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# Evaluation of plasma PCSK9 concentrations, transcript of LDL receptor, as well as the total number of monocyte LDL receptors in acute coronary syndrome patients

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## Abstract

**Background:** *Before our study, there were no data concerning complex evaluation of: plasma PCSK9 concentrations, transcript LDL receptor (LDLR), as well as the total amount of monocytes' LDLR in acute coronary syndrome (ACS) patients. PCSK9 levels in a few cohort studies were found to correlate with the number of white blood cells (WBC) or platelets (PLT). The study aims to evaluate PCSK9-LDLR concentrations, as well as to find any association between PCSK9 and WBC or PLT.*

**Methods:** *The study group included 95 consecutive patients with acute myocardial infarction, in whom angiography/angioplasty of the culprit vessel was performed. The control group consisted of 10 healthy young volunteers. Thirty patients from the studied group were qualified for further percutaneous revascularization after 3 months. Laboratory tests were performed using commercially available kits. LDLR expression on monocyte surface was measured by flow cytometry, but the mRNA level for LDLR was established by real time polymerase chain reaction. The PCSK9 plasma concentration was measured by ELISA kits.*

**Results:** *Higher concentration of PCSK9 and amount of LDLR on monocytes surface were observed in patients with ACS compared with healthy young volunteers (number of LDLRs on monocytes [reaction units] 10.8 ± 9.6 vs. 41.8 ± 11.8, $p < 0.001$, PCSK9 [ng/mL] 295.4 ± 76.4 vs. 213 ± 63.2, $p < 0.001$). A similar relationship was observed after application of 3-month intensive lipid-lowering therapy in patients with ACS ($n = 30$, PCSK9 [ng/mL] 281.1 ± 59.5 vs. 358.5 ± 74.7, $p < 0.001$, LDLR transcript [reaction units] 0.6 ± 0.32 vs. 1.87 ± 0.24, $p < 0.001$, number of LDLRs on monocytes [reaction units] 5.9 ± 3.1 vs. 22.3 ± 3.8, $p < 0.001$). There were no significant differences in levels of PCSK9, LDLR between patients with ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI). There was no relation of the PCSK9 with WBC as well as with PLT.*

**Conclusions:** *We observed significantly higher concentration of PCSK9, and significantly higher levels of mRNA LDLR transcript in patients with ACS compared with healthy young volunteers. A similar pattern was observed after 3 months of intensive statin therapy among patients with ACS. There were no differences in these parameters between patients with STEMI vs. NSTEMI. The results of the study require confirmation in a larger population of patients.* (Cardiol J 2016; 23, 6: 604–609)

**Key words: PCSK9, LDL receptor, acute coronary syndrome**

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## Introduction

Cardiovascular diseases (CVD) are the leading cause of death worldwide. It is estimated that in 2008, 17.3 million people died from the CVD, representing 30% of all deaths (World Health Organization, 2011). Approximately 7.3 million among this group suffered from coronary artery disease (CAD). Atherosclerosis is responsible for 95% cases of CAD [1]. We know so much about the etiology and risk factors of CAD that the concept of the atherosclerotic plaque formation as a result of activation of local inflammation (in the vessel wall) was proposed several years ago [2–4]. Recent data seem to confirm this hypothesis [5]. There are in fact several papers suggesting that atherosclerosis can be considered not a local but also a systemic inflammation in the course of increased bone marrow activation [5–7]. The exponent of this process is increased white blood cell (WBC) count during acute coronary syndrome (ACS), lasting up to several months after the ACS, what seems to be a poor prognostic factor. The indirect proof of the above could be i.e. increased C-reactive protein (CRP) levels [1, 5, 8–10].

According to classical risk factors of atherosclerosis, it turns out that still, the most effective prevention is the reduction of low-density lipoprotein (LDL) plasma concentration [10–12]. These molecules are responsible for carrying components of atherosclerotic plaque such as free cholesterol, its esters, and other fatty compounds [1]. Exaggerated LDL biosynthesis, its impaired uptake by disturbed cellular receptor, or too small number of LDL receptors (LDLR), are dangerous due to the fact that they increase the plasma LDL concentrations [1]. These situations are often caused by abnormalities of genes coding sequence of: the LDLR protein, apoB100 protein (ligand for LDLR), or discovered several years ago — proprotein convertase subtilisin/kexin type 9 (PCSK9) protein [13].

After reviewing their biological role, the quantitative and qualitative disturbances of LDLR, PCSK9, and apoB100 were associated with the severity of atherosclerosis and inflammation in large number of population studies [14]. Additionally, in a few cohort studies PCSK9 levels were found to correlate with the number of white blood cells or platelets [15, 16].

It is surprising that the evaluation of PCSK9 and LDLR was usually conducted separately, in the population of hypercholesterolemic subjects with stable CAD [14]. The evaluation of these proteins has not been studied yet in ACS population in humans. This led us to create a prospective project, where plasma concentrations of PCSK9, the real time LDLR transcript levels and the amount of the LDLR particles on the monocytes were measured in patients with ACS. An additional objective was to evaluate a possible link of PCSK9 concentrations with WBCs, as well as with platelet counts.

## Methods

The study included 95 consecutive patients with ACS (ST-segment elevation myocardial infarction [STEMI] and non-ST-segment elevation myocardial infarction [NSTEMI]). The control group consisted of 10 healthy volunteers. ACS patients were subjected to routine clinical management specified in the guidelines of the European Society of Cardiology. Therefore, patients had performed urgent coronary angiography and angioplasty of the infarct related artery. Morphology of peripheral blood and full lipid profile, as well as evaluation of glucose, troponin, aminotransferases, electrolytes, creatinine, and urea concentrations were determined routinely for every patient during hospital admission. Furthermore, during coronary angiography, blood samples for analysis of PCSK9, as well as for LDLR were obtained. Thirty patients from the study group were qualified for further percutaneous revascularization after 3 months. During second hospitalization, the same panel of laboratory tests was carried out with a molecular assessment of PCSK9 and LDLR.

The control group consisted of 10 healthy, anonymous volunteers, who had blood samples obtained to assess only concentrations of PCSK9, total amount of monocytes surface LDLRs, and evaluation of monocytes mRNA LDLR expression. The study protocol was approved by the Local Bioethics Committee.

Inclusion criteria to the study was ACS scheduled for urgent coronary angiography.

Exclusion criteria was the suspected patient's life long less than 3 months.

Only 13% of patients (in ACS group) received statins before ACS hospitalization, but in low or intermediate dose regimen. Every subject who presented with myocardial infarction received 80 mg of atorvastatin during hospital stay, and atorvastatin therapy was continued from discharge till the follow-up visit in a reduced dose of 40 mg. There were no patients who received ezetimibe during follow-up. At discharge each patient had been recommended an optimal medical therapy (with additional comorbidities), including high dose

statin thrapy (atorvastatin 40 mg daily) according to European Society of Cardiology guidelines [13].

No healthy volunteers received hypolipemic treatment at baseline.

## Laboratory tests

Laboratory tests were performed using commercially available diagnostic kits. The serum creatinine concentration was determined according to Jaffe's reaction using Roche Cobas C (Hitachi, Germany). Creatinine clearance and glomerular filtration rate were estimated using Cockroft-Gault formulas. Potassium and sodium concentrations were determined by potentiometry using the Cobas System 6000 (Roche Diagnostics, Germany). Assessments of peripheral blood counts were performed using Sysmex XT2000i (USA). Concentration of urea, uric acid, total cholesterol, triglycerides and high-density lipoproteins (HDL) were quantified using enzymatic colorimetric method (Cobas C Roche/Hitachi, Germany) with specific reagents. The concentration of LDL was calculated using the Friedewald formula.

## LDLR, PCSK9 analysis

Monocytes derived from blood were selected in flow cytometry (Cytomics FC500MPL, Beckmann Coulter) using Flow-Count (Beckmann Coulter) and then 'positive isolation' by anty-CD14. LDLR amount on monocyte surface was studied by flow cytometry (Cytomics FC500MPL, Beckmann Coulter) using average fluorescence of antibodies against LDLR. RNA isolated from recruited monocytes (in flow cytometry) was used to receive cDNA in reverse transcription-polymerase chain reaction (PCR). Then, synthetized cDNA was used to establish mRNA transcript level for LDLR by real time PCR with the usage of sophisticated starters. The starters were designed with OLIGO 6.65 software (Rychlik&Rhoads 1989-2002) based on LDLR gene sequence (ENSG00000130164) and Porphobilinogen deaminase sequence (ENSG00000256269) derived from Ensemble Gene View. The starters were prepared by Tib Molbiol.

The PCSK9 plasma concentration was measured by commercially available ELISA kits.

## Statistical analysis

Normality was tested with the Shapiro-Wilk's W test. At normal distribution of variables we used the Student's t-test (for 2 independent and dependent variables). Mann-Whitney test (for 2 independent variables) and Sign test, as well as the Wilcoxon matched pairs test (for 2 dependent variables) were used at abnormal variables of distribution. The results were given as mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$. All analyzes were performed with STATISTICA 7.0 (Statsoft, USA) and SPSS-20 (IBM, USA).

# Results

The studied group included consecutive ACS patients with STEMI (n = 58), and NSTEMI (n = 37). The characteristics of the group are presented in Tables 1–3. All patients in the studied group had coronary-angiography performed. In 99% cases, percutaneous coronary intervention of the culprit vessel was conducted. NSTEMI patients did not significantly differ from STEMI subjects with respect to risk factors, clinical management and laboratory findings except for the levels of WBCs and blood glucose assessed at admission (NSTEMI vs. STEMI for WBC: 10.35 ± 3.7 vs. 12.5±3.9 μl/L; p = 0.006, for glucose: 7.38 ± 3.43 vs. 8.5 ± 3 mmol/L, p = 0.006, respectively).

The control group had blood taken only to assess: the PCSK9 concentrations, total amount of leucocytes surface LDLRs and monocytes LDLR transcript (mRNA) expression.

The value of PCSK9, LDLRs mRNA, and total monocytes LDLR amount (measured as an average fluorescence of anty-LDLR antibodies) in ACS patients vs. healthy volunteers were as follows: PCSK9 295.4 ± 76.4 vs. 213 ± ± 63.2 ng/mL, $p < 0.001$, number of LDLR transcript [reaction units] 1.8 ± 10.6 vs. 1.02 ± 0.1, p = NS, total leucocytes LDLR amount [reaction units] 10.8 ± 9.6 vs. 41.8 ± 11.8, $p < 0.001$ (Fig. 1).

The value of PCSK9, LDLRs mRNA, and total monocytes LDLR amount (measured as an average fluorescence of anti-LDLR antibodies) in STEMI patients vs. NSTEMI subjects were as follows: PCSK9 290.4 ± 74.4 vs. 303. 8 ± ± 80.2 ng/mL, p = NS, number of LDLR transcript [reaction units] 2.5 ± 13.5 vs. 0.8 ± 0.22, p = NS, total leucocytes LDLR amount [reaction units] 11 ± 10.3 vs. 10.4 ± 8.2, p = NS.

The value of PCSK9, LDLRs mRNA, and total monocytes LDLR amount (measured as an average fluorescence of anty-LDLR antibodies) during ACS vs. 3 months after episode of myocardial infarction were as follows: n = 30, PCSK9 281.1 ± 59.5 vs. 358.5 ± 74.7 ng/mL, $p < 0.001$, number of LDLR transcript [reaction units] 0.6 ± 0.32 vs. 1.87 ± 0.24, $p < 0.001$, total leucocytes LDLR amount [reaction units] 5.9 ± ± 3.1 vs. 22.3 ± 3.8, $p < 0.001$ (Fig. 2).

**Table 1.** Characteristics of the studied group.

| Parameter | Mean | SD |
|---|---|---|
| Age [years] | 66.5 | 11.7 |
| Body mass index [kg/m$^2$] | 27.5 | 4.9 |
| Hemoglobin [mmol/L] | 8.9 | 0.96 |
| Hematocrit [%] | 38.4 | 12 |
| Erythrocytes [10$^6$/$\mu$L] | 4.6 | 0.5 |
| Leucocytes [10$^3$/$\mu$L] | 11.7 | 3.9 |
| Platelets [10$^3$/$\mu$L] | 252.1 | 78.4 |
| Glucose [mmol/L] | 8.1 | 3.2 |
| Creatinine [mmol/L] | 82.2 | 27.5 |
| Urea [mmol/L] | 6.2 | 3.9 |
| Uric acid [mmol/L] | 337.4 | 102.8 |
| Troponin [ng/L] | 199.4 | 345.2 |
| ASPAT [U/L] | 61 | 73 |
| ALAT [U/L] | 33.5 | 24.8 |
| Creatine phosphokinase [U] | 448.1 | 556.1 |
| Creatine kinase MB [U] | 65.9 | 101.8 |
| Total cholesterol [mg/dL] | 219.4 | 56 |
| HDL [mg/dL] | 52.2 | 14.1 |
| Non-HDL [mg/dL] | 167.2 | 55.11 |
| LDL [mg/dL] | 139.6 | 51.4 |
| Triglycerides [mg/dL] | 141 | 74 |
| $Na^+$ [mmol/L] | 138.7 | 2.8 |
| $K^+$ [mmol/L] | 4.2 | 0.49 |

ALAT — alanine aminotransferase; ASPAT — aspartate aminotransferase; HDL — high density lipoproteins; K — potassium; LDL — low density lipoproteins; Na — sodium; SD — standard deviation

**Table 2.** Characteristics of the studied group.

| | |
|---|---|
| Women | 33.68% |
| Death | 1.05% |
| Coronary artery disease | 24.21% |
| MI history | 23.16% |
| CABG | 2.11% |
| Heart failure | 2.11% |
| Aortic aneurysm | 1.05% |
| PAD (atherosclerosis of leg arteries) | 2.11% |
| PAD (carotid atherosclerosis) | 1.05% |
| Ischemic stroke | 5.26% |
| Diabetes | 32.63% |
| Hypertension | 56.84% |
| Overweight | 64.20% |
| Obesity | 27.16% |
| Prior statin use in ACS subjects | 13.83% |
| Chronic renal insufficiency | 5.26% |
| COPD | 7.37% |

ACS — acute coronary syndrome; CABG — coronary artery by-pass graft; COPD — chronic obstructive pulmonary disease; MI — myocardial infarction; PAD — peripheral artery disease

**Table 3.** Characteristics of patients qualified for further percutaneous revascularization (n = 30), 3 months after episode of myocardial infarction (n = 30).

| Parameter | Mean | SD |
|---|---|---|
| Hemoglobin [mmol/L] | 8.8 | 1.1 |
| Hematocrit [%] | 28.2 | 2 |
| Erythrocytes [10$^6$/$\mu$L] | 4.7 | 0.6 |
| Leucocytes [10$^3$/$\mu$L] | 7.4 | 1.9 |
| Platelets [10$^3$/$\mu$L] | 239.4 | 78.4 |
| $Na^+$ [mmol/L] | 139.4 | 2.5 |
| $K^+$ [mmol/L] | 4.4 | 0.4 |
| Glucose [mmol/L] | 5.95 | 1.2 |
| Creatinine [mmol/L] | 77 | 14 |
| ASPAT [U] | 21.7 | 5.3 |
| ALAT [U] | 29. 8 | 12.8 |
| Total cholesterol [mg/dL] | 172.2 | 46 |
| HDL [mg/dL] | 56 | 14.8 |
| Non-HDL [mg/dL] | 116.5 | 43.2 |
| LDL [mg/dL] | 93.3 | 39.6 |
| Triglycerides [mg/dL] | 115.2 | 43 |

ALAT — alanine aminotransferase; ASPAT — aspartate aminotransferase; HDL — high density lipoproteins; K — potassium; LDL — low density lipoproteins; Na — sodium; SD — standard deviation

There were no significant differences in PCSK9 concentration, level of LDLR mRNA transcript expression, and total monocytes surface LDLR amount between diabetic vs. non-diabetic subjects, those receiving vs. those not taking statin (prior to the episode of ACS), between people with strongly severe CAD (3-vessel disease) vs. less severe CAD (1-vessel disease), or between obese (body mass index [BMI] > 25 kg/m$^2$) vs. non-obese (BMI < < 25 kg/m$^2$). No significant associations of the mRNA for LDLR, and total leucocytes surface LDLR with any of the analyzed parameter were found. Only a weak correlation between the concentration of PCSK9 and urea levels was noticed (R = 0.3, p < < 0.05). The significant reduction of total cholesterol, LDL, WBC, hemoglobin, and hematocrit levels wase observed after 3 months from ACS episode among patients qualified for further percutaneous coronary revascularization (n = 30) (Table 4).

## Discussion

Our project is probably a unique one to rank the simultaneous expression of LDLR and PCSK9 concentration levels in patients with ACS. In addition, it is one of few trials evaluating (after

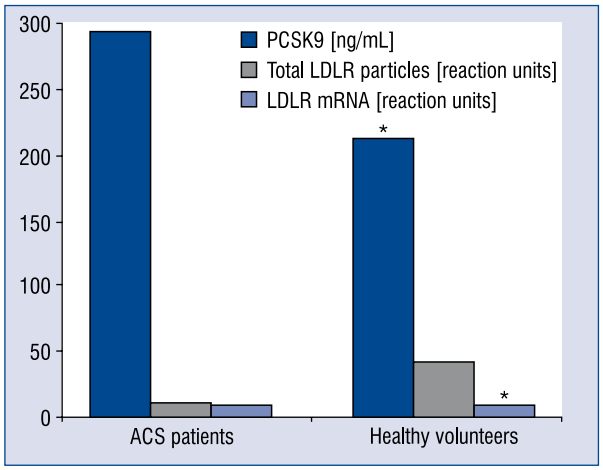


**Figure 1.** Comparison of acute coronary syndrome (ACS) vs. healthy volunteers according to value of PCSK9, LDLRs mRNA, and total monocytes LDLR amount (measured as an average fluorescence of anti-LDLR antibodies (see text); *p < 0.05.

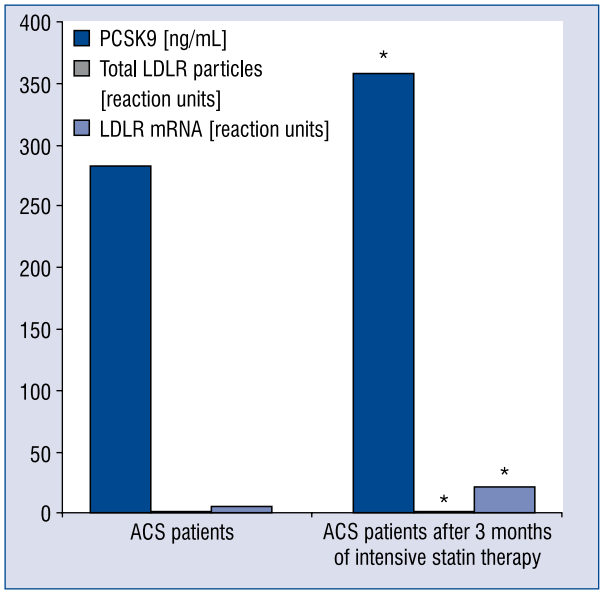


**Figure 2.** Comparison of the same patients during acute coronary syndrome (ACS) vs. 3-month period of intensive statin therapy (initiated during ACS) according to value of PCSK9, LDLRs mRNA, and total monocytes LDLR amount (measured as an average fluorescence of anti-LDLR antibodies (see text); *p < 0.05.

3 months) the effect of intensive lipid lowering therapy (initiated during ACS) on the expression of LDLR and PCSK9. We observed significantly higher concentration of PCSK9 and levels of mRNA LDLR transcripts among ACS patients compared

**Table 4.** Significant differences among (the same patients) during acute coronary syndrome versus 3-month period after episode of myocardial infarction (n = 30).

| Parameter | Mean ± SD | P |
|---|---|---|
| Hemoglobin [mmol/L] | 9.4 ± 0.6 | 0.024 |
| Hemoglobin after 3 months | 8.7 ± 1.1 | |
| Hematocrit [$10^3/\mu L$] | 30 ± 0.2 | 0.04 |
| Hematocrit after 3 months | 28 ± 0.2 | |
| Leucocytes [$10^3/\mu L$] | 10.9 ± 3.5 | < 0.001 |
| Leucocytes after 3 months | 7.4 ± 1.9 | |
| TC [mg/dL] | 219.4 ± 45.6 | 0.003 |
| TC after 3 months | 173 ± 47.3 | |
| Non-HDL [mg/dL] | 165.9 ± 45 | 0.024 |
| Non-HDL after 3 months | 116.6 ± 44.6 | |
| LDL [mg/dL] | 134.8 ± 39.2 | < 0.001 |
| LDL after 3 months | 94 ± 40.7 | |

HDL — high density lipoprotein; LDL — low density lipoprotein; SD — standard deviation

to healthy volunteers. On the other hand, the total amount of monocytes LDLR (functional assessment of receptor) were significantly higher in the group of healthy individuals. The results were not surprising and should be explained by the severity of atherosclerosis, as well as pathobiochemical effects due to myocardial infarction. We can only speculate that the expression of PCSK9 is higher in ACS due to enhanced expression of LDLR, which in turn results in the necessity of absorbing increased amount of cholesterol (progression and activation of atherosclerosis) [1]. It is not surprising that ACS patients generally have lower number of LDLR particles, which is due to two facts. Firstly, ACS group is at highest cardiovascular risk. Secondly, one should remember that the control group consisted of young, healthy people, and as it is known, the metabolism of LDL is age-dependent.

There were no differences in the expression of LDLR and concentrations of PCSK9 between patients with different types of myocardial infarction (STEMI, NSTEMI), between people with severe CAD (3-vessel disease) vs. less severe CAD (1-vessel disease), between obese (BMI > 25) vs. non-obese (BMI < 25), and diabetic vs. non-diabetic patients.

We observed significantly higher concentration of PCSK9, levels of mRNA LDLR transcripts and total amount of surface monocyte LDLRs after 3 months of intensive statin therapy, which were parallel to significant reduction in total cholesterol, LDL

and non-HDL fraction. This result can be explained by physiological reaction to a statin [1]. Firstly, according to the reduction of endogenous cholesterol biosynthesis, plasma LDL concentration decreases, which results in overexpression of LDLR expression (upregulation) [17–20]. In turn, the greater expression of the LDLR, the greater its counter-regulation is potentiated. Function of PCSK9 is to attach to LDLR, which results in changing the LDLR conformation and prevention of the LDLR release from the endosome (the LDLR recirculation is impaired) [20, 21].Therefore, increased expression of the protein PCSK9 occurs to diminish the overexpression of LDLR, to establish the balance [21]. To summarize, our clinical observations are consistent with the mentioned biochemical data regarding the regulation of metabolism of LDL [18–20]. This effect was independent from statin dose, because every subject during hospital stay received 80 mg of atorvastatin, and atorvastatin therapy was continued from discharge till the follow-up visit in reduced dose of 40 mg. Only 13% of patients received statins before ACS hospitalization in low or intermediate dose regimen.

There was no association of PCSK9 with the number of platelets and WBC, but the reduction of WBC at 3 months after the infarction was observed. This is definitely a beneficial observation, in the light of current knowledge, where the worse prognosis of ACS patients is strictly associated with severity of systemic inflammatory response (the exponent of the phenomenon is increased activation of the bone marrow, and massive leukocyte formation) [5].

The weakness of the project is a small studied group, which explains discrepancies with other authors' results concerning the association between PCSK9 and the number of WBC. Additionally, results of laboratory parameters among studied patients should be interpreted with caution due to the fact that clinical conditions of STEMI and NSTEMI are characterized by various dynamics of assessment (different time from "door to balloon"). There were no available data concerning CRP levels during episode of ACS. We had it during second hospitalization and there was no association between CRP, PCSK9, and LDLR cascade. We decided not to present these results due to the fact that the group was very small.

## Conclusions

In conclusion, we observed significantly higher concentration of PCSK9, and significantly higher levels of mRNA LDLR transcript in patients with ACS compared with healthy young volunteers. A similar pattern was observed after 3 months of intensive statin therapy among patients with ACS. There were no differences in these parameters between patients with STEMI vs. NSTEMI. The results of the study require confirmation in a larger population of patients.

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**Conflict of interest:** None declared

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