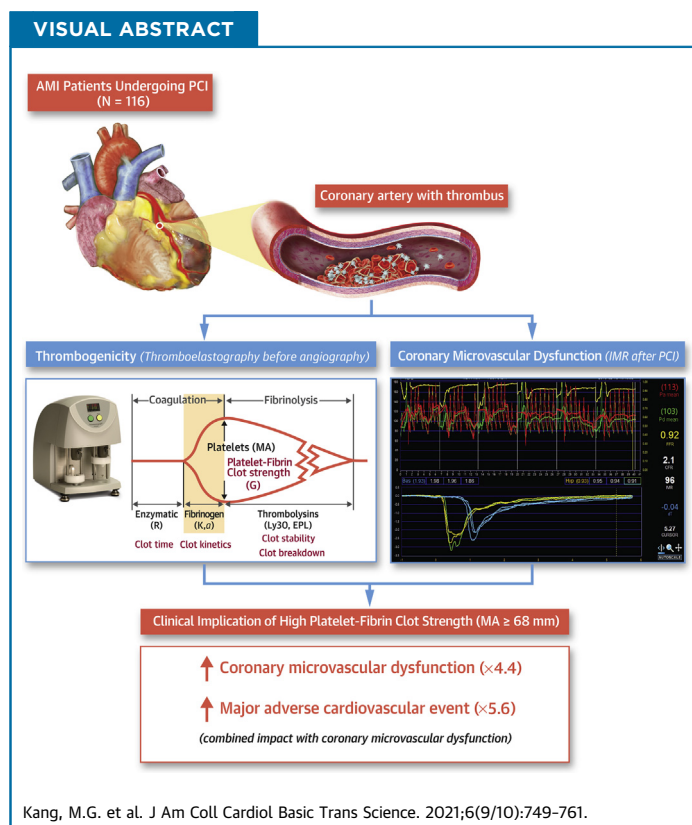


CLINICAL RESEARCH

Association Between Thrombogenicity Indices and Coronary Microvascular Dysfunction in Patients With Acute Myocardial Infarction



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HIGHLIGHTS

- Our study supports the feasibility of measuring thrombogenicity in patients with acute myocardial infarction, with intent to predict periprocedural and long-term clinical events, in real-world clinical practice.
- Preprocedural assessment of ex vivo thrombogenicity (eg, platelet-fibrin clot strength by thromboelastography) was significantly associated with the risk of coronary microvascular dysfunction.
- A combined risk stratification with coronary microvascular dysfunction and platelet-fibrin clot strength increased the prognostic implication to predict the rates of long-term clinical outcomes.

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

CFR = coronary flow reserve

CMD = coronary microvascular dysfunction

IMR = index of microcirculatory resistance

LASSO = least absolute shrinkage and selection operator

MA = maximum amplitude

MACE = major adverse cardiovascular events

OR = odds ratio

PCI = percutaneous coronary intervention

P-FCS = platelet-fibrin clot strength

PRU = P2Y₁₂ reaction units

R = reaction time

TEG = thromboelastography

TIMI = Thrombolysis in Myocardial Infarction

Tmn = mean transit time

SUMMARY

The association between thrombogenicity and coronary microvascular dysfunction (CMD) has been poorly explored in patients with acute myocardial infarction (AMI). In our real-world clinical practice (N = 116), thrombogenicity was evaluated with thromboelastography and conventional hemostatic measures, and CMD was defined as index of microcirculatory resistance of >40 U using the invasive physiologic test. High platelet-fibrin clot strength (P-FCS) (≥ 68 mm) significantly increased the risk of postprocedural CMD (odds ratio: 4.35; 95% CI: 1.74-10.89). Patients with both CMD and high P-FCS had a higher rate of ischemic events compared to non-CMD subjects with low P-FCS (odds ratio: 5.58; 95% CI: 1.31-23.68). This study showed a close association between heightened thrombogenicity and CMD and their prognostic implications after reperfusion in acute myocardial infarction patients. (J Am Coll Cardiol Basic Trans Science 2021;6:749-761) © 2021 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Acute myocardial infarction (AMI) is one of the significant causes of death and is associated with high morbidity despite recent advances in procedural and pharmacologic modalities. Over the last few decades, the focus has been on timely reperfusion of the epicardial coronary artery in patients with AMI (1). Decreased myocardial perfusion has been associated with extensive myocardial infarction leading

to adverse left ventricular remodeling and increased cardiac mortality (2). Coronary microvascular dysfunction (CMD) after reperfusion, such as microvascular obstruction, is also associated with poor clinical outcome. The index of microcirculatory resistance (IMR) is a quantitative and reproducible wire-based method to assess the CMD (3). High IMR following percutaneous coronary intervention (PCI) predicted the risk of atherothrombotic events in patients with acute coronary syndromes (4-8).

Restoration of perfusion triggers a cascade of events encompassing myocyte swelling, development of endothelial projections that occlude capillaries, platelet-neutrophil recruitment, and fibrin deposition (1). In an experimental model, coronary microembolization by atherothrombotic debris has

been shown to contribute to the occurrence of microvascular obstruction (9). Because intrinsic hemostatic properties may affect the risk of microvascular obstruction (10), proper assessment and pharmacologic modulation of these properties could be an effective therapeutic strategy to prevent the risk of CMD (11-14).

Hemostasis is a complex process involving both cellular elements (eg, platelet function and inflammatory cells) and plasma proteins (inflammatory, procoagulant, anticoagulant, and fibrinolytic factors) (15). However, few comprehensive studies have evaluated the impact of hemostatic components on CMD occurrence in patients with AMI. Moreover, there is a lack of strong clinical evidence to suggest a close relationship between ex vivo thrombogenicity properties and the occurrence of CMD assessed by an intracoronary physiologic test. The aim of this study was to evaluate the association between thrombogenicity indices and CMD, as well as its prognostic implications in patients with AMI.

METHODS

PATIENT POPULATION. The study population was derived from the Gyeongsang-National University

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the [Author Center](#).

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Hospital registry (NCT04650529), which was a prospective, 2-center database that enrolled PCI-treated patients with significant coronary artery disease, and evaluated multiple hemostatic, vascular, and physiologic parameters if indicated (16). In this retrospective analysis, we enrolled AMI patients who underwent uneventful PCI with available invasive physiologic assessments and thrombogenicity indices between January 2013 and December 2017 (Figure 1). AMI was defined as increased cardiac troponin values with ischemic symptoms or ischemic changes on electrocardiogram or imaging evidence of recent loss of viable myocardium or new regional wall motion abnormality (17).

Major inclusion criteria were timely PCI for AMI with standard pharmacologic therapy based on the current guidelines (18). Exclusion criteria were: 1) post-PCI Thrombolysis in Myocardial Infarction (TIMI) flow grade of ≤ 2 ; 2) previous history of myocardial infarction (MI); 3) cardiogenic shock; 4) New York Heart Association class 4; and 5) fibrinolytic therapy.

Baseline demographic, angiographic, and procedural characteristics and clinical outcome data were collected prospectively. Patients were routinely followed up at 1, 6, and 12 months after the index procedure and annually thereafter. Further information was collected by medical records or telephone contact if necessary. The Institutional Review Board of the hospital approved the study protocol (GNUH 2020-04-026) and waived the requirement for written informed consent for access to an institutional registry. The study was performed in accordance with the Good Clinical Practice Guidelines and the principles of the Declaration of Helsinki.

PROCEDURES. After arrival at the emergency department, patients with suspected AMI planned for emergent PCI were treated with standard loading doses of aspirin and a P2Y₁₂ receptor inhibitor. For anticoagulation, unfractionated heparin was administered with dosage according to label instructions (100 IU/kg). Glycoprotein IIb/IIIa inhibitor use was allowed during the procedure at the discretion of attending interventionists for bailout cases.

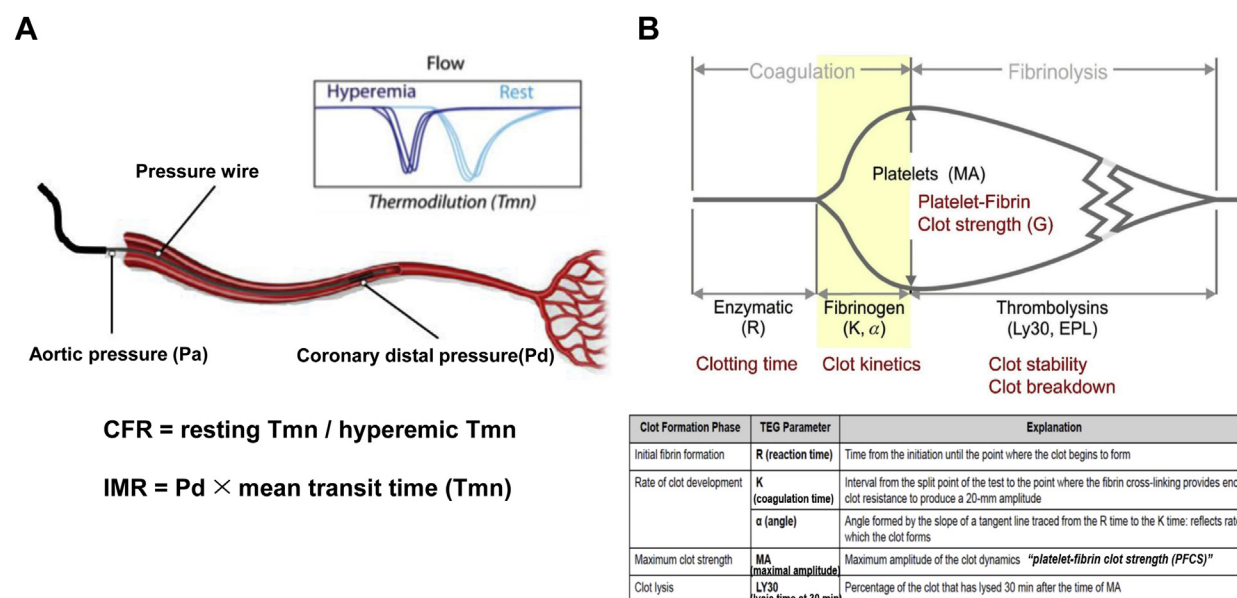
All the angiographic variables for calculating the SYNTAX (Synergy Between PCI With Taxus and Cardiac Surgery) score were obtained by reviewing the diagnostic angiograms acquired before the procedure. All PCI procedures were performed according to the standard technique (19). After uneventful PCI, we evaluated post-PCI TIMI flow. In cases of post-PCI TIMI flow 3, an invasive physiologic testing was conducted according to the predefined protocol. All

patients were treated with guideline-recommended pharmacologic therapy.

INTRACORONARY PHYSIOLOGIC TESTING. Coronary angiography was performed by standard techniques. All angiograms were analyzed at a local core laboratory in a blinded fashion. At the end of PCI, IMR was measured by 1 of 2 dedicated physicians (Drs Koh and Kang), using a pressure- and temperature-sensing guidewire (Abbott Vascular) through a guiding catheter (5-F to 7-F) without side holes (3). All patients received 200 mg of intracoronary nitroglycerin before the physiologic assessment. The calibrated wire was equalized to guide catheter pressure and then advanced to the distal third of the culprit artery. Intravenous adenosine infusion (140 μ g/kg/min for 2 minutes) was used for hyperemia induction. Continuous recordings of aortic pressure and distal pressure were obtained during baseline and drug infusion periods. During maximal hyperemia, the fractional flow reserve was calculated as the ratio of mean distal to aortic pressure. Mean transit time (Tmn) during a hand-injected room temperature saline (3-mL bolus) was measured using the standard thermodilution methodology, in triplicate at rest and during steady-state maximal hyperemia.

IMR was quantified by distal coronary pressure $\times$ Tmn during hyperemia. Coronary flow reserve (CFR) was quantified by dividing resting Tmn by hyperemic Tmn (Figure 1A). All data were extracted from the RadiAnalyzer Xpress instrument and then analyzed offline using Coroflow software (Coroventis Research AB) by an investigator blinded to the results of hemostatic measurements.

HEMOSTATIC MEASUREMENTS. According to the predetermined protocol, baseline biochemical assessment was performed in whole blood samples drawn immediately after arrival to the emergency department. Fibrinogen level was measured by the Clauss method with STA-Fibrinogen reagent (Diagnostica Stago), and D-dimer levels were measured by the immunoturbidimetric method with the STA-Liatest D-DI reagent (Diagnostica Stago), using a Stago STA-R Evolution hemostasis system (Diagnostica Stago). High-sensitivity C-reactive protein level was measured with a commercially available enzyme-linked immunosorbent assay using UniCel Dx C 800 Synchron Clinical System (Beckman Coulter). Other biochemical measurements, including complete blood count, lipid profile, and chemistry profile, were performed immediately after arrival to the emergency department using standard laboratory assays.

FIGURE 1 Measurements from Intracoronary Physiologic Testing and Thromboelastography

(A) Index of microcirculatory resistance. (B) Thromboelastography. α = the angle in degrees formed by the tangent line to the thromboelastography tracing measure at the reaction time; CFR = coronary flow reserve; EPL = estimated percent lysis; IMR = index of microcirculatory index; K = coagulation time; LY30 = percentage of the clot that has lysed 30 minutes after the time of maximum amplitude; MA = maximum amplitude; R = reaction time; Tmn = mean transit time.

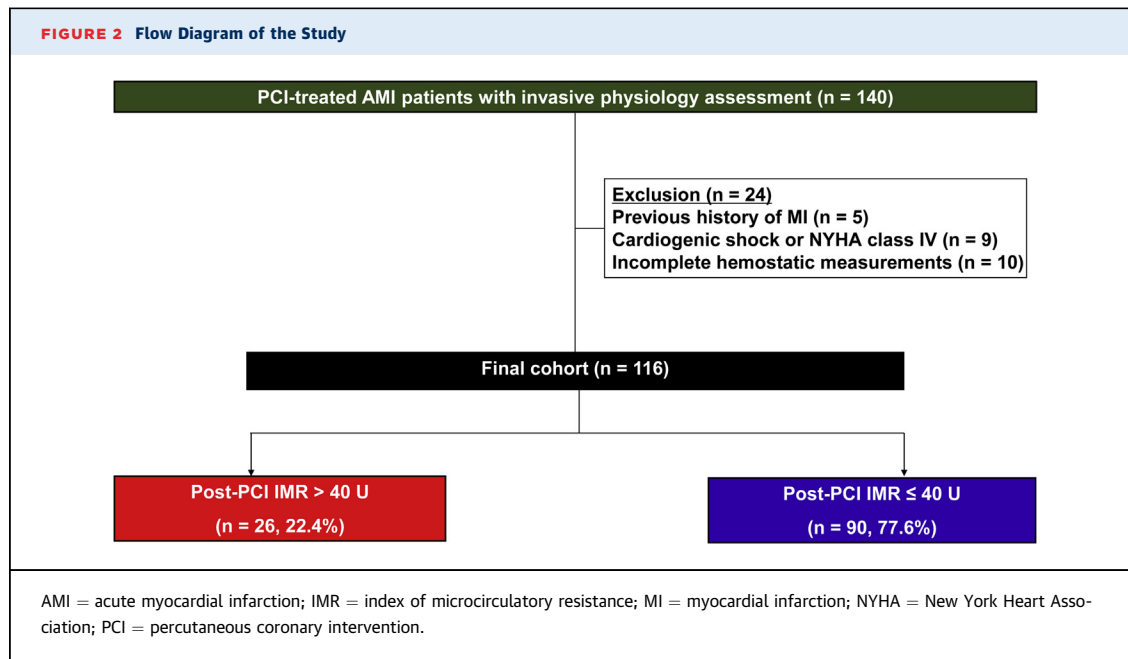
Platelet function testing. Immediately after the arterial sheath insertion for coronary angiography, blood samples for platelet function analysis by VerifyNow assay (Accriva) were collected in 3.2% citrate Vacuette tubes (Greiner Bio-One Vacuette North America, Inc). The VerifyNow P2Y₁₂ assay is a whole-blood, point-of-care, turbidimetric-based optical detection assay designed to measure platelet aggregation in response to an agonist that is based on the ability of activated platelets to bind to fibrinogen (20).

The first channel contains fibrinogen-coated polystyrene beads, 20 μ mol/L adenosine diphosphate, and 22 nmol/L prostaglandin E₁; the optical signal of this channel is reported as P2Y₁₂ reaction units (PRU). The second channel contains fibrinogen-coated polystyrene beads, 3.4 mmol/L isothrombin receptor-activating peptide (protease-activated receptor-1 [PAR-1] agonist), and PAR-4-activating peptide; the optical signal of this channel is reported as the Base, which indicates the maximal platelet function independent of P2Y₁₂ receptor blockade.

Thromboelastography. Immediately after the arterial sheath insertion for coronary angiography, blood samples for global hemostasis by thromboelastography (TEG) were drawn into Vacutainer tubes containing 3.2% trisodium citrate (Becton Dickinson).

The assay was performed using the TEG 5000 Hemostasis Analyzer System (Haemonetics Corp) with automated analytical software (21) (Figure 1B), which provides measurements of the viscoelastic properties of a clot. Briefly, 500 μ L of citrated blood is mixed with kaolin by inversion, and 340 μ L of the activated blood is then transferred to a reaction cup, to which 20 μ L of 200 mmol/L calcium chloride is added. In heparin-pretreated cases, a vial containing heparinase is used to neutralize the heparin effect. A stationary pin is suspended into an oscillating cup that contains the whole blood sample. As the blood clots, it links the pin to the cup. Pin movement is converted into an electrical signal by a transducer and is interpreted by the computer to create a tracing. The degree of platelet contribution to the clot strength through platelet-fibrin bonding directly influences the magnitude of pin movement and, ultimately, the amplitude of the tracing.

Reaction time (R, in minutes), a representative of the initiation phase of enzymatic clotting, is the time from the start of the sample run to the point of the first significant clot formation corresponding to an amplitude of 2 mm reading on the TEG tracing. K is a measure of the time to reach 20-mm clot strength from R. Angle (α) is reflective of fibrinogen activity



and is the degrees of the angle formed by the tangent line to TEG tracing measure at R. Kaolin-induced maximum amplitude (MA, in millimeters) represents the maximum platelet-fibrin clot strength (P-FCS) and is influenced by changes in fibrinogen, platelet count, and platelet function. Multiple clinical studies have suggested the influence of P-FCS on the occurrence of clinical events in patients with coronary artery disease undergoing PCI (16,21). LY30 indicates the percentage of the clot that has lysed 30 minutes after the occurrence of MA and indicates the level of fibrinolytic activity.

ENDPOINTS AND DEFINITIONS. The primary endpoint was P-FCS level between the CMD and non-CMD groups (16,21). The secondary endpoints were: 1) other thrombogenicity indices between the groups; and 2) the impact of major thrombogenicity indices on clinical events according to CMD status.

Major adverse cardiovascular events (MACE) were defined as a composite of death, MI, revascularization, or rehospitalization caused by heart failure (22). All-cause death included death from cardiovascular and noncardiovascular causes during the follow-up. MI was defined as increased cardiac troponin values with ischemic symptoms or ischemic changes on electrocardiography or imaging evidence of recent loss of viable myocardium or new regional wall motion abnormality (17).

STATISTICAL ANALYSIS. A previous trial suggested that patients with an IMR of >40 U were observed in

28% of AMI patients after PCI (23). Our previous study demonstrated a greater P-FCS in high-risk patients with versus without peripheral artery disease (68.8 ± 9.8 vs 62.0 ± 10.0 mm; $P < 0.001$) (16). We assumed that the level of P-FCS would be similar according to CMD status. Based on previous results, at least 114 patients were needed to detect an absolute difference of 6.8 mm, with a power of 90% and a 2-sided alpha error of 0.05.

The Kolmogorov-Smirnov test was performed to analyze the normal distribution of continuous variables. Continuous variables are presented as mean $\pm$ SD or as median (interquartile range), as appropriate, and categorical variables were reported as frequencies (percentages). The unpaired Student's *t*-test for parametric continuous variables and the Mann-Whitney *U* test for nonparametric continuous variables are used to compare the CMD versus non-CMD groups. Comparisons between categorical variables were performed using the chi-square or Fisher exact test, as appropriate.

The least absolute shrinkage and selection operator (LASSO) method, which is suitable for a regression model with high-dimensional data, was used to determine the risk factors for CMD. With larger penalties, the estimates of weaker factors shrink toward 0 (minimal λ value) so that only the strongest predictors remain in the model. Then, the odds ratios (ORs) for the risk factors were calculated by the exponential of coefficients and are presented with the c-index. Receiver-operating characteristic (ROC)

TABLE 1 Baseline Characteristics

	Total (N = 116)	CMD Group (n = 26)	Non-CMD Group (n = 90)	P Value
Index presentation with AMI	116 (100)	26 (100)	90 (100)	>0.999
Time from symptom onset to balloon, min	205 ± 123	189 ± 94	211 ± 132	0.491
Age, y	62 ± 12	67 ± 14	60 ± 10	0.006
Female	28 (24.1)	12 (46.2)	16 (17.8)	0.008
Body mass index, kg/m ²	24.1 ± 3.3	23.1 ± 3.6	24.4 ± 3.2	0.089
Risk factor or past medical history				
Diabetes mellitus	26 (22.4)	5 (19.2)	21 (23.3)	0.793
Hypertension	47 (40.5)	13 (50.0)	34 (37.8)	0.365
Dyslipidemia	54 (46.6)	9 (34.6)	45 (50.0)	0.187
Chronic kidney disease	13 (11.2)	2 (7.7)	11 (12.2)	0.730
Anemia	20 (17.2)	6 (23.1)	14 (15.6)	0.385
Previous MI	0 (0)	0 (0)	0 (0)	>0.999
Previous PCI	6 (5.2)	1 (3.8)	5 (5.6)	>0.999
Previous stroke	6 (5.2)	2 (7.7)	4 (4.4)	0.615
Laboratory findings				
WBC count, ×10 ³ /mm ³	9.9 ± 3.6	9.9 ± 2.6	9.8 ± 3.8	0.877
Hemoglobin, g/dL	14.2 ± 1.8	13.4 ± 1.7	14.4 ± 1.8	0.012
Platelet count, ×10 ³ /mm ³	248 ± 70	263 ± 69	244 ± 70	0.231
Hemoglobin A1c, %	6.3 ± 1.6	6.1 ± 1.7	6.3 ± 1.5	0.466
GFR, mL/min/1.73 m ² (MDRD)	87 ± 23	85 ± 15	88 ± 25	0.671
Total cholesterol, mg/dL	190 ± 51	186 ± 33	191 ± 55	0.599
HDL cholesterol, mg/dL	44 ± 12	43 ± 12	44 ± 12	0.680
LDL cholesterol, mg/dL	133 ± 41	127 ± 30	135 ± 43	0.407
Left ventricular ejection fraction, %	53 ± 10	49 ± 10	54 ± 8	0.008
Concomitant medications				
Aspirin	112 (96.6)	25 (96.2)	87 (96.7)	>0.999
P2Y ₁₂ receptor inhibitor				0.238
Clopidogrel	75 (64.7)	20 (76.9)	55 (61.1)	
Ticagrelor	27 (23.3)	3 (11.5)	24 (26.7)	
Prasugrel	4 (3.4)	1 (3.8)	3 (3.3)	
Beta blocker	87 (75.0)	15 (57.7)	72 (80.0)	0.037
Angiotensin blockade	80 (69.0)	13 (50.0)	67 (74.4)	0.029
Statin	113 (98.3)	25 (96.2)	88 (98.9)	0.403
Proton pump inhibitor	83 (72.2)	19 (73.1)	64 (71.9)	>0.999

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curve analysis was performed to find the optimal cutoff for P-FCS to further evaluate the association between CMD status and MACE. Kaplan-Meier methods were used to both compare MACE over time (log-rank test) and estimate the rate of MACE at 3 years (chi-square test). Cox proportional hazards models were used to estimate HRs with 95% CIs. Penalized Cox proportional hazards regression models were used to determine independent predictors of MACE. A *P* value of <0.05 was considered statistically significant, and statistical analyses were performed using SPSS software, version 24.0 (SPSS Inc) and R software, version 4.0.3 (R Core Team).

RESULTS

PATIENT CHARACTERISTICS. During the study period, 140 patients who presented with AMI

underwent invasive physiologic testing. After exclusion, 116 patients were enrolled in the final cohort (Figure 2). CMD (IMR: >40 U) was observed in 26 patients (22.4%). Patients with CMD were older and had lower levels of hemoglobin and left ventricular ejection fraction compared to patients without CMD (Table 1). There were no differences in lesional and procedural characteristics between the groups. Patients with CMD showed lower CFR levels than those without CMD (1.5 ± 1.0 vs 2.0 ± 0.9 ; *P* = 0.039) (Table 2).

TEG PARAMETERS. Compared with the non-CMD group, the CMD group had higher P-FCS (MA: 69.9 ± 5.8 vs 62.5 ± 8.8 mm; *P* < 0.001) (Figure 3). In addition, the CMD group showed a different dynamic profile of clot development as compared to the non-CMD group (α : $68.6^\circ \pm 10.6^\circ$ vs $62.1^\circ \pm 14.0^\circ$; *P* = 0.032). However, there were no significant differences in reaction time (R) and clot lysis (LY30) between the groups.

CONVENTIONAL HEMOSTATIC MEASUREMENTS. D-dimer value was greater in patients with versus without CMD (787 ± 646 vs 473 ± 345 μg/L; *P* = 0.001) (Table 2). Platelet reactivity was numerically greater in the CMD group than in the non-CMD group (235 ± 110 vs 196 ± 89 PRU; *P* = 0.065). Other hemostatic profiles did not show any statistical differences between the groups (Table 2).

DETERMINANTS OF CMD OCCURRENCE. The 46 variables evaluated at hospital admission were included in the LASSO regression. After LASSO regression selection (Supplemental Figure 1), 5 variables remained as predictors of CMD (c-index: 0.809): age (OR: 1.005), sex (OR: 1.298), left ventricular ejection fraction (OR: 0.991), D-dimer (OR: 1.472), and P-FCS (OR: 1.058).

To further evaluate the relationship between CMD and P-FCS, we categorized P-FCS using both the quartiles and the optimal cutoff based on the ROC curve analysis. The prevalence of CMD was positively correlated with P-FCS (3.6% vs 21.9% vs 25.9% vs 41.4% by quartile distribution; *P* for trend <0.001) (Figure 4A). P-FCS of ≥ 68 mm was identified as the optimal cutoff of high P-FCS to predict CMD occurrence (c-index: 0.747; 95% CI: 0.644-0.850; *P* < 0.001) (Figure 4B). Patients with P-FCS of ≥ 68 mm had an approximate 4-fold increased odds of CMD (OR: 4.35; 95% CI: 1.74-10.89; *P* = 0.001) (Figure 4C).

CLINICAL OUTCOMES. The median follow-up duration was 40 months (interquartile range 20-60). During the total follow-up period, 17 patients experienced a MACE occurrence, including 12 patients by the 3-year follow-up (3 deaths, 2 nonfatal

MIIs, 5 revascularizations, and 2 rehospitalizations caused by heart failure). Patients with high P-FCS did not show a statistically significant increased hazard of MACE (16.0% vs 8.7% at 3 years; OR: 2.23; 95% CI: 0.71-7.02; $P = 0.172$). The CMD group had a statistically significantly increased likelihood of MACE versus the non-CMD group (25.3% vs 7.8% at 3 years; OR: 4.20; 95% CI: 1.22-14.40; $P = 0.022$) (Figure 5A). However, patients with high CFR (>2) did not show a statistically significant increased risk of MACE compared with subjects with low CFR (≤ 2) (14.6% vs 6.0% at 3 years; OR: 2.76; 95% CI: 0.57-13.27; $P = 0.205$) (Figure 5B). In the LASSO regression selection, 3 variables still remained as predictors of MACE (c-index: 0.858): age (OR: 1.019), left ventricular ejection fraction (OR: 0.991), and IMR (OR: 1.149).

Risk stratification with CMD and P-FCS criteria appeared to be synergistic to predict 3-year MACE (Table 3). Patients with both high IMR and high P-FCS showed a higher risk of MACE occurrence compared to those with both low IMR and low P-FCS (HR: 5.58; 95% CI: 1.31 to 23.68; $P = 0.024$) (Figures 5C and 5D).

DISCUSSION

This is the first study, to our knowledge, to suggest the influence of thrombogenicity on CMD occurrence and its prognostic implications in patients with AMI. The main findings of the study are as follows: 1) preprocedural P-FCS levels were associated with the risk of CMD indicated by IMR at the end of PCI (P-FCS of ≥ 68 mm increased the risk for CMD by about 4-fold); 2) preprocedural levels of platelet reactivity showed the limited influence on the occurrence of post-PCI CMD risk; and 3) a combined risk stratification with CMD and high P-FCS increased the prognostic power to predict 3-year MACE.

Small arteries (100-400 μm) are most responsive to intravascular perfusion and flow changes, whereas arterioles (10 to <100 μm) are sensitive to tissue metabolites (1). These structures constitute a major resistance conduit in myocardial blood flow (indicated by IMR) and maintain a constant coronary blood flow through dynamic changes in lumen diameter. Therefore, prompt re-establishment of coronary blood flow after prolonged coronary occlusion always does not lead to positive changes at the microvascular level.

During the coronary recanalization procedure, multiple biochemical factors (eg, ischemia-reperfusion injury, endothelial abnormality, and external compression) may induce structural changes in resistance vessels (24). The activity of the

TABLE 1 Continued

	Total (N = 116)	CMD Group (n = 26)	Non-CMD Group (n = 90)	P Value
Procedural characteristics				
Pre-PCI TIMI grades 0-2	96 (82.8)	22 (84.6)	74 (82.2)	>0.999
Thrombus grades 4 or 5	94 (81.0)	21 (80.8)	73 (81.1)	>0.999
Post-PCI TIMI grade 3	116 (100)	26 (100)	90 (100)	>0.999
Infarct-related artery				0.234
Left anterior descending	73 (62.9)	15 (57.7)	58 (64.4)	
Left circumflex	16 (13.8)	2 (7.7)	14 (15.6)	
Right coronary artery	27 (23.3)	9 (34.6)	18 (20.0)	
Collateral flow grade				0.372
0	83 (71.6)	17 (65.4)	66 (73.3)	
I	19 (16.4)	7 (26.9)	12 (13.3)	
II	9 (7.8)	1 (3.8)	8 (8.9)	
III	5 (4.3)	1 (3.8)	4 (4.4)	
I-III	33 (28.4)	9 (34.6)	24 (26.7)	0.289
II-III	14 (12.1)	2 (7.7)	12 (13.3)	0.348
Multivessel disease	60 (51.7)	16 (61.5)	44 (48.9)	0.275
ACC/AHA lesion type B2/C	66 (56.9)	15 (57.7)	51 (56.7)	>0.999
Chronic total occlusion lesion	3 (2.6)	1 (3.8)	2 (2.2)	0.536
SYNTAX score				
Culprit vessel score	11.9 $\pm$ 2.2	12.3 $\pm$ 2.2	11.8 $\pm$ 2.2	0.255
Total score	21.8 $\pm$ 11.3	24.5 $\pm$ 9.7	21.0 $\pm$ 11.7	0.174
Multivessel PCI	11 (9.5)	2 (7.7)	9 (10.0)	0.534
Aspiration thrombectomy	58 (50.0)	16 (61.5)	42 (46.7)	0.265
Bailout use of tirofiban	31 (26.7)	9 (34.6)	22 (24.4)	0.321
Intervention method				0.615
Everolimus-eluting stent	57 (49.1)	15 (57.7)	42 (46.7)	
Zotarolimus-eluting stent	31 (26.7)	6 (23.1)	25 (27.8)	
Sirolimus-eluting stent	11 (9.5)	1 (3.8)	10 (11.1)	
BVS	17 (14.7)	4 (15.4)	13 (14.4)	
Number of stents	1.2 $\pm$ 0.4	1.1 $\pm$ 0.4	1.2 $\pm$ 0.5	0.591
Stent diameter, mm	3.2 $\pm$ 0.4	3.2 $\pm$ 0.4	3.2 $\pm$ 0.5	0.909
Stent length, mm	30 $\pm$ 16	28 $\pm$ 14	31 $\pm$ 16	0.318
Postdilation	90 (77.6)	18 (69.2)	72 (80.0)	0.288

Values are n (%) or mean $\pm$ SD.

ACC/AHA = American College of Cardiology/American Heart Association; AMI = acute myocardial infarction; BVS = bioresorbable vascular scaffold; GFR = glomerular filtration rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MDRD = modification of diet in renal disease; MI = myocardial infarction; PCI = percutaneous coronary intervention; SYNTAX = Synergy Between PCI With Taxus and Cardiac Surgery; TIMI = thrombolysis in myocardial infarction; WBC = white blood cell.

thromboinflammatory axis may be related to the initiation and progression of clot formation and migration in the injured vessel and its downstream microvessels. Therefore, microembolization via atherosclerotic debris, platelet-neutrophil recruitment, and the deposition of fibrin can determine the degree of microvessel occlusion (1). Because microvascular obstruction itself contributes to the occurrence of CMD, this complex process related with CMD may be associated with a worse prognosis in patients with AMI (25).

It is uncertain which ex vivo measured thrombogenicity indices can reflect better a in vivo

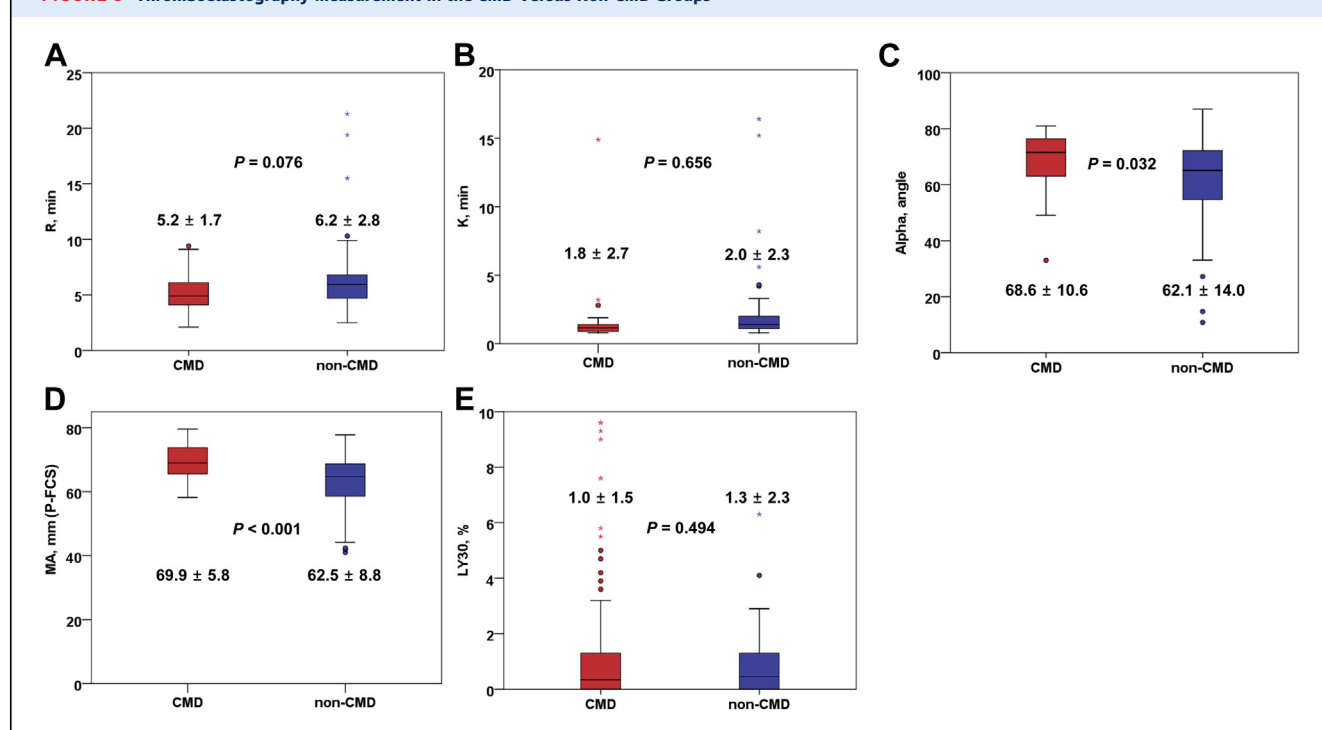
TABLE 2 Hemostatic and Physiologic Tests According to Coronary Microvascular Dysfunction

	Total (N = 116)	CMD Group (n = 26)	Non-CMD Group (n = 90)	P Value
Hemostatic measurement				
NT-proBNP, pg/mL	337.6 ± 587.2	417.9 ± 786.2	313.1 ± 515.3	0.438
hs-CRP, mg/L	4.7 ± 11.2	5.0 ± 9.3	4.7 ± 11.6	0.915
Fibrinogen, mg/dL	331 ± 88	334 ± 94	331 ± 87	0.863
D-dimer, µg/L	543 ± 447	787 ± 646	473 ± 345	0.001
VerifyNow P2Y ₁₂ assay				
PRU	204 ± 95	235 ± 110	195 ± 89	0.065
Base	249 ± 64	253 ± 70	247 ± 62	0.678
Post-PCI physiologic test				
FFR	0.89 ± 0.06	0.88 ± 0.07	0.89 ± 0.05	0.225
CFR	1.9 ± 0.9	1.5 ± 1.0	2.0 ± 0.9	0.039
IMR, U	29.2 ± 21.1	60.3 ± 23.3	20.2 ± 7.8	<0.001
Pd	78.1 ± 18.8	79.9 ± 16.7	77.6 ± 19.4	0.609
1/Tmn	3.7 ± 1.9	1.5 ± 0.6	4.3 ± 1.7	<0.001

Values are mean ± SD.
 CFR = coronary flow reserve; CMD = coronary microvascular dysfunction; FFR = fractional flow reserve; hs-CRP = high-sensitivity C-reactive protein; IMR = index of microcirculatory resistance; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PCI = percutaneous coronary intervention; Pd = distal coronary pressure; Tmn = mean transit time; PRU = P2Y₁₂ reaction units.

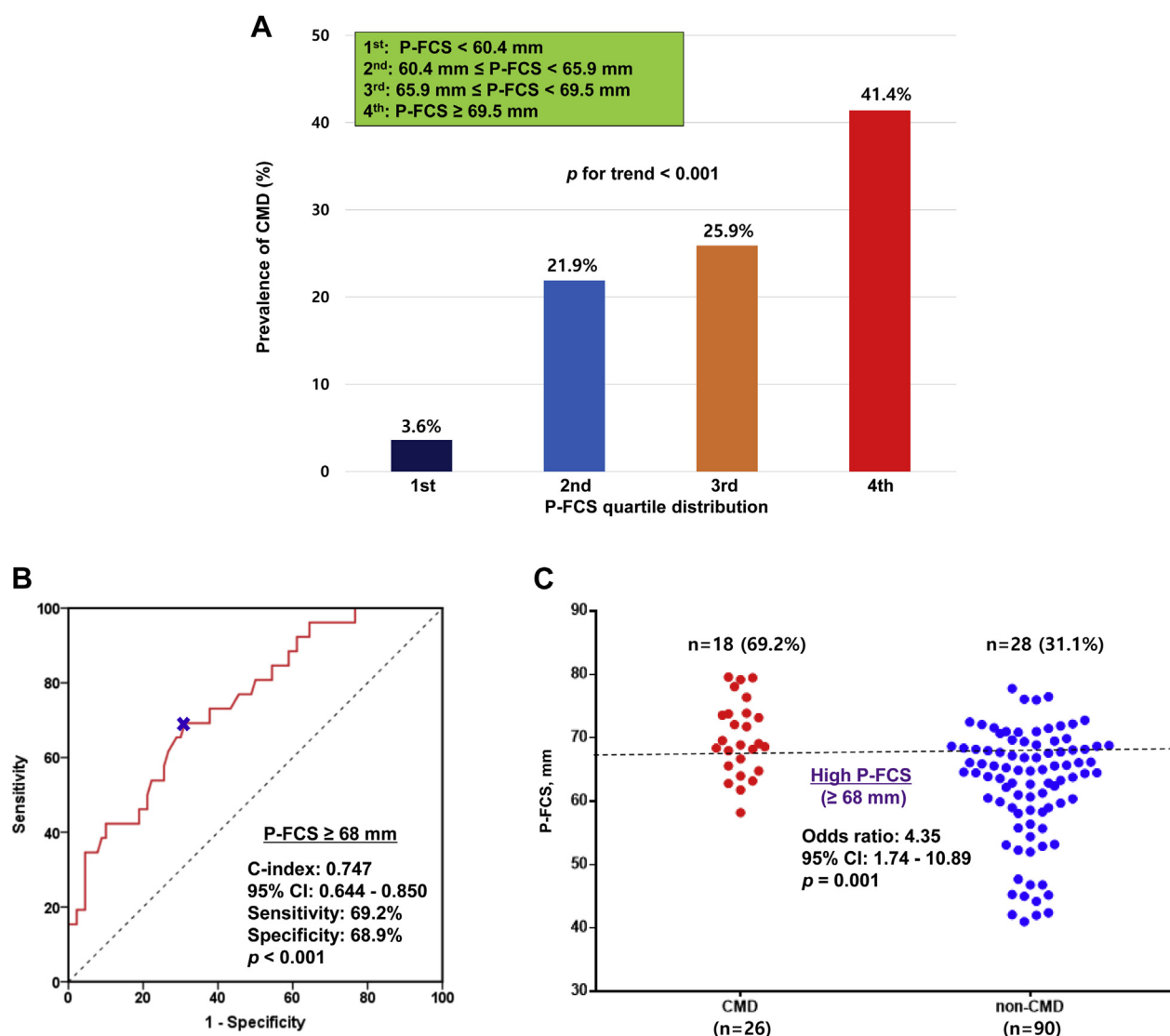
thromboembolic cascade in humans. Along this line, the present analysis suggested important evidence regarding this issue. Hemostasis is a finely regulated process composed of approximately 80 biochemical reactions including both cellular elements and plasma proteins (15). Because routine hemostatic tests mostly ignore these interactions and focus on assessing a specific pathway, their values on predicting the clinical manifestation of thromboembolic events would be very limited. In this analysis, several biomarkers and surrogates (eg, hemoglobin, white blood cell count, platelet count, cholesterol, N-terminal pro-B-type natriuretic peptide, high-sensitivity C-reactive protein, fibrinogen, and PRU) did not show considerable relevance to predicting CMD occurrence. In line with this observation, a recent randomized trial showed a similar value of post-PCI IMR following clopidogrel versus ticagrelor loading in patients with acute coronary syndrome (26).

In the current analysis, a marked elevation in D-dimer levels was observed in patients with versus

FIGURE 3 Thromboelastography Measurement in the CMD Versus Non-CMD Groups

CMD = coronary microvascular dysfunction; P-FCS = platelet-fibrin clot strength; other abbreviations as in Figure 1.

FIGURE 4 Association Between P-FCS and CMD Occurrence



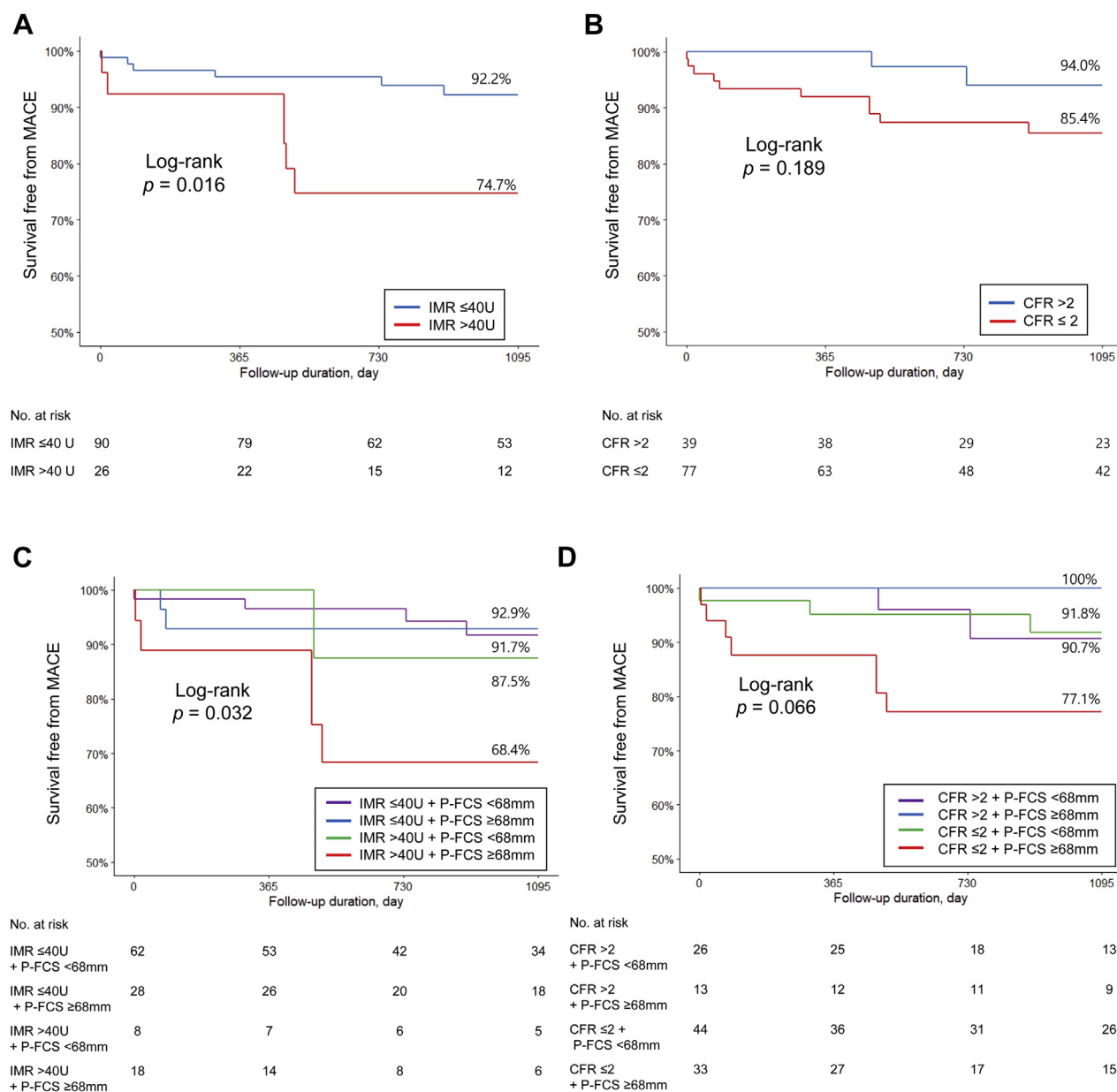
(A) Distribution of P-FCS and prevalence of CMD. (B) Receiver-operating characteristic curve analysis: the optimal cutoff of P-FCS for CMD. (C) Risk of high P-FCS for CMD occurrence. OR = odds ratio; other abbreviations as in Figures 1 and 3.

without CMD. D-dimers indicate the hypercoagulation activity and the presence of a thrombus (27). Coagulopathy in patients with COVID-19 increases the risk of thromboembolic events. An elevated level of D-dimer has been related to the risk of death and the level of thrombus burden in AMI patients (27,28). High D-dimer in COVID-19 may be associated with a prothrombotic state, a hypofibrinolytic state, or both. Therefore, this biomarker may have the potential to monitor COVID-19 coagulopathy and CMD occurrence. Our study also demonstrated the significant value of

D-dimer in predicting important thromboembolic occlusion.

The TEG assay is a whole-blood ex vivo assay that measures the dynamic viscoelastic properties of clot generation from the time of initial plasmatic thrombin generation, to platelet-fibrin dependent clot formation, to clot strengthening, and finally to clot lysis (21). The TEG assay also provides comprehensive information on thrombin generation, the interaction between fibrinogen/fibrin and platelets to form a platelet-fibrin clot, the contribution of

FIGURE 5 Kaplan-Meier Curves of 3-Year MACE



(A) Groups divided by IMR. **(B)** Groups divided by CFR. **(C)** Groups divided by IMR and P-FCS. **(D)** Groups divided by CFR and P-FCS. CFR = coronary flow reserve; MACE = major adverse cardiovascular events; other abbreviations as in [Figures 1 and 3](#).

platelet count and function as well as fibrinogen level and activity to clot strength, and the stability of the clot (15). P-FCS (ie, MA measured by TEG) levels are affected by changes in fibrinogen level and activity as well as platelet count and function. In addition, our previous reports suggested close relationships with coagulation activity (prothrombin

time), fibrinogen level, hemoglobin, and platelet count (21,29).

Because P-FCS can reflect the global hemostatic potential including both cellular elements and plasma proteins, this surrogate may better demonstrate in vivo thromboembolic processes and the risk of CMD occurrence following PCI in AMI patients.

TABLE 3 Univariable Risk Ratio of 3-Year MACE Occurrence

	HR	95% CI	P Value
IMR ≤40 U + P-FCS <68 mm vs			
IMR ≤40 U + P-FCS ≥68 mm	1.12	0.19 - 6.48	>0.999
IMR >40 U + P-FCS <68 mm	2.07	0.20 - 21.24	0.465
IMR >40 U + P-FCS ≥68 mm	5.58	1.31 - 23.68	0.024
CFR >2 + P-FCS <68 mm vs			
CFR >2 + P-FCS ≥68 mm	0.92	0.83 - 1.03	0.544
CFR ≤2 + P-FCS <68 mm	0.88	0.14 - 5.63	>0.999
CFR ≤2 + P-FCS ≥68 mm	3.23	0.61 - 17.10	0.274

CFR = coronary flow reserve; IMR = index of microcirculatory resistance; MACE = major adverse cardiovascular event; OR = odds ratio; P-FCS = platelet-fibrin clot strength.

Because both CFR and fractional flow reserve reflect the physiologic status of the proximal epicardial coronary artery (>400 μm in diameter), the influence of TEG measurement on these indicators appeared insignificant in the present study. It's a matter of course to observe the synergistic risk stratification with IMR and P-FCS, not with CFR and P-FCS.

Our study showed that post-PCI IMR of >40 U (the classic criterion of CMD) (4) was well correlated with pre-PCI P-FCS of ≥68 mm (16,21,29), indicating a strong association between intrinsic hypercoagulability and CMD risk. Moreover, thrombogenic phenotype (ie, elevated P-FCS) significantly increased the risk of MACE only in AMI patients with CMD. In line with these observations, the combined assessment of CMD and P-FCS may enhance the prognosis assessment and assist in potential personalization of adjunctive therapeutic strategy in high-risk AMI patients (25). High P-FCS may be a therapeutic target to determine the risks of CMD and long-term ischemic events (30). Hence, more intensified antithrombotic therapy, such as a glycoprotein IIb/IIIa inhibitor during PCI and adjunctive use of vascular-dose rivaroxaban for long-term treatment, can be considered in patients with this risk factor (31). Future clinical trials of novel adjunctive therapies are needed to lessen the risk of CMD in AMI patients treated with PCI.

STUDY LIMITATIONS. The study was a single-center experience with a limited number of AMI patients having heterogeneous demographics and treatments, which was related with the character of this registry. Because post hoc testing to adjust type I error for multiple testing was not done, the results should be interpreted with caution. Because there has been critical concern regarding the clinical safety of potent P2Y₁₂ inhibitors versus clopidogrel in East Asian patients presented with AMI (32), the type of antiplatelet regimen appeared not consistent

in this analysis. However, the level of platelet reactivity did not show a significant relationship with CMD risk. One would indicate the limitation of the TEG assay with kaolin activator in measuring an antiplatelet effect. However, the present study did not show any difference in fibrin level according to CMD occurrence, and previous reports demonstrated that P-FCS level is significantly associated with multiple hemostatic components, including prothrombin time, fibrinogen, hemoglobin, and platelet count and function (21,29). The study is underpowered to suggest the definite clinical impact of CMD and P-FCS criteria. In addition, currently available hemostatic measurements were selected by the investigators' experiences and updated knowledge; other important hemostatic biomarkers related to microvascular obstruction may have been missed.

CONCLUSIONS

This is the first study to suggest a close relation of hypercoagulability (high P-FCS) assessed by TEG to CMD after reperfusion in AMI patients. In addition, the combined measurements of P-FCS and CMD may enhance the risk stratification and facilitate future targeting of adjunctive antithrombotic therapies.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: This study offers novel evidence to evaluate the influence of thrombogenicity on microvascular obstruction. This is the first study, to our knowledge, to demonstrate the close association between P-FCS (indicated with a thromboelastography-based parameter) and coronary microvascular dysfunction (indicated with the wire-based IMR). Furthermore, combined risk stratification with P-FCS and IMR could more precisely predict long-term clinical outcomes. Future studies to expand on this pilot study would yield insightful knowledge regarding the relationship between thrombogenicity indices and invasive physiologic profiles after reperfusion.

TRANSLATIONAL OUTLOOK: Coronary microvascular dysfunction is a common complication following acute myocardial infarction, presenting an unmet therapeutic need. The translational outlook will involve the large-scale evaluation of patients with enhanced thrombogenicity for preventing the occurrence of coronary microvascular dysfunction. Future therapeutic interventions may involve periprocedural and long-term control of hypercoagulability in high-risk patients with coronary artery disease (eg, glycoprotein IIb/IIIa inhibitor and oral anticoagulant) aimed at reducing the risks of coronary microvascular dysfunction and long-term adverse clinical events.

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KEY WORDS acute myocardial infarction, cardiovascular event, clot strength, coronary microvascular dysfunction, thrombogenicity

APPENDIX For a supplemental figure, please see the online version of this paper.