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RESEARCH ARTICLE

Clinical Significance of PCSK9 and Soluble P-selectin in Predicting Major Adverse Cardiovascular Events After Primary Percutaneous Coronary Intervention in Patients with Acute Coronary Syndrome

Yao Yao[1,a], Qining Qiu[1,2,a], Xiaoye Li[1], Zi Wang[1], Shikun Xu[3] and Qianzhou Lv[1]

[1]*Department of Pharmacy, Zhongshan Hospital, Fudan University, Shanghai, China*
[2]*Department of Pharmacy, Zhongshan Hospital, Fudan University, Xiamen, Fujian, China*
[3]*Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai, China*

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Abstract

Objective: This study aimed at investigating the association of proprotein convertase subtilisin/kexin type 9 (PCSK9) with soluble P-selectin (sP-selectin), and their values in predicting major adverse cardiovascular events (MACE) at 1-year follow-up in patients with acute coronary syndrome (ACS) receiving dual antiplatelet therapy after primary percutaneous coronary intervention (PCI).

Methods: A total of 563 patients with ACS who underwent primary PCI were prospectively recruited from March 2020 to June 2021. The baseline levels of PCSK9, sP-selectin, and other platelet reactivity biomarkers were determined using enzyme-linked immunosorbent assays.

Results: sP-selectin and ox-LDL levels significantly increased with increasing PCSK9 tertiles. High sP-selectin was associated with high PCSK9 levels, and PCSK9 was positively correlated with sP-selectin. Patients with both PCSK9 >17.4 ng/mL and sP-selectin >7.2 ng/mL had a significantly higher incidence of MACE than patients with lower levels. Multivariate analysis indicated that high sP-selectin and PCSK9 levels were independent risk factors for MACE, and the combination of PCSK9 and sP-selectin had better predictive value than each biomarker alone.

Conclusion: PCSK9 and sP-selectin may be potential predictive biomarkers for 1-year prognosis in patients with ACS after primary PCI.

Keywords: PCSK9; sP-selectin; acute coronary syndrome; percutaneous coronary intervention; major adverse cardiovascular event

[a]Yao Yao and Qining Qiu have contributed equally to this work.

Correspondence: **Shikun Xu**, Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai, China, Tel.: +86-18616881171, E-mail: xu.shikun@zs-hospital.sh.cn; and **Qianzhou Lv**, Department of Pharmacy, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai 200032, China, Tel.: +86-13761160988, E-mail: 13916088938@163.com

Introduction

Cardiovascular diseases are a heterogeneous group of disorders that affect the heart and vasculature. These diseases primarily include coronary artery disease (CAD), peripheral artery disease, and

cerebrovascular disease; CAD is the predominant form, accounting for 30–50% of all cases [1]. The etiology of CAD is generally considered to stem from the accumulation of lipid-rich atherosclerotic plaques in the coronary arterial walls, thus, eventually leading to myocardial infarction (MI) under conditions of sustained inflammatory responses and platelet activation [2, 3]. According to data from the World Health Organization, MI remains the leading cause of death globally, despite a decline in the mortality rate over the past two decades, largely because of advancements in interventional revascularization techniques [4].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) participates in lipid metabolism by binding hepatic low-density lipoprotein receptors and promoting the degradation of the receptors [5]. As PCSK9 inhibitors (PCSK9i) entered the market, intensive research on PCSK9 has gradually entered the public view. Existing evidence indicates that the predominant role of PCSK9 is lipid regulation, yet studies are increasingly implicating PCSK9 pleiotropy in the pathogenesis of atherosclerosis [5, 6]. In prospective studies, PCSK9 has been demonstrated to be positively correlated with the severity of coronary artery stenosis, independently of low-density lipoprotein cholesterol (LDL-C), among patients with CAD or with acute coronary syndrome (ACS) [7, 8]. The assessment of plasma PCSK9 in populations of diverse ethnicities has indicated substantial variations among seemingly healthy individuals. Furthermore, the association between circulating PCSK9 levels and plasma low-density lipoprotein (LDL) concentration varies across these populations [9]. Further assays of plaque vulnerability and plaque components have shown that PCSK9 levels are linearly associated with both the proportion and absolute volume of necrotic core tissue, despite statin use [10].

The above studies suggest roles of PCSK in promoting inflammation, platelet activation, and thrombosis – effects independent of LDL-C. Direct binding of PCSK9 to platelet CD36 has been reported in animal experiments [11]. Activated platelets increase adhesion to impaired endothelial cells and release platelet-activating factors that stimulate inflammation. Therefore, dual antiplatelet therapy (DAPT) is a cornerstone of the management of both acute and long-term ACS. However, recurrent cardiovascular events cannot be completely avoided after DAPT, thus suggesting platelet activation of other pathways.

Soluble P-selectin (sP-selectin) is a stable marker indicative of persistent platelet activation. A study has revealed significant associations between PCSK9 and platelet activation markers, including sP-selectin and soluble CD40 ligand (sCD40L). In patients with diabetes mellitus, PCSK9 levels have shown positive associations with sP-selectin and sCD40L levels. This association is particularly pronounced in patients with diabetes mellitus, thus underscoring the link between PCSK9 and platelet activation [12]. Animal experiments have demonstrated a significant decrease in sCD40L and sP-selectin levels in rabbits with dyslipidemia treated with a cholesteryl ester transfer protein inhibitor that suppresses PCSK9 expression. Importantly, the decrease in these markers was positively correlated with the suppression of PCSK9, thereby providing further evidence of PCSK9's role in platelet activation [13]. Another notable study by von Brühl et al. has demonstrated that thrombi formed in PCSK9 knockout mice show diminished leukocyte recruitment – a phenomenon mediated by sP-selectin. These findings provide additional support for the involvement of PCSK9 in platelet activation [14]. Although prior research has established a link between PCSK9 and sP-selectin, limited knowledge exists regarding their combined prognostic relevance in patients with ACS. This study was aimed at assessing the link between PCSK9 and sP-selectin, and their roles in predicting major adverse cardiovascular events (MACE) within 1 year among patients with ACS who underwent primary percutaneous coronary intervention (PCI).

Methods

Study Design and Population

This was a single-center, prospective, observational study, which sequentially enrolled patients diagnosed with ST-elevation myocardial infarction (STEMI) or high-risk/very high-risk non-ST-elevation myocardial infarction (NSTEMI). These individuals had undergone primary PCI within 24 hours of experiencing symptoms, between March 2020 and June 2021. Diagnosis of STEMI and NSTEMI was determined according to most

current guidelines established by the European Society of Cardiology [15, 16]. Risk stratification for NSTEMI was performed with the GRACE score, and patients with scores >140 were defined as high-risk and requiring primary PCI [17, 18]. On the basis of high-risk NSTEMI, very high-risk patients may exhibit hemodynamic instability, cardiogenic shock, and recurrent or refractory angina despite medical therapy, malignant arrhythmias, mechanical complications of MI, acute heart failure attributed to NSTEMI, or ST-segment depression >1 mm in more than six leads, with ST-segment elevation in aVR and/or V1 leads [19]. Basic demographic information and baseline characteristics were recorded at admission. Comorbidities of hypertension and diabetes were defined according to self-reports or self-reported history of associated medications.

Individuals 18 years or above were assessed for inclusion in this study if they had received pretreatment involving a 300 mg dose of aspirin and 180 mg of ticagrelor, followed by the standard administration of DAPT, comprising daily aspirin at 100 mg and ticagrelor at 90 mg twice daily, continuing for a minimum of 1 year post-primary PCI without any alterations to the regimen. Other standard therapies including lipid lowering were instituted in accordance with clinical guidelines at the attending physicians' discretion. The exclusion criteria were patients meeting any of the following conditions: 1) those with a prior history of PCI or coronary artery bypass grafting (CABG); 2) individuals scheduled for elective CABG; 3) patients diagnosed with non-obstructive coronary atherosclerosis, as confirmed through coronary angiography; 4) those who had received anti-platelet or lipid-lowering therapies within the 3 months before screening; 5) participants prescribed PCSK9 inhibitors or oral anticoagulants after enrollment in the study; 6) individuals with severe kidney, liver, or multivesicular insufficiency; and 7) patients presenting with hemoglobin levels below 100 g/L or a total platelet count below 100×10^9/L, to avoid erroneous results of platelet biomarkers due to thrombocytopenia or anemia [20, 21].

Blood Sampling

Fasting peripheral venous blood was collected on admission for the measurement of lipid indices, glucose, HbA1c, other laboratory parameters, PCSK9, sP-selectin, CD40L, platelet activating factor (PAF), lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), and oxidized low-density lipoprotein (ox-LDL).

Plasma was isolated by centrifugation at 4000 ×g for 10 min at 4 °C from blood in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. PCSK9, sP-selectin, CD40L, PAF, LOX-1, and ox-LDL concentrations were determined using enzyme-linked immunosorbent assay kits purchased from Yaji Biological (Shanghai, China). All assay procedures followed the manufacturer's protocol. Each sample was assayed in duplicate, and the averages are reported.

Routine testing on blood from EDTA tubes was performed with a Sysmex XS 500i hematology analyzer. Biochemical assessments were performed on serum samples in tubes designed to promote coagulation, with a Hitachi 7600-120 automated biochemistry analyzer (Hitachi Corporation, Tokyo, Japan). Coagulation function tests were performed with citrate vacuum tubes and a STA-R Evolution Coagulation Analyzer. All measurements were promptly processed after blood collection at the ISO 15189-certified Center of Laboratory Medicine at Zhongshan Hospital.

Study Endpoints and Follow-Up

The study's primary outcome, referred to as MACE, was defined as a composite event encompassing all-cause mortality, non-fatal MI recurrence, ischemic stroke, heart failure necessitating rehospitalization, stent thrombosis, and non-elective revascularization. Follow-up was performed by well-trained physicians routinely at 1, 3, 6, and 12 months after discharge through clinic visits, telephone calls, or online outpatient services until the last day of the follow-up period or the first MACE occurrence.

Statistical Analysis

For clinical data presentation, continuous variables are expressed as either mean ± standard deviation (SD) or median [interquartile range (IQR)], depending on their distribution, as determined by the Kolmogorov-Smirnov test. Statistical comparisons among or between groups were conducted with

the Student's t-test or one-way ANOVA, as appropriate. Categorical data are reported as frequencies (percentages), and were compared with either the chi-square test or Fisher's exact test.

Spearman's rank test was used to evaluate correlations among PCSK9, sP-selectin, platelet activation biomarkers, and other clinical parameters. Subsequently, a heat map was generated to provide a comprehensive and visually intuitive representation of the data. Simple linear regression analyses were conducted to examine the associations between sP-selectin and PCSK9. To account for potential confounding factors, confounders were introduced into univariate linear regression analyses. Factors with P-values less than 0.10 in the univariate analysis were included in the multivariate regression analyses. The cumulative incidence of MACE and the individual components of composite endpoints were computed using Kaplan-Meier (KM) curves and compared using the log-rank test within subgroups with different PCSK9 and sP-selectin levels.

To identify predictors of MACE, the analysis initially comprised univariate Cox regression, followed by multivariate Cox regression, including variables with a P-value below 0.10 in the univariate Cox analysis. Receiver operating characteristic (ROC) curves were generated for PCSK9 and sP-selectin, and the area under the ROC curve (AUC) was used to quantify their predictive value for MACE.

All statistical analyses were performed in SPSS (version 26.0), and statistical significance was established as a two-tailed P-value of less than 0.05.

Results

Baseline Characteristics of Study Populations

A total of 563 patients were screened for enrollment (Figure 1). Table 1 presents the baseline characteristics of patients stratified by PCSK9 and sP-selectin levels. Across PCSK9 tertiles, the mean age of patients significantly differed: the lowest tertile had a mean age of 62.08 ± 11.25 years, whereas the highest tertile had a mean age of 64.52 ± 10.48 years (P = 0.045). The percentage of men was highest in the high PCSK9 group (81.4%) and lowest in the median PCSK9 group (73.8%), although the difference was not statistically significant (P = 0.097). The ox-LDL and sP-selectin levels increased significantly with increasing PCSK9 tertile (ox-LDL: P = 0.035; sP-selectin: P < 0.001). No other statistical differences were observed among other parameters except TG.

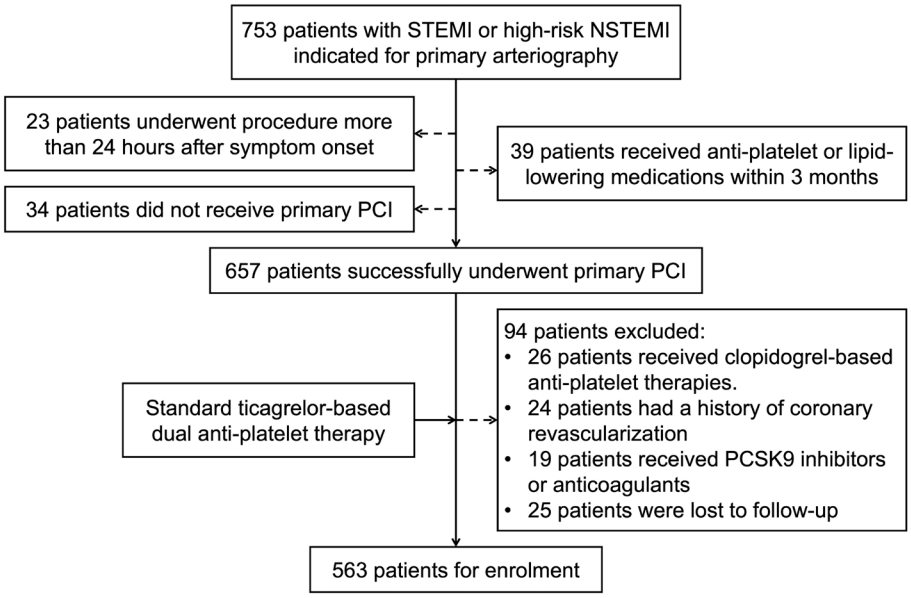

Figure 1 Flow Chart for Patient Recruitment.
STEMI, ST elevation myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; PCI, percutaneous coronary intervention; PCSK9, proprotein convertase subtilisin/kexin type 9.

Table 1 Baseline Characteristics of Patients with Different PCSK9 and sP-Selectin Levels Undergoing Primary PCI.

	PCSK9			P	sP-selectin			P
	Low (<13.3 ng/mL)	Median 13.3–17.4 ng/mL	High (>17.4 ng/mL)		Low <5.3 ng/mL	Median 5.3–7.2 ng/mL	High >7.2 ng/mL	
Age, years	62.08 ± 11.25	64.45 ± 10.52	64.52 ± 10.48	0.045	64.13 ± 10.48	63.70 ± 11.35	63.21 ± 10.57	0.710
Sex, male	154 (81.9%)	138 (73.8%)	153 (81.4%)	0.097	145 (77.1%)	145 (77.1%)	155 (82.9%)	0.286
BMI, kg/m^2	25.02 ± 3.19	25.40 ± 3.57	24.53 ± 3.52	0.172	24.81 ± 2.91	25.32 ± 3.91	24.82 ± 3.43	0.256
Current smoker	123 (65.4%)	105 (56.1%)	114 (60.6%)	0.184	118 (62.8%)	105 (55.9%)	119 (63.8%)	0.239
Hypertension	90 (47.9%)	87 (46.5%)	85 (45.2%)	0.875	99 (52.7%)	80 (42.6%)	83 (44.4%)	0.112
Diabetes mellitus	65 (34.6%)	57 (30.5%)	56 (29.8%)	0.559	68 (36.2%)	56 (29.8%)	54 (28.9%)	0.254
History of stroke	9 (4.8%)	7 (3.7%)	7 (3.7%)	0.837	7 (3.7%)	10 (5.3%)	6 (3.2%)	0.560
Family history of coronary artery disease	2 (1.1%)	6 (3.2%)	2 (1.1%)	0.217	0	4 (2.1%)	6 (3.2%)	0.057
SBP, mmHg	130.20 ± 22.96	131.24 ± 24.39	128.30 ± 21.62	0.500	129.31 ± 21.66	128.80 ± 22.39	129.86 ± 22.97	0.569
TC, mmol/L	3.96 [3.33–4.61]	4.51 [3.88–5.07]	5.12 [4.44–5.95]	<0.001	3.96 [3.33–4.61]	4.51 [3.88–5.07]	5.12 [4.44–5.95]	0.345
TG, mmol/L	1.41 [1.02–2.14]	1.55 [1.04–2.13]	1.38 [0.98–1.96]	0.123	1.41 [1.02–2.14]	1.55 [1.04–2.13]	1.38 [0.98–1.96]	0.712
LDL-C, mmol/L	2.26 ± 0.77	2.67 ± 0.80	3.39 ± 0.96	<0.001	2.66 ± 0.95	2.82 ± 0.98	2.85 ± 0.97	0.122
HDL-C, mmol/L	1.08 ± 0.39	1.09 ± 0.44	1.10 ± 0.26	0.832	1.09 ± 0.34	1.09 ± 0.47	1.08 ± 0.28	0.758
Non-HDL-C, mmol/L	2.88 [2.33–3.46]	3.43 [2.88–3.93]	3.95 [3.42–4.70]	<0.001	2.88 [2.33–3.46]	3.43 [2.88–3.93]	3.95 [3.42–4.70]	0.145
Lp(a), nmol/L	37.0 [15.0–74.5]	29.5 [14.0–66.3]	32.0 [14.0–66.0]	0.919	37.0 [15.0–74.5]	29.5 [14.0–66.3]	32.0 [14.0–66.0]	0.216
sdLDL, mmol/L	0.65 [0.44–0.87]	0.80 [0.53–1.07]	0.82 [0.59–1.17]	0.613	0.65 [0.44–0.87]	0.80 [0.53–1.07]	0.82 [0.59–1.17]	0.602
HbA1c, %	6.61 ± 1.61	6.56 ± 1.46	6.62 ± 1.46	0.944	6.63 ± 1.62	6.62 ± 1.38	6.53 ± 1.39	0.846
CK-MB, U/L	37.0 [19.0–115.0]	46.5 [20.0–144.3]	47.0 [18.0–175.0]	0.141	42.0 [18.0–164.0]	48.0 [19.0–127.5]	45.0 [20.0–142.8]	0.633
cTnT, ng/mL	1.45 [0.48–3.67]	1.58 [0.52–3.61]	1.55 [0.44–4.94]	0.219	1.69 [0.51–3.89]	1.57 [0.41–4.05]	1.35 [0.47–3.54]	0.395
NT-proBNP, pg/mL	954.0 [412.2–1817.3]	118.5 [427.5–2270.5]	1046.0 [454.6–2774.5]	0.334	1070.0 [398.9–2628.0]	1068.0 [413.3–2303.5]	977.0 [460.6–2070.5]	0.420
eGFR, ml min^{-1}·1.73 m^2	79.92 ± 20.04	77.78 ± 21.98	80.81 ± 20.86	0.421	78.04 ± 19.54	79.94 ± 20.76	80.33 ± 22.53	0.608
hs-CRP, mg/L	7.60 [3.10–22.60]	8.75 [3.00–27.60]	8.60 [2.55–19.15]	0.959	7.55 [2.90–18.35]	9.10 [3.50–28.30]	7.20 [2.25–25.60]	0.404
Fibrinogen, mg/dL	382.0 [302.0–474.0]	367.5 [307.5–484.0]	368.0 [301.0–486.0]	0.977	378.0 [304.0–487.0]	384.0 [314.8–475.0]	353.0 [292.0–460]	0.805
D-dimer, μg/mL	0.28 [0.46–0.82]	0.48 [0.29–0.88]	0.47 [0.25–0.99]	0.298	0.46 [0.28–0.92]	0.48 [0.26–0.83]	0.48 [0.27–0.94]	0.183
WBC, ×10^9/L	9.02 [7.03–10.58]	8.50 [6.93–10.31]	8.98 [6.91–11.16]	0.221	8.64 [6.91–10.40]	9.00 [6.96–10.81]	8.78 [6.92–10.83]	0.420
PLT, ×10^9/L	217.74 ± 63.93	216.33 ± 68.04	210.47 ± 59.48	0.514	221.70 ± 63.38	210.74 ± 57.48	211.98 ± 69.98	0.194
MPV, fL	10.75 ± 0.91	10.66 ± 1.11	10.76 ± 1.22	0.617	10.74 ± 1.08	10.64 ± 1.10	10.79 ± 1.08	0.399
PDW, %	12.72 ± 2.08	12.61 ± 2.32	12.79 ± 2.39	0.741	12.78 ± 2.07	12.52 ± 2.34	12.81 ± 2.37	0.407
PCSK9, ng/mL	11.59 ± 1.13	15.22 ± 1.22	19.93 ± 1.59	<0.001	14.65 ± 3.59	15.51 ± 3.51	16.57 ± 3.67	<0.001
sP-selectin, ng/mL	5.89 ± 1.45	6.47 ± 1.43	6.52 ± 1.42	<0.001	4.61 ± 0.40	6.30 ± 0.55	7.99 ± 0.43	<0.001
CD40L. ng/mL	11.79 ± 2.54	11.89 ± 2.79	11.43 ± 2.63	0.207	11.69 ± 2.68	11.89 ± 2.69	11.53 ± 2.60	0.417
PAF, ng/mL	16.62 ± 5.08	16.82 ± 4.85	16.80 ± 4.96	0.910	16.58 ± 5.19	17.00 ± 4.84	16.67 ± 4.84	0.690
LOX-1, pg/mL	270.36 ± 58.36	255.68 ± 63.40	267.46 ± 63.79	0.053	265.95 ± 64.93	262.73 ± 61.73	264.87 ± 59.84	0.878
Ox-LDL, mmol/L	120.58 ± 29.35	126.52 ± 30.38	128.24 ± 30.24	0.035	122.85 ± 29.43	125.13 ± 31.76	127.37 ± 29.08	0.348
Statins	183 (97.3%)	185 (98.9%)	185 (98.4%)	0.499	186 (98.9%)	185 (98.4%)	182 (97.3%)	0.491

Table 1 (continued)

	PCSK9			P	sP-selectin			P
	Low (<13.3 ng/mL)	Median 13.3–17.4 ng/mL	High (>17.4 ng/mL)		Low <5.3 ng/mL	Median 5.3–7.2 ng/mL	High >7.2 ng/mL	
β-blockers	132 (70.2%)	145 (77.5%)	145 (77.1%)	0.184	133 (70.7%)	143 (76.1%)	146 (78.1%)	0.238
ACEI or ARB	139 (69.1%)	133 (71.1%)	142 (75.5%)	0.370	124 (66.0%)	143 (76.1%)	138 (73.8%)	0.073

Continuous data are presented as the mean ± standard deviation (SD) or median [interquartile range], and were compared with appropriate statistical tests, including ANOVA or non-parametric tests, on the basis of PCSK9 or sP-selectin tertiles. Categorical data were analyzed with either the chi-square test or Fisher's exact test, as applicable. PCI: Percutaneous Coronary Intervention; PCSK9: Proprotein Convertase Subtilisin/Kexin Type 9; BMI: Body Mass Index; SBP: Systolic Blood Pressure; TC: Total Cholesterol; TG: Triglyceride; LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; Lp(a): Lipoprotein(a); sdLDL-C: Small Dense Low-Density Lipoprotein Cholesterol; CK: Creatine Kinase; cTnT: Cardiac Troponin T; NT-proBNP: N-terminal Pro-B-type Natriuretic Peptide; eGFR: Estimated Glomerular Filtration Rate; hs-CRP: High-Sensitivity C-Reactive Protein; WBC: White Blood Cell Count; PLT: Platelet Count; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PAF: Platelet Activating Factor; LOX-1: Lectin-like Oxidized Low-Density Lipoprotein Receptor-1; ox-LDL: Oxidized Low-Density Lipoprotein; ACEI: Angiotensin-Converting Enzyme Inhibitors; ARB: Angiotensin Receptor Blocker.

Concerning the sP-selectin tertiles, we observed no noteworthy variations in age, sex distribution, or other demographic factors. The high sP-selectin group had the highest PCSK9 levels, whereas the low sP-selectin group had the lowest PCSK9 levels ($P < 0.001$).

Biomarker Correlation

The relationships among PCSK9, sP-selectin, CD40L, PAF, LOX-1, and ox-LDL, alongside a comprehensive array of metabolism, inflammation, and platelet-associated markers, are presented in Figure 2. Notably, PCSK9 exhibited a significant and positive correlation with LDL-C ($r = 0.493$, $P < 0.001$), ox-LDL ($r = 0.156$, $P < 0.001$), and sP-selectin ($r = 0.232$, $P < 0.001$). However, PCSK9 did not demonstrate substantial correlations with any other parameters examined in this analysis.

To identify the independent variables for PCSK9, we used linear regression for both univariate and multivariate analyses (Table 2). The results indicated that the relationship between sP-selectin and PCSK9 could be adjusted for potential confounders, including age, TC, LDL-C, non-HDL-C, NT-proBNP, and ox-LDL. Notably, PCSK9 remained independently and positively associated with sP-selectin after the adjustment ($\beta = 0.179$, $P < 0.001$). Additionally, age and LDL-C were independently associated with elevated PCSK9 levels.

Associations of PCSK9 and sP-selectin Levels with Clinical Endpoints

During the 12-month follow-up, MACE occurred in 119 patients, constituting 21.1% of the cohort. This composite endpoint comprised 25 cases of all-cause mortality, 30 instances of recurrent MI, nine occurrences of ischemic stroke, 15 cases requiring rehospitalization due to heart failure, five episodes of stent thrombosis, and 35 non-elective revascularizations. As detailed in Table 3, individuals who experienced MACE were characterized by several distinct features: they were generally older ($P = 0.028$), and had elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP) ($P < 0.001$) and high-sensitivity C-reactive protein (hsCRP) ($P = 0.028$). Additionally, this group had a lower estimated glomerular filtration rate (eGFR) ($P < 0.001$).

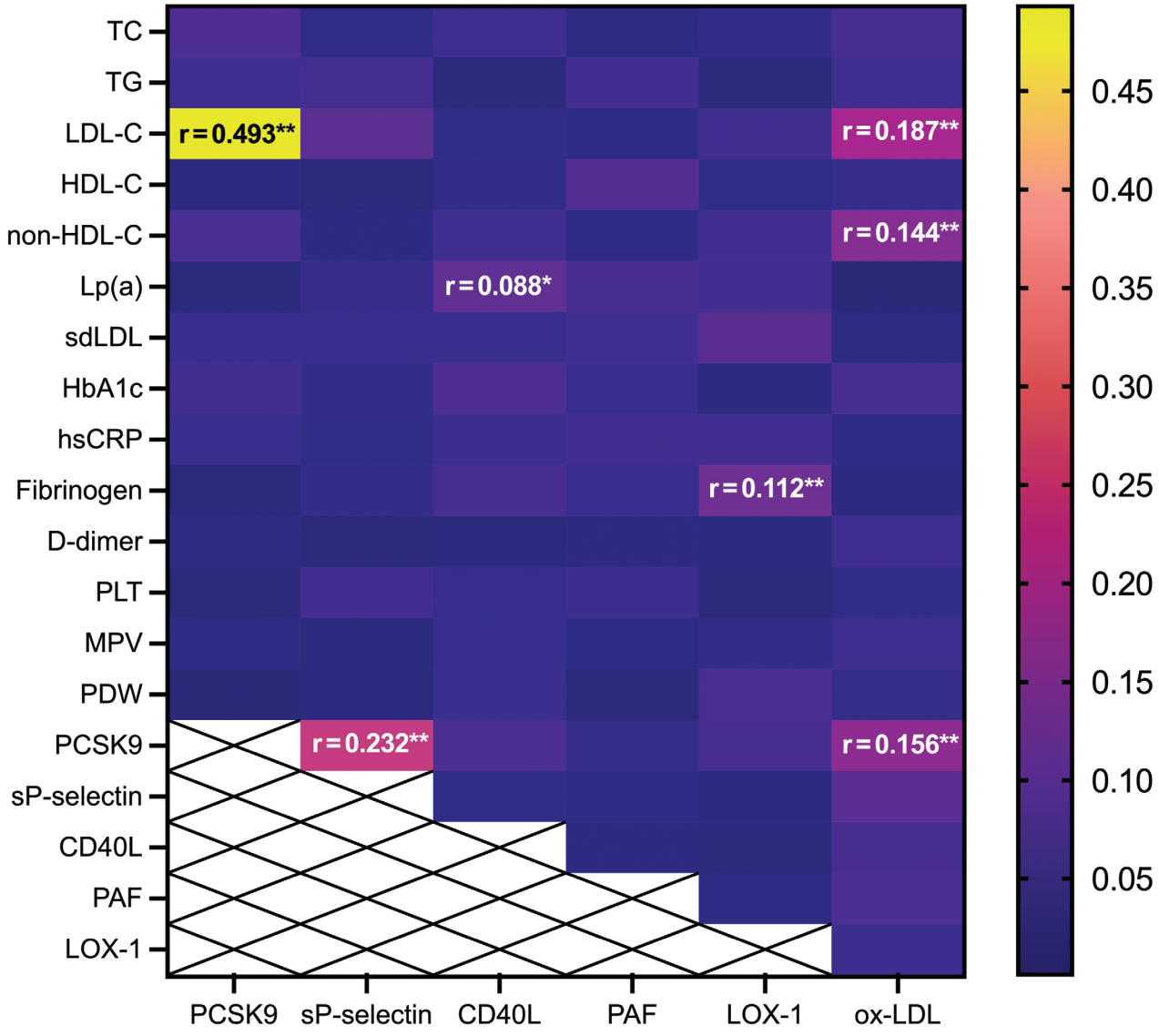

Figure 2 Heat Map Based on the Relationship between Lipid Metabolism and Platelet Markers, Assessed with Spearman's Rank Correlation Coefficient (Spearman's Rho).
Data were analyzed with Spearman's correlation. The heat map was generated on the basis of the absolute values of Spearman correlation coefficients (r). Significant data are presented with exact values of r on the heat map. *P < 0.05, **P < 0.01.

Notably, the MACE cohort had higher levels of sP-selectin (P < 0.001), ox-LDL (P = 0.014), and PCSK9 (P < 0.001). Although this cohort was also less likely to have hypertension and diabetes, those trends did not reach statistical significance.

KM survival curve analysis was performed according to the highest tertiles of PCSK9 (>17.4 ng/mL) and sP-selectin (>7.2 ng/mL) as the segmentation points. Patients with both high PCSK9 and sP-selectin levels had higher incidences of MACE than those with lower PCSK9 (≤17.4 ng/mL) and sP-selectin (≤7.2 ng/mL) levels (48.7% vs. 5.3%, HR = 11.34, 95% CI: 5.63–22.85, log-rank P < 0.001; Figure 3A). Additional analysis for each clinical endpoint component showed significant differences among all groups, except for stent thrombosis (Figure 3B-G). Further detailed subgroup analysis combining low, moderate, and high levels of PCSK9 and sP-selectin yielded consistent results with the aforementioned findings (Figure 4).

In separate univariate Cox regression models, age, NT-proBNP, eGFR, hsCRP, sP-selectin, ox-LDL, and PCSK9 were significant risk factors (P < 0.05) (Table 4). After adjustment for confounding factors

Table 2 Association of sP-selectin with PCSK9.

Variables	Univariate		Multivariate	
	β	P value	β	P value
Age	0.079	0.062	0.077	0.043
Sex (male)	0.031	0.459		
Current smoker	−0.060	0.154		
Hypertension	−0.033	0.428		
Diabetes mellitus	−0.060	0.155		
TC	0.227	<0.001	0.026	0.513
TG	0.007	0.863		
LDL-C	0.493	<0.001	0.496	<0.001
HDL-C	0.010	0.819		
Non-HDL-C	0.434	<0.001	−0.029	0.751
Lp(a)	0.046	0.282		
sdLDL	0.019	0.663		
HbA1c	0.026	0.619		
CK-MB	0.056	0.193		
cTnT	0.066	0.126		
NT-proBNP	0.081	0.059	0.057	0.129
eGFR	0.014	0.763		
hs-CRP	−0.013	0.755		
Fibrinogen	0.014	0.745		
D-dimer	0.050	0.247		
WBC	0.011	0.794		
PLT	−0.023	0.590		
MPV	−0.004	0.928		
PDW	0.013	0.753		
sP-selectin	0.225	<0.001	0.179	<0.001
CD40L	−0.063	0.136		
PAF	0.025	0.560		
LOX-1	−0.046	0.275		
Ox-LDL	0.156	<0.001	0.038	0.310

Confounding factors were introduced into univariate analyses, with variables having P-values less than 0.10 in univariate analysis subsequently included in multivariate regression analyses.

with $P < 0.10$, i.e., age, diabetes, NT-proBNP, eGFR, hs-CRP, fibrinogen, MPV, PCSK9, and sP-selectin, a significant association of high sP-selectin and PCSK9 levels with increased risk of MACE was found (Table 4). Furthermore, multivariate analysis underscored the significance of age as an autonomous risk factor for MACE (HR = 1.031, 95% CI: 1.005–1.059, P = 0.021).

To assess the predictive potential of PCSK9, sP-selectin, and their joint influence on MACE, we conducted ROC analyses (Figure 5A). PCSK9 and sP-selectin showed adequate discrimination between patients with or without MACE. The AUC for their combination was greater than that of curves representing each biomarker alone. Furthermore, when the risk factors for MACE established from univariable Cox regression with $P < 0.10$ were added, similar results were observed in the ROC curves, and the AUC significantly increased (Figure 5B).

Discussion

In this study, we prospectively enrolled 563 patients with ACS from March 2020 to June 2021.

Table 3 Patient baseline characteristics, stratified by MACE status.

	MACE	No MACE	P
Age, years	65.61 ± 11.06	63.16 ± 10.68	0.028
Sex, male	92 (77.3%)	353 (79.5%)	0.602
BMI, kg/m^2	24.65 ± 3.31	25.07 ± 3.48	0.235
Current smoker	73 (61.3%)	269 (60.6%)	0.880
Hypertension	47 (39.5%)	215 (48.4%)	0.083
Diabetes mellitus	29 (24.4%)	149 (33.6%)	0.056
History of stroke	2 (1.7%)	21 (4.7%)	0.218
Family history of coronary artery disease	1 (0.8%)	9 (2.0%)	0.632
Systolic blood pressure, mmHg	129.41 ± 23.35	129.98 ± 22.89	0.825
TC, mmol/L	4.77 [4.08–5.59]	4.45 [3.70–5.16]	0.104
TG, mmol/L	1.40 [1.01–1.85]	1.45 [1.02–2.10]	0.951
LDL-C, mmol/L	3.10 ± 1.03	2.68 ± 0.93	0.081
HDL-C, mmol/L	1.07 ± 0.28	1.10 ± 0.39	0.603
Non-HDL-C, mmol/L	3.72 [3.01–4.29]	3.37 [2.65–4.04]	0.763
Lp(a), nmol/L	30.0 [14.8–65.3]	35.0 [14.0–70.0]	0.377
sdLDL,	0.71 [0.52–1.08]	0.76 [0.52–1.12]	0.708
HbA1c, %	6.77 ± 1.62	6.55 ± 1.41	0.251
CK-MB, U/L	41.0 [16.0–168.0]	46.0 [20.0–131.0]	0.663
cTnT, ng/mL	1.51 [0.27–5.03]	1.51 [0.53–3.64]	0.679
NT-proBNP, pg/mL	1710.0 [547.0–4265.0]	940.5 [409.0–1894.8]	<0.001
eGFR, ml min^{-1}·1.73 m^2	70.39 ± 28.48	81.84 ± 17.90	<0.001
hs-CRP, mg/L	11.75 [2.95–37.78]	7.60 [2.90–20.43]	0.028
Fibrinogen, mg/dL	377.0 [307.0–521.0]	373.0 [303.5–470.5]	0.145
D-dimer, μg/mL	0.61 [0.29–1.52]	0.44 [0.27–0.80]	0.342
WBC, ×10^9/L	8.45 [6.68–11.38]	8.86 [7.05–10.54]	0.797
PLT, ×10^9/L	213.79 ± 73.62	215.17 ± 61.09	0.851
MPV, fL	10.89 ± 1.17	10.68 ± 1.06	0.064
PDW, %	12.95 ± 2.49	12.64 ± 2.19	0.189
PCSK9, ng/mL	18.15 ± 3.05	14.89 ± 3.51	<0.001
sP-selectin, ng/mL	7.11 ± 1.37	6.08 ± 1.40	<0.001
CD40L. ng/mL	11.80 ± 2.62	11.68 ± 2.67	0.664
PAF, ng/mL	17.05 ± 4.85	16.67 ± 4.99	0.463
LOX-1, pg/mL	263.58 ± 60.00	264.77 ± 62.73	0.853
Ox-LDL, mmol/L	131.78 ± 30.07	124.36 ± 28.88	0.014
Statins	116 (97.5%)	437 (98.4%)	0.489
β-blockers	89 (74.8%)	333 (75.0%)	0.963
ACEI or ARB	90 (75.6%)	315 (70.9%)	0.313

The data are presented as n (%), mean ± SD, or median [interquartile range]. Student's t-test or Mann-Whitney U test was used to analyze continuous data between different groups, whereas categorical data were assessed with the chi-square test or Fisher's exact test.

Importantly, high levels of sP-selectin were associated with elevated PCSK9 levels in patients with ACS after primary PCI. Patients with both PCSK9 levels above 17.4 ng/mL and sP-selectin levels above 7.2 ng/mL exhibited a significantly higher incidence of MACE than those with lower levels.

The combination of PCSK9 and sP-selectin had better predictive value for MACE than either

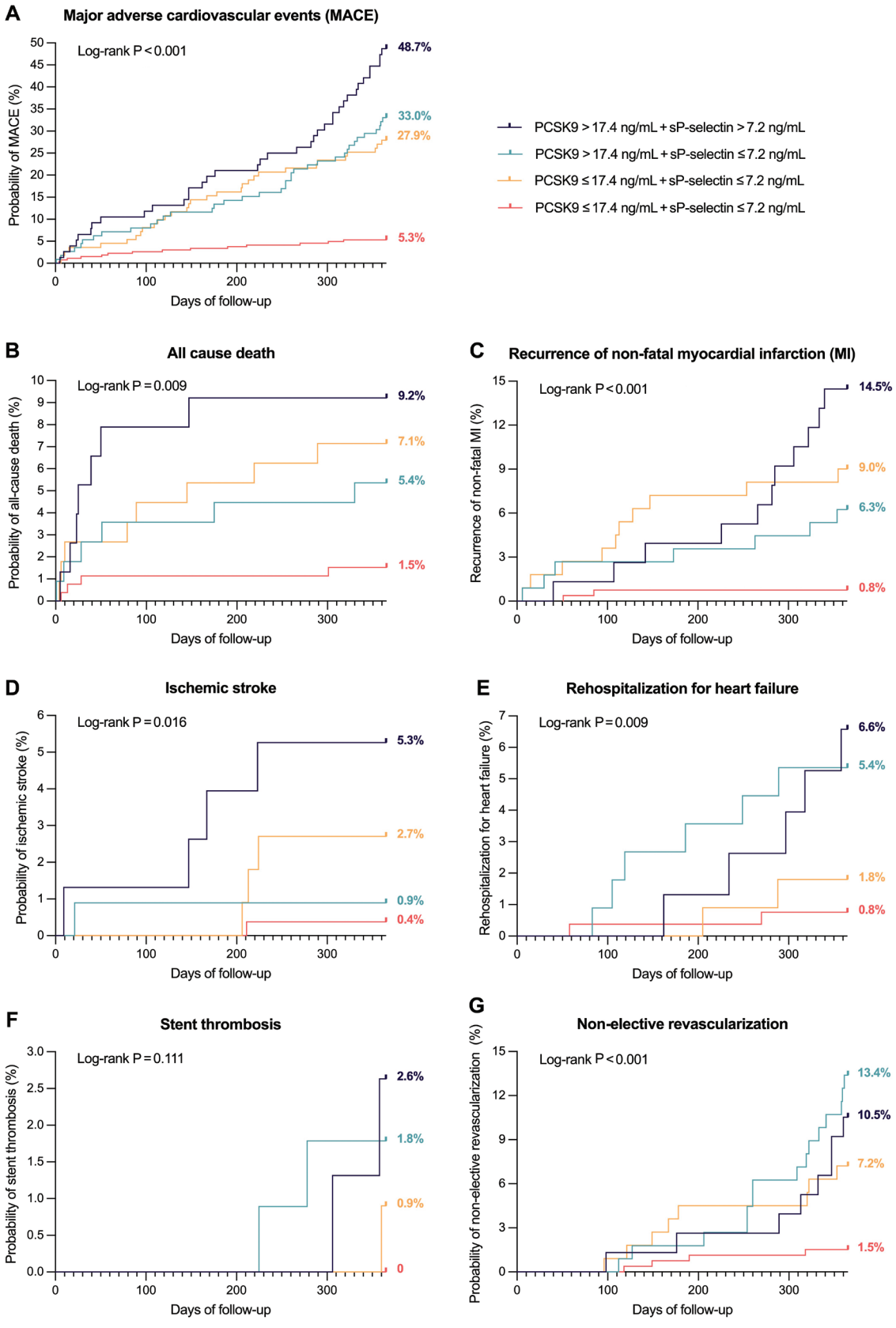

Figure 3 Kaplan–Meier 1-Year Survival Curves of MACE in Patients Stratified by the Highest Tertiles of PCSK9 and sP-selectin.

MACE, major adverse cardiac events; PCSK9, proprotein convertase subtilisin/kexin type 9; sP-selectin, soluble P-selectin.

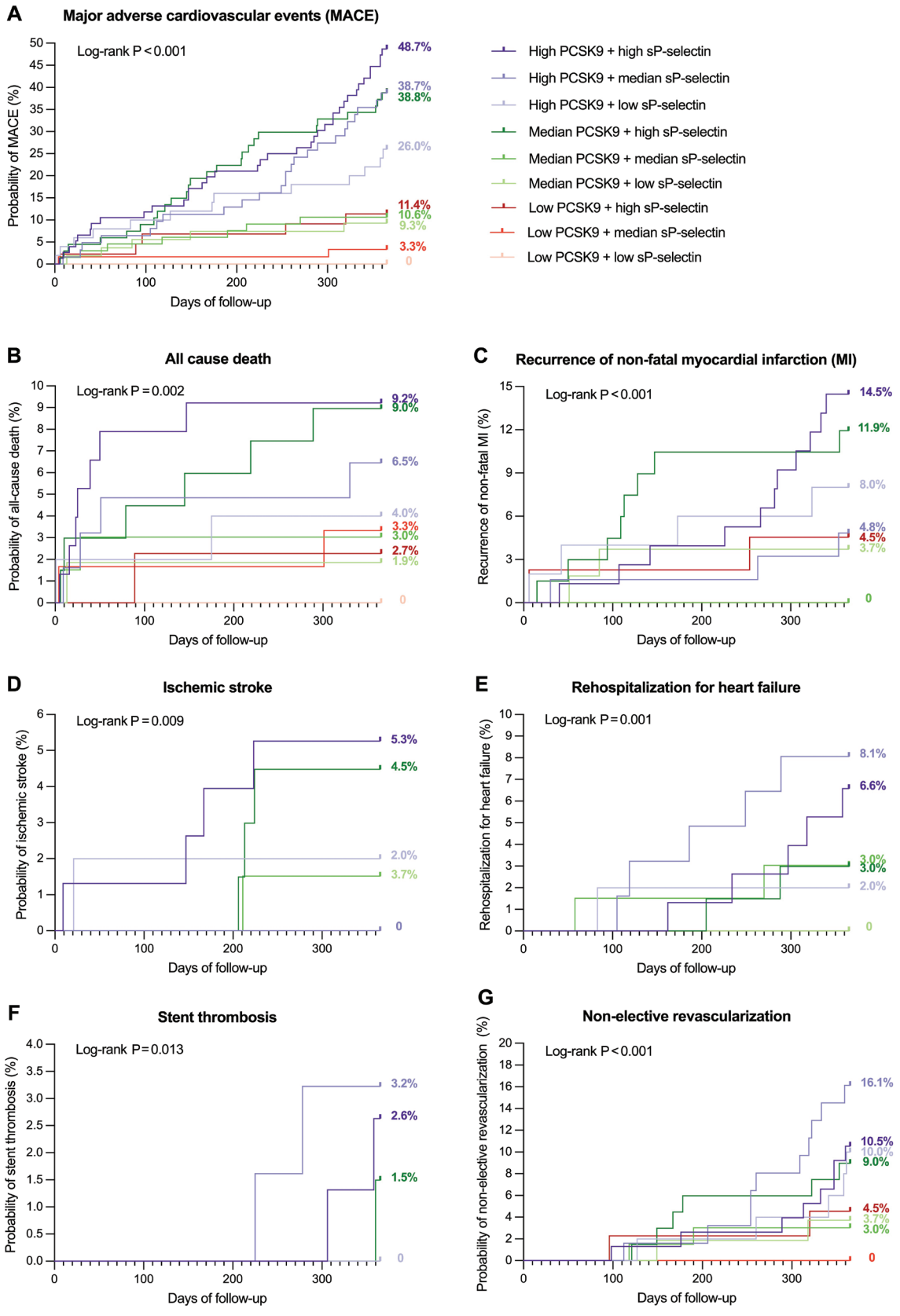

Figure 4 Kaplan–Meier 1-Year Survival Curves of MACE in Patients Stratified by PCSK9 and sP-selectin Tertiles.
MACE, major adverse cardiac events; PCSK9, proprotein convertase subtilisin/kexin type 9; sP-selectin, soluble P-selectin.

Table 4 Univariate and multivariate Cox regression analysis of patient characteristics and incidence of MACE.

	Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P
Age	1.022 (1.004–1.041)	0.015	1.031 (1.005–1.059)	0.021
Sex (male)	0.878 (0.572–1.348)	0.551		
Current smoker	1.022 (0.707–1.478)	0.907		
Hypertension	0.727 (0.503–1.050)	0.089		
Diabetes mellitus	0.663 (0.436–1.007)	0.054	0.863 (0.525–1.418)	0.561
TC	1.006 (0.964–1.051)	0.779		
TG	0.991 (0.902–1.088)	0.848		
LDL-C	0.935 (0.774–1.129)	0.484		
HDL-C	1.528 (0.820–3.050)	0.171		
Non-HDL-C	0.918 (0.770–1.096)	0.343		
Lp(a)	1.002 (0.998–1.004)	0.736		
sdLDL	0.988 (0.952–1.024)	0.500		
HbA1c	1.088 (0.946–1.252)	0.238		
CK-MB	1.000 (0.999–1.002)	0.616		
cTnT	0.998 (0.978–1.018)	0.826		
NT-proBNP	1.004 (1.003–1.005)	<0.001	1.000 (0.999–1.001)	0.096
eGFR	0.976 (0.968–0.984)	<0.001	0.989 (0.975–1.002)	0.101
hs-CRP	1.006 (1.001–1.010)	0.013	1.002 (0.996–1.009)	0.491
Fibrinogen	1.001 (0.999–1.002)	0.080	1.000 (0.999–1.002)	0.809
D-dimer	1.024 (0.977–1.073)	0.326		
WBC	1.015 (0.955–1.079)	0.628		
PLT	1.000 (0.997–1.003)	0.856		
MPV	1.164 (0.987–1.372)	0.070	1.044 (0.859–1.271)	0.663
PDW	1.048 (0.971–1.130)	0.229		
PCSK9	1.235 (1.173–1.300)	<0.001	1.160 (1.092–1.232)	<0.001
sP-selectin	1.615 (1.402–1.861)	<0.001	1.269 (1.083–1.487)	0.003
CD40L	1.014 (0.948–1.085)	0.677		
PAF	1.015 (0.979–1.052)	0.415		
LOX-1	1.000 (0.977–1.003)	0.854		
Ox-LDL	0.998 (0.993–1.004)	0.587		

The hazard ratio (HR) was computed, and the corresponding 95% confidence interval (CI) was determined to assess the risk of major adverse cardiovascular events (MACE). Univariate Cox regression was initially used, and variables with statistical significance at a threshold of $P < 0.10$ were subsequently included in the multivariate stepwise Cox proportional hazard regression model.

biomarker alone, thereby facilitating more precise risk stratification. This synergy suggests a broader role of PCSK9 extending beyond its influence on lipid levels, and potentially affecting platelet reactivity and exaggerated inflammation.

The PCSK9-sP-selectin link in patients with ACS has substantial clinical applications that may directly affect patient care. These insights might enable personalized treatment strategies and closer monitoring. Notably, for patients with ACS with normal lipid profiles during the acute phase of MI, elevated PCSK9 levels may indicate the potential benefits of PCSK9 inhibitors in stabilizing coronary plaques and ultimately improving long-term outcomes. Our findings also underscored the critical role of antiplatelet therapy. Furthermore, our results hint at the possibility of developing targeted therapies that specifically modulate PCSK9 and sP-selectin, thereby offering exciting prospects for innovative drug development in cardiovascular

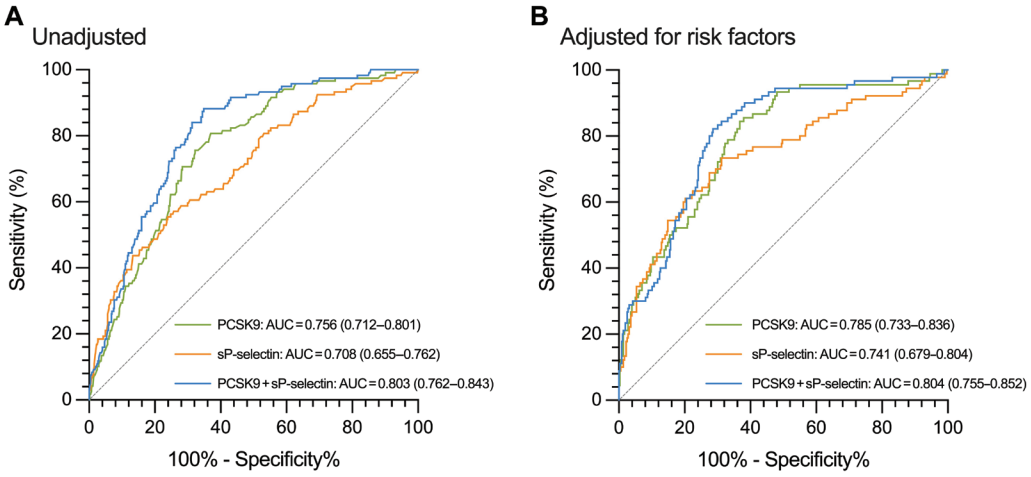

Figure 5 Receiver Operating Characteristic Curves of PCSK9 and sP-selectin for Predicting MACE, With or Without Adjustment for Established Risk Factors.
PCSK9, proprotein convertase subtilisin/kexin type 9; sP-selectin, soluble P-selectin; MACE, major adverse cardiac events; AUC, area under the receiver operating characteristic curve.

pharmacology. Policymakers and guideline committees might consider integrating PCSK9 and sP-selectin assessments into risk algorithms for patients with ACS. This integration could lead to more standardized and effective high-risk management, and potentially improve overall healthcare quality and outcomes.

Ischemia in the myocardium results in a significant increase in local or systemic concentrations of PCSK9; consequently, PCSK9 levels are tightly associated with the incidence of ischemic myocardial injury [22]. The HUNT study has demonstrated that low PCSK9 is an independent predictive factor for coronary heart disease after adjustment for sex and age [23, 24]. In addition to its use in predicting new-onset myocardial ischemia, a high PCSK9 level has been positively associated with the recurrence of cardiovascular events. These findings have been confirmed in the NSTEMI population undergoing PCI and expanded upon by Wang et al., who have shown that serum PCSK9 concentration is associated with a hypercoagulable state and hyperinflammation [25]. This phenomenon is particularly pronounced in the subgroup of patients with diabetes undergoing primary PCI for STEMI [12]. Additionally, our investigation revealed that individuals with high PCSK9 levels had elevated risk of requiring rehospitalization due to heart failure after MI. Notably, the BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF) has provided further evidence revealing a positive linear correlation between PCSK9 levels and the risk of death, mortality rates, and unplanned hospitalizations due to heart failure, through multivariate analysis [26]. Consequently, heightened PCSK9 levels were hypothesized to potentially play a role in the progression of heart failure. Importantly, current clinical guidelines do not recommend lipid-lowering therapy involving PCSK9 inhibitors for patients with heart failure [27]. Consequently, the precise role of PCSK9 in heart failure awaits clarification within the framework of these established guidelines.

P-selectin has a critical role in the pathological progression of atherosclerosis. Notably, patients with ACS exhibit markedly greater P-selectin exposure than individuals diagnosed with stable angina pectoris [28]. In a case-control study in premenopausal women, elevated sP-selectin levels have been associated with a twofold elevated risk of MI [29]. Moreover, sP-selectin concentrations have been established as a harbinger of unfavorable outcomes in patients with ACS as well as those with peripheral artery disease [30, 31]. Our study corroborated that concurrent elevations in the concentrations of PCSK9 and sP-selectin are associated with poorer outcomes. Several possible explanations may account for the findings.

Accumulating evidence suggests that platelet reactivity is enhanced after elevated PCSK9 levels. Wang et al. have examined the independent contribution of PCSK9 to platelet reactivity, as

measured by impedance aggregometry in healthy Chinese populations, and shown that PCSK9 does not mediate arachidonic acid-induced platelet aggregation [32]. Many studies have confirmed that high adenosine diphosphate-induced platelet reactivity is associated with substantial risk of ischemic events, regardless of arachidonic acid agonism [33–36].

Under activation conditions, P-selectin, contained in platelet α-granules, migrates to the outer cell membrane and is released as a soluble protein into the circulation [37]. In agreement with the present study, PCSK9 has been found to have a direct relationship with sP-selectin [13, 38, 39]. The augmentation of platelet activation is likely to be facilitated predominantly via CD36 platelet receptors and, to some extent, the expression of lectin-like ox-LDL receptor-1 in various tissues, including cultured vascular endothelial and smooth muscle cells [11, 40]. We stratified patients into groups according to the highest tertiles of PCSK9 and sP-selectin to achieve a robust risk stratification approach. Risk stratification with tertiles is widely used within the cardiovascular literature [41–43], and provides a straightforward way to divide data into three equally sized categories, thus facilitating clinical interpretation and research analysis. This approach enables the identification of profiles associated with the highest or lowest risks for specific outcomes, mirroring how clinicians routinely categorize patients into risk groups.

Historically, the 6-month post-procedure mortality rates in 2015 for these groups have been 5.3% and 6.3%, respectively [44]. Advances in interventional techniques, standardized DAPT, and proactive post-discharge care have contributed to improved outcomes for patients with ACS. Recent Asian data have shown encouraging outcomes for patients undergoing primary PCI, with 1-year rates of a composite adverse event (comprising death, non-fatal MI, and non-fatal ischemic stroke, and bleeding events) of 4.1% for STEMI and 5.1% for NSTEMI, along with respective all-cause mortality rates of 2.5% and 2.7% [45]. Our KM survival curves indicated an exceptionally high predictive ability of the PCSK9 and sP-selectin combination at higher risk of adverse events, thus underscoring its clinical and research relevance.

Associations of PCSK9 with lipid parameters remain controversial in existing studies. Elevated LDL-C is widely acknowledged as one of the most important components in the atherosclerotic process. Atherosclerosis is caused by chronic progressive endothelial injury and inflammation, during which PCSK9 is secreted by endothelial cells, and an oxidative stress state is simultaneously induced [46, 47]. LDL in the oxidized state tends to be readily taken up by macrophages through a repertoire of scavenger receptors [48]. Ox-LDL is considered a potent pro-inflammatory mediator inducing the activation of Toll-like receptors, the NLRP3 inflammasome, NF-κB, IL-1β, and IL-18, which in turn enhance the expression of PCSK9 [49]. Animal experiments have demonstrated that PCSK9 stimulates ox-LDL uptake via upregulation of scavenger receptor expression [50]. In a multicenter clinical study, PCSK9i treatment has been found to decrease ox-LDL formation in patients with familial hypercholesterolemia [51]. Our study also indicated a stepwise increase in ox-LDL by PCSK9 tertile, a finding attributable to platelet reactivity [51]. Furthermore, PCSK9 may also correlate with other serum lipid profiles. In a cross-sectional study recruiting patients with chronic kidney disease, PCSK9 has been directly associated with TG and Lp(a) levels [52]. Lp(a) contains an LDL-like particle to which apolipoprotein(a) is linked by an apolipoprotein B [53]. Subgroup analysis of FOURIER trial data has indicated that Lp(a) is causally linked to the development of coronary diseases, whereas PCSK9i directly mitigates the risk of cardiovascular events by decreasing Lp(a) levels [54]. However, no associations were found based on Lp(a) in our study.

This study has several limitations. First, this was a single-center study recruiting a relatively small sample size of patients. Patients with hemoglobin levels below 100 g/L or a total platelet count below 100×10^9/L were excluded, to minimize potential confounding effects of underlying hematological conditions, because the primary focus was on acute MI [20]. Notably, in our univariate Cox analysis, diabetes appeared to be a protective factor in relation to MACE. However, univariate analyses often uncover multiple signals, which can be misleading because of the absence of consideration

of confounding variables, thus underscoring the importance of conducting multivariate analysis. Herein, we used multivariate analysis to disentangle the complex relationships among various factors and provide a more accurate assessment of the predictors of MACE risk. Thus, the results might not be generalizable to other populations. Second, the current study selected only patients undergoing primary PCI. Future studies will be necessary to extend the findings to a more diverse cohort with a spectrum of coronary artery diseases. Third, we did not directly compare the predictive ability of the combination of PCSK9 and sP-selectin with various well-established risk scores. This omission was primarily because currently established risk scores have been designed for assessing short-term prognosis, and their value has been consistently demonstrated in this regard [17, 55]. Moreover, although the relationship between PCSK9 and sP-selectin was strong, the associations of PCSK9 and other platelet reactivity biomarkers were relatively low. The possible effects of PCSK9 on more platelet indices must be explored. In summary, further large prospective studies on the effects of PCSK9 in regulating platelet reactivity to predict adverse cardiovascular events are warranted to address these limitations.

Conclusion

In conclusion, our study sheds light on the relationship between PCSK9 and sP-selectin levels in the context of MACE risk among patients with ACS who have undergone primary PCI. Notably, our findings underscore the enhanced predictive value of combining PCSK9 and sP-selectin as biomarkers for MACE, thus surpassing the utility of each biomarker alone. From a clinical standpoint, these findings prompt consideration of PCSK9 inhibitors in normolipidemic ACS populations. Such an approach could offer dual benefits of enhancing plaque stability and protecting endothelial function, thereby contributing to improved patient outcomes in this high-risk group. However, the limitations of our study must be acknowledged, including its small sample size and single-center design, which might affect the generalizability of the results. Despite these limitations, our study highlights the multifaceted role of PCSK9 and sP-selectin in ACS prognosis, and provides a promising avenue for further research and potential therapeutic interventions in the management of ACS.

Declarations

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Authors' Contributions

Yao Yao was responsible for the materials, data collection, analysis, and writing of the article. Qining Qiu performed data analysis, and wrote and revised the article. Xiaoye Li contributed to the analysis and interpretation of the data, and writing of the article. Zi Wang conducted the literature review. Shikun Xu supervised the research and provided critical review. Qianzhou Lv conceptualized and designed the study, and provided critical review. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

Ethics Statement

The study was approved by the Ethics Committee of Zhongshan Hospital, Fudan University, with approval number B2022–078B. The study protocol complied with ethical guidelines and standards for human research, and was conducted in accordance with the Declaration of Helsinki. Signed informed consent was obtained from all patients. The privacy and confidentiality of patient data were also rigorously maintained.

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