PCSK9 in relation to coronary plaque inflammation: Results of the ATHEROREMO-IVUS study

Rohit M. Oemrawsingh*, Jin M. Cheng*, Hector M. Garcia-Garcia, Eric Boersma, Robert-Jan M. van Geuns, Patrick W. Serruys, Isabella Kardys, K. Martijn Akkerhuis

** These authors contributed equally to this work.*

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ABSTRACT

Background and aims: Experimental studies have suggested that proprotein convertase substilisin/kexin type 9 (PCSK9) might directly promote inflammatory processes contributing to atherosclerosis by mechanisms independent of low-density lipoprotein (LDL) cholesterol levels. This study aims to investigate the association between serum PCSK9 levels and the fraction and amount of necrotic core tissue in coronary atherosclerotic plaque as assessed by intravascular ultrasound virtual histology (IVUS-VH) imaging.

Methods: Between 2008 and 2011, IVUS-VH imaging of a non-culprit coronary artery was performed in 581 patients who underwent coronary angiography for acute coronary syndrome (ACS) or stable angina. PCSK9 concentrations were measured in serum samples that were drawn prior to coronary angiography. None of the patients received PCSK9 inhibitors.

Results: After adjustment for established cardiac risk factors, statin use and serum LDL cholesterol, serum PCSK9 levels were linearly associated with the fraction of plaque consisting of necrotic core tissue (β=1.24 percent increase per 100µg/L increase in PCKS9, 95%CI 0.55-1.94, p=0.001) and with the absolute volume of necrotic core tissue (β=0.09, 95%CI 0.01-0.18, p=0.033), but were not significantly associated with plaque burden (p=0.11), plaque volume (p=0.22) or the presence of IVUS-VH-derived thin-cap fibroatheroma lesions (p=1.0).

Conclusion: Serum PCSK9 levels were linearly associated with the fraction and amount of necrotic core tissue in coronary atherosclerosis, independently of serum LDL cholesterol levels and statin use. Therefore, PCSK9 may be an interesting therapeutic target for the treatment of atherosclerotic disease beyond LDL cholesterol regulation.

Key words: PCSK9, atherosclerosis, inflammation, intravascular ultrasound, prognosis

INTRODUCTION

Proprotein convertase substilisin/kexin type 9 (PCSK9) has an important role in the degradation of low-density lipoprotein (LDL) receptors, resulting in increased serum LDL cholesterol concentrations.[1,2] Novel drugs targeting PCSK9 are currently being investigated in large phase II and phase III clinical trials.[2-9] Most of these trials investigate the effects of PCSK9 inhibition on LDL cholesterol reduction. However, recent experimental studies have also demonstrated that PCSK9 might directly promote inflammation, apoptotic cell death and endothelial dysfunction in atherosclerosis by mechanisms that are independent of its effect on the LDL receptor.[2,10-12] Therefore, it has been hypothesized that PCSK9 contributes directly to the progression of atherosclerotic disease, beyond its indirect role in cholesterol homeostasis.[2]

Intravascular ultrasound virtual histology (IVUS-VH) is an in-vivo imaging technique that analyzes radiofrequency backscatter.[13] IVUS-VH imaging allows for accurate measurement of the extent of coronary atherosclerosis and of the type of plaque tissue, including necrotic core tissue which is considered to be a result of continuous inflammation.[13-17] Previous studies have demonstrated that the amount of necrotic core tissue on IVUS-VH is predictive of cardiovascular outcome.[14-16] This study aims to investigate the association between serum PCSK9 levels and the fraction and amount of necrotic core tissue in coronary atherosclerotic plaque as assessed by IVUS-VH imaging.

METHODS

Study population

The design of The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described in detail elsewhere (ClinicalTrials.gov NCT01789411).[14,18] In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) or stable angina pectoris were included. None of the patients were treated with drugs targeting PCSK9 during the study period. This study was approved by the medical ethics committee of Erasmus MC. Written informed consent was obtained from all included patients.

Serum proprotein convertase substilisin/kexin type 9

Blood samples were drawn from the arterial sheath prior to coronary angiography, and were stored at a temperature of −80°C within 2 hours after blood collection. PCSK9 concentrations were measured in the stored serum samples (n=576) using an enzyme-linked immunosorbent assay (Human PCSK9 Quantikine ELISA, R&D Systems Inc., Min-

neapolis, MN, USA). The minimum detectable concentration of this assay was 0.096 µg/L with a coefficient of variation of 4.1% at a mean value of 27.9 µg/L. In 5 patients, serum samples were not available for PCSK9 measurement.

Coronary intravascular ultrasound imaging

Following the standard coronary angiography procedure, IVUS-VH imaging of the most proximal part of a non-culprit coronary artery was performed. Offline analysis of the IVUS-VH images was performed by an independent core laboratory (Cardialysis bv, Rotterdam, the Netherlands) that was blinded for patient characteristics, PCSK9 levels and clinical outcome data. Extent and phenotype of the atherosclerotic plaque were assessed (Figure 1). Plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area. Plaque volume was adjusted for the imaged segment length (adjusted plaque volume = plaque volume / imaged segment length * median segment length in study population). The composition of atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core (Figure 1).[13] The fraction of coronary atherosclerosis consisting of necrotic core tissue was a priori defined as primary outcome measure. A coronary lesion was defined as a segment with a plaque burden of more than 40% in at least 3 consecutive frames. An IVUS-VH-derived TCFA lesion was defined as a lesion with presence of >10% confluent necrotic core in direct contact with the lumen in at least three consecutive frames.[14-16,18-20]

Statistical analysis

The distributions of the continuous variables, including PCSK9 levels and the IVUS-VH parameters, were assessed for normality by visual examination of the histogram. The non-normally distributed variables (i.e. plaque volume and necrotic core volume) were root-transformed. Linear regression analyses were performed to evaluate the associations of serum PCSK9 levels with 1. plaque burden; 2. plaque volume; 3. fraction of plaque consisting of necrotic core tissue; and 4. necrotic core volume. The results are presented as β with 95% confidence interval (95% CI). Logistic regression analyses were performed to evaluate the association between serum PCSK9 levels and the presence of IVUS-VH-derived TCFA lesions. The results are presented as odds ratios (OR) with 95% CI. First, all analyses were performed univariably. In subsequent multivariate analyses, the variables age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, indication for coronary angiography (ACS or stable CAD) and statin use at time of hospital admission were a priori defined as potential confounders. Hereafter, baseline serum LDL cholesterol levels was additionally entered into the model to evaluate whether the associations between PCSK9 and coronary plaque characteristics were independent of serum LDL cholesterol levels. Additionally, stratified analyses were performed to evaluate

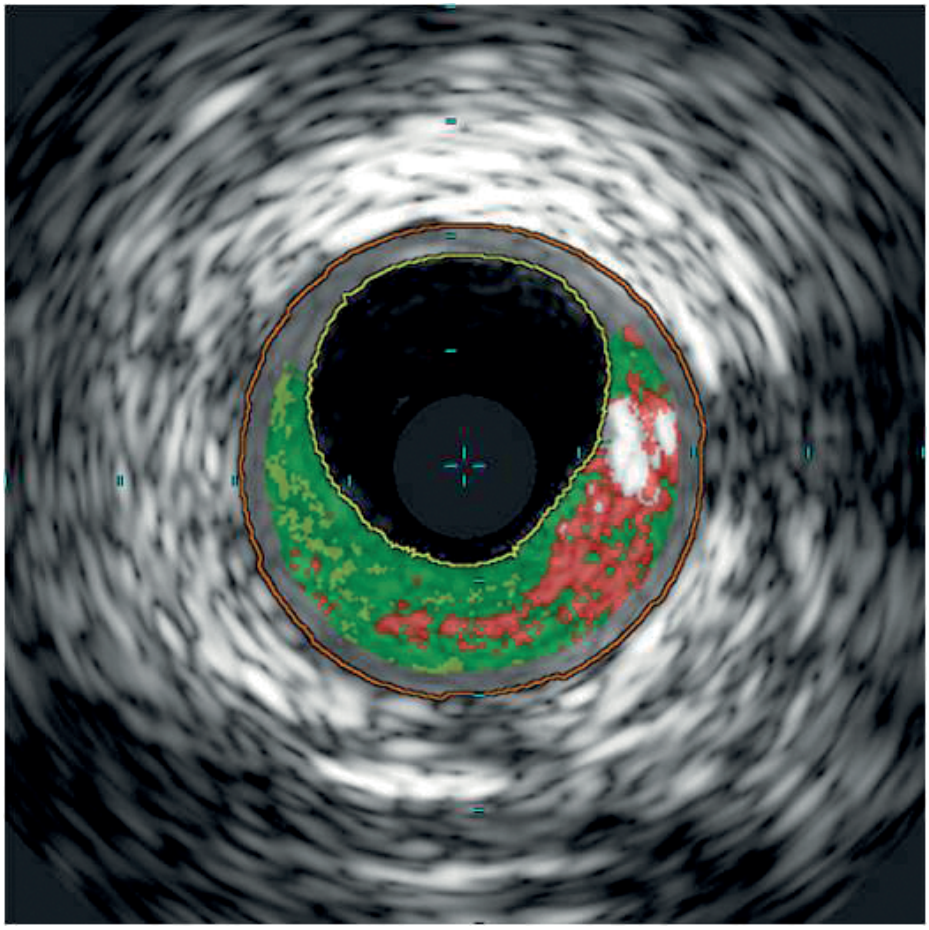

Figure 1. Intravascular ultrasound virtual histology imaging
Intravascular ultrasound virtual histology imaging was used to characterize atherosclerotic plaques into 4 different tissue types: fibrous (dark green), fibro-fatty (light green), dense calcium (white) and necrotic core (red).

whether the observed associations between PCSK9 and coronary plaque characteristics in the total study population were applicable for all patient subgroups (including statin users and non-statin users, as well as patients with low and high LDL cholesterol). All data were analyzed with SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant

RESULTS

Patient characteristics

The patient characteristics are described in Table 1. Median serum PCSK9 level was 270 µg/L and ranged from 91 to 804 µg/L [interquartile range 217-336]. PCSK9 levels were higher in patients with hypertension (median 283 [227-347] versus 255 [215-335] µg/L, p=0.005), hypercholesterolemia (median 281 [228-350} versus 255 [210-328] µg/L, p=0.001), and statin use at time of hospital admission (median 280 [221-342] versus 253 [208-323] µg/L, p=0004). PCSK9 levels did not differ between patients admitted with ACS and patients with stable CAD (p=0.19). The median length of the imaged coronary segment was 44.3 [33.8-55.5] mm. Mean plaque burden in the imaged coronary segment was 38.2 ± 11.5 percent and median plaque volume was 222 [147-326] mm^3. Mean fraction of plaque that consisted of necrotic core was 21.4 ± 8.0 percent and median necrotic core volume was 21.1 [8.6-41.6] mm^3. A total of 241 (42%) patients had at least one IVUS-VH-derived TCFA lesion.

Association between PCSK9 level and coronary plaque characteristics

In univariate analysis, higher serum PCSK9 levels were linearly associated with a higher necrotic core fraction (β = 1.31 percent increase in necrotic core per 100 µg/L increase

Table 1. Baseline characteristics

	n = 576 patients
Patient characteristics	
Age, years	61.5 ± 11.3
Men, n (%)	435 (75.5)
Diabetes Mellitus, n (%)	99 (17.2)
Hypertension, n (%)	300 (52.1)
Hypercholesterolemia, n (%)	320 (55.6)
Current smoking, n (%)	167 (29.0)
Positive family history, n (%)	300 (52.1)
Previous MI, n (%)	183 (31.8)
Previous PCI, n (%)	186 (32.3)
Previous CABG, n (%)	18 (3.1)
Previous stroke, n (%)	26 (4.5)
History of peripheral artery disease, n (%)	36 (6.2)
History of renal insufficiency, n (%)	32 (5.6)
History of heart failure, n (%)	19 (3.3)
Serum LDL cholesterol, mmol/L	2.72 [2.12-3.54]

Table 1. *(continued)*	
Serum PCSK9, µg/L	270 [217-336]
Statin use at time of hospital admission, n (%)	359 (62.3)
Procedural characteristics	
Indication for coronary angiography	
ACS, n (%)	314 (54.5)
ST-elevation MI	164 (28.5)
Non-ST-elevation ACS	150 (26.0)
Stable coronary artery disease, n (%)	262 (45.5)
Number of diseased coronary vessels *	
No significant stenosis, n (%)	42 (7.3)
1-vessel disease, n (%)	306 (53.1)
2-vessel disease, n (%)	167 (29.0)
3-vessel disease, n (%)	61 (10.6)
PCI performed, n (%)	507 (88.0)
IVUS-VH imaging	
Imaged coronary artery	
Left anterior descending, n (%)	207 (35.9)
Left circumflex, n (%)	193 (33.5)
Right coronary artery, n (%)	176 (30.6)
Segment length, mm	44.3 [33.8-55.5]
Plaque burden, %	38.2 ± 11.5
Plaque volume †, mm^3	222 [147-326]
Fibrous tissue fraction, %	57.8 ± 11.6
Fibro-fatty tissue fraction, %	8.9 [5.7-12.6]
Dense calcium fraction, %	9.3 [5.1-15.1]
Necrotic core fraction, %	21.4 ± 8.0
Fibrous tissue volume †, mm^3	56.2 [26.9-95.9]
Fibro-fatty volume †, mm^3	7.7 [3.4-17.2]
Dense calcium volume †, mm^3	8.9 [2.9-20.7]
Necrotic core volume †, mm^3	21.1 [8.6-41.6]
≥1 IVUS-VH-derived TCFA lesion, n (%)	241 (41.8)

Data are presented as mean ± standard deviation or as median [interquartile range].

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

† Adjusted for imaged segment length.

ACS = acute coronary syndrome; CABG = coronary artery bypass grafting; IVUS-VH = intravascular ultrasound virtual histology; LDL = low-density lipoprotein; MI = myocardial infarction; PCI = percutaneous coronary intervention; PCSK9 = proprotein convertase substilisin/kexin type 9; TCFA = thin-cap fibroatheroma.

Table 2. Association between serum PCSK9 level and coronary plaque characteristics

	Unadjusted	P-value	Adjusted for cardiac risk factors + ACS or stable CAD + statin use *	P-value	Adjusted for cardiac risk factors + ACS or stable CAD + statin use + serum LDL *	P-value
Plaque burden †	β 0.62 (−0.36;1.59)	0.22	β 0.77 (−0.19;1.73)	0.12	β 0.78 (−0.19;1.74)	0.11
Plaque volume ‡	β 0.03 (−0.06;0.12)	0.48	β 0.05 (−0.03;0.14)	0.22	β 0.05 (−0.03;0.14)	0.22
Necrotic core fraction §	β 1.31 (0.63;1.99)	<0.001	β 1.22 (0.52;1.91)	0.001	β 1.24 (0.55;1.94)	0.001
Necrotic core volume ||	β 0.08 (−0.01;0.16)	0.075	β 0.09 (0.01;0.18)	0.036	β 0.09 (0.01;0.18)	0.033
≥1 IVUS-VH-derived TCFA lesion	OR 1.01 (0.85-1.20)	0.93	OR 1.00 (0.84-1.20)	0.99	OR 1.00 (0.84-1.20)	1.0

* Cardiac risk factors include: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking. Statin use was registered at the time of hospital admission.
† β (95% confidence interval) is increase in plaque burden (%) per 100 µg/L increase in PCKS9.
‡ β (95% confidence interval) is increase in standard deviations of square root transformed plaque volume (mm^3) per 100 µg/L increase in PCKS9. Plaque volume is adjusted for imaged segment length.
§ β (95% confidence interval) is increase in necrotic core fraction (%) per 100 µg/L increase in PCKS9.
|| β (95% confidence interval) is increase in standard deviations of square root transformed necrotic core volume (mm^3) per 100 µg/L increase in PCKS9. Necrotic core volume is adjusted for imaged segment length.
ACS = acute coronary syndrome; CAD = coronary artery disease; IVUS-VH = intravascular ultrasound virtual histology; LDL = low-density lipoprotein; PCSK9 = proprotein convertase substilisin/kexin type 9; TCFA = thin-cap fibroatheroma.

in PCSK9, 95% CI 0.63;1.99, p<0.001) and tended to be associated with a higher absolute necrotic core volume (β = 0.08, 95%CI −0.01;0.16, p=0.075) (Table 2 and Figure 2). Furthermore, PCSK9 levels were inversely associated with fractions of fibrous tissue (β = −1.45, 95%CI −2.43;−0.47, p=0.004) and fibro-fatty tissue (β = −0.83, 95%CI −1.36;−0.30, p=0.002), and positively associated with dense calcium fraction (β 0.97, 95%CI 0.32;1.62, p=0.004) (Supplemental Figure 1). PCSK9 levels were not associated with overall plaque burden (β = 0.62, 95%CI −0.36;1.59, p=0.22), plaque volume (β = 0.03, 95%CI −0.06;0.12, p=0.48) or the presence of IVUS-VH-derived TCFA lesions (OR 1.01, 95%CI 0.85-1.20, p=0.93). After adjustment in multivariate analysis, PCSK9 levels remained significantly associated with both necrotic core fraction (β = 1.22 percent increase in necrotic core per 100 µg/L increase in PCSK9, 95% CI 0.52-1.91, p<0.001) and absolute necrotic core volume (β = 0.09, 95%CI 0.01;0.18, p=0.036). These associations did not

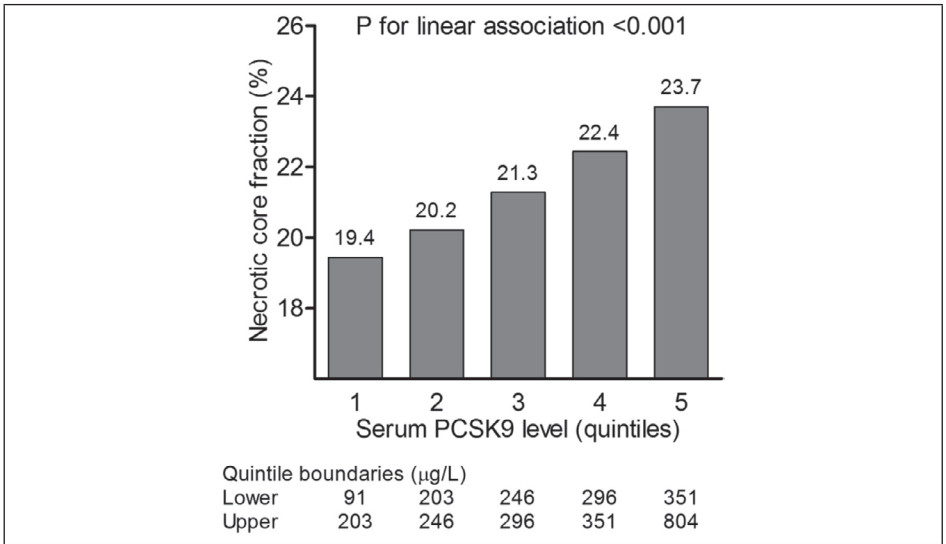

Figure 2. Association between serum PCSK9 level and fraction of coronary plaque that consists of necrotic core tissue

PCSK9, proprotein convertase substilisin/kexin type 9.

change materially after additional adjustment for serum LDL cholesterol levels (Table 2). The full univariate and multivariate predictors of necrotic core fraction are presented in Supplemental Table 1. Subgroup analysis showed that the positive association between serum PCSK9 levels and necrotic core fraction was present in all patient subgroups, including statin users and non-statin users as well as patients with low and high LDL cholesterol. (Figure 3). There was no significant heterogeneity in the β estimate between the evaluated patient subgroups.

PCSK9 level and cardiovascular outcome

Although this study was not primarily designed to investigate the association between serum PCSK9 levels and cardiovascular outcome, 1-year follow-up was available for (99.7%) of patients. A total of 28 patients died or had an ACS (definite culprit lesion-related events were not counted). Serum PCSK9 levels were significantly associated with the composite of death or ACS when PCSK9 was analyzed as a categorical variable (event rate 3.1% in patients with PCSK9 below median versus event rate 6.6% in patients with PCSK9 above median, p=0.049) (Supplemental Figure 2).

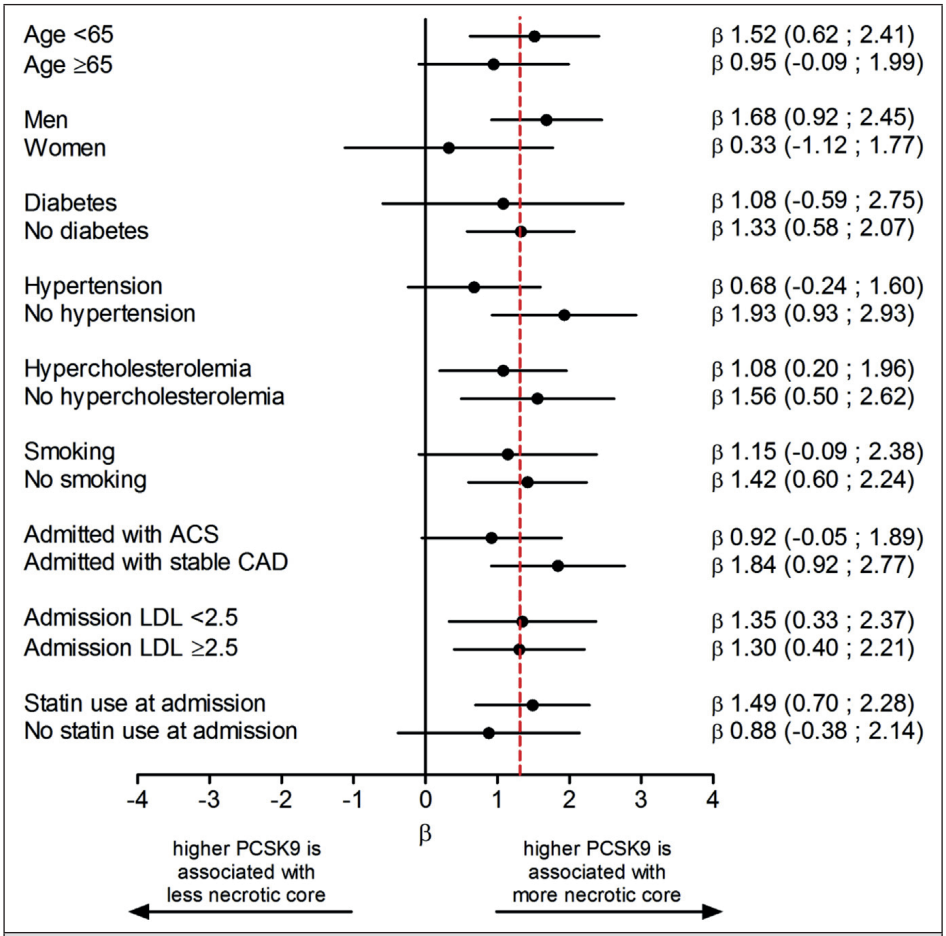

Figure 3. Association between PCSK9 level and necrotic core fraction stratified by patient subgroups

β (95% confidence interval) is increase in necrotic core fraction (%) per 100 μg/L increase in PCKS9. Red dotted line indicates the β estimate in the total study population.

ACS, acute coronary syndrome; CAD, coronary artery disease; LDL, low-density lipoprotein.

DISCUSSION

This study investigated the association of serum PCSK9 levels with the fraction and amount of necrotic core tissue in coronary atherosclerotic plaque as assessed by IVUS-VH imaging in patients with established CAD undergoing coronary angiography. The main finding was that higher serum PCSK9 levels were linearly associated with a higher necrotic core fraction in coronary atherosclerosis. This association was independent of serum LDL cholesterol levels and statin use, and was observed in all patient subgroups, including statin users and non-statin users as well as patients with low and high LDL cho-

lesterol. To the best of our knowledge, this is, as yet, the first study that has investigated the relation between serum PCSK9 levels and atherosclerotic plaque characteristics.

Serum PCSK9 levels vary between individuals.[21] The median PCSK9 level in our patient population with established CAD (270 µg/L) was higher than that in healthy individuals in previously published studies, for example in the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin trial (JUPITER) trial (71 µg/L).[21] Currently, PCSK9 is mostly known for its role in the regulation of cholesterol homeostasis.[2] It enhances the degradation of hepatic LDL receptors, resulting in an increase in LDL cholesterol levels.[2] Gain-of-function mutations in the PCSK9 gene are linked to familiar hypercholesterolemia, while loss-of-function mutations of the PCSK9 gene are linked to low LDL cholesterol levels and low cardiovascular risk, without currently known adverse effects on health.[22-25] Furthermore, statin treatment is known to increase PCSK9 levels by a negative feedback mechanism in response to lower cholesterol levels, making it even more interesting to investigate the effects of new PCSK9 inhibiting drugs on top of statin treatment.[21,26-28] Recent phase II clinical trials have reported promising results on serum LDL cholesterol levels by administration of monoclonal antibodies against PCSK9.[3-9]

Although a previous study did not find a significant association between serum PCSK9 levels and carotid intima-media thickness in healthy men,[29] other studies have suggested that PCSK9 may have a direct role in inflammatory processes contributing to atherosclerotic disease by mechanisms that are independent of LDL cholesterol levels.[2] Recent experimental studies have shown that PCSK9 is expressed in human atherosclerotic plaques.[30] PCSK9 enhances the expression of pro-inflammatory genes through activation of nuclear factor kappa beta (Nf-κB).[10] Inhibition of PCSK9 has been shown to suppress this pro-inflammatory pathway.[10] Furthermore, PCSK9 also targets apolipoprotein E receptor 2, which is a family member of the LDL receptor.[11] Degradation of apolipoprotein E receptor 2 is accompanied with loss of its known anti-inflammatory function.[11,31] Finally, PCSK9 is also associated with increased oxidized LDL-induced apoptosis of human endothelial cells, which may lead to endothelial dysfunction.[12] Inhibition of PCSK9 has been shown to suppress such endothelial apoptosis.[12] Our finding that serum PCSK9 levels were linearly associated with the amount of necrotic core by IVUS-VH imaging, independently of serum LDL cholesterol levels and in all patient subgroups (including statin users and non-statin users as well as patients with low and high LDL cholesterol), supports the hypothesis that PCSK9 has a direct role in plaque inflammation.

Although this study was not primarily designed to investigate the association between serum PCSK9 levels and cardiovascular outcome, a significant association between PCSK9 (below vs. above median level) and 1-year death or ACS was present. Previous studies have demonstrated that the presence of IVUS-VH-derived TCFA lesions and the amount of IVUS-VH-derived necrotic core tissue in coronary atherosclerosis are

both independent predictors of adverse coronary events.[14-16] Rupture of a TCFA lesion is believed to be a major cause of ACS.[32] Although we did not find an association between PCSK9 and the presence of IVUS-VH-derived TCFA lesions, we did find an association with its precursor, namely necrotic core. Plaque erosion due to chronic inflammation is another major cause of ACS.[33] It may be possible that PCSK9 has a role in plaque erosion through its involvement in the pro-inflammatory pathways and endothelial apoptosis as described above. The exact mechanism underlying the relation between PCSK9, the amount of necrotic core tissue and cardiovascular outcome (beyond its role in LDL cholesterol homeostasis) requires further elucidation in future research.

Some limitations of this study need to be acknowledged. Firstly, a single non-culprit coronary vessel was imaged in this study. This approach was eventually chosen to test the hypothesis that the phenotype of a non-culprit artery segment may indicate the patient's systemic atherosclerotic disease burden.[18] This hypothesis is supported by our previous finding that IVUS-VH imaging in only one non-culprit vessel appeared relevant for prognostication.[14] However, necrotic core-rich plaques (e.g. TCFA lesions) elsewhere in the coronary tree (including the culprit lesion) were not assessed in our study. This may have lead to an underestimation of the association between PCSK9 and necrotic core-rich plaques in the coronary tree. Secondly, repeated intracoronary imaging with IVUS-VH was not performed. Therefore, the association between PCSK9 and actual progression of necrotic core tissue and atherosclerotic plaque could not be investigated. Finally, this study was not primarily designed to investigate the association between PCSK9 and clinical outcome, and the number of clinical endpoints was relatively small.

CONCLUSIONS

In patients with established CAD, the range in serum PCSK9 levels is wide. Higher PCSK9 levels were linearly associated with a higher fraction and amount of IVUS-VH-derived necrotic core tissue in coronary atherosclerotic plaque. These associations were independent of serum LDL cholesterol levels and were observed in all patient subgroups, including statin users and non-statin users, as well as patients with low and high LDL cholesterol. Our results support the hypothesis that PCSK9 is directly involved in promoting inflammatory processes contributing to atherosclerosis by mechanisms independent of LDL cholesterol levels. Therefore, PCSK9 may be an interesting therapeutic target for the treatment of atherosclerotic disease beyond LDL cholesterol regulation (i.e. on top of statin treatment). Further research is warranted to investigate the effects of PCSK9 inhibiting therapies on the composition of atherosclerosis and on clinical outcome.

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Conflict of interest

None.

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SUPPLEMENTAL MATERIAL

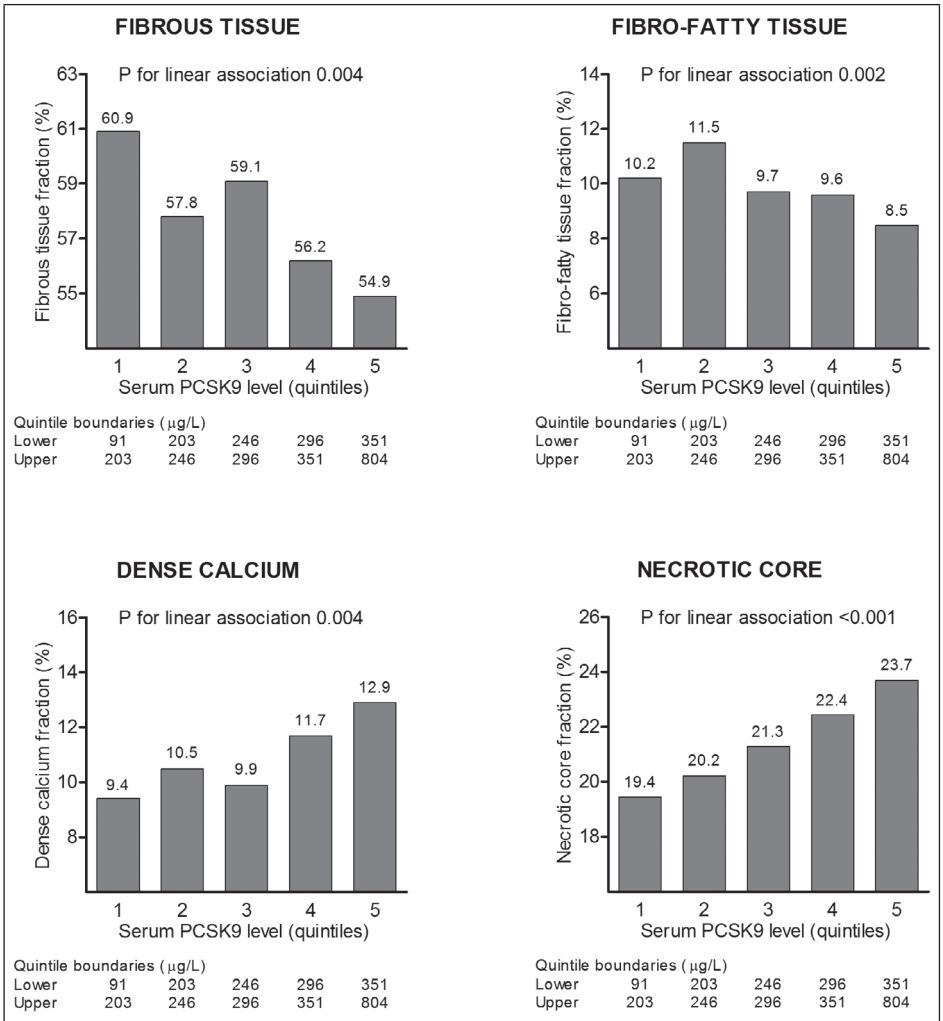
Supplemental Table 1. Predictors of necrotic core fraction

	Unadjusted	P-value	Adjusted*	P-value
Age	β -0.02 (-0.07;0.04)	0.62	β -0.02 (-0.08;0.05)	0.61
Gender (man)	β -0.73 (-2.3;0.79)	0.35	β -0.46 (-2.04;1.12)	0.57
Diabetes mellitus	β -0.86 (-2.60;0.89)	0.34	β -0.61 (-2.42;1.20)	0.51
Hypertension	β 0.60 (-0.72;1.91)	0.37	β 0.57 (-0.91;2.05)	0.45
Hypercholesterolemia	β 0.26 (-1.06;1.58)	0.70	β -0.28 (-1.87;1.31)	0.73
Smoking	β -0.25 (-1.70;1.20)	0.73	β -0.64 (-2.23;0.95)	0.43
ACS (vs stable CAD)	β -0.65 (-1.96;0.67)	0.34	β -0.95 (-2.40;0.50)	0.20
LDL cholesterol	β 0.28 (-0.33;0.90)	0.36	β 0.56 (-0.17-1.28)	0.13
Statin use	β 0.99 (-0.37;2.34)	0.15	β 1.71 (-0.02;3.44)	0.052
PCSK9†	β 1.31 (0.63;1.99)	<0.001	β 1.24 (0.55;1.94)	<0.001

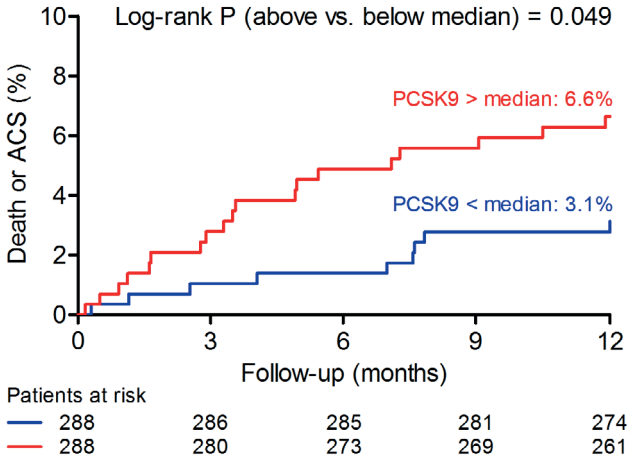
* Full model includes: age, gender, diabetes mellitus, hypertension, hypercholesterolemia, smoking, clinical presentation (ACS or stable CAD), LDL cholesterol, statin use (registered at the time of hospital admission) and PCSK9.

† β (95% confidence interval) is increase in necrotic core fraction (%) per 100 µg/L increase in PCKS9.

ACS = acute coronary syndrome; CAD = coronary artery disease; LDL = low-density 9 = proprotein convertase substilisin/kexin type 9.



Supplemental Figure 1. Association between serum PCSK9 and plaque composition
PCSK9 = proprotein convertase substilisin/kexin type 9.



Supplemental Figure 2. Association of PCSK9 level with clinical outcome
Definite culprit lesion-related events were not counted as endpoint.
ACS = acute coronary syndrome.