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PCSK9 and atherosclerosis burden in the coronary arteries of patients undergoing coronary angiography

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ABSTRACT

Aims: To investigate the association between plasma proprotein convertase subtilisin/kexin type 9 (PCSK9) concentrations, current acute coronary syndrome (ACS), coronary artery disease (CAD) presence, severity and extension and the burden of coronary calcifications in patients with suspected CAD.

Methods and results: One hundred and one patients, with or without current ACS, were recruited for this cross-sectional study. CAD presence was defined based on either the presence or absence of at least one significant (≥50%) CAD lesion (SCAD). CAD severity was classified according to the absence of coronary lesions, the presence of non-significant (< 50%) CAD (MCAD) or SCAD in at least one major coronary artery. Patients with one, two or three significantly diseased major coronary arteries were defined as 1-SCAD, 2-SCAD and 3-SCAD, respectively. The cumulative length of SCAD lesions and the amount of calcifications in coronary arteries were estimated. Plasma PCSK9 concentrations were higher in patients with SCAD as compared to those without ($p = .012$). A significant increase in plasma PCSK9 concentrations was observed with greater CAD severity ($p = .042$). Higher plasma PCSK9 concentrations were found in 3-SCAD patients as compared to either 2-SCAD or 1-SCAD ($p < .001$). PCSK9 increased with the cumulative length of SCAD lesions and the burden of calcifications ($p < .05$ for both comparisons). Multivariable adjustment abolished the association between PCSK9 and either CAD presence or severity, but not the association between PCSK9 and the number of significantly diseased vessels, SCAD lesion length and the burden of coronary calcifications. ACS was associated with a borderline significant increase of plasma PCSK9 concentrations among patients not taking statins ($p = .05$).

Conclusion: Circulating PCSK9 concentrations discriminate patients with greater coronary atherosclerotic lesion extension and calcification, and are increased in patients with current ACS.

1. Introduction

Coronary heart disease (CHD) is the leading cause of death worldwide, accounting for nearly 9 millions of deaths in 2015 [1]. Among the multitude of traditional and emerging cardiovascular (CV) risk factors, hypercholesterolemia, particularly elevated plasma low-density lipoprotein cholesterol (LDL-C), has been established as an unequivocal causative factor for CHD [2].

Albeit different functions and roles of proprotein convertase subtilisin/kexin type 9 (PCSK9) have been proposed [3–5], its role in regulating plasma cholesterol concentrations by promoting LDL receptor (LDLR) degradation is remarkably consistent [6]. Thus, a higher expression of the *PCSK9* gene has been found to be associated with increased LDL-C concentrations and poor CV prognosis. Accordingly, autosomal dominant severe hypercholesterolemia has been related to gain-of-function *PCSK9* gene variants, the latter being associated with a dramatic increase in CHD risk [7]. Also, a prospective association between plasma PCSK9 concentrations and CHD risk has been found in some but not all studies [8–10], as clearly depicted in a meta-analysis of 9 studies including 12,081 participants followed for an average of

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6.62 years [11]. Importantly, some studies have remarked the dependence of this association on plasma lipids [12,13], whereas other studies showed independence [14,15].

The controversial scenario emerging from prospective studies performed in different clinical settings underlies the debate on whether PCSK9 may exert a proatherogenic effect that is independent of lipid pathways and, more specifically, of plasma LDL-C [16]. In this regard, inconsistent data on the association between plasma PCSK9 concentrations and carotid intima-media thickness have been published [17,18]. At the coronary district, the association between plasma PCSK9 concentrations and either calcium score [19,20] or plaque necrotic core [21] was reported to be independent of several CV risk factors and LDL-C as well. Plasma PCSK9 concentrations was associated with the presence and complexity of CAD in patients with suspected CAD undergoing coronary angiography [22]. The association between plasma PCSK9 concentrations and CAD presence and severity, as assessed by the Gensini score system, was found to be mediated at least in part by circulating lipids and inflammation [23]. More recently, Nose et al. [24] reported a significant and independent association between plasma PCSK9 concentrations and the presence of CAD in 393 Japanese patients undergoing multidetector-row computed tomography coronary angiography; however, this association was lost in the 134 statin-treated patients. Because lipid-lowering medications typically increase plasma PCSK9 concentrations [25], a subgroup of participants not taking lipid-lowering medications was selected in the Ottawa Heart Genomics Study with the aim to explore retrospectively the drug-unbiased association between PCSK9 and CAD [26]; in this sub-study, including 492 patients with CAD and 279 controls, plasma PCSK9 did not differ among groups [26]. Finally, elevated PCSK9 concentrations did not predict obstructive CAD, coronary calcium score, major acute coronary events and mortality in patients with chronic kidney disease [27], nor mortality in patients with acute coronary syndrome (ACS) [28].

Based on the available studies, the association between PCSK9 and CAD presence and severity remains still unclear and requires further clarification. Hence, we performed a cross-sectional study in patients with clinically suspected CAD undergoing coronary angiography, with the aim to shed further light on the association between plasma PCSK9 concentrations, the presence of CAD, its severity, its extension and the burden of coronary calcifications. The association between plasma PCSK9 concentrations and clinical features of the study population, particularly current ACS, was also evaluated.

2. Methods

2.1. Study subjects

This cross-sectional study was conducted during the year 2018. One hundred and one patients who were referred to the Baqiyatallah Hospital of Tehran for coronary angiography because of clinically suspected CAD were included in this study. Exclusion criteria were the presence of valvular and/or congenital heart disease. The methods were performed in accordance with the Declaration of Helsinki. The locally appointed ethics committee approved the research protocol and the informed consent was obtained from each patient. A data checklist including demographic information, medical history [diabetes, hypertension, dyslipidemia, statin therapy, current smoking, chronic kidney disease (CKD), and family history of CAD], body mass index (BMI), systolic and diastolic blood pressure, left ventricular ejection fraction (LVEF) according to echocardiography [29], blood tests and angiographic results was completed for each patient. Blood tests included fasting blood glucose (FBG), lipid profile [total cholesterol, LDL-C (using a direct method), high-density lipoprotein cholesterol (HDL-C), triglycerides] and creatinine, which were measured using routine enzymatic and immuno-assays (BioSystems S.A, Spain). Troponin-I was measured using a high sensitivity assay (CV: 7.0%; VIDAS®, bioMérieux, France). C-reactive protein (CRP) was measured using a latex immunoturbidimetric method (Bionik Co., Germany). Estimated glomerular filtration rate (eGFR) was calculated with the Cockcroft-Gault equation from age, weight, serum creatinine, and gender. PCSK9 assay was carried out using a sandwich ELISA technique with a commercial kit (abcam, MA, USA) having intra-assay and inter-assay variations of 4.4% and 4.6%, respectively. The sensitivity of the assay as well as the specific recovery (for serum sample) were 68 pg/mL and 104–107%, respectively.

2.2. Coronary angiography assessment of CAD

According to the result of coronary angiography, subjects having normal coronary arteries, those having non-significant CAD lesions (< 50% coronary stenosis) in one or more major coronary arteries and those having at least one significant CAD lesion (≥ 50% coronary stenosis) in major coronary arteries were classified as Controls, minimal CAD (MCAD) and significant CAD (SCAD), respectively. CAD presence was defined according to the absence (no-CAD, including Controls and MCAD patients) or the presence of SCAD. CAD severity was defined according to the absence of coronary lesions, the presence of MCAD in one or more coronary arteries or the presence of SCAD in at least one major coronary artery. SCAD was further classified as single-vessel (1-SCAD), two-vessel (2-SCAD) or three-vessel SCAD (3-SCAD). Patients were stratified according to the cumulative length of SCAD lesions (LL groups). The reference group for lesion length (Group A-LL) included no-CAD patients, while Group B-LL included patients with a cumulative SCAD lesion length ≤ 20 mm, and Group C-LL included patients with a cumulative SCAD lesion length > 20 mm. CAD extent was defined based on the number of major coronary vessels with SCAD and by the cumulative length of SCAD lesions. In addition, patients were stratified according to the amount of coronary calcifications (CC groups), which was determined based on the visual judgment of angiography reports by a cardiologist. The reference group for calcification burden (Group A-CC) included patients without calcifications, while Group B-CC included patients with a mild calcification burden and Group C-CC those with a moderate/severe amount of coronary calcifications, respectively.

2.3. Statistical analysis

SPSS statistical package, release 17.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses. Values are expressed as the mean ± SD or median and interquartile range. Sample size was calculated considering Z-value of 1.96 (corresponding to $\alpha = 0.05$), a confidence limit of 0.05 and an estimated proportion of 0.07 based on the overall prevalence of CAD (7%). Base 10 logarithmic (LG) transformation was performed for skewed variables and the LG-variables were used when appropriate. Independent samples t-test and Wilcoxon rank-sum test were used for 2-group comparisons of the study variables, whereas analysis of variance (ANOVA) with Bonferroni's post-hoc test or Kruskal–Wallis test were used for 3-group comparisons. Correlation analyses were performed using the Pearson's and Spearman's coefficients of correlations. Multivariable analysis was performed with measures of CAD presence (SCAD *versus* no-CAD), severity (Controls *versus* MCAD *versus* SCAD groups), extent (1-SCAD, 2-SCAD, 3-SCAD and LL groups, respectively) and calcifications (CC groups) as dependent variables. Statistical significance was assumed if a null hypotesis could be rejected at $p = .05$.

3. Results

3.1. Angiographic findings

Out of the 300 hundred patients that were initially evaluated, 101 cases were selected. Exclusions were due to incomplete medical history information, incomplete laboratory data, not meeting the inclusion

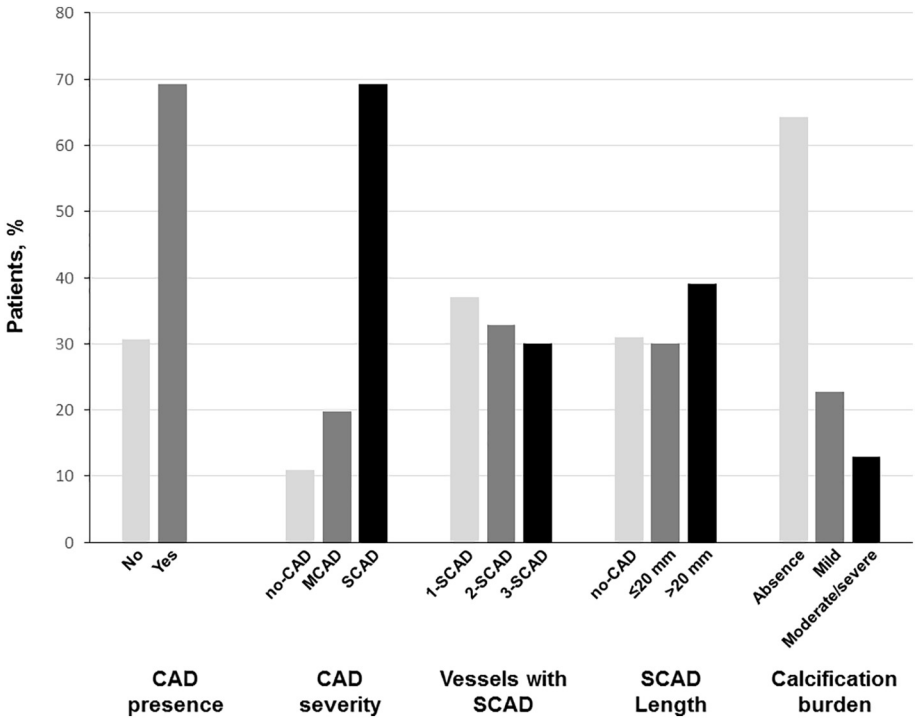

Fig. 1. Angiographic findings in the overall study population.
noCAD: patients with normal angiographic findings and those with minimal coronary artery disease; MACD: minimal coronary artery disease (*i.e.*, patients with evidence of at least one coronary stenosis < 50% in diameter in one or more vessels); SCAD: significant coronary artery disease (*i.e.*, patients with at least one coronary stenosis ≥50% in diameter in one or more vessels). SCAD patients were further classified as single vessel (1-SCAD), two vessel (2-SCAD) or three vessel SCAD (3-SCAD).

criteria and not consenting to participate in the study. Among the 101 studied subjects, coronary angiography showed normal angiographic findings in 11 (10.9%) patients, MCAD in 20 (19.8%), and SCAD in 70 patients (69.3%), the latter group of patients being further defined as having 1-SCAD, 2-SCAD or 3-SCAD in 26 (37.1%), 23 (32.9%) and 21 (30%) patients, respectively. After patients were stratified according to the cumulative length of SCAD lesions, Group A-LL included 32 patients (31.7%), Group B-LL 30 patients (29.7%) and Group C-LL 39 patients (38.6%). Coronary calcifications were absent in 65 patients (64.3%) (Group A-CC), mild in 23 (22.8%) (Group B-CC) and moderate/severe in 13 (12.9%) (Group C-CC) (Fig. 1).

3.2. Clinical characteristics of the study population and angiographic findings

Table 1 shows the clinical characteristics of the study participants, stratified as Controls ($n = 11$), MCAD patients ($n = 20$) or SCAD patients ($n = 70$). The mean age, LVEF, plasma troponin-I, CRP and total cholesterol were significantly different between the three groups of patients. SCAD patients were more likely to take statin therapy than Controls and patients with MCAD. ACS was diagnosed exclusively in patients with SCAD. The mean age, plasma troponin-I concentrations and statin use were significantly higher ($p = .013$, $p = .013$, and $p = .002$, respectively) while total cholesterol and LVEF were significantly lower ($p = .014$ and $p = .005$, respectively) in SCAD patients as compared to MCAD patients. There were no additional significant intergroup differences. In the minimally age-adjusted regression analysis, the ability of LG-CRP to predict CAD severity was not significant ($\beta = 0.16$, $p = .09$).

When SCAD patients were classified according to the number of diseased vessels (*i.e.*, 1-SCAD, 2-SCAD, 3-SCAD), the intergroup comparison resulted in significant differences of LVEF, plasma troponin-I and CRP concentrations (Table 2). A significant trend toward an increase in ACS events was recorded across the 3 groups of patients. No additional significant intergroup differences were found. No significant differences were found when comparing 3-SCAD patients with 2-SCAD patients.

Table 1
Characteristics of the study population stratified according to CAD severity.

	Controls ($n = 11$)	MCAD patients ($n = 20$)	SCAD patients ($n = 70$)	*P*
Age, years	54 ± 6	59 ± 10	65 ± 9	0.001
Gender, % men	64	50	70	0.251
BMI, kg/m^2	27 ± 2	28 ± 5	27 ± 4	0.353
Current smoking, %	100	5	9	0.541
Dyslipidemia, %	55	60	70	0.481
Diabetes, %	36	45	46	0.844
Hypertension, %	36	65	53	0.305
CKD, %	9	5	6	0.890
Family history of CAD, %	64	40	50	0.447
SBP, mmHg	122 ± 7	123 ± 14	123 ± 15	0.982
DBP, mmHg	75 ± 5	75 ± 8	76 ± 10	0.903
Total cholesterol, mg/dL	157 ± 30	185 ± 28	161 ± 35	**0.033**
LDL-C, mg/dL	95 ± 18	108 ± 26	93 ± 29	0.165
HDL-C, mg/L	41 ± 8	39 ± 8	39 ± 8	0.539
Triglycerides, mg/dL	170 (110–188)	172 (133–215)	139 (110–171)	0.066
FBG, mg/dL	94 (87–134)	109 (94–163)	118 (100–156)	0.198
CRP, mg/L	1.8 (1.3–4.5)	5.2 (2.8–8.4)	4.4 (2.4–6.6)	**0.030**
Troponin-I, ng/mL	0.001 (0.001–0.002)	0.002 (0.001–0.003)	0.003 (0.001–0.016)	**0.004**
LVEF, %	51 ± 3	53 ± 5	47 ± 9	**0.001**
Statin therapy, %	55	45	80	**0.005**
Current ACS, %	0	0	13.9	**0.027**

Values are expressed as percentage, mean ± SD or median (IQR). ACS, acute coronary syndrome; BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; DBP, diastolic blood pressure; FBG, fasting blood glucoe; HDL-C, high-density lipoprotein cholesterol; CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MCAD, minimal coronary artery disease; SBP, systolic blood pressure; SCAD, severe coronary artery disease. Significant p-values are bolded to be more distinguishable.

Table 2
Characteristics of SCAD patients according to the number of diseased vessels.

	1-SCAD patients (n = 26)	2-SCAD patients (n = 23)	3-SCAD patients (n = 21)	P
Age, years	62 ± 9	66 ± 9	66 ± 9	0.186
Gender, % men	62	70	81	0.352
BMI, kg/m^2	28 ± 4	27 ± 3	26 ± 3	0.332
Current smoking, %	4	9	14	0.446
Dyslipidemia, %	65	65	81	0.424
Diabetes, %	35	52	52	0.358
Hypertension, %	42	61	57	0.385
CKD, %	100	4	14	0.104
Family history of CAD, %	54	44	52	0.744
SBP, mmHg	122 ± 11	122 ± 17	126 ± 15	0.562
DBP, mmHg	75 ± 9	75 ± 11	77 ± 9	0.640
Total cholesterol, mg/dL	164 ± 30	163 ± 38	156 ± 38	0.726
LDL-C, mg/dL	95 ± 18	97 ± 31	87 ± 35	0.491
HDL-C, mg/L	39 ± 6	39 ± 9	38 ± 9	0.863
Triglycerides, mg/dL	133 (109–177)	133 (88–164)	158 (119–193)	0.193
FBG, mg/dL	111 (98–148)	118 (104–170)	127 (93–152)	0.784
CRP, mg/L	3.5 (2.0–5.7)	5.4 (3.4–12.5)	3.2 (2.2–6.0)	**0.050**
Troponin-I, ng/mL	0.002 (0.001–0.003)	0.008 (0.003–0.055)	0.005 (0.002–0.050)	**0.002**
LVEF, %	51 ± 7	43 ± 11	44 ± 7	**0.003**
Statin therapy, %	69	91	81	0.155
Current ACS, %	1.4	8.5	10	**0.029**

Values are expressed as percentage, mean ± SD or median (IQR). ACS, acute coronary syndrome; BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; SCAD, severe coronary artery disease. Significant p-values are bolded to be more distinguishable.

3.3. PCSK9, clinical characteristics of the study population and angiographic findings

Plasma PCSK9 concentrations were significantly associated with age (rho = 0.22, p = .025), systolic blood pressure (rho = 0.21, p = .034), troponin-I (rho = 0.22, p = .028) and LVEF (rho = −0.30, p = .002). The association between LG-PCSK9 and either plasma troponin-I or LVEF was abolished after adjustment for CAD severity (β = −0.10, p = .318 and β = −0.16, p = .093, respectively). In the overall study population plasma PCSK9 concentrations were almost doubled in patients with CKD (mean eGFR: 38 ± 22 mL/min) as compared to those without CKD (mean eGFR: 75 ± 21 mL/min), and showed a trend toward an increase in statin-treated as compared to statin-untreated patients (Table 3).

In addition, plasma PCSK9 concentrations did not differ significantly between patients with or without current ACS in the overall study population (Table 3) but when restricting the analysis to statin-untreated patients it resulted a borderline significant difference in plasma PCSK9 concentrations between those with current ACS and those without current ACS (Table 3). Plasma troponin-I and CRP were significantly higher in patients with current ACS than in those without (p < .001 and p = .001, respectively), whereas LVEF was significantly lower in current ACS patients (p < .001).

Plasma PCSK9 concentrations were higher in patients with SCAD as compared to those without (no-CAD) (Table 3). A significant trend toward an increase in plasma PCSK9 concentrations was observed with greater severity of CAD (Table 3). In the SCAD group-restricted analysis, higher plasma PCSK9 concentrations were found in patients with 3-SCAD as compared to patients with either 2-SCAD or 1-SCAD (Table 3). Plasma PCSK9 concentrations increased significantly with the

Table 3
Plasma PCSK9 concentrations according to the angiographic features (A) and to the clinical characteristics (B) of the study population.

(A)	CAD presence		CAD severity		SCAD vessels			SCAD length			Calcification burden		
	No-CAD	SCAD	Controls	MCAD	SCAD	1-SCAD	2-SCAD	3-SCAD	No-CAD	≤ 20 mm	> 20 mm	Absence	Mild	Moderate/severe
PCSK9 ng/mL	87.7 (76.9–107.5)	97.7 (87.9–155.3)	87.5 (74.3–114.9)	89.2 (78.9–105.4)	97.7 (87.9–155.3)	88.5 (87.4–97.4)	97.9 (87.6–99.8)	165.7 (121.4–180.6)	87.7 (76.9–107.5)	89.3 (83.5–98.2)	108.8 (89.6–165.9)	89.5 (78.9–103.9)	99.8 (89.6–165.1)	134.3 (91.9–167.1)
P value	0.012		0.042 (p for trend)			0.000 (1-SCAD *versus* 3-SCAD)			0.000 (no-CAD *versus* > 20 mm)			0.020 (absence *versus* mild)		
						0.000 (2-SCAD *versus* 3-SCAD)			0.000 (≤ 20 mm *versus* > 20 mm)			0.006 (absence *versus* moderate/severe)		

(B)	Statin therapy (overall study population, n = 101)		Current ACS (overall study population, n = 101)		Current ACS (statin-untreated patients, n = 30)		CKD (overall study population, n = 101)	
	Yes	No	Yes	No	Yes	No	Yes	No
PCSK9 ng/mL	97.4 (87.6–142)	90.3 (78.9–101.4)	104.3 (87.6–176)	94.8 (86.8–114.9)	108.7 (98.9–..)	87.9 (78.9–98.9)	164.6 (104.6–187)	94.8 (86.8–114.9)
P value	0.129		0.079		0.050		0.006	

PCSK9 is expressed as median (IQR). ACS, acute coronary syndrome; CAD, coronary artery disease; CKD, chronic kidney disease; MCAD, minimal coronary artery disease; PCSK9, proprotein convertase subtilisin/kexin type 9; SCAD, severe coronary artery disease.

Table 4
Predictors of CAD presence (Model A), CAD severity (Model B), vessels with SCAD (Model C), SCAD lenght (Model D), and calcification burden (Model E).

Model	Dependent variable	Independent variables	B, SE	β	*p*
A	CAD presence	LG-PCSK9	3.85, 2.06	–	0.061
		Age	0.08, 0.03	–	**0.002**
B	CAD severity	LG-PCSK9	–	0.158	0.098
		Age	–	0.342	< **0.001**
C	SCAD vessels	LG-PCSK9	–	0.481	< **0.001**
		Age	–	0.151	0.151
		Gender	–	−0.153	0.157
		CKD	–	0.085	0.425
		LDL-C	–	0.011	0.921
		Statin therapy	–	0.124	0.254
		Current ACS	–	0.159	0.146
D	SCAD length	LG-PCSK9	–	0.285	**0.001**
		Age	–	0.306	**0.001**
		Gender	–	−0.224	**0.011**
		CKD	–	−0.076	0.367
		LDL-C	–	0.001	0.988
		Statin therapy	–	0.239	**0.008**
		Current ACS	–	0.215	**0.014**
E	Calcification burden	LG-PCSK9	–	0.244	**0.013**
		Age	–	0.316	**0.001**
		Gender	–	−0.014	0.878
		CKD	–	−0.112	0.232
		LDL-C	–	−0.089	0.339
		Statin therapy	–	0.096	0.324
		Current ACS	–	0.230	**0.017**

ACS, acute coronary syndrome; CAD, coronary artery disease; CKD, chronic kidney disease; LDL-C, low-density lipoprotein cholesterol; LG, logarithmic; LVEF, left ventricular ejection fraction; PCSK9, proprotein convertase subtilisin/kexin type 9; SCAD, severe coronary artery disease. Significant p-values are bolded to be more distinguishable.

cumulative length of significant atherosclerotic lesions and with the burden of calcifications (Table 3). In the minimally age-adjusted regression analysis, the ability of LG-PCSK9 to predict CAD presence and severity was not significant (B = 3.85, SE = 2.06, $p = .06$ and β = 0.16, $p = .098$, respectively) (Table 4). In the fully-adjusted (*i.e.*, adjusted for age, sex, CKD, LDL-C, statin use and current ACS) regression analysis, LG-PCSK9 was significantly associated with the number of diseased coronary arteries (β = 0.48, $p < .001$) (Table 4). Regression models with either LL groups or CC groups as dependent variables, showed that LG-PCSK9 was significantly associated with both the dependent variables, independent of multiple confounders (β = 0.28, $p = .001$ and β = 0.24, $p = .013$, respectively) (Table 4).

4. Discussion

In this cross-sectional study the association between plasma PCSK9 concentrations and different angiographic features of CAD (presence, severity, number of major coronary arteries with SCAD, cumulative length of SCAD lesions, and burden of coronary calcifications) was investigated. Also, plasma PCSK9 concentrations were compared between patients with current ACS and those without.

4.1. PCSK9 and angiographic features of CAD

In the overall study population, plasma PCSK9 concentrations were higher in patients with CAD as compared to those without; in addition, an increase of plasma PCSK9 concentrations was found with increasing CAD severity. However, both these differences were abolished after adjustment for confounders. This suggests that PCSK9 cannot be considered as a reliable biomarker in the prediction of the presence of CAD and its severity, the latter being merely defined by the presence of any SCAD lesion. In agreement with our results, in most of previous studies the association between plasma PCSK9 concentrations and SCAD presence was either absent or strongly impaired by multivariable adjustment for confounders [22,23,26,27].

Noteworthy, when the study population was stratified according to CAD severity, significant intergroup differences in total cholesterol, CRP, troponin-I and LVEF were recorded (Table 1). However, CRP was not significantly different between SCAD patients and MCAD patients, and the association between CRP and CAD severity was abolished after the adjustment for confounders. Accordingly, previous studies investigating the independent predictive value of CRP for the burden of atherosclerotic CV disease showed inconsistent results [30], fueling the debate on the utility of targeting systemic inflammation as anti-atherosclerotic strategy [31–35]. Instead, higher plasma troponin-I concentrations, lower LVEF and lower plasma total cholesterol concentrations were recorded in SCAD patients as compared to MCAD patients. This was consistent with a higher prevalence of current ACS and statin use in SCAD patients as compared to MCAD patients.

When the analyses were restricted to the subgroup of patients with SCAD, the ability of plasma PCSK9 concentrations to predict the number of diseased coronary arteries was significant and independent from potential confounders. Particularly, higher plasma PCSK9 concentrations were found in patients with 3-SCAD than in those with either 2-SCAD or 1-SCAD, with the association between plasma PCSK9 concentrations and either the number of vessels with SCAD or the presence of 3-SCAD resulting significant after multivariable adjustment (Table 4). This suggests that PCSK9 may be considered as a useful biomarker to predict the presence of extensive SCAD. Further supporting this notion, we found a significant independent association between plasma PCSK9 concentrations and the cumulative length of SCAD lesions. Previous observational studies investigating the association between plasma PCSK9 concentrations and SCAD extension showed discordant results [22,24,26]. However, it is likely that different demographic and clinical characteristics of the enrolled populations, potentially influencing plasma PCSK9 concentrations and CV risk (*e.g.*, gender, ethnicity, concomitant CV risk factors, and previous CV events) [36–38], might explain, at least partially, such inconsistency.

Finally, in our study, plasma PCSK9 concentrations were independently associated with the burden of coronary calcifications, in line with some previous clinical (observational and intervention) studies [19,29,39].

Overall, the association between plasma PCSK9 concentrations and angiographich features of CAD suggests a possible direct pro-atherogenic role of PCSK9 along with a possible promoting effect of PCSK9 on coronary calcifications. Regarding the putative direct pro-atherogenic role of PCSK9, it will be extremely hard to distinguish the effects of PCSK9 from those of LDL-C, just because PCSK9 interfere very rapidly with cholesterol metabolism *via* the enhanced LDLR degradation at different tissue levels [40,41]. Regarding the putative calcification-promoting effect of PCSK9, available evidence from experimental studies supports the notion that possible cholesterol-dependent mechanisms might mediate it [42–44]. Specifically, the PCSK9-mediated LDLR endocytosis might induce the preferential binding of LDLR-related protein 5 (Lrp5), an LDLR coreceptor, to the frizzled receptors. This in turn may activate the Wnt3a/β-catenin pathway and trigger a cascade of downstream signaling events leading to the upregulation of different mediators of calcium deposition and matrix mineralization (*e.g.*, alkaline phosphatase, bone morphogenetic protein 2, osteocalcin) [43–46]. However, whether PCSK9 may exert a pro-calcifying effect on arterial wall remains a matter of debate in light of the neutral effect of evolocumab, a potent anti-PCSK9 monoclonal antibody, on coronary plaque calcification [47], as reported in the large-scale intravascular ultrasound (IVUS)-based Glagov trial.

4.2. PCSK9 and ACS

In our study plasma PCSK9 concentrations showed a trend toward

an increase in patients with current ACS as compared to those with stable CAD when considering the overall population, but they showed a borderline significant difference in patients with current ACS as compared to those with stable CAD when restricting the analysis to the subgroup of patients not taking statins. This result is in line with emerging evidence reporting that plasma PCSK9 concentrations show a trend toward an increase in the first hours/days following an ACS [26,28,48]. However, it remains to be elucidated as to whether plasma PCSK9 concentrations may increase following the ACS (*i.e.*, as an acute phase reactant) or before the ACS (*i.e.*, as a trigger factor inducing plaque instability and complication). The confounding effect of statin therapy on the association between PCSK9 and current ACS may be attributed, at least partially, to the inhibition of cholesterol synthesis by statins therapy, which is known to upregulate *PCSK9* transcription through the activation of regulatory elements in the *PCSK9* promoter by sterol-regulatory element binding protein (SREBP)-2 (SREBP-2) and SREBP-1c [25,49]. However, it cannot be excluded that additional mechanisms may underline the confounding effect of statin therapy on PCSK9 increase during ACS.

5. Conclusions

Overall, this study suggests that the measurements of plasma PCSK9 concentrations may be useful for predicting both SCAD extension and the burden of coronary calcifications and for discriminating patients with current ACS from those without. This may have important implications. In fact, initial clinical evaluation of individuals with suspected CAD is often a challenge and the availability of biomarkers reflecting specific CAD features, particularly the extension and the calcification of one or more coronary atherosclerotic lesions, may be particularly useful in order to plan the most appropriate intervention strategy.

Limitations of the present study need to be acknowledged. First, the small sample size of the enrolled population, which was recruited in a single center, limited our ability to perform reliable subgroup analyses and the generalizability of the results. Second, the association between plasma PCSK9 concentrations was adjusted only for traditional CV risk factors; thus, a residual confounding effect of other clinical variables cannot be excluded. Third, the cross-sectional design of this study did not allow to elucidate a possible causal role of plasma PCSK9 concentrations in CAD progression nor the timing of plasma PCSK9 increase in ACS.

In conclusion, our study contributes to the evidence of a relationship between circulating PCSK9 concentrations, SCAD extension and the burden of coronary calcification. Moreover, we provide supporting evidence of an increase of plasma PCSK9 during ACS. Whether PCSK9 may have direct pro-atherogenic and pro-calcifying effects and/or its increase in SCAD patients may be considered an epiphenomenon of the coronary atherosclerotic burden need to be established. Similarly, whether the acute increase of plasma PCSK9 concentrations during ACS may be the result of causality (*e.g.*, PCSK9 induces plaque destabilization/thrombosis) or reverse causation (*e.g.*, PCSK9 behaves as an acute phase reactant or PCSK9 is released from ruptured atherosclerotic plaque) remains an unresolved issue.

Declaration of Competing Interest

None.

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