Association of serum PCSK9 with NIRS-derived lipid core burden index and long-term cardiac outcome

(ATHEROREMO-NIRS substudy)

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ABSTRACT

Aims: We investigated the association between serum proprotein convertase subtilisin/kexin type 9 (PCSK9) levels, and near-infrared spectroscopy (NIRS)-derived lipid core burden index (LCBI) and the occurrence of major adverse cardiac events (MACE) in patients with coronary artery disease (CAD).

Methods: Serum PCSK9 levels were measured in 576 CAD patients who underwent diagnostic coronary angiography (CAG). NIRS imaging was performed in a subset of 203 patients, in a non-culprit coronary artery segment. Data on all-cause mortality, nonfatal ACS or unplanned coronary revascularization was collected during a median follow-up of 4.7 years.

Results: In multivariable analysis, serum PCSK9 was positively associated with LCBI (mean increase of 0.390 (95% CI [0.011-0.769] ZlnLCBI per unit increase in lnPCSK9 level p =0.044)). During a median follow-up of 4.7 years, 155 patients (27%) had MACE. After multivariable adjustment, serum PCSK9 levels showed a tendency towards an association with MACE (HR [95%CI]: 1.64[0.99-2.71], p=0.055) and a positive association with the composite of death or ACS (HR [95%CI]: 1.88[1.01-3.51], p=0.047). Patients admitted with serum PCSK9 levels above the median of 270 µg/L had 53% higher risk of MACE and 67% higher risk of death or ACS than those with levels below the median.

Conclusion: CAD patients with elevated serum PCSK9 levels had a higher NIRS-derived LCBI and higher incidence of adverse cardiac outcome than those with lower levels.

INTRODUCTION

The proprotein convertase subtilisin/kexin type 9 (PCSK9) enzyme plays a central role in the regulation of cholesterol homeostasis by increasing the endosomal and lysosomal degradation of hepatic low-density lipoprotein (LDL) receptors, resulting in increased serum LDL cholesterol (LDL-C) concentrations (1). In the last decade, PCSK9 enzyme has received substantial attention. Genetic studies have shown that loss-of-function mutations in the PCSK9 gene are associated with hypocholesterolemia and a decreased cardiovascular risk (2, 3). Recently, large phase 2 and phase 3 clinical trials have shown that PCSK9 inhibitors effectively reduce LDL-C levels, decrease plaque burden as assessed by intravascular ultrasound (IVUS) and reduce the risk of cardiovascular events (4-8). Moreover, in a previous study, we have shown that the serum PCSK9 level displayed a positive association with the amount of necrotic core tissue in coronary atherosclerotic plaque as assessed by IVUS, as well as with adverse cardiovascular outcome during 1 year follow-up, independent of serum LDL-C levels and statin use (9).

The catheter based near-infrared spectroscopy system (NIRS) is an intracoronary imaging technique capable of identifying lipid rich core-containing plaques in the coronary artery wall (10). Lipid rich core-containing plaques have been shown to be more vulnerable to rupture than plaques without a lipid rich core (11). Furthermore, in previous studies, we have demonstrated a strong association between a high NIRS-derived lipid core burden index (LCBI) and adverse cardiovascular events during short-term, as well as long-term follow-up (12, 13). However, there are currently no data on the association between serum PCSK9 levels and NIRS-derived LCBI.

Therefore, the primary aim of the current study is to investigate the relationship between serum PCSK9 levels and NIRS-derived LCBI in patients with coronary artery disease (CAD) undergoing coronary angiography (CAG). Secondly, we investigated whether the association between serum PCSK9 levels and the occurrence of major adverse cardiac events (MACE) persists during longer-term follow-up (9).

METHODS

Study population and design

The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis–intravascular Ultrasound (ATHEROREMO-IVUS) study, and its NIRS sub-study have been described in detail elsewhere (12-14). In brief, 768

patients were included in the ATHEROREMO-IVUS study between 2008 and 2011 at Erasmus MC, Rotterdam, the Netherlands. All patients included were 18 years or older and had an indication for CAG or percutaneous coronary intervention (PCI) due to an acute coronary syndrome (ACS) or stable angina pectoris (SAP). The flow chart of the study is shown in Figure 1. After the initial procedure, NIRS of a non-culprit vessel was performed in a subset of 203 patients. The medical ethics committee of Erasmus MC approved the ATHEROREMO-IVUS study and its NIRS sub-study and written informed consent was obtained from all patients. The ATHEROREMO-IVUS study and its NIRS sub-study were performed in accordance with the declaration of Helsinki.

Serum PCSK9 levels

Prior to the CAG or PCI, blood samples were collected from the arterial sheath and transported to the clinical laboratory of Erasmus MC within 2 hours for storage at a temperature of −80 °C. Serum samples were available for PCSK9 measurements

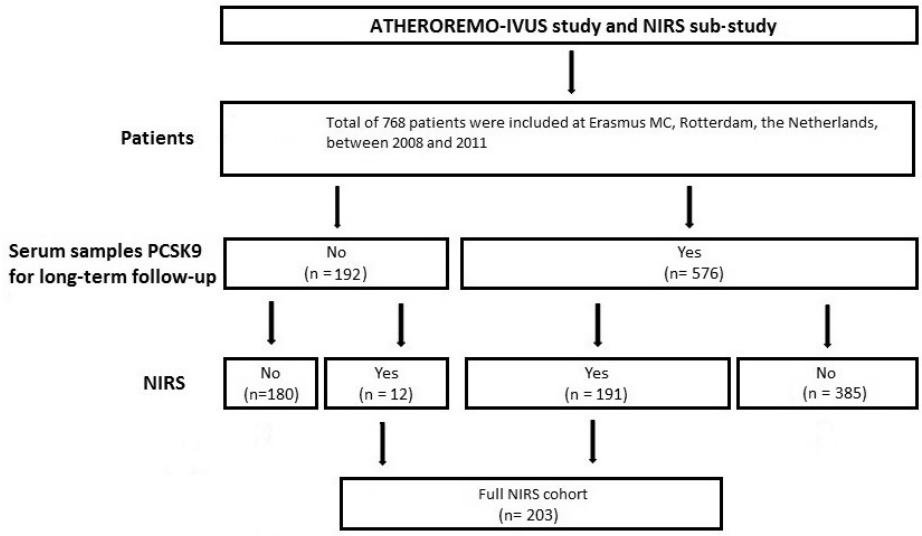

Figure 1. Flow chart ATHEROREMO-IVUS study and NIRS sub-study
Serum samples PCSK9 were measured in 576 CAD patients who underwent diagnostic coronary angiography for stable angina pectoris or acute coronary syndrome. NIRS imaging was performed in a subset of 203 patients, in a non-culprit coronary artery segment of at least 40 mm in length and without a reduction in lumen diameter >50%.
CAD, coronary artery disease; NIRS, Near-infrared spectroscopy; PCSK9, proprotein convertase subtilisin/kexin type 9.

in 203 patients with intracoronary NIRS imaging (NIRS cohort). For the long term follow-up, serum samples for PCSK9 measurements were available in 576 patients (full cohort). PCSK9 levels were measured in the stored serum samples using an enzyme linked immunosorbent assay (Human PCSK9 Quantikine ELISA, R&D systems Inc., Minneapolis, MN, USA). The minimum detectable level of this essay was 0.096 µg/L with a coefficient of variation of 4.1% at a mean value of 27.9 µg/L. The laboratory was blinded to clinical and intracoronary imaging data.

Near-infrared spectroscopy

Subsequent to the index CAG or PCI, invasive imaging with IVUS and NIRS was performed in one non-culprit coronary artery segment. The study protocol predefined the order of preference for the selection of the non-culprit study vessel: 1) left anterior descending artery; 2) right coronary artery; and 3) left circumflex artery. The non-culprit coronary artery segment had to be at least 40 mm in length and without a reduction in lumen diameter >50% by online angiographic visual assessment. The NIRS system, which was approved by the U.S. Food and Drug Administration, included a 3.2-F rapid exchange catheter, a pullback and rotation device, and a console (InfraReDx, Burlington, Massachusetts, USA). A motorized catheter pullback was performed at a speed of 0.5mm/s and 240rpm, starting distal to a side branch. Immediately after a pullback, the data in the scanned coronary artery segment were displayed in a chemogram. The probability of the presence of lipid rich core-containing plaques in the scanned coronary artery segment was calculated by means of a prediction algorithm and was displayed using colors, ranging from red (low probability of lipid content) to yellow-coded plaque (high probability of lipid content) (15)(Figure 2). LCBI was determined for the entire segment, as well as for the 10 mm and 4 mm long segments with the highest LCBI ($MaxLCBI_{4mm}$ and $MaxLCBI_{10mm}$). The NIRS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands) that was blinded to the clinical and PCSK9 data of the patients.

Follow-up and study endpoints

Clinical and vital status of patients were collected from medical charts, civil registries or by written or telephone contacts with the patients or relatives. Follow-up questionnaires as a screening tool for identifying probable adverse events were sent to all living patients participating in this study. For patients with any hospitalization

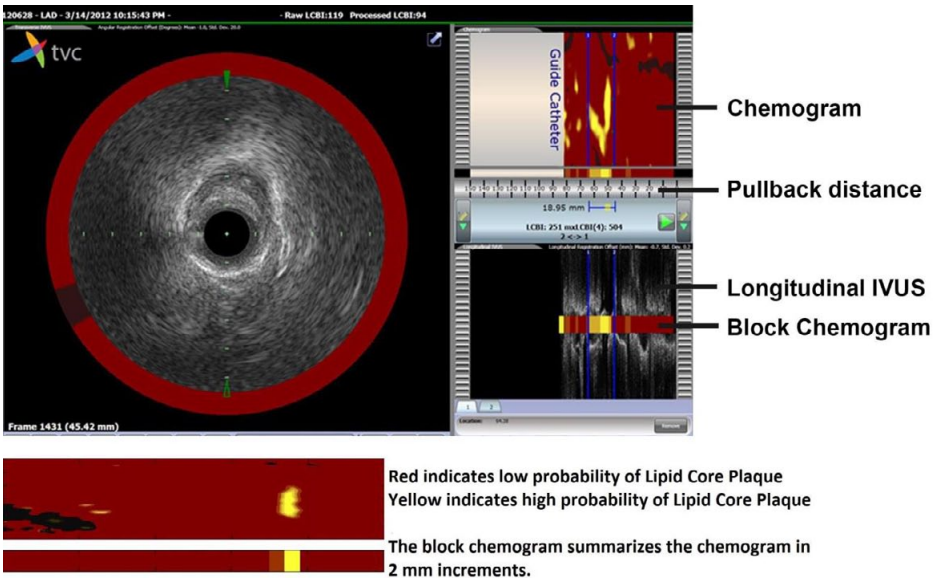

Figure 2. Intracoronary near-infrared spectroscopy
The figure displays an example of coronary wall imaging by NIRS. Spectral characteristics of lipid core plaques (LCP) are displayed on a chemogram along the length (x-axis, in mm) and circumference (y-axis, 0 to 360 degrees) of the scanned coronary artery. Yellow regions in the chemogram represent high probability of LCP while red regions represent those with low probability of LCP. The LCBI quantifies the amount of LCP in the entire scanned artery segment on the block chemogram, and is computed as the fraction of valid pixels that exceed an LCP probability of 0·6, multiplied with 1000. LCBI, Lipid Core Burden Index; NIRS, Near-infrared spectroscopy.

or a possible adverse event, additional information was obtained from hospital discharge letters.

The primary clinical endpoint was the occurrence of major adverse cardiac events (MACE), defined as the composite of all-cause mortality, nonfatal ACS or unplanned coronary revascularization. The secondary endpoint was the composite of all-cause mortality or nonfatal ACS. ACS was defined as the clinical diagnosis of ST-segment elevation myocardial infarction (STEMI), non-STEMI or unstable angina pectoris (UAP) in accordance with the guidelines of the European Society of Cardiology (16, 17). Unplanned coronary revascularization was defined as any PCI or coronary artery bypass grafting (CABG) that was not foreseen at the index procedure. Endpoints were adjudicated by a clinical events committee that was blinded to the serum PCSK9 levels and imaging data.

Statistical analysis

Categorical variables are presented as numbers and percentages. Normally-distributed continuous variables are presented as mean ± standard deviation (SD), while non-normally distributed continuous variables are presented as median with 25^{th}-75^{th} percentile. The distribution of continuous variables was examined for normality by visual inspection of the histogram. Serum PCSK9 levels (measured in µg/L) were not normally distributed and were therefore ln-transformed for further analyses (LnPCSK9). NIRS-derived LCBI, $MaxLCBI_{4mm}$, $MaxLCBI_{10mm}$ were first ln-transformed and then standardized as a z-score (Z_{lnLCBI}). Comparisons were done using the Chi-square test for categorical variables and Student's t-test and the Mann-Whitney U test for continuous variables.

Linear regression was used to examine the associations between PCSK9 levels (independent variable) and the NIRS findings (dependent variables) in the NIRS cohort (n= 203 patients). Results of linear regression are presented as the mean (95% confidence interval (CI)) change in Z_{lnLCBI} per unit change in lnPCSK9 level. We conducted multivariable analysis, including the following covariates: age, gender, hypertension, diabetes mellitus, LDL-C level, statin use at time of hospital admission and indication for index CAG.

Cumulative event rates were estimated according to the Kaplan-Meier method. Patients lost to follow-up were censored at the date of last contact. Cox proportional hazards models were used to evaluate the associations between PCSK9 levels and clinical study endpoints in the full cohort (n= 576 patients). PCSK9 level was analyzed as a categorical variable (serum PSCK9 levels above versus below the median) and as continuous variable. The final results are presented as unadjusted and multivariable adjusted hazard ratios (HRs) with 95% CIs.

To account for possible effect modification by baseline indication for CAG, all statistical analyses were performed in the overall study population with and without the interaction term on indication for CAG (i.e. SAP or ACS). Furthermore, stratified analysis by age, gender, diabetes, hypertension, hypercholesteremia, LDL-C level and statin use at hospital admission were also performed to assess effect modification. All statistical tests were two-tailed and p-values <0.05 were considered statistically significant. Data were analyzed using IBM SPSS software (SPSS 23.0 IBM corp., Armonk, NY, USA).

Table 1. Baseline clinical and procedural characteristics of the NIRS cohort

	Total (n=203)	ACS patients (n= 95)	SAP patients (n= 108)	P value
Clinical characteristics				
Age, years, mean ± SD	63.4 ± 10.9	62 ± 11.7	64.7 ± 10.2	0.082
Male, n (%)	148 (72.9)	63 (66.3)	85 (78.7)	0.048
Diabetes Mellitus, n (%)	41 (20.2)	17 (17.9)	24 (22.2)	0.443
Hypertension, n (%)	114 (56.2)	51 (53.7)	63 (58.3)	0.505
Hypercholesterolemia, n (%)	115 (56.7)	43 (45.3)	72 (66.7)	0.002
Smoking, n (%)	50 (24.6)	30 (31.6)	20(18.5)	0.067
Positive family history of CAD, n (%)	120 (59.1)	51 (54.3)	69 (63.9)	0.164
Previous MI, n (%)	79 (38.9)	34 (35.8)	45 (41.7)	0.391
Previous PCI, n (%)	78 (38.4)	27 (28.4)	51 (47.2)	0.006
Previous CABG, n (%)	6 (3.0)	2 (2.1)	4 (3.7)	0.502
Previous stroke, n (%)	6 (3.0)	4 (4.2)	2 (1.9)	0.322
Peripheral artery disease, n (%)	11 (5.4)	5 (5.3)	6 (5.6)	0.927
History of heart failure, n (%)	9 (5.9)	3 (3.2)	6 (5.6)	0.408
Serum PCSK 9 µg/L	278.3[217.5-343.9]	269.2[191.5-336.8]	280.4[222.4-358.7]	0.319
Statin use at baseline, n (%)	181 (89.2)	82 (86.3)	99 (91.7)	0.261
Serum TC mmol/L	4.20[3.60-4.90]	4.40[3.68-5.33]	4.00[3.40-4.80]	0.015
Serum LDL-C mmol/L	2.49[1.98-3.34]	2.82[2.08-3.60]	2.37[1.94-3.00]	0.007
Serum HDL-C mmol/L	1.06[0.87-1.33]	1.11[0.87-1.35]	1.06[0.87-1.31]	0.507
Serum TG mmol/L	1.30[0.93-1.87]	1.19[0.79-1.76]	1.42[1.08-2.10]	0.002
Procedural characteristics				
Indication for coronary angiography				
ACS, n (%)	95 (46.8)	95 (100)	0 (0)	
STEMI, n (%)	28 (13.8)	28 (29.5)	0 (0)	
Non ST-ACS/ UAP, n (%)	67 (33.0)	67 (70.5)	0 (0)	
Stable angina pectoris, n (%)	108 (53.2)	0 (0)	108 (100)	
PCI performed, n (%)	179 (88.2)	88 (92.6)	91 (84.3)	
Coronary artery disease [52]				
No significant stenosis, n (%)	16 (7.9)	8 (8.4)	8 (7.4)	
1-vessel disease, n (%)	106 (52.2)	49 (51.6)	57 (52.8)	
2-vessel disease, n (%)	58 (28.6)	26 (27.4)	32 (29.6)	
3-vessel disease, n (%)	23 (11.3)	12 (12.6)	11 (10.2)	
NIRS characteristics				
LCBI [25th-75th]	43.0[15.0-90.0]	47.0[16.0-90.0]	35.0[14.0-85.5]	0.441
LCBI $_{4mm}$ [25th-75th]	234[93-377]	267[100-387]	201[85-377]	0.431
LCBI $_{10mm}$[25th-75th]	131[60-247]	153[68-253]	239[47-121]	0.435
Imaged coronary artery				
Left anterior descending, n (%)	74 (36.5)	41 (43.2)	33 (30.6)	

Left circumflex, n (%)	70 (34.5)	30 (31.6)	40 (37.0)
Right coronary artery, n (%)	59 (29.1)	24 (25.3)	35 (32.4)

Continuous variables are presented as mean± standard deviation (SD) or as median with 25th-75th percentile. Categorical variables are presented in numbers and percentages n (%).
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ACS, acute coronary syndrome; CABG, coronary artery bypass grafting; CAD, coronary artery disease; HDL-C, high-density lipoprotein cholesterol; LCBI, Lipid Core Burden Index; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; NIRS, Near-infrared spectroscopy; Non ST-ACS, non ST segment elevation acute coronary syndrome ; PCI, percutaneous coronary intervention; PCSK9, proprotein convertase subtilisin/kexin type 9 SAP, stable angina pectoris; TG, triglycerides; UAP, unstable angina pectoris.

52 A significant stenosis was defined as a stenosis ≥ 50% of the vessel diameter by visual assessment of the coronary angiogram.

RESULTS

Baseline characteristics

A total of 203 patients were enrolled in the NIRS cohort (Figure 1). Mean age was 63.4 years and 72.9% were men (Table 1). The median PCSK9 level was 278 µg/L and ranged from 91 to 804 µg/L [25th-75th percentile; 218-344 µg/L] with no differences between patients admitted with ACS and patients with SAP. In contrast, the median serum LDL-C level was 2.49 [25th-75th percentile: 1.98-3.34] mmol/L and was higher in patients admitted with ACS when compared with patients with SAP (2.82 [2.08-3.60] versus 2.37 [1.94-3.00] mmol/l, p=0.007). At the time of hospital admission, statin use was 89.2%. A total of 46.8% of the patients were diagnosed with ACS. During the index CAG, PCI was performed in 88.2% of the patients. The median LCBI of the imaged coronary segment was 43 [25th-75th percentile: 15-90].

Association between PCSK9 level and NIRS-derived LCBI

Patients with higher serum PCSK9 levels also had higher NIRS-derived LCBI with a mean increase of 0.381 (95% CI [0.004-0.757]) in Z_{lnLCBI} per unit increase in lnPCSK9 level ('beta'; p=0.047; Table 2). This association remained statistically significant after multivariable adjustment for cardiac risk factors and statin use (Beta= 0.404; 95% CI [0.024-0.783], p=0.037), as well as after additional adjustment for baseline serum LDL-C level (Beta= 0.390 (95% CI [0.011-0.769] p =0.044)). Furthermore, in multivariable analysis, serum PCSK9 levels were also significantly associated with $LCBI_{10mm}$ (Beta= 0.406 (95% CI [0.014-0.797], p=0.042). Results were similar in patients admitted with ACS or SAP.

Table 2. Association between serum PCSK9 and LCBI

NIRS characteristics	Model1 Beta (95%CI)	P-value	Model 2 Beta (95%CI)	P-value	Model3 Beta (95%CI)	P-value
NIRS cohort (n=203)						
LCBI	0.381 (0.004-0.757)	0.047	0.404 (0.024-0.783)	0.037	0.390 (0.011-0.769)	0.044
LCBI $_{4\,mm}$	0.251 (-0.137-0.640)	0.203	0.292 (-0.099-0.684)	0.142	0.281 (-0.111-0.673)	0.159
LCBI $_{10\,mm}$	0.370 (-0.016-0.756)	0.060	0.411 (0.021-0.801)	0.039	0.406 (0.014-0.797)	0.042

The results are presented as beta coefficients (B) that indicate the mean (95% confidence interval (CI)) change in Z_{lnLCBI}, $Z_{lnLCBI4mm}$ or $Z_{lnLCBI10mm}$ per unit change in lnPCSK9.
Model 1 includes serum PCSK9 levels.
Model 2 includes serum PCSK9 levels, age, gender, diabetes mellitus, hypertension, indication for index CAG and statin use. [53]
Model 3 includes serum PCSK9 level, age, gender, diabetes mellitus, hypertension, indication for index CAG, statin use and serum LDL cholesterol.[54]
CAG, coronary angiography; LCBI, Lipid Core Burden Index; LDL, low-density lipoprotein; NIRS, Near-infrared spectroscopy; PCSK9, proprotein convertase subtilisin/kexin

Subgroup analyses are presented in Figure 3. A significant interaction for the association between serum PCSK9 levels and LCBI was found for statin use at hospital admission (p=< 0.001). In line with this, in patients without statin use at hospital admission, higher serum PCSK9 levels were significantly associated with a higher NIRS-derived LCBI. In patients that used statins at admission, no statistically significant association was found.

PCSK9 and cardiovascular outcome during long-term follow-up

During a median follow-up time of 4.7 [25th-75th percentile: 4.2-5.6] years, MACE occurred in 157 patients. In these patients, the median PCSK9 level was 283 µg/L [25th-75th percentile: 229-339 µg/L]. In patients without events, the median PCSK9 level was 263 µg/L [25th-75th percentile: 213-345 µg/L] p= 0.119.

The cumulative incidence of MACE, stratified according to PCSK9 levels, is depicted in Figure 4. Patients with serum PCSK9 levels above the median of 278 µg/L had significantly higher incidence of MACE (30% versus 23% at 4.7 years follow-up; HR [95%CI]: 1.42[1.03-1.94], p=0.031) as well as significantly higher incidence of death or ACS (21% versus 15 % at 4.7 years follow-up); HR [95%CI]: 1.50[1.01-2.21], p=0.044) than their counterparts with lower levels. After adjustment for cardiac risk factors, statin use and baseline serum LDL-C level, these associations persisted: patients with PCSK9 above the median had 53% higher incidence of MACE (HR

53 Statin use was registered at the time of hospital admission.
54 Serum LDL cholesterol concentration was measured prior to CAG.

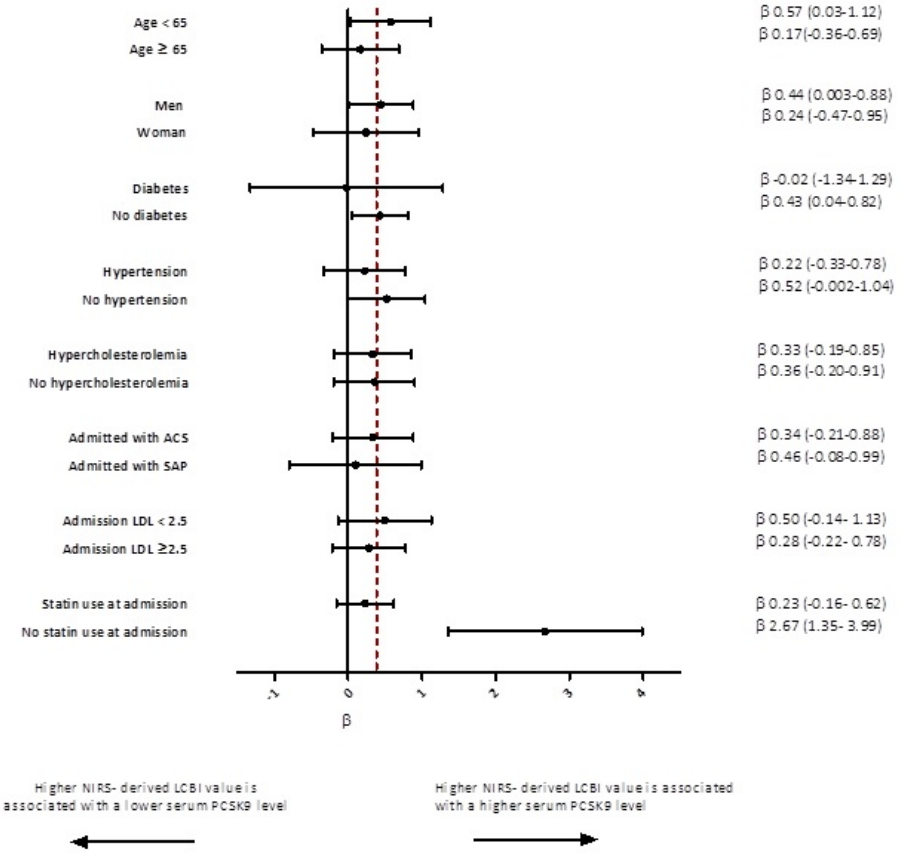

Figure 3. Association between serum PCSK9 concentration and NIRS-derived LCBI stratified by patient subgroups

Beta coefficients (B) indicate the mean (95% confidence interval (CI)) change in ZlnLCBI per unit change in lnPCSK9.

ACS, acute coronary syndrome; CAD, coronary artery disease; LCBI, Lipid Core Burden Index; LDL, low- density lipoprotein cholesterol level; NIRS, Near-infrared spectroscopy; PCSK9, proprotein convertase subtilisin/kexin type 9; SAP, stable angina pectoris.

[95%CI]: 1.53 [1.10-2.12], p=0.011), and 67% higher incidence of death or ACS (HR [95%CI]: 1.67 [1.12-2.50], p=0.013). Results were similar in SAP and ACS patients.

When serum PCSK9 level was analyzed as a continuous variable, and after multivariable adjustment, there was a tendency towards an association between serum PCSK9 levels and MACE (HR: 1.64; per unit increase in lnPCSK9 levels (95%CI [0.99-2.71], p=0.055)) and an association between PCSK9 levels and the composite

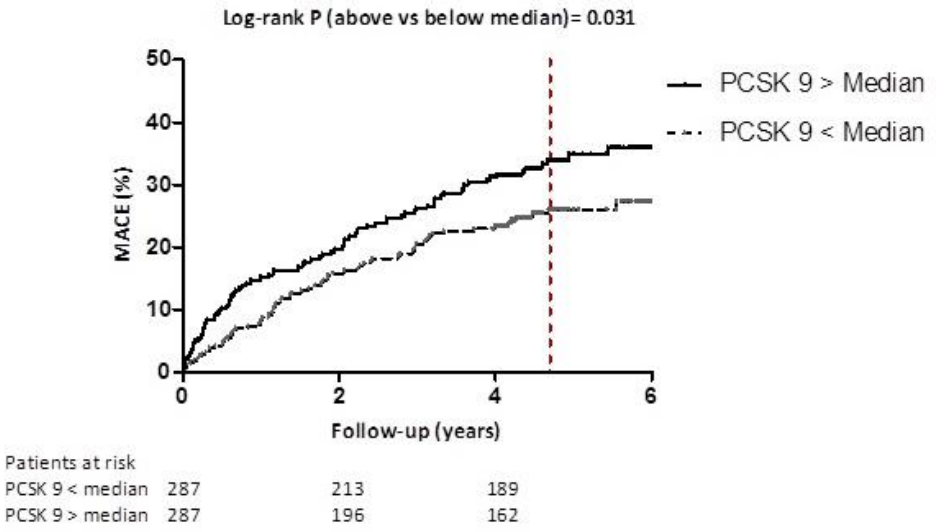

Figure 4. Association of PCSK9 level above vs below the median with clinical outcome in the full cohort (n=576).
MACE, major adverse cardiac events; PCSK9, proprotein convertase subtilisin/kexin type 9.

of death or ACS (HR: 1.88; per unit increase in lnPCSK9 levels 95%CI [1.01-3.51, p=0.047).

DISCUSSION

In patients undergoing CAG because of ACS or SAP, higher serum PCSK9 levels were associated with higher NIRS-derived LCBI. This association was independent of established cardiac risk factors, serum LDL-C level and statin use. Furthermore, serum PCSK9 levels were associated with the incidence of adverse cardiovascular outcomes as long as 4.7 years after the index procedure. Again, this association was independent of cardiac risk factors, serum LDL-C level and statin use.

The PCSK9 enzyme is a member of the proprotein convertase family of proteases, most closely related to proteinase-K. Previous genetic studies have shown that mutations in the PCSK9 gene are associated with either hypercholesterolemia with increased cardiovascular risk (gain-of-function mutations) or with hypocholesterolemia with decreased cardiovascular risk (loss-of-function mutations) (1-3). During the past decade, PCSK9 enzyme has been an intensively studied target for lipid lowering therapy in cardiovascular disease (4, 5, 7). It has been demonstrated that PCSK9 inhibitors suppress serum PCSK9 levels and consistently and substantially

reduce LDL-C levels (7). The FOURIER study showed that inhibition of PCSK9 on a background of statin therapy effectively decreased LDL-C levels and reduced the risk of cardiovascular events during long-term follow-up (8). Moreover, the GLAGOV study demonstrated that addition of PCSK9 inhibitors to stable statin therapy resulted in a greater decrease in plaque burden as assessed by IVUS (6). In our previous study in the current patient population, serum PCSK9 levels were associated with the fraction and amount of necrotic core tissue in coronary atherosclerotic plaques as assessed by IVUS (9). Our current finding that serum PCSK9 level is also associated with NIRS-derived LCBI extends and corroborates our previous findings. This association is independent of serum LDL-C level and statin use, which is important as statin treatment is known to increase PCSK9 levels by a negative feedback mechanism in reaction to lower cholesterol levels (24).

The PCSK9 enzyme induces the LDL-R degradation in the liver, resulting in an increase in circulating serum LDL-C levels that promote atherosclerosis (2). Therefore, it has been increasingly appreciated that PCSK9 plays a key role in the development of atherosclerosis through a lipid pathway. However, our current finding that serum PCSK9 levels were associated with NIRS-derived LCBI, independent of serum LDL-C levels, may also suggest a non-lipid pathway for serum PCSK9 levels in atherosclerosis. In fact, it is well known that inflammatory mechanisms play an important role in the pathophysiology of atherosclerosis and plaque vulnerability by mechanisms that are independent of LDL-C levels (18). In this respect, it is important to note that it has been shown that PCSK9 enzyme positively influences the expression of LOX-1 and mitochondrial reactive oxygen species (msROS), resulting in endothelial inflammation and damage (19, 20). In turn, msROS inhibition reduced the expression of both PCSK9 and LOX-1 (21). LOX-1 is a scavenger receptor in vascular cells and contributes to the development of atherosclerosis via increasing the uptake of oxidized-LDL (oxLDL), a major pro-inflammatory factor in atherosclerosis (20, 21). OxLDL and tumour necrosis factor-α (TNF-α) regulate PCSK9 expression that is mediated by the NF-κB signalling pathway (22). PCSK9 overexpression also upregulates TLR4 expression and promotes the activation of NF-kB (22). The TLR4/NF-kB signalling pathway is critical for atherogenesis since it regulates vascular inflammatory responses (22). PCSK9 may also increase the expression of VCAM-1 and ICAM-1 in endothelian cells and promote the adhesion of circulating inflammatory monocytes to the endothelium (19). Finally, previous studies have also demonstrated that serum PCSK9 levels were independently associated with higher levels of high-sensitivity CRP (hsCRP) and white blood cell count, both markers of

inflammation and mediators of atherosclerosis (23, 24). Our current finding, that serum PCSK9 levels are associated with NIRS-derived LCBI, independent of serum LDL-C levels, indirectly provides further support to these previous observations that serum PCSK9 levels contribute directly to the inflammation in the atherosclerotic plaque and may reflect the vulnerability of the entire coronary tree. Therefore, PCSK9 inhibition may exert its beneficial therapeutic effects not only by means of its LDL-C lowering, but also by its anti-inflammatory properties in CAD.

In prior studies, conflicting results have been observed on the relationship of serum PCSK9 levels with adverse cardiovascular outcome (23, 25-27). Although Gencer et al.(23) did not find a significant association between serum PCSK9 levels and 1-year follow-up, other studies found a significant association of serum PCSK9 levels with adverse cardiovascular outcome (25-27). Clinical studies in patients with CAD on the association of serum PCSK9 levels with cardiovascular outcome during long-term follow-up are scarce. Werner et al. demonstrated in a prospective observational study that serum PCSK9 levels predict cardiovascular outcome during 4-year follow-up in statin treated patients with stable CAD (27). The other studies have mostly used healthy populations during long-term follow-up (25, 26) or are with only short-term follow-up (23).

This study extends our previous 1-year follow-up data on the relationship between serum PCSK9 levels and adverse cardiac outcome in the ATHEROREMO study (9). The current study confirms these previous findings and extents these results to long-term follow-up. The adverse prognostic implications associated with higher PCSK9 levels may reflect the vulnerability of the entire coronary tree through a higher lipid content of coronary plaques as reflected by associated higher LCBI values, but also a more direct role in coronary plaque inflammation. The latter is supported by a recent analysis of the FOURIER study that showed that patients with higher hsCRP levels experienced a greater absolute risk reduction in cardiovascular events with the PCSK9 inhibitor evolocumab (28).

Some limitations of the present study need to be acknowledged. Firstly, by design of the study, repeated blood samples of serum PCSK9 levels and intracoronary NIRS imaging were not performed. Therefore, the effects of changes in serum PCSK9 levels and their effect on NIRS-derived LCBI over time could not be investigated. Secondly, NIRS imaging was limited to a pre-specified target segment of a non-culprit coronary artery. This method was chosen under the hypothesis that such a non-culprit coronary artery segment reflects the vulnerability of the entire coronary tree (14). In fact, we have indirectly supported this hypothesis in our previous studies by

showing that IVUS and NIRS imaging of the composition of coronary atherosclerosis in a non-culprit coronary artery segment predicts adverse outcome throughout the entire coronary tree (9, 12, 13). Finally, the NIRS chemogram only represents plaque information in a 2-dimensional manner and does not provide data on the depth of the cholesterol accumulation within the coronary artery wall. Nevertheless, it has previously been demonstrated that LCBI values obtained in a non-culprit coronary artery segment are strongly and independently associated with increased risk of cardiovascular outcome within the current study population (12, 13).

In conclusion, higher serum PCSK9 levels were associated with a higher NIRS-derived LCBI in a single non-culprit coronary artery segment in patients undergoing CAG because of ACS or SAP. This association was independent of established cardiac risk factors, as well as of serum LDL-C levels and statin use. Furthermore, serum PCSK9 levels were associated with the incidence of adverse cardiovascular outcomes during a median follow-up period of 4.7 years after the index procedure. Again this association was independent of cardiac risk factors, serum LDL-C levels and statin use.

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