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Proprotein Convertase Subtilisin/Kexin type 9, C-Reactive Protein, Coronary Severity, and Outcomes in Patients With Stable Coronary Artery Disease

A Prospective Observational Cohort Study

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Abstract: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is suggested as a novel factor associated with coronary artery disease (CAD). However, few studies have comprehensively evaluated plasma PCSK9 with cardiovascular risk till now. Hence, we aimed to prospectively investigate the association between baseline PCSK9 and cardiovascular risk graded with number of risk factors (RFs), coronary severity, and outcomes in patients with stable CAD.

Baseline characteristics and biomarkers were measured in 616 consecutive, nontreated patients with stable CAD. Coronary severity was measured using SYNTAX, Gensini, and Jeopardy scoring systems. Patients were then received treatment and followed for a median of 17 months. The primary endpoints were cardiac death, stroke, myocardial infarction (MI), post-discharge revascularization, or unstable angina (UA).

Overall, follow-up data were obtained from 603 patients. A total of 72 (11.9%) patients presented with at least 1 major adverse cardiovascular event (MACE) (4 cardiac deaths, 4 strokes, 6 MIs, 28 revascularizations, and 30 UAs). At baseline, PCSK9 was increased with an increasing number of RFs and positively associated with coronary severity scores ($P < 0.05$, all). After follow-up, those with MACE had a higher baseline PCSK9, hs-CRP, and coronary scores than those without ($P < 0.05$, all). Multivariate analysis showed that PCSK9, hs-CRP, and coronary scores were independently predictive for MACEs ($P < 0.05$, all). Interestingly, more significant predictive values of PCSK9 in medical-alone-treated population but no such associations in revascularization-treated patients were found.

Together, plasma PCSK9, as well as hs-CRP and coronary scores, could independently predict MACEs in patients with stable CAD.

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Abbreviations: ACS = acute coronary syndrome, Apo = apolipoprotein, BMI = body mass index, CABG = coronary artery bypass grafting, CAD = coronary artery disease, CAG = coronary angiogram, DM = diabetes mellitus, HbA1C = hemoglobin A1C, HDL = high-density lipoprotein, HF = heart failure, Hs-CRP = high sensitivity C-reactive protein, LDL-C = low-density lipoprotein cholesterol, LDLR = low-density lipoprotein receptor, LVEF = left ventricular ejection fraction, MACE = major adverse cardiovascular event, MI = myocardial infarction, NHDL-C = nonhigh-density lipoprotein cholesterol, NT-proBNP = N-terminal pro brain natriuretic peptide, PCI = percutaneous coronary intervention, PCSK9 = proprotein convertase subtilisin/kexin type 9, RF = risk factor, TC = total cholesterol, TG = triglyceride, UA = unstable angina.

INTRODUCTION

Proprotein convertase subtilisin/kexin 9 (PCSK9) is a circulating serine protease that has been well-described to efficiently bind low-density lipoprotein (LDL) receptor (LDLR) leading to its intracellular degradation,[1,2] thus increasing plasma LDL-cholesterol (LDL-C) levels, a major causal risk factor of coronary artery disease (CAD). Subsequently, a growing body of discoveries in genetic,[3–6] experimental,[7,8] and epidemiologic data[9,10] formed a clear association between PCSK9 function and cardiovascular risk.

However, few studies have comprehensively evaluated the association of plasma PCSK9 with cardiovascular risk till now.[9,11] So far, there are only 2 studies that investigate the predictive value of plasma PCSK9 levels for cardiovascular risk and suggest a positive association of PCSK9 levels with incidence of cardiovascular events (CVEs).[9,11] They both enrolled the statin-treated patients with stable CAD as study population. Additionally, it is well-known that statins up-regulate PCSK9 levels;[12,13] inhibiting PCSK9 is thus a logical strategy for

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enhancing statin-induced LDL-C lowering to maximize the reduction in cardiovascular risk.[14,15] The association of plasma PCSK9 levels with major adverse cardiovascular events (MACEs) in treated patients may not reflect the ''real world'' of predictive value of baseline PCSK9 levels.

Hence, in the present study, we aimed to prospectively investigate the association between baseline PCSK9 and cardiovascular risk graded with a number of risk factors (RFs), coronary severity using the Synergy between percutaneous coronary intervention with Taxus and cardiac surgery (SYNTAX), Gensini, and Jeopardy scoring systems, and outcomes in nontreated patients with stable CAD. We also compared the predictive value of PCSK9 levels in different treatment modalities after the baseline diagnostic coronary angiogram (CAG).

MATERIALS AND METHODS

Study Population

The present study as well as our previous studies[16–18] was approved by the Ethics Committee of the Fu Wai Hospital. Among the patients who were scheduled for CAG because of their angina-like chest pain and had not been started the regular second-level prevention drugs in our division during October 2012 to January 2015, we consecutively selected 1076 patients in the present study. The inclusion and exclusion criteria were described as our previous studies,[16–18] as well as showed in Figure 1. After the initial enrollment and the collection of clinical characteristics and blood specimen based on a fully informed consent, the patients were then received the standard medical treatment from their cardiologists. Hence, in the present study, we analyzed a total of 616 nontreated, eligible patients with stable CAD.

Laboratory Examinations

The clinical characteristics and fasting blood samples were collected from all patients before the diagnostic/interventional CAG and medication treatment as our previous studies.[10,16–18] The laboratory examinations evaluated the concentrations of lipids including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), LDL-C, apolipoproteinA-1 (apoA1), and apoB, hs-CRP, and PCSK9 and used the same approaches or methods as described previously.[16,17] As lipid-lowering drugs were known to influence lipids, PCSK9, and hs-CRP, we recorded these data before the treatment in our patients.

Definition of Conventional Cardiovascular RFs

To assess the presence and severity of cardiovascular RFs in the study population, we collected the information from each patient before the admission. Together, we investigated the histories of hypertension, diabetes mellitus (DM), hypercholesterolemia, hypo HDL cholesterolemia, obesity, risk age of onset, smoking, and the family history of CAD. The definition of hypertension and DM was described as previous studies.[16,17] Hypercholestrolemia was defined by medical history or fasting TC $\geq$ 200 mg/dL. Hypo HDL cholesterolemia was defined as HDL-C $<$ 40 mg/dL for men or $<$50 mg/dL for women. Obesity was considered as patients with the body mass index (BMI) $\geq$30 kg/m^2. The risk age of onset was $<$55 years for men or 65 years for women. Smoking was ascertained as subjects who had smoked regularly within the previous 12 months. Positive family history of CAD was present when a first-degree relative suffered an MI or PCI/CABG before 55 years for men or 65 years for women.

Assessment for the Severity of CAD

All patients subjected to a diagnostic CAG, which performed using the standard Judkin's technique with filming of multiple views of each vessel according to our previous studies.[10,16,17] The severity of CAD was assessed according to the SYNTAX, Gensini, and Jeopardy scoring systems. In patients who underwent PCI or CABG, the angiographic severity was measured before the revascularization procedures.

As reported previously, the SYNTAX scoring system provides a helpful approach for therapy decision regarding the complexity of CAD.[19] In the SYNTAX scoring system, a

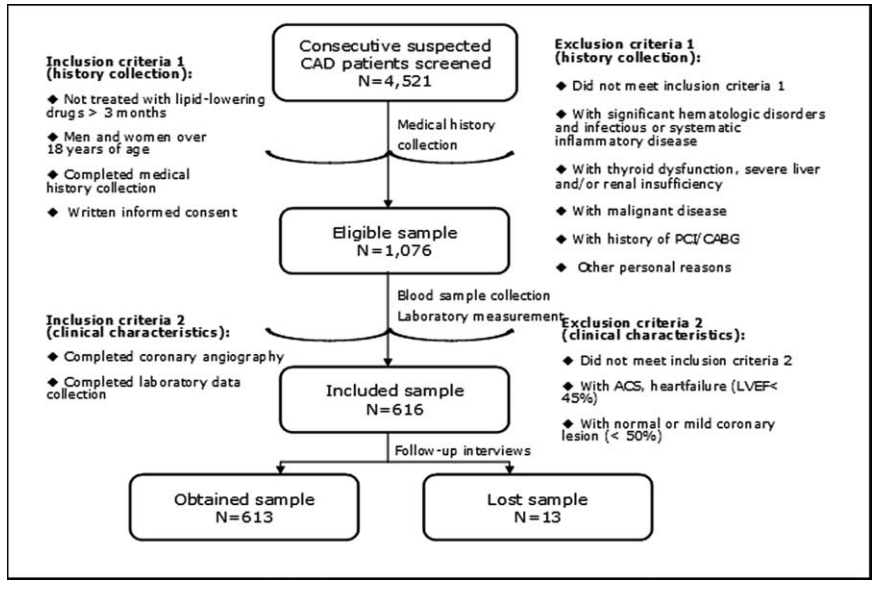

FIGURE 1. The study flowchart. Flowchart of the selection of the present study population including inclusion and exclusion criteria was showed.

diameter stenosis of ≥50% in vessels with a minimum diameter of ≥1.5 mm was recognized as the significant lesion. Then, the individual lesion was visually assessed and given a corresponding point value. Finally, we added up the individual values as the total score of the patient.[19] The Gensini scoring system, which is established to define the severity of coronary stenosis,[20] was also used for evaluating the coronary severity. The exact performance had reported in our previous studies.[10,17] Additionally, we evaluated the patients with the Jeopardy scoring system. It is designed to estimate the myocardium at risk or in jeopardy[21] and is recognized as the simplest and easiest assessment of the coronary severity. Briefly, 6 major segments including left anterior descending (LAD), major diagonal branch, major septal branch, left circumflex (LCX), major obtuse marginal branch, and right coronary artery (RCA) were defined and 2 points were given to each stenotic segment of >75% in the Jeopardy scoring system.[21] In the present study, we put on the 3, SYNTAX, Gensini, and Jeopardy score, considering that all of them evaluate predominantly anatomy and angiographic findings.

Follow-Up

According to the CAG results, all patients with stable CAD were received appropriate treatment modality (including PCI, CABG, medical-alone) from their cardiologists. Generally, patients were prospectively followed up at 6, 12, 24, 36 months using telephone and/or interview after the initial appointment by trained nurses or cardiologists, who were blinded to the results of the laboratory tests. If there was no MACE in the follow-up, the censorship was counted till the last traceable telephone and/or interview before April 2015. Hence, the follow up time interval (months) was counted from the admission for CAG and study blood sample collection till the last traceable hospital outpatient or inpatient record or telephone interview before April 2015.

The primary outcome was the composite of cardiac death, stroke, myocardial infarction (MI), postdischarge revascularization (PCI/CABG), or unstable angina (UA).[22] Cardiac death was primarily confirmed by the death from the cardiac causes including sudden cardiac death, congestive HF, acute MI, severe arrhythmia, stroke, or other structural/functional cardiac diseases. The definition of stroke was the acute cerebral infarction on the basis of the imaging or typical symptoms. MI was diagnosed by a comprehensive evaluation combining the chest pain or equivalent symptom complex, the diagnostic changes in cardiac enzyme levels, and the electrocardiogram. Postdischarge revascularization was the PCI and/or CABG performing during the follow-up. UA was considered if patients reported the chest pain, which was characterized with the new-onset angina and/or rest symptoms and/or increasing duration/severity compared to the previous stable symptoms, the dynamic electrocardiogram changes, but without abnormal change in cardiac enzyme levels.[23]

Statistical Analysis

The statistical analysis was performed with SPSS version 19.0 software (SPSS Inc, Chicago, IL). A P value <0.05 was considered statistically significant.

The values were expressed as the mean ± SD or median (Q1–Q3 quartiles) for the continuous variables and the number (percentage) for the categorical variables. The differences of clinical and biochemical parameters between groups were analyzed using independent sample t test, Mann–Whitney U test, χ^2-tests, and Fisher's exact test where appropriate. Non HDL-C (NHDL-C) was calculated as TC minus HDL-C. Cox regression analysis for event-free survival was performed to investigate the predictive value of PCSK9 levels and severity scores in different treatment modality after diagnostic CAG with adjustment for age, sex, BMI, hypertension, diabetes, hypercholesterolemia, hypo HDL cholesterolemia, current smoking, family history of CAD, uric acids, and hs-CRP, whereas the adjusted models investigating the predictive value of hs-CRP were with adjustment for the above factors except hs-CRP. Time-to-event curves were constructed using the Kaplan–Meier method to describe the incidence of death over time, and log-rank tests were applied to evaluate differences between groups that were divided by the medium of PCSK9 levels or severity scores.

RESULTS

Baseline Characteristics

We recruited 616 consecutive, nontreated patients with stable CAD for the present study. Follow-up data were not obtained in 13 patients for several reasons (declined to answer and wrong telephone number). Therefore, follow-up analysis was confined to 603 patients with CAD (Figure 1). Baseline characteristics according to occurrence of MACEs were listed in Table 1.

PCSK9 Levels and the Number of Coronary RFs

To investigate whether PCSK9 levels associated with the number of RFs, we calculated the number of RFs (hypertension, DM, hypercholesterolemia, hypo HDL cholesterolemia, obesity, risk age of onset, smoking, family history of CAD) for each patient. Then, patients were divided into the 3 groups according to previous studies (those with 0–2, 3–5, ≥6 RFs).[24] As shown in Figure 2, with increasing number of RFs, PCSK9 levels increased (222.12 [118.40–273.91] vs 230.19 [190.38–277.77] vs 271.06 [211.33–327.19] ng/mL; $P < 0.05$). The positive relationship of PCSK9 levels with number of RFs remained exist in both men and women.

PCSK9 Levels and Coronary Severity

The correlations were examined between baseline SYNTAX and Gensini ($r = 0.792$, $P < 0.001$), SYNTAX and Jeopardy ($r = 0.704$, $P < 0.001$), Gensini and Jeopardy (0.857, $P < 0.001$). The associations of PCSK9 levels with the 3 coronary severity scores, which were grouped as tertiles, were depicted in Figure 3. We found that PCSK9 levels significantly increased with the severity scores (SYNTAX, 221.35 [183.30–260.13] vs 228.00 [190.13–285.44] vs 246.93 [201.22–302.92] ng/mL; Gensini, 219.58 [181.65–271.65] vs 234.98 [194.66–280.86] vs 237.63 [192.57–283.63] ng/mL; Jeopardy, 224.97 [185.02–271.52] vs 236.89 [194.64–290.45] vs 237.90 [193.38–287.44] ng/mL; all P for trend <0.05).

PCSK9, as well as hs-CRP levels and Coronary Severity Scores With MACEs

Overall, follow-up data were obtained from 603 patients. Within a median follow-up of 17 months (3–31 months), 72 (11.9%) patients presented with at least 1 MACE (4 cardiac deaths, 4 nonfatal strokes, 6 MIs, 28 revascularizations, and 30 UAs). As shown in Table 1, patients with MACE had a higher baseline PCSK9, hs-CRP, and severity scores than those without (all $P < 0.05$).

TABLE 1. Baseline Characteristics in Patients With Stable CAD, With or Without MACE

	All Patients (n = 603)	Without MACE (n = 531)	With MACE (n = 72)	*P* Value
Cardiovascular risk factors				
Age (years)	57.88 ± 9.82	57.87 ± 9.76	58.04 ± 10.52	0.890
BMI (kg/m^2)	25.73 ± 3.39	25.68 ± 3.40	26.08 ± 3.22	0.347
Gender, men% (n)	72.0 (434)	71.8 (381)	73.6 (53)	0.782
Hypertension, % (n)	65.0 (392)	64.2 (341)	70.8 (51)	0.294
DM, % (n)	16.3 (98)	16.0 (85)	18.1 (13)	0.613
Hypercholesterolemia, % (n)	32.3 (195)	32.2 (171)	33.3 (24)	0.893
Hypo HDL cholesterolemia, % (n)	61.4 (370)	61.4 (326)	61.1 (44)	0.998
Current smoking, % (n)	45.6 (275)	44.8 (238)	51.4 (37)	0.315
Family history of CAD, % (n)	19.2 (116)	20.3 (108)	11.1 (8)	0.078
Biomarkers				
TG (mmol/L)	1.64 (1.18–2.32)	1.62 (1.18–2.31)	1.71 (1.16–2.35)	0.661
TC (mmol/L)	4.86 ± 1.02	4.85 ± 1.01	4.89 ± 1.15	0.738
HDL-C (mmol/L)	1.07 ± 0.33	1.07 ± 0.38	1.06 ± 0.38	0.793
LDL-C (mmol/L)	3.24 ± 0.95	3.23 ± 0.95	3.26 ± 0.98	0.853
NHDL-C (mmol/L)	3.79 ± 0.98	3.77 ± 0.96	3.83 ± 1.14	0.663
Apo A1 (g/L)	1.30 ± 0.30	1.30 ± 0.29	1.31 ± 0.34	0.838
Apo B (g/L)	1.04 ± 0.27	1.03 ± 0.27	1.03 ± 0.26	0.823
Glucose (mmol/L)	5.67 ± 1.81	5.62 ± 1.49	6.08 ± 3.40	0.041
HA1bC (%)	6.25 ± 1.10	6.24 ± 1.12	6.34 ± 1.01	0.461
Hs-CRP, mg/L	1.49 (0.70–3.02)	1.41 (0.66–3.02)	2.01 (0.90–3.56)	**0.021**
Uric acid (umol/L)	360.04 ± 90.10	358.23 ± 89.78	366.24 ± 91.72	0.479
NT-proBNP, pg/mL	49.77 (35.4–77.51)	49.31 (34.92–76.61)	53.36 (39.10–82.66)	0.162
PCSK9 (ng/mL)	230.11 (190.45–277.83)	228.62 (187.85–275.73)	246.06 (203.08–297.77)	**0.047**
Prior drug treatment				
Statin, % (n)	0 (0)	0 (0)	0 (0)	–
Aspirin, n (%)	40.5 (244)	41.4 (220)	33.3 (24)	0.203
Beta-blocker, % (n)	21.1 (127)	21.8 (116)	15.3 (11)	0.221
CCB, n (%)	22.9 (138)	22.4 (119)	26.4 (19)	0.456
ARB/ACEI, n (%)	17.5 (106)	18.5 (98)	11.1 (8)	0.316
Coronary severity scores				
SYNTAX score	10 (6–21)	9 (5–21)	13 (7–17)	**0.009**
GENSINI score	26 (12–58)	24 (11–58)	31 (20–56)	**0.042**
Jeopardy score	2 (2–6)	2 (0–4)	4 (2–6)	**0.006**

Data shown are mean ± SD, median (Q1-Q3 quartiles) or n (%). The bold values indicate statistical significance and are bolded to improve the readability of the table.

Apo = apolipoprotein, BMI = body mass index, CAD = coronary artery disease, DM = diabetes mellitus, HbA1C = hemoglobin A1C, HDL = high-density lipoprotein, Hs-CRP = high sensitivity C-reactive protein, LDL-C = low-density lipoprotein-cholesterol, MACE = major adverse cardiovascular event, NHDL-C = nonhigh-density lipoprotein cholesterol, NT-proBNP = N-terminal probrain natriuretic peptide, PCSK9 = proprotein convertase subtilisin/kexin type 9, TC = total cholesterol, TG = triglyceride.

Of note, the therapeutic modalities after the CAG in our cohort included PCI, CABG, and medical-alone treatment, which could complicate the interpretation of PCSK9, hs-CRP, and severity scores versus outcomes. Therefore, we grouped the patients based on the treatment modality and found that the associations of PCSK9, hs-CRP, and severity scores with the incidence of MACE not necessarily the same case in different treatments (Table 2). In the group of patients received PCI treatment after the diagnostic CAG, there were no differences in PCSK9, hs-CRP levels, and severity scores except SYNTAX, between patients with and without MACE. In the groups of patients received CABG treatment, PCSK9, hs-CRP levels, and severity scores were found no association with the presence of MACE, although there was an increasing trend of PCSK9 levels in patient with MACE. In the group of patients received medical-alone treatment, PCSK9, hs-CRP levels, and severity scores all increased significantly in those who had MACE.

Multivariable Cox regression analysis was performed to test whether PCSK9, hs-CRP levels, and severity scores were independent risk predictors of cardiovascular outcomes (Table 3). Results showed that PCSK9, hs-CRP levels, and severity scores including SYNTAX and Jeopardy scores, were predictive for MACEs independently from confounding factors in the total population or in the subgroup of medical-alone population in both univariate and multivariate models ($P < 0.05$). The association of Gensini score with MACE was only found in medical-alone population ($P < 0.05$).

The time-to-event analysis was performed using Kaplan–Meier estimates and compared between groups that were divided by the medium of PCSK9 levels (Figure 4) or severity scores (supplemental Figure 1, http://links.lww.com/MD/A590 2, http://links.lww.com/MD/A590 and 3, http://links.lww.com/MD/A590). Overall, 72 events were documented within a median of 17 months. PCSK9 levels and coronary scores were all positively associated with the time to the first primary

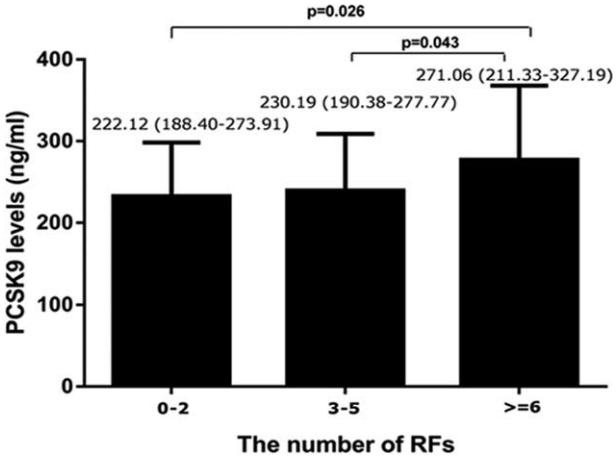

FIGURE 2. PCSK9 levels in relation to the number of coronary risk factors (RFs). Patients were divided into 3 groups (0–2, 3–5, ≥6 RFs). PCSK9 levels increased (222.12 [118.40–273.91] vs 230.19 [190.38–277.77] vs 271.06 [211.33–327.19] ng/mL; $P < 0.05$) with increasing number of RFs.PCSK9 = proprotein convertase subtilisin/kexin type 9; RFs = risk factors.

MACE (p = 0.018, 0.001, 0.031, and 0.015 for PCSK9, SYNTAX, Gensini, and Jeopardy scores, respectively). In the subgroup of patients received PCI/CABG treatment after the CAG, 45 events (41 in PCI-treated patients and 4 in CABG-treated patients) were occurred, and only SYNTAX score was shown an association with the time to the first primary MACE ($P = 0.047$). In the subgroup of patients received medical-alone treatment, 27 events were recorded, and the associations of PCSK9 and severity scores with the time to the first MACE were all detected in the present study ($P < 0.001$, = 0.011, 0.018, and 0.001 for PCSK9, SYNTAX, Gensini, and Jeopardy scores, respectively). Multivariate Cox regression analysis showed that a high PCSK9 level was independent predictor of MACE after adjustment for confounding factors including conventional risk factors and uric acids, and hs-CRP in total population and in the subgroup of medical-alone population (both $P < 0.05$).

DISCUSSION

In this study of patients who were not taking a lipid-lowering drug until the enrollment and had angiographically proven stable CAD, we demonstrated for the first time that across categories of increasing cardiovascular risk, there was a graded, positive association of plasma PCSK9 with number of RFs, severity of CAD, as well as adverse cardiovascular prognosis. Baseline PCSK9 levels, as well as hs-CRP and coronary scores could independently predict MACEs in patients with stable CAD. What's more, among patients who had different treatment modalities after the diagnostic CAG, plasma PCSK9 was associated with MACE in medial-alone-treated patients rather than in PCI- or CABG-treated patients.

The association of PCSK9 function with CAD development and prognosis has attracted a lot of attention from gene to protein in the past decades.[25] Since its discovery in 2003, PCSK9 has become a genetically validated target for autosomal dominant hypercholesterolemia (ADH), a form of familial hypercholesterolemia (FH).[4] Shortly thereafter, continued associations between PCSK9 function, LDL-C level, and

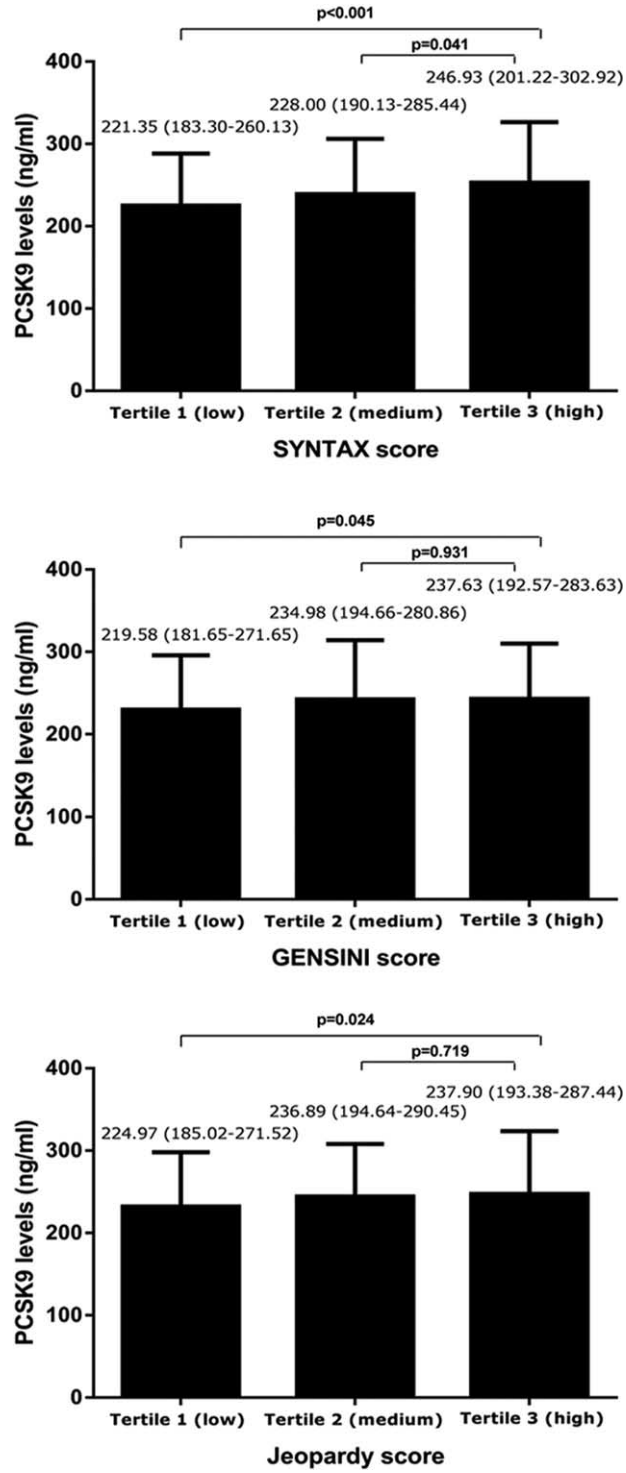

FIGURE 3. PCSK9 levels in relation to coronary severity. The coronary severity was assessed by SYNTAX, Gensini, and Jeopardy scoring systems and patients were divided into 3 groups according to the tertiles of the 3 scores respectively. PCSK9 levels were all significantly associated with coronary severity assessed by SYNTAX, Gensini, and Jeopardy scoring systems (all $P < 0.05$). PCSK9 = proprotein convertase subtilisin/kexin type 9.

TABLE 2. PCSK9 and Coronary Severity Scores in Different Treatment Modality After Diagnostic CAG, With or Without MACE

	All Patients (n = 603)	Without MACE (n = 531)	With MACE (n = 72)	*P* Value
Treatment modality				
PCI, % (n)	40.0 (241)	37.7 (200)	56.9 (41)	**<0.001**
PCSK9 (ng/mL)	234.58 (191.65–277.56)	234.58 (190.38–276.50)	216.87 (192.63–283.14)	0.940
Hs-CRP (mg/L)	1.77 (0.72–3.44)	1.71 (0.68–3.38)	2.05 (0.97–3.93)	0.118
SYNTAX score	9 (5–16)	9 (5–15)	13 (8–16)	**0.019**
GENSINI score	32 (20–49)	32 (20–51)	30 (20–46)	0.947
Jeopardy score	2 (2–4)	2 (2–4)	4 (2–6)	0.197
CABG, % (n)	12.4 (75)	13.4 (71)	5.6 (4)	**<0.001**
PCSK9 (ng/mL)	230.13 (194.93–285.47)	227.24 (193.38–285.47)	254.19 (226.88–299.90)	0.354
Hs-CRP (mg/L)	1.69 (0.76–3.61)	1.69 (0.73–3.79)	1.70 (0.82–7.82)	0.794
SYNTAX score	24 (16–33)	24 (17–33)	24 (10–34)	0.794
GENSINI score	75 (54–95)	73 (52–95)	85 (74–122)	0.235
Jeopardy score	6 (4–8)	6 (4–8)	7 (5–8)	0.829
Medical alone, % (n)	47.6 (287)	49.0 (260)	37.5 (27)	**<0.001**
PCSK9 (ng/mL)	226.25 (187.48–278.99)	222.69 (186.20–273.91)	266.75 (213.43–309.60)	**0.010**
Hs-CRP (mg/L)	1.29 (0.65–2.85)	1.20 (0.64–2.82)	1.67 (0.86–3.44)	0.048
SYNTAX score	7 (4–18)	7 (4–17)	13 (7–24)	**0.041**
GENSINI score	13 (7–32)	12 (6–27)	24 (14–56)	**0.004**
Jeopardy score	2 (0–4)	2 (0–4)	2 (2–6)	**0.001**

Data shown are medians (Q1–Q3 quartiles). The bold values indicate statistical significance and are bolded to improve the readability of the table.
CABG = coronary artery bypass grafting, CAG = coronary angiogram, Hs-CRP = high sensitivity C-reactive protein, MACE = major adverse cardiovascular event, PCI = percutaneous coronary intervention, PCSK9 = proprotein convertase subtilisin/kexin type 9.

cardiovascular risk are reported, presenting that the gain-of-function mutations in PCSK9 lead to higher levels of LDL-C and increased risk of CAD whereas loss-of-function PCSK9 variants are associated with reductions in both LDL-C and cardiovascular risk.[25] On the other hand, as a secret protein mainly by the liver, PCSK9 binds the LDLR at the surface of cells to prevent its recycling but enhance its degradation, presenting a key role in LDL-C metabolism.[2,25] It takes 9 years to elaborate powerful new PCSK9-based therapeutic approaches to reduce circulating levels of LDL-cholesterol.

TABLE 3. Cox Regression Models of Predictors for MACE in Different Treatment Modality After Diagnostic CAG

Variables	Unadjusted HR (95%CI)	*P* Value	Adjusted HR (95%CI)	*P* Value
All patients				
PCSK9 (ng/mL)	1.004 (1.001–1.007)	**0.016**	1.005 (1.001–1.008)	**0.005**
Hs-CRP (mg/L)	1.076 (1.010–1.147)	0.023	1.073 (1.005–1.145)	0.034
SYNTAX score	1.016 (1.000–1.042)	**0.046**	1.019 (1.000–1.041)	**0.043**
GENSINI score	1.003 (0.996–1.010)	0.362	1.003 (0.997–1.010)	0.339
Jeopardy score	1.104 (1.020–1.195)	**0.014**	1.111 (1.023–1.207)	**0.012**
PCI/CABG				
PCSK9 (ng/mL)	1.001 (0.997–1.006)	0.529	1.002 (0.997–1.006)	0.501
Hs-CRP (mg/L)	1.079 (0.994–1.173)	0.073	1.084 (0.995–1.182)	0.066
SYNTAX score	0.990 (0.962–1.019)	0.511	0.991 (0.962–1.021)	0.540
GENSINI score	0.995 (0.986–1.004)	0.295	0.995 (0.985–1.005)	0.295
Jeopardy score	0.993 (0.885–1.115)	0.907	0.992 (0.878–1.120)	0.892
Medical alone				
PCSK9 (ng/mL)	1.007 (1.002–1.012)	**0.005**	1.008 (1.003–1.013)	**0.003**
Hs-CRP (mg/L)	1.076 (1.002–1.188)	0.047	1.086 (1.003–1.203)	0.031
SYNTAX score	1.044 (1.008–1.081)	**0.016**	1.056 (1.016–1.098)	**0.006**
GENSINI score	1.009 (1.000–1.020)	**0.028**	1.010 (1.000–1.022)	**0.012**
Jeopardy score	1.227 (1.079–1.395)	**0.002**	1.262 (1.101–1.448)	**0.001**

Cox regression analysis was performed. Hazard ratios (HR) with 95% confidence intervals (95%CI) for the time to the first primary outcome event of the study cohort, stratified by PCSK9, hs-CRP levels, and coronary severity scores. The adjusted models investigating the predictive value of PCSK9 levels and severity scores were with adjustment for age, sex, BMI, hypertension, diabetes, hypercholesterolemia, hypo HDL cholesterolemia, current smoking, family history of CAD, uric acids, and hs-CRP, whereas the adjusted models investigating the value of hs-CRP were with adjustment for the above factors except hs-CRP. The bold values indicate statistical significance.
CABG = coronary artery bypass grafting, CI = confidence interval, HR = hazard ratios, Hs-CRP = high sensitivity C-reactive protein, MACE = major adverse cardiovascular event, PCI = percutaneous coronary intervention, PCSK9 = proprotein convertase subtilisin/kexin type 9.

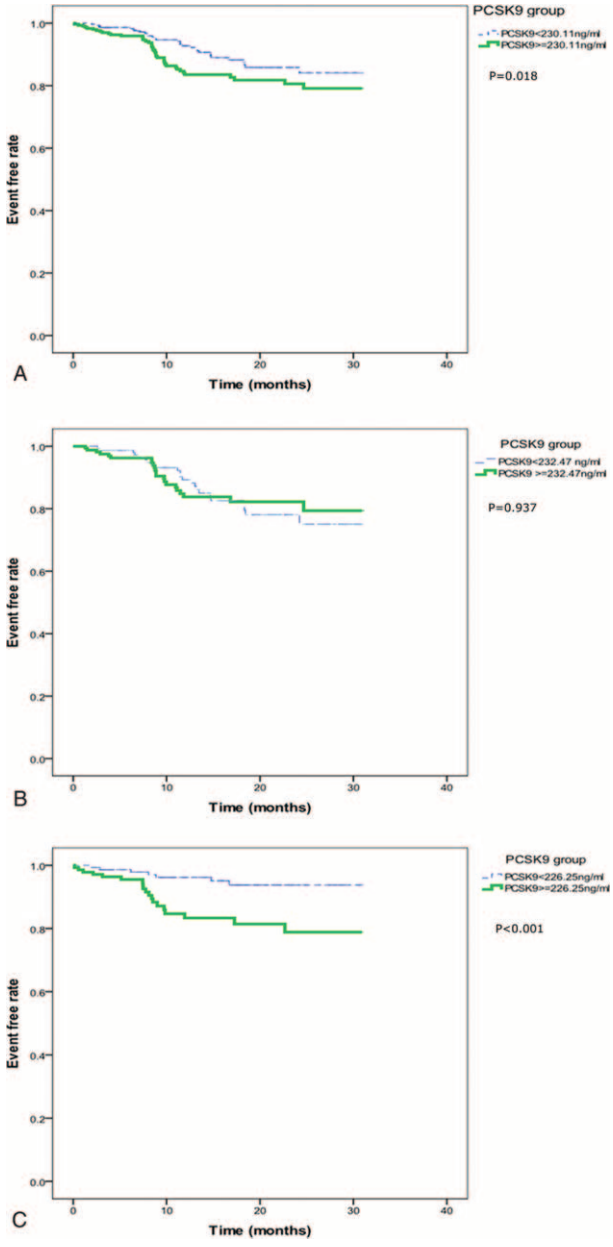

FIGURE 4. Time-to-event analysis was performed using Kaplan–Merier estimates comparing between low versus high PCSK9 levels groups with the log-rank test used for the *P* value in total population (A), PCI/CABG-treated subgroup population (B), and medical-alone-treated subgroup population (C).CABG = coronary artery bypass grafting; PCI = percutaneous coronary intervention; PCSK9 = proprotein convertase subtilisin/kexin type 9.

Presently, PCSK9 inhibitors including alirocumab[15] and evolocumab[14] are proven to lower CVEs in the background of stain-treatment. During ~1 year of therapy, the use of evolocumab, as compared with standard therapy alone, significantly reduced LDL-C levels by 61% and reduced the incidence of CVEs from 2.18% in the standard-therapy group to 0.95% in the evolocumab group. A reduction in the rate of cardiovascular events with alirocumab from 3.3% to 1.7% was detected over a period of 78 weeks.

However, so far, there are few prospective studies regarding plasma PCSK9, whether measuring baseline plasma PCSK9 as a useful biomarker could help predict future MACE in CAD remains to be determined.[9,11] In the present study, we first found a quantitative difference in baseline PCSK9 levels (prior the lipid-lowering treatment) between patients with and without MACE, and hence corroborating the usefulness of measuring PCSK9 levels from a prospective view. The novel findings and clinical implications of this study were that measuring PCSK9 might be emerged as a useful tool for improved risk prediction or in the quest for individualized medicine. Indeed, just 2 previous studies have reported the predictive role of plasma PCSK9 in a consistent statin-treated population. One study is from Werner, et al,[9] they enrolled 504 well-treated patients with stable CAD and defined the primary outcomes using the composite of cardiovascular death and unplanned cardiovascular hospitalization. Of note, the population treated with statin heterogeneously for at least 2 reasons. First, they reported 95% of the patients with statin-treatment but 5% left free of statin. Second, a certain proportion of the patients with a nonuniformed regimen of simvastatin 40 mg received other statins and dosages. Nevertheless, they found that PCSK9 levels in the presence of statins could predict CVEs in patients with stable CAD. Another study is from Huijgen, et al.[11] They were also interested in the association between PCSK9 levels and clinical outcomes in statin-treated CAD patients. But it was with regret that the study was retracted with unknown reason.

It is well established in humans and animal models that various lipid-lowering medications, particularly statins, increase plasma PCSK9 levels[12,13] and conversely that attenuation of PCSK9 function enhances the hypolipemic and cardiovascular protective effects of statins.[14,15] According to the previous data, the degree of PCSK9 elevation was shown to be various with the certain statins and the treatment term.[12,26] It is necessary to exclude individuals on lipid-lowering medication to evaluate the association of PCSK9 level with CAD in a ''real world.'' However, given the high prevalence of lipid-lowering prescriptions in the era of statins, it is challenge to obtain a large sample size of nontreated patients. As mentioned above, most of the studies regarding PCSK9 and CAD risk are performed in statin-treated patients.[9,14,15,27] The data concerning only patients who were not taking a lipid-lowering medication is limited. Therefore, we aimed to prospectively recruit a group of nontreated patients prior to the enrollment.

In the present study, we comprehensively evaluated plasma PCSK9 with cardiovascular risk in nontreated patients with stable CAD. First, our data indicated the high likelihood of a greater number of measurable RFs prevalence, which was suggested to be biologically plausible in association with atherosclerotic progression of disease,[28] in patients with a high PCSK9 level. Second, we extended the prior studies to investigate the association of PCSK9 levels with different assessment systems of coronary severity. The present study showed that PCSK9 was not only associated with a SYNTAX scoring, which illustrates the complexities of coronary lesions and difficulties of PCI, but also associated with the Gensini and Jeopardy scoring, which present the coronary diameter stenosis. Finally, the present study is among the first to investigate the predictive value of baseline PCSK9, as well as hs-CRP and coronary severity scores in patients with stable CAD, a ''real'' and ''pure'' population for PCSK9 analysis.

These results might be of particular importance in view of the clinical studies showing the selective PCSK9 inhibitors significantly reduced plasma PCSK9 activity and prevented

CAD progression, suggesting that PCSK9 inhibitors may be a useful therapeutic agent for the reduction of cardiovascular risk. However, the synergistic effect of PCSK9 inhibitor and traditional standard therapy (statin treatment) was investigated, and the clinical significance of PCSK9 inhibitors in cardiovascular health remained to be proven.

There were several limitations in the present study. The main limitation was the relatively small numbers of the study population. A larger study is necessary to confirm our results and improve the prognostic power of PCSK9 levels before definitive conclusions can be made. Another limitation was a single-center nature and the relatively short-term follow-ups. Further large clinical studies or longer follow-ups are required to validate our findings.

CONCLUSIONS

Collectively, these results suggested that baseline PCSK9 levels, as well as hs-CRP and coronary severity scores could independently predict MACE in patients with stable CAD.

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