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Prognostic value of PCSK9 levels in patients with acute coronary syndromes

Baris Gencer[1]*, Fabrizio Montecucco[1,2], David Nanchen[3], Federico Carbone[1,2], Roland Klingenberg[4], Nicolas Vuilleumier[5], Soheila Aghlmandi[6], Dik Heg[6], Lorenz Räber[7], Reto Auer[3], Peter Jüni[8,9], Stephan Windecker[7], Thomas F. Lüscher[4], Christian M. Matter[4], Nicolas Rodondi[10], and François Mach[1]

[1]Cardiology Division, Geneva University Hospitals, Rue Gabrielle-Perret Gentil 4, Geneva 14 1211, Switzerland; [2]First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa School of Medicine and IRCCS Azienda Ospedaliera Universitaria San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, 6 viale Benedetto XV, 16132 Genoa, Italy; [3]Department of Ambulatory Care and Community Medicine, Lausanne University, Lausanne, Switzerland; [4]Department of Cardiology, University Heart Center, University of Zurich, Zurich, Switzerland; [5]Laboratory Medicine Division, Geneva University Hospitals, Geneva, Switzerland; [6]Institute of Social and Preventive Medicine, Clinical Trials Unit, Department of Clinical Research, University of Bern, Bern, Switzerland; [7]Department of Cardiology, University Hospital of Bern, Bern, Switzerland; [8]Institute of Primary Health Care, University of Bern, Bern, Switzerland; [9]Applied Health Research Centre (AHRC), Li Ka Shing Knowledge Institute of St Michael's Hospital, University of Toronto, Toronto, ON M5S, Canada; and [10]Department of General Internal Medicine, University Hospital of Bern, Bern, Switzerland

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Aims Proprotein convertase subtilisin kexin 9 (PCSK9) is an emerging target for the treatment of hypercholesterolaemia, but the clinical utility of PCSK9 levels to guide treatment is unknown. We aimed to prospectively assess the prognostic value of plasma PCSK9 levels in patients with acute coronary syndromes (ACS).

Methods and results Plasma PCSK9 levels were measured in 2030 ACS patients undergoing coronary angiography in a Swiss prospective cohort. At 1 year, the association between PCSK9 tertiles and all-cause death was assessed adjusting for the Global Registry of Acute Coronary Events (GRACE) variables, as well as the achievement of LDL cholesterol targets of $<$1.8 mmol/L. Patients with higher PCSK9 levels at angiography were more likely to have clinical familial hypercholesterolaemia (rate ratio, RR 1.21, 95% confidence interval, CI 1.09–1.53), be treated with lipid-lowering therapy (RR 1.46, 95% CI 1.30–1.63), present with longer time interval of chest pain (RR 1.29, 95% CI 1.09–1.53) and higher C-reactive protein levels (RR 1.22, 95% CI 1.16–1.30). PCSK9 increased 12–24 h after ACS (374 $\pm$ 149 vs. 323 $\pm$ 134 ng/mL, $P < 0.001$). At 1 year follow-up, HRs for upper vs. lower PCSK9-level tertiles were 1.13 (95% CI 0.69–1.85) for all-cause death and remained similar after adjustment for the GRACE score. Patients with higher PCSK9 levels were less likely to reach the recommended LDL cholesterol targets (RR 0.81, 95% CI 0.66–0.99).

Conclusion In ACS patients, high initial PCSK9 plasma levels were associated with inflammation in the acute phase and hypercholesterolaemia, but did not predict mortality at 1 year.

Keywords Lipids • PCSK9 • Acute coronary syndromes • Cohort studies • Familial hypercholesterolaemia

Introduction

Proprotein convertase subtilisin kexin 9 (PCSK9) has received considerable attention in the last decade as a promising target for lipid-lowering therapy.[1,2] Proprotein convertase subtilisin kexin 9 reduced the uptake of LDL cholesterol by increasing the endosomal and lysosomal degradation of LDL receptor, yielding an increase of circulating LDL.[3] Observational studies have shown that loss-of-function mutations of PCSK9 were associated with low levels of LDL cholesterol and lower risk of coronary heart disease (CHD).[4] Several phase III randomized controlled trials have shown inhibition of PCSK9 using monoclonal antibodies to decrease levels of LDL by 60–70% compared with standard therapy (maximally tolerated doses of statins).[5,6] Recently, *post hoc* analyses of PCSK9

* Corresponding author. Tel: +41 79 553 35 33, Fax: +41 22 372 72 29, Email: baris.gencer@hcuge.ch

inhibitor trials demonstrated up to 50% reduction in major adverse cardiovascular (CV) events in high-risk patients after acute coronary syndromes (ACS) or familial hypercholesterolaemia.[7,8]

We have shown clinical familial hypercholesterolaemia to be highly prevalent among patients with ACS (20%), and LDL cholesterol to be poorly controlled 1 year after ACS in this group.[9,10] Only one-third of patients reached the long-term recommended target for LDL cholesterol of $\leq$1.8 mmol/L, although most were on high-intensity statin therapy.[9–11] Among the potential mechanisms leading to statin resistance is the up-regulation of PCSK9 levels at therapy initiation.[12] Moreover, myocardial infarction (MI) causes a rapid decline in LDL cholesterol levels, making their interpretation 24 h post-MI be unreliable for the steady-state levels.[13] One study reported PCSK9 levels to be predictive of CV events in patients with stable CHD patients and receiving statin treatment for up to 4 years' follow-up.[14] Measuring circulating plasma PCSK9 levels in humans may therefore be clinically relevant in secondary prevention to identify patients at risk of CV events. However, the prognostic value of PCSK9 plasma levels assessed during the acute phase of ACS remains undetermined.

Thus, we hypothesized that higher PCSK9 levels would be associated with pre-specified baseline characteristics of patients, such as clinical familial hypercholesterolaemia, inflammation, and chest pain duration. Thus, using a large Swiss multicentre cohort of patients hospitalized with ACS, we aimed to assess the association between PCSK9 plasma levels measured at coronary angiography with 1-year all-cause or cardiac death. Moreover, we assessed the association between PCSK9 plasma levels and the achievement of recommended LDL cholesterol targets 1 year after the index event.

Methods

Study population

The Special Program University Medicine Acute Coronary Syndromes and Inflammation (SPUM-ACS) supported by the Swiss National Science Foundation was established by four Swiss university hospitals (Bern, Geneva, Lausanne, and Zurich) to prospectively recruit and analyse a real-life cohort of patients with ACS (NCT01000701). Two thousand one hundred sixty eight consecutive patients with a diagnosis of ACS that underwent coronary angiography were enrolled between December 2009 and October 2012.[15] Inclusion criteria were: subjects aged $\geq$18 years presenting within 5 days (preferably within 72 h) after pain onset with a main diagnosis of non-ST elevation myocardial infarction (NSTEMI), ST-elevation myocardial infarction (STEMI), or unstable angina. Enrolled patients had symptoms compatible with angina pectoris (chest pain, dyspnoea) and at least one of the following criteria: (i) ECG ischaemic changes such as persistent or dynamic ST-segment deviation, T-waves inversion, new left bundle branch block; (ii) evidence of positive conventional or high-sensitive troponin by local laboratory reference values with a rise and/or fall in enzyme levels; (iii) known CHD defined by pre-existing MI, coronary artery bypass graft (CABG), or percutaneous coronary intervention or documented $\geq$50% stenosis of coronary artery in a previous angiography.[16] Exclusion criteria comprised severe physical disability, inability to give consent (dementia), and life expectancy $<$1 year (for non-cardiac reason). The protocol was approved by the local institutional review board and all study participants gave written informed consent.

Familial hypercholesterolaemia classification

Based on a previous study, we used the validated Dutch Lipid Clinic Network algorithm for familial hypercholesterolaemia classification.[10] In this algorithm, familial hypercholesterolaemia was considered possible when scoring 3–5 points, probable for 6–8 points and definite for $>$8 points.[17] The diagnosis of possible familial hypercholesterolaemia required both an elevated LDL cholesterol $>$4.9 mmol/L along with family or personal history of premature atherosclerosis.[17] Lipid-lowering therapy was included use of statins, fibrates, niacin, ezetimibe, or resins.

Biomarker measurements

Blood was drawn from the arterial sheath at coronary angiography (time-point 1, [T1]) and centrifuged at 2700 $\times$ *g* for 10 min at room temperature to obtain serum, and then frozen and stored in aliquots at -80°C. Additional blood sampling with venous puncture was performed at time-point (T2) 12–24 h after ACS, as well as 1 year after ACS at time-point (T3). Aliquots were measured blinded to the patients' data by means of numbered codes and merged with the clinical dataset. PCSK9 was measured in one serum aliquot from each patient using EDTA plasma by colorimetric enzyme-linked immunosorbent assay from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. The minimal limit of detection was 0.625 ng/mL, the mean intra- and inter-assay coefficient of variation was at the accepted threshold of $<$8%. Cardiac troponin T (hs-TnT), N-terminal-pro B-type natriuretic peptide (NT-proBNP), high-sensitive C-reactive protein (hs-CRP) were measured by a high-sensitive electrochemiluminescence immunoassay in one serum aliquot from each patient on a cobase 602® reader (all Roche Diagnostics, Mannheim, Germany) with assay characteristics as reported by the manufacturer at the Zürich Core Laboratory.

Study endpoints

Participants were first followed up by telephone by a trained study nurse at 30 days post-ACS, and again at 1 year post-ACS to attend a clinical visit. If patients were unable to attend the clinic visit, follow-up was performed in the following order: (i) phone call, (ii) postal mail or email, (iii) through family members, or (iv) via primary care physician or cardiologist. The primary endpoint comprised the composite of all-cause death (cardiac, vascular, and non-CV) as done in previous publication in the ACS patients.[18] In secondary analyses, other major adverse CV events comprised recurrence of MI,[16] ischaemic stroke (including transient ischaemic attack) and clinically indicated coronary revascularization. Clinical endpoints were adjudicated by a panel of independent experts (three certified cardiologists) blinded to the results of PCSK9. A subgroup of patients of the SPUM-ACS had LDL cholesterol measurement performed at 1-year follow-up visit.

Statistical methods

The data were expressed as medians $\pm$ interquartile range for continuous variables and for categorical variables as numbers and percentages. The correlation between PCSK9 and other biomarkers was evaluated by nonparametric test (Spearman rank correlation). Paired *t*-test was used to assess changes of PCSK9 levels between T1 and T2, as well as between T1 and T3. Continuous PCSK9 levels were categorized into tertiles to assess the association with clinical outcomes as done in previous publication.[18] Generalized linear regression with a log-link function and Poisson pseudo-maximum likelihood estimator was used in a multivariable model to assess the association between elevated PCSK9 levels, defined by upper PCSK9 tertile and compared with lower tertile, with covariates of interest: chest pain onset, familial

hypercholesterolaemia classification (dichotomized), use of lipid-lowering therapy, hs-CRP, and lipid levels. Continuous variables (hs-CRP and triglycerides) were divided by two times its standard deviation to scaling the regression inputs.[19] Based on this analysis, rate ratio (RR) and the corresponding 95% confidence intervals (CIs) were presented using forest plots.[20] Given that familial hypercholesterolaemia classification was based on age, gender, and history of MI, those variables were not included in the model. Time-to-first event or composite events were analysed censoring patients at 365 days, at death, or last valid contact date. The univariable association of tertiles of PCSK9 with all-cause and cardiac death was evaluated using Cox proportional hazards models and expressed with hazard ratios (HRs) and 95% CI. To increase statistical power, we additionally considered continuous PCSK9 concentrations, estimating the effect on clinical outcome per 1 SD increase. Effect estimates were presented as HR and 95% confidence interval (95% CI). In sensitivity analyses, we used restricted cubic splines to detect a possible non-linear association with three pre-specified knots located at the 10th, 50th, and 90th percentiles.[18] In the multivariable model, we adjusted for the long-term Global Registry of Acute Coronary Events (GRACE) score variables (age, heart rate, systolic blood pressure, initial serum creatinine, Killip class, cardiac arrest at admission, elevated cardic markers, and ST-segment deviation).[21] Further adjustment was performed for CV risk factors such as gender, history of diabetes, history of hypertension, smoking, and total cholesterol. To analyse the incremental value of adding continuous PCSK9 variable to the recommended GRACE score mortality risk prediction model, we calculated C-index and integrated discrimination improvement (IDI).[22] Two GRACE scores were calculated for each patient: (1) in-hospital GRACE score[23] used for the 30 days outcomes analysis (referred to as "GRACE 30 days"); (2) and 6 months GRACE score[24] used for the 1 year outcomes analyses (referred to as "GRACE 1 year"). The association between tertiles of PCSK9 levels and the achievement of LDL cholesterol target was expressed using RR and 95% CI and adjusted for previously published predictors [age, sex, body mass index (BMI), LDL cholesterol, lipid-lowering therapy, history of diabetes, history of MI, and attendance to cardiac rehabilitation].[9] All hypothesis tests were two sided and the significance level set at 5%. Statistical analyses were performed using STATA software® (Version 13, STATA Corp, College Station, TX, USA).

Results

Patient characteristics

Among the 2168 patients recruited, 2030 had available data for PCSK9 measurements at baseline (T1), 1665 at T2 (12–24 h after angiography for ACS) and 1171 at T3 (1 year after ACS) (*Figure 1*). Mean age was 63.6 ± 12.5 years old, 14.5% had a history of MI, 30.0% received statins and 2.2% were on other lipid-lowering therapy. Using the Dutch Lipid Clinic Network algorithm, 18.1% of patients were classified as possible familial hypercholesterolaemia and 1.6% as probable or definite familial hypercholesterolaemia (*Table 1*). In total, 66.9% of patients were admitted to the catheterization lab within 24 h after chest pain onset, 24.5% between 24–72 h and 8.5% beyond 72 h.

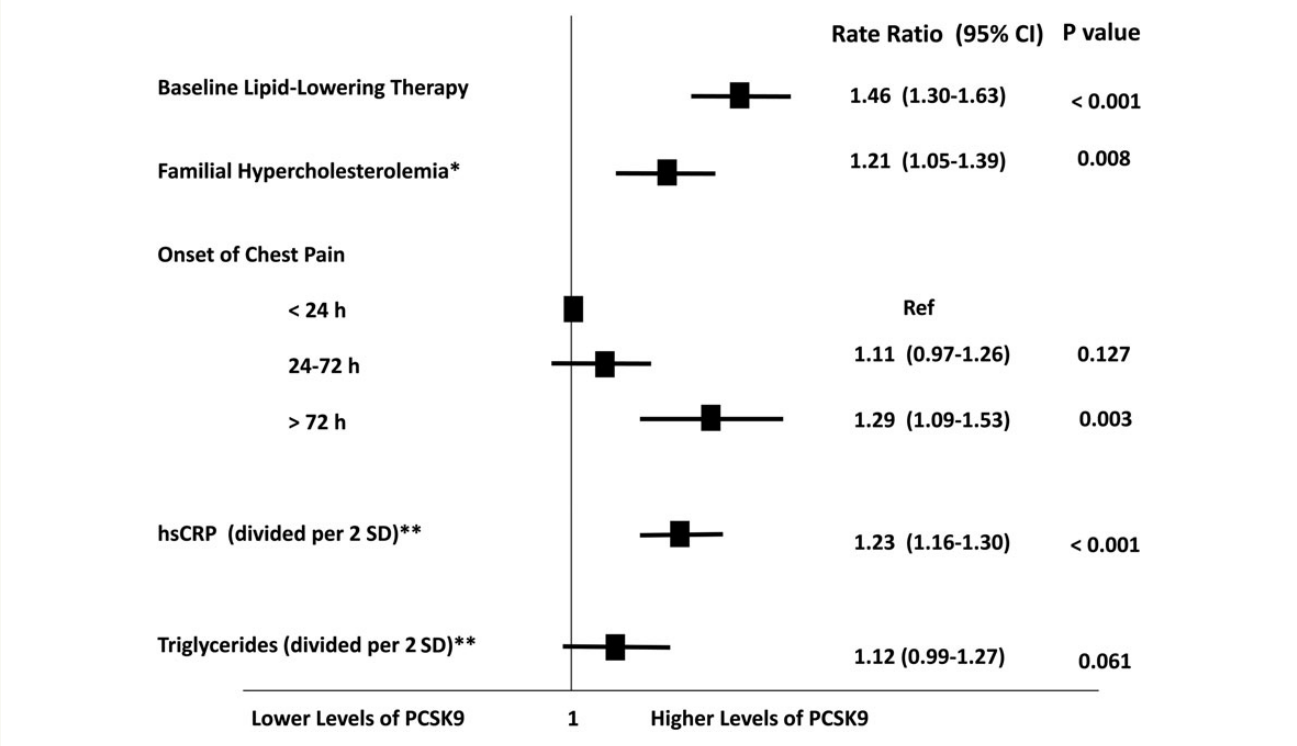

Figure 1 Factors associated with elevated proprotein convertase subtilisin kexin 9 plasma levels (upper tertile compared with lower tertile) in patients with acute coronary syndromes undergoing coronary angiography. *The definition of familial hypercholesterolaemia was based on the Dutch Clinical network algorithm.[10] Possible, probable, and definite familial hypercholesterolaemia was merged ($n = 393$) for this category. **Continuous variables were divided by 2 standard deviation as done in a previous publication[19] and presented using forest plots.[20] hs-CRP, high-sensitive C-reactive protein; PCSK9, proprotein convertase subtilisin kexin 9.

Table 1 Baseline characteristics (*N* = 2030)

Variables	Value
Demographics	
Age (years), mean ± SD	63.6 ± 12.5
Women, *n* (%)	529 (21.1)
BMI (kg/m^2), mean ± SD	27.1 ± 4.3
Clinical variables	
Familial hypercholesterolaemia[a], *n* (%)	
Possible, *n* (%)	361 (18.1)
Probable, *n* (%)	32 (1.6)
Familial history of CAD[b], *n* (%)	517 (26.0)
History of hypertension[c], *n* (%)	851 (41.9)
History of diabetes[d], *n* (%)	370 (18.2)
History of MI, *n* (%)	294 (14.5)
Current smoker, *n* (%)	810 (40.6)
ACS diagnosis	
STEMI, *n* (%)	1073 (52.9)
NSTEMI, *n* (%)	872 (43.0)
Unstable angina, *n* (%)	83 (4.1)
Chest pain onset	
<24 h, *n* (%)	1348 (66.9)
24–72 h, *n* (%)	494 (24.5)
>72 h, *n* (%)	172 (8.5)
Killip at presentation	
Class I, *n* (%)	1756 (89.3)
Classes II–IV, *n* (%)	211 (10.7)
Fasting plasma glucose, mean (±SD)	7.1 (±2.9)
eGFR, mean (±SD)	90.9 ± 27.5
Index procedure	
Stenting	1756 (92.2)
Ballooning	202 (10.6)
CABG	75 (3.9)
Baseline medication	
Aspirin, *n* (%)	650 (32.2)
Statin, *n* (%)	604 (30.0)
Other lipid-lowering therapy ‖, *n* (%)	43 (2.1)
β-Blockers, *n* (%)	506 (25.1)
ACEI/ARB, *n* (%)	713 (35.4)

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery by-pass grafting; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate using the MDRD formula; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; STEMI, ST-elevation myocardial infarction; TIA, transient ischaemic attack. Missing values: 20 for BMI, 9 for LVEF, 39 for family history of CAD, 2 for history of MI, 33 for smoking, 2 for ACS diagnosis, 16 for chest pain onset, 63 for Killip and 129 for fasting plasma glucose. Ten for aspirin, 13 for statin or other lipid-lowering therapy, 16 for β-blockers, 18 for ACEI/ARB, 16 for CCB, and 15 for diuretic.
[a]Diagnosis of familial hypercholesterolaemia was based on the Dutch Lipid Clinic Network algorithm. A possible diagnosis was considered when the score was 3–5, and a probable/definite FH when the score was 6 or higher.
[b]Based on a cardiovascular event in a brother or father younger than 55 years old, or a mother or sister younger than 60 years old.
[c]Defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or use of blood pressure lowering drugs.
[d]Based on patients' self-report or use of anti-diabetic therapy. Other lipid-lowering therapy comprised fibrates, niacin, ezetimibe, and resins.

Table 2 Correlations between proprotein convertase subtilisin kexin 9 and other biomarkers

	All ACS patients *N* = 2030		After excluding lipid-lowering therapy[a] *N* = 1417	
Biomarkers	Rho[b]	*P*-value	Rho[b]	*P*-value
hsTnT (μg/L)	−0.032	0.172	−0.013	0.657
CK (U/L)	−0.061	0.010	−0.041	0.151
NT-proBNP (ng/L)	0.027	0.252	0.009	0.761
Hs-CRP (mg/L)	0.038	0.104	0.077	0.006
Total cholesterol (mmol/)	0.031	0.173	0.143	<0.001
LDL cholesterol (mmol/L)	−0.015	0.511	0.106	<0.001
HDL cholesterol (mmol/L)	0.030	0.179	0.038	0.158
Triglycerides (mmol/L)	0.092	<0.001	0.106	<0.001

Missing values: 203 for hsTnT, 212 for hs-CRP, 203 for NT-proBNP, 264 for CK, 55 for cholesterol, 80 for LDL cholesterol, 76 for HDL cholesterol, and 65 for triglycerides.
CK, creatinine kinase; HDL, high-density lipoprotein; hs-CRP, high-sensitive-C-reactive-protein; hsTnT, high-sensitive troponin T; LDL, low-density lipoprotein; NT-proBNP, N-terminal-pro B-type natriuretic peptide; PCSK9, proprotein convertase subtilsin kexin 9.
[a]Lipid-lowering therapy included statin, ezetimibe, niacine, fibrates, and resins.
[b]Spearman's rank correlation rho for continuous variables; rho = 1 denotes perfect positive correlation; rho = −1 denotes perfect negative correlation, and rho = 0 absent correlation.

Correlation of plasma proprotein convertase subtilisin kexin 9 levels with other biomarkers

Proprotein convertase subtilisin kexin 9 levels were correlated with other lipid markers, such as total cholesterol, LDL cholesterol, and triglycerides, especially after excluding patients on lipid-lowering therapy ($P < 0.001$, *Table 2*). An association was also observed with hs-CRP ($r = 0.077$, $P = 0.006$), but not with other cardiac markers, such as hs-TnT, NT-proBNP, and creatinine kinase (CK).

Clinical factors associated with high plasma proprotein convertase subtilisin kexin 9 levels and proprotein convertase subtilisin kexin 9 kinetics

Mean values of PCSK9 were 203.3 ± 40.4 in the lower tertile, 302 ± 27.4 in the middle tertile, and 477.6 ± 134.4 in the upper tertile. In our multivariable model, the use of lipid-lowering therapy prior ACS (RR 1.46, 95% CI 1.30–1.63), the presence of familial hypercholesterolaemia (RR 1.21, 95% CI 1.05–1.39), longer chest pain onset (RR 1.29, 95% CI 1.09–1.53), higher triglycerides levels, and higher hs-CRP levels were all independently associated with the highest tertile of PCSK9 levels at coronary angiography for ACS (*Figure 2*). Similar association were found when PCSK9 levels were considered as a continuous variable (see Supplementary material online, *Table S1*). We found a significant increase in mean PCSK9 levels over time at the three specified time-points (*Figure 3*). Mean PCSK9 levels were 323 ± 134 ng/mL at T1, 374 ± 149 ng/mL

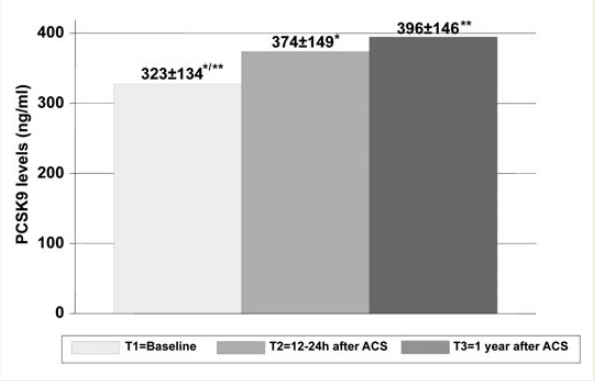

Figure 2 Changes in the mean of proprotein convertase subtilisin kexin 9 levels over time: (1) T1 at baseline, (2) T2 12–24 h and (3) T3 1 year after angiography for acute coronary syndromes. *P-value using paired t-test was <0.001 with available data for 1665 participants for T1 and T2. **P-value using paired t-test was <0.001 with available data for 1171 participants for T1 and T3. PCSK9, proprotein convertase subtilisin kexin 9.

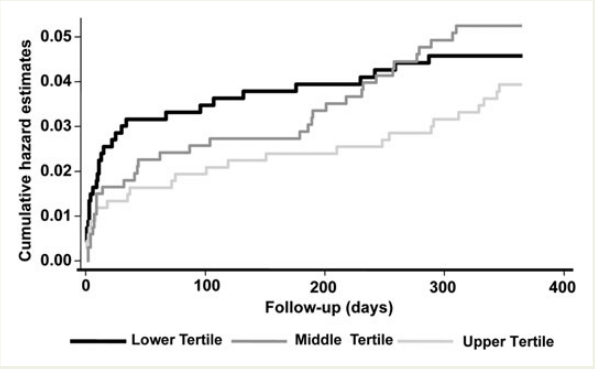

Figure 3 Cumulative hazards for all-cause death according to the proprotein convertase subtilisin kexin 9 tertiles in patients with acute coronary syndromes undergoing coronary angiography.

at T2, and 396 ± 146 ng/mL at T3 with significant paired P-values while comparing T1 and T2 ($P < 0.001$), as well T1 and T3 ($P < 0.001$).

Association between proprotein convertase subtilisin kexin 9 plasma levels and 1-year all-cause or cardiac death

No significant association was found between PCKS9 tertiles at coronary angiography for ACS and 1-year all-cause death (*Figure 3*). Thirty ACS patients died in the lowest tertile (4.4%), 26 in the middle tertile (3.8%), and 34 in the highest tertile (5.0%, *Table 3*) Comparing the highest vs. the lowest PCSK9 tertile, crude HR was 1.08 (95% CI 0.60–1.85), similar to HR adjusted for the GRACE score (HR 1.08, 95% CI 0.65–1.85) and HR adjusted for CV risk factors (HR 1.33, 95% CI 0.75–2.34). Results were similar when considering PCSK9 levels as a continuous variable or as a restricted cubic spline. After excluding patients treated with lipid-lowering therapy prior to ACS, results remained similar (HR 1.43, 95% CI 0.71–2.86 for the model adjusted for the GRACE score variables and traditional risk factors), as well as after excluding those with familial hypercholesterolaemia. The incremental value of the addition of PCSK9 levels to the 1-year GRACE score prediction model was not significant (IDI 0.0005, $P = 0.606$, *Table 4*). Results were similar when censoring all-cause death at 30 days (see Supplementary material online, *Table S2*), IDI was 0.009, $P = 0.177$. The association between higher PCSK9 levels and cardiac death at 1-year was not significant (see Supplementary material online, *Table S3*): crude HR 0.85 (95% CI 0.45–1.63), HR adjusted for the GRACE score (HR 0.84, 95% CI 0.41–1.72), and HR adjusted for the GRACE score and traditional risk factors (95% CI 0.93, 95% CI 0.43–2.01). No significant association was found between the highest PCSK9 tertile and major adverse CV events: crude HR was 0.75 (95% CI 0.52–1.08) for the association with cardiac death or recurrent MI or stroke events, while HR was 0.88 (95% CI 0.63–1.22) for the association with cardiac death or recurrent MI or coronary revascularization events (see Supplementary material online, *Tables S4–S6*).

Association between proprotein convertase subtilisin kexin 9 plasma levels and 1-year LDL cholesterol target achievement

A total of 35.1% of patients reached the recommended target of LCL cholesterol <1.8 mmol/L 1 year post-ACS. Proprotein convertase subtilisin kexin 9 levels were significantly higher in those patients who did not reach the recommended target (355.0 ± 146.1 vs. 328.8 ± 146.1, $P = 0.008$, see Supplementary material online, *Table S7*). In the multivariable logistic regression model, the association between the highest tertile of PCKS9 levels and non-achievement of LDL target (OR 0.81, 95% CI 0.66–0.99) persisted when adjusted for known predictors, such as age, gender, BMI, baseline LDL cholesterol, use of lipid-lowering therapy, history of diabetes, history of MI, or attendance to cardiac rehabilitation (*Table 5*). We found a similar association when we selected an alternate recommended LDL-C target of ≥50% decrease (OR 0.72, 95% CI 0.54–0.96, $P = 0.023$, see Supplementary material online, *Table S8*).

Discussion

This large multicentre prospective cohort study of patients with ACS adds new evidence on the prognostic role of circulating PCSK9 in patients with ACS: (i) patients with higher PCSK9 levels at coronary angiography were less likely to reach the recommended target of LDL cholesterol <1.8 mmol (<70 mg/dL) at 1 year, independently of baseline LDL cholesterol levels. (ii) Proprotein convertase subtilisin kexin 9 levels obtained at the time of coronary angiography were not significantly associated with all-cause or cardiac mortality at 1 year. (iii) Higher PCSK9 levels were associated with a higher degree of inflammation as measured by hs-CRP, but not with cardiac biomarkers, such as hsTnT or CK. (iv) Patients with ACS who underwent coronary angiography 72 h after chest pain onset presented higher PCSK9 levels. (v) Patients who had usual statin therapy prior to the index ACS event had significantly

Table 3 Association between proprotein convertase subtilisin kexin 9 tertiles and all-cause death at 1 year

	Per SD increase	1st Tertile	2nd Tertile	3rd Tertile	*P* for trend
Hazard ratios (95% CI) in all ACS patients ($N = 2030$)					
Number events/participants		30/676	26/679	34/675	
Crude	0.98 (0.79–1.21)	1 (Ref)	0.85 (0.50–1.43)	1.13 (0.69–1.85)	0.547
Adjusted for GRACE score	0.98 (0.78–1.24)	1 (Ref)	0.95 (0.54–1.67)	1.21 (0.71–2.05)	0.659
Adjusted for GRACE score + CVRF	1.02 (0.81–1.29)	1 (Ref)	0.80 (0.43–1.50)	1.41 (0.81–2.46)	0.180
Hazard ratios (95% CI) after excluding lipid-lowering therapy[a] ($N = 1407$)					
Number events/participants		20/473	17/472	23/472	
Crude	1.02 (0.79–1.30)	1 (Ref)	0.84 (0.44–1.60)	1.16 (0.63–2.10)	0.604
Adjusted for GRACE score	1.03 (0.79–1.34)	1 (Ref)	1.01 (0.50–2.05)	1.31 (0.68–2.52)	0.651
Adjusted for GRACE score + CVRF	1.05 (0.81–1.36)	1 (Ref)	0.91 (0.42–1.96)	1.49 (0.76–2.94)	0.339

CIs, confidence intervals; CVRF, cardiovascular risk factor (sex, history of hypertension, history of diabetes, smoking status, baseline total cholesterol, and baseline use of statin). GRACE, Global Registry of Acute Coronary Events (ST segment deviation, age, heart rate, systolic blood pressure, creatinine, Killip class, cardiac arrest at admission, and elevated biomarkers of necrosis); PCSK9, proprotein convertase subtilisin kexin 9.
[a]Lipid-lowering therapy included statin, ezetimibe, niacine, fibrates, and resins.

Table 4 Relative contribution of proprotein convertase subtilsin kexin 9 levels to the Global Registry of Acute Coronary Events prediction model for all-cause death at 30 days and at 1 year among 2030 patients with acute coronary syndromes

Model	Model χ^2	LR *P*-value	C-Index[a]	IDI (*P*-value)
GRACE 30 days	84.5	0.032	0.886	0.009 ($P = 0.177$)
+PCSK9	89.1		0.881	
GRACE 1 year	107.6	0.361	0.785	0.0005 ($P = 0.606$)
+PCSK9	108.4		0.784	

GRACE, Global Registry of Acute Coronary Events; IDI, integrated discrimination improvement; PCSK9, proprotein convertase subtilsin kexin 9. GRACE score variables are ST segment deviation, age, heart rate, systolic blood pressure, creatinine, Killip class, cardiac arrest at admission, and elevated biomarkers of necrosis.
[a]The C-index is an adaptation of the C statistic or area under the ROC curve used with logistic regression models. Higher values indicate better discrimination.

Table 5 Associated factors with 1 year LDL cholesterol target achievement (<1.8 mmol/L)

Characteristics	Rate ratios (95% CI)	Multivariate[a] *P*-value
Upper vs. lower PCSK9 tertile (ng/mL)	0.81 (0.66–0.99)	0.037
Age (per year)	1.01 (1.01–1.02)	<0.001
BMI (per 5 kg/m^2)	1.09 (0.99–1.19)	0.091
LDL cholesterol (per 1 mmol/L)	0.67 (0.60–0.73)	<0.001
Women	0.83 (0.65–1.06)	0.144
On lipid-lowering therapy, *n* (%)	0.99 (0.81–1.21)	0.941
History of diabetes, *n* (%)	1.24 (1.02–1.51)	0.032
History of MI, *n* (%)	0.68 (0.51–0.90)	0.007
Attendance to CR, *n* (%)	1.27 (1.05–1.54)	0.014

BMI, body mass index; CIs, confidence intervals; CR, cardiac rehabilitation; LDL, low-density lipoprotein; MI, myocardial infarction; PCSK9, proprotein convertase subtilisin kexin 9.
[a]Multivariate model included age, sex BMI, LDL cholesterol, lipid-lowering therapy, history of diabetes, history of MI, and attendance to CR.

higher PCSK9 levels when compared with patients untreated with statins. (vi) Proprotein convertase subtilisin kexin 9 levels increased 12–24 h after angiography for ACS suggesting a possible upregulation of PCSK9 levels in the acute context of ACS.

Our study is the first one to evaluate PCSK9 levels just in patients with ACS undergoing coronary angiography. Our findings indicate that assessing PCSK9 levels in the ACS setting is likely to be devoid of clinical utility in terms risk stratification to avoid recurrence of adverse event. Because *in vivo* models demonstrated that hepatic PCSK9 expression is enhanced in the context of MI and inflammation,[25,26] we cannot exclude that the blood sampling in the acute clinical setting could have biased the prognostic value of PCSK9 levels. Nevertheless, as it was previously shown in 504 stable CHD patients under statin therapy that high PCSK9 levels were no longer predictive of CV complications when adjusted for triglyceride levels.[14] Furthermore, clinical data indicate that LDL cholesterol may not be accurate in predicting CV events during the acute phase of inflammation.[13] For these reasons, it still remains uncertain how the timing of blood sampling could represent a key pre-analytical factor for PCSK9 levels. Furthermore, as the present study confirms our previous observation, indicating that PCSK9 levels are higher in

patients classified as having familial hypercholesterolaemia compared with no familial hypercholesterolaemia,[10] we cannot exclude that assessing PCSK9 could represent an additional tool for detection for familial hypercholesterolaemia in top of conventional lipid and genetic profiling. This hypothesis requires further investigation.

Another interesting finding is the impact of lipid-lowering therapy on PCSK9 levels. If experimental studies among humans showed that the use of statins is associated with an elevation in PCSK9 levels,[12,27,28] the impact of PCSK9 levels on the effects of statin therapy has been poorly described.[29] In the present study, we observed that higher levels of PCSK9 levels at coronary angiography were associated with poorer controlled LDL cholesterol levels 1-year post-ACS, after adjustment for baseline LDL cholesterol levels and use of lipid-lowering therapy. Our data suggest that circulating PCSK9 levels are predictors for the achievement of LDL cholesterol targets post-ACS, independently of the use of lipid-lowering therapy. We have previously shown that PCSK9 levels were correlated to physical activity in healthy volunteers, and could be used as a marker of preventive interventions.[30] However, it is a source of controversy whether PCSK9 can give additional information beyond current available risk stratification post-ACS to become an independent marker for clinical outcomes.[31]

Limitations

First, blood collection was performed at the time of angiography in the management of patients with ACS. Therefore, our study did not address whether PCSK9 would help to diagnose ACS or help in decision making, i.e. with respect to invasive strategy. The study sample might not represent all patients hospitalized with ACS. Indeed, not all patients with cardiogenic shock or post-resuscitation had been included, in part explaining the low mortality rate. As with all biomarker studies, pre-analytical (long-term stability of PCSK9 at −80°C is poorly known) as well as analytical aspects may have affected our findings. As samples were stored for 2–3 years prior to analysis, degradation of PCSK9 may have occurred. Although we have a large sample size of ACS patients, we cannot exclude a lack of statistical power to detect a possible association given the low event rate. More prospective data are needed to consider the potential of PCSK9 in the prediction of mortality. Finally, we cannot exclude other possible confounding factors, such as physical activity, which our group has previously demonstrated to affect PCSK9 levels.[30]

Conclusions

In conclusion, even if PCSK9 levels in ACS seem to be modulated by inflammation, lipid-lowering therapy, and the clinical onset of ACS, high PCSK9 levels were not associated with mortality at 1 year. However, the clinical utility of PCSK9 remains to be further evaluated, because high PCSK9 levels were associated with suboptimal controlled LDL cholesterol 1-year after ACS, independently of baseline hypercholesterolaemia, suggesting a potential role of PCSK9 for medical decisions and management of lipids in secondary prevention.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contributions

B.G., D.H., P.J. performed statistical analysis. B.G., F.M., T.F.L., C.M.M., N.R., P.J., S.W. handled funding and supervision. B.G., Fa.Mo., F.C., D.N. R.K., L.R., R.A. acquired the data. B.G., F.M. conceived and designed the research. B.G., F.M. drafted the manuscript. B.G., Fa.Mo., D.N., F.C., R.K., N.V., D.H., L.R., R.A., P.J., S.W., T.F.L., C.M.M., N.R., F.M. made critical revision of the manuscript for key intellectual content.

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