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Short communication

Serum PCSK9 levels predict the occurrence of acute coronary syndromes in patients with severe carotid artery stenosis

Luca Liberale [a,b,*], Federico Carbone [a], Maria Bertolotto [a], Aldo Bonaventura [a], Alessandra Vecchié [a], François Mach [c], Fabienne Burger [c], Aldo Pende [d], Giovanni Spinella [e], Bianca Pane [e], Giovanni G. Camici [b], Domenico Palombo [e], Franco Dallegri [a,f], Fabrizio Montecucco [a,f,g]

[a] First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, 6 viale Benedetto XV, 16132 Genoa, Italy
[b] Center for Molecular Cardiology, University of Zürich, Wagistrasse 12, 8952 Schlieren, Switzerland
[c] Division of Cardiology, Foundation for Medical Researches, Department of Medical Specialties, University of Geneva, 64 Avenue de la Roseraie, 1211 Geneva, Switzerland
[d] Clinic of Emergency Medicine, Department of Emergency Medicine, University of Genoa and Ospedale Policlinico San Martino, 10 Largo Rosanna Benzi, 16132 Genoa, Italy
[e] Vascular and Endovascular Surgery Unit, Department of Surgery, University of Genoa and Ospedale Policlinico San Martino, 10 Largo Rosanna Benzi, 16132 Genoa, Italy
[f] Ospedale Policlinico San Martino, 10 Largo Benzi, 16132 Genoa, Italy
[g] Centre of Excellence for Biomedical Research (CEBR), University of Genoa, 9 viale Benedetto XV, 16132 Genoa, Italy

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ABSTRACT

Background: The pharmacological inhibition of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) has shown to dramatically impact on low-density lipoprotein-cholesterol (LDL-C) levels and associated cardiovascular (CV) diseases. However, the potential use of PCSK9 serum levels as a CV risk biomarker remains to be clarified.
Methods: 189 patients with severe carotid artery atherosclerosis undergoing carotid endarterectomy (CEA) and whose clinical records and serum sample aliquots for PCSK9 level measurement were available both directly before CEA and at 24-month follow-up were included in the present pilot study. The study endpoint was to determine whether PCSK9 serum levels prior to CEA would predict the occurrence of acute coronary syndromes (ACS) at 24-month follow-up.
Results: PCSK9 serum levels were significantly accurate in predicting ACS at 24-month follow-up, as assessed by ROC curve analysis (AUC: 0.719 [95% CI 0.649–0.781]). According to the cut-off point indicated by Youden's index, PCSK9 values >431.3 ng/mL were correlated with a higher risk of ACS occurrence (Log Rank test, $p = 0.0003$). At Cox regression analysis, the predictive ability of high serum PCSK9 was confirmed also after adjustment for age, gender, baseline statin treatment and active smoking, dyslipidemia, and chronic coronary artery disease (HR 17.04 [95% CI 3.34–86.81]; $p = 0.001$).
Conclusions: High serum PCSK9 levels predict ACS occurrence at 24-month follow-up after CEA in patients with severe carotid artery stenosis. Larger clinical studies are needed to evaluate whether PCSK9 serum levels could be used towards predicting the risk of ACS in patients with advanced carotid atherosclerosis.

1. Introduction

Several inflammatory molecules (such as interleukin [IL]-1β, IL-6, C-reactive protein [CRP]) have proven to play a pathophysiological role in atherogenesis [1]. Although promising results from observational studies have highlighted the potential clinical relevance of these mediators, interventional clinical trials only partially confirmed their prognostic value. For this reason, additional pro-atherosclerotic pathways (mainly targeting low-density lipoprotein cholesterol [LDL-C]) have recently been "re-evaluated" for the critical role they might play in atherogenesis [2]. Originally described as a regulator of the neuronal differentiation pathway [3], Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) was inhibited pharmacologically resulting in a strong and safe LDL-C-lowering effect, which did beneficially impact on CV events [4]. In addition to its now well-described anti-LDL-C activity, PCSK9 inhibition is now also being investigated for potential pleiotropic effects on different organs. Interestingly, PCSK9 plays an active part in the regulation of several genes involved in apoptosis, proliferation, and inflammation [5]. In this context, PCSK9 levels have been associated with chronic low-grade inflammation and may be considered as a marker of disease severity [6–8] with a potential role in atherogenesis and the acute cardiac complications associated with it, such as acute coronary syndrome (ACS) [4]. On the basis of these premises, the aim of our study was to investigate the predictive value of circulating PCSK9 levels in relation to the occurrence of ACS at 24-month follow-up after carotid endarterectomy (CEA) in a previously published cohort

* Corresponding author at: First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, 6 viale Benedetto XV, 16132 Genoa, Italy.
E-mail address: luca.liberale@uzh.ch (L. Liberale).

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of patients with advanced atherosclerosis (in the form of severe carotid artery stenosis).

2. Methods

2.1. Patients and clinical assessment

As previously described [9,10], we consecutively enrolled 269 patients suffering from extra-cranial high-degree internal carotid stenosis (>70% luminal narrowing) undergoing CEA from March 2008 to June 2011 at the Vascular Surgery Unit of Ospedale Policlinico San Martino in Genoa, Italy. In the present pilot sub-study analysis, we were able to include 189 patients that had both available records on 24-month follow-up and remaining serum sample aliquots to measure PCSK9 levels. Serum samples were obtained by venopuncture under fasting condition the day prior the surgical intervention to collect serum and to evaluate blood parameters. Exclusion criteria and patients' characteristics were previously reported [9]. The protocol adheres to the principles of the Declaration of Helsinki and has been approved by the Ethics Committee of Ospedale Policlinico San Martino. All patients gave informed consent prior to entering the study.

2.2. Study endpoint

The present report has to be considered as a pilot study. The primary end-point was to determine whether serum PCSK9 concentrations could predict the occurrence of ACS (defined as fatal and non-fatal acute myocardial infarction, and unstable angina) over a 24-month follow-up period in patients with severe carotid artery stenosis undergoing CEA. Two independent investigators who were blinded to the biochemical analysis adjudicated the study endpoint.

2.3. PCSK9 serum level detection

Serum levels of PCSK9 were measured by colorimetric enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (R&D Systems, Minneapolis, MN). The lower limit of detection was 0.625 ng/mL; mean intra- and inter-assay coefficients of variation were <8%.

2.4. Statistical analysis

Analyses were performed with IBM SPSS Statistics for Windows, Version 23.0 (IBM CO., Armonk, NY) and MedCalc 12.5 (MedCalc Software, Ostend, Belgium). Continuous variables were expressed as median and interquartile range (IQR). The prognostic ability of PCSK9 serum levels was assessed in a post-hoc manner based upon the receiver operator characteristic (ROC) curve. The area under the curve (AUC) was given with 95% confidence interval (CI) and the cut-off level for serum PCSK9 was calculated maximizing the sensitivity in accordance to Youden's index. Kaplan–Meier survival analysis with Log Rank test was performed to estimate the cumulative event rate during the 24 months following CEA and to calculate the corresponding risk difference according to PCSK9 levels >431 ng/mL and PCSK9 levels ≤431 ng/mL. Finally, the effect of PCSK9 concentration on ACS risk was estimated by the Cox proportional hazards model and expressed by hazard ratios (HR) and 95% CI. In the multivariate model, we adjusted for age, gender, chronic coronary artery disease (CAD), baseline statin treatment and active smoking and dyslipidemia. For all statistical analyses, a two-sided p-value <0.05 was considered as significant.

3. Results

3.1. PCSK9 serum levels exhibit prognostic accuracy towards ACS

In the overall cohort, the median value of PCSK9 was 272.84 ng/mL (193.28–348.40 ng/mL). According to the ROC curve analysis, PCSK9 serum levels had significant prognostic accuracy to predict ACS at 24-month follow-up (AUC: 0.719 [95% CI 0.649–0.781]) (Fig. 1A). By using Youden's index, a serum PCSK9 concentration >431.3 ng/mL provided the best cut-off point for this endpoint, displaying a sensitivity of 55.6% (95% CI 21.2–86.3) and a specificity of 87.8% (95% CI 82.1–92.2) (Fig. 1A). After dividing the cohort, 27 patients were categorized as belonging to the high PCSK9 group (>431.3 ng/mL) and 162 to the low PCSK9 group (≤431.3 ng/mL).

3.2. High PCSK9 serum levels predict ACS occurrence at 24-month follow-up

The Kaplan–Meier analysis indicated that patients with high serum PCSK9 levels had a higher rate of ACS. Among 9 events observed over the 24-month follow-up period, 5 were reported in the high PCSK9 group, whereas 4 occurred in the other one (18.51% vs. 2.46%; Log Rank test: $p = 0.0003$) (Fig. 1B). Cox proportional hazard regression analysis further confirmed the ability of PCSK9 levels >431.3 ng/mL (as compared with PCSK9 levels ≤431.3 ng/mL) to predict ACS (HR 7.67 [95% CI 2.06–28.58], $p = 0.002$). Of importance, this result remained statistically significant also after adjustment for age, male gender, chronic CAD, baseline statin treatment and active smoking and dyslipidemia (HR 12.41 [95% CI 2.91–52.94]; $p = 0.001$) (Table 1).

4. Discussion

The present pilot sub-study shows that serum PCSK9 concentrations at the time of CEA can predict ACS in patients with severe carotid artery stenosis. This prognostic role of PCSK9 levels towards ACS persisted after adjusting for known CV risk factors, such as age, male sex, and CV comorbidities (such as chronic CAD and dyslipidemia), suggesting a potential pro-atherosclerotic activity of PCSK9 that could be independent of LDL-C levels. Alongside its lipid modulatory effect, PCSK9 has been reported to influence the "response-to-stress" axis in humans [4]. Accordingly, potential effects of PCSK9 as a circulating pro-inflammatory molecule have been recently hypothesized. Patients with ACS presenting with higher circulating PCSK9 levels showed a higher degree of inflammation [7]. In addition, PCSK9 have been associated with subclinical carotid atherosclerosis irrespective of traditional risk factors and inflammation biomarkers [11]. Also, the prognostic role of circulating PCSK9 levels in the CV field is nowadays under deep investigation. In line with our results, Leander and co-workers recently reported that baseline PCSK9 levels are able to predict major adverse CV events (fatal and non-fatal myocardial infarction, unstable angina, deaths from coronary heart disease, fatal and non-fatal ischemic strokes) in a cohort of 60-year-old men and women. This statistical association was independent of other known CV risk factors [12]. Conversely, Ridker and colleagues did not confirm any association between PCSK9 concentration and CV events in a nested case–control study enrolling 716 subjects [13]. These apparent controversial results from preliminary studies investigating PCSK9 circulating levels as a novel CV biomarker may be due to the use of different PCSK9 assay kits, concomitant treatments (i.e. statin use), and heterogeneity of the study populations. For instance, our study includes a highly selective population with high CV risk, that excluded very common comorbidities (i.e. cancer, inflammatory diseases). Another issue for discussion is represented by PCSK9 cut-off point (431.3 ng/mL) that is very specific, but with a quite low sensibility. This aspect might limit the clinical use of PCSK9 in the non-selective populations. Therefore, larger population studies are clearly needed to validate these recent promising observations.

4.1. Study limitations

Some limitations have to be acknowledged when analyzing our findings. First, this is a single-center study based on a relative small cohort with a consequent small event rate, which needs to be confirmed by larger multicenter studies. Second, we did not collect the information on changes in medications at 24-month follow-up. However, we carefully checked treatment with PCSK9 inhibitors and we can confirm that no patient was administered with a PCSK9 inhibitor during the study. Third, we did not consider PCSK9 polymorphisms, which already showed ability to influence serum PCSK9 levels. In addition, the post-hoc definition of the cut-off by Youden's index should be taken into account as a limitation, but this was a forced choice due to the lack of previous studies reporting abnormal values. Furthermore, taking into account the observational aim of our study, any pathophysiological explanation would be highly speculative and miss underlying molecular mechanisms. Finally, the role of PCSK9 for risk stratification in early stages of atherosclerosis remains to be investigated.