct one or mediated through independent fibrinogen effects.

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Key Words: clopidogrel ■ fibrinogen ■ periprocedural myocardial infarction ■ platelet reactivity ■ VerifyNow.