Research Article

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PCSK9 concentrations in different stages of subclinical atherosclerosis and their relationship with inflammation

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Abstract: The aim of this study was to detect the concentrations of PCSK9 in various subclinical stages of atherosclerosis and to highlight its relationship with inflammation. One hundred and fifty-nine healthy patients were divided into three groups, based on the extent of atherosclerotic changes in the carotid artery: a group without identifiable atherosclerosis, $cIMT_{>75‰}$ and an asymptomatic plaque group. The PCSK9 was measured by ELISA and hsCRP by the immunoturbidimetric method. Vascular changes were identified by a carotid ultrasound. PCSK9 was elevated, when comparing the healthy group with the $cIMT_{>75‰}$ group; however, no significant increase was detected between $cIMT_{>75‰}$ and the asymptomatic plaque group. A positive linear correlation of the PCSK9 concentration and atherosclerotic changes was found; however, after the re-analysis in each group, this correlation persisted only in the group with still normal values. Additionally, a significant linear correlation was found between the PCSK9 concentrations and lipid parameters. However, no significant association was found with hsCRP. PCSK9 was found to be elevated only in $cIMT_{>75‰}$, but not in the later plaque stage. A linear correlation of PCSK9 values was detected only in the group with still reference values. Based on this fact, we assumed the direct linear role of PCSK9 in initiating atherosclerosis; however, in the later phases, the relationship, which highlights other risk factors such as inflammation, is not linear.

Keywords: PCSK9, inflammation, atherosclerosis, plaque, initiation

1 Introduction

Atherosclerosis is an inflammatory-degenerative disease characterized by the accumulation of lipids and white blood cells and the proliferation of smooth muscle into the intima of the vascular wall. The lesion is called an atheroma/plaque that affects the vascular flow through the luminal constriction and, in case of rupture, can cause occlusion and ischaemia, and eventually necrotic changes in the target organ [1,2]. The recognition that dyslipidaemia is one of the most critical risk factors for cardiovascular disease has caused researchers to tightly focus on the lowering of cholesterol (LDL-C) in both primary and secondary prevention, by inhibiting formation, increased uptake or reduced resorption [3]. In addition to the lipid factors involved in the initiation and progression of atherosclerotic lesions, the inflammatory components of the atherosclerotic process are well known, and they manifest as an intravascular inflammation affecting the plaque progression and stability itself [4]. An essential role of the inflammatory process, which is offset by the lipid risk factor, has been outlined by the results of a recent CANTOS study. This study provided evidence of a significant reduction in the primary end

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points in patients treated with statins in combination with canakinumab, which has a crucial anti-atherogenic effect mediated independently of lipid metabolism, facilitated by the anti-inflammatory pathways [5].

Proprotein convertase subtilisin/kexin-9 (PCSK9) has gained a lot of focus as a therapeutic target in the field of cardiology. PCSK9 acts as an indirect regulator of LDL-C. This action is rooted in the regulation of the number of LDL receptor (LDL-R) molecules expressed at the plasma membrane of various cells. The human PCSK9 gene is located on chromosome 1p32.3, is ~22 kb long and comprises 12 exons encoding a 692 amino acid protein. PCSK9 is expressed mainly in the liver, small intestine and kidney. Once the PCSK9 is secreted, it binds to the epidermal growth factor (EGF)-like repeat A of the LDL-R [6]. This region is thought to be central for recycling the LDL-R from endosomes to the cell surface. A crystal structure of PCSK9 bound to the EGF-like repeat A revealed that the binding site on PCSK9 for the LDL-R is distant from its catalytic site. The binding of PCSK9 intriguingly results in the redistribution of LDL-R from the cell surface to lysosomes. A demonstration of the exact molecular mechanisms for these changes remains elusive. However, it is possible that this binding induces conformational changes in the LDL-R that ultimately render PCSK incapable of being sorted to recycling endosomes. Alternatively, the PCSK9–LDL-R complex could be actively recognized and directed towards lysosomes. PCSK9 is regulated by the sterol-regulatory element binding protein (SREBP) through a sterol-regulatory element motif in the promoter region [7]. The regulation of cholesterol synthesis, LDL-R and PCSK9 by the action of SREBP results in a paradoxical scenario, whereby the depletion of intracellular cholesterol levels leads to a simultaneous upregulation of both LDL-R and PCSK9 expression. This SREBP-mediated upregulation of PCSK9 attenuates the LDL-C-lowering effect of medications, such as statins and ezetimibe. The PCSK9 promoter also contains a hepatocyte nucleic factor 1 motif (between the sterol-regulatory element and Sp1 sites), which likely functions as a liver-specific regulatory sequence. Clinically, this stimulation of the LDL-R degradation results in a reduction in the liver cholesterol recirculation/absorption and an increase in the concentration and circulation time of LDL particles that are vulnerable to oxidation and modification. They are later captured in the vascular wall, resulting in the manifestation of several forms of atherosclerotic disease in the long term [6,7].

Inhibition of PCSK9 has demonstrated significant reductions in LDL-C, a higher availability of cell surface LDL-R and an improved clearance of LDL particles [7]. The administration of PCSK9 inhibitor to patients already on statin therapy may additionally reduce the LDL-C by up to 60% from baseline [8]. Clinical trials studying the PCSK9 inhibition have shown that the administration of PCSK9 inhibitors can significantly reduce cardiovascular morbidity and mortality [9] as well as atheroma volume and thus cause regression of atherosclerotic changes [10]. In addition to the lipid effects of PCSK9, the role of the mediator in viral and bacterial inflammation and sepsis was described in previous studies [11]. Despite the significant effect of anti-PCSK9 treatment on LDL-C concentrations and cardiovascular mortality, there was no significant decrease in hs-CRP concentrations following the anti-PCSK9 antibody administration. Some studies describe the persistence of a residual inflammatory risk [12]. Although the evaluation of systemic inflammation was performed in most studies on anti-PCSK9 therapy [13], there are no data to analyse PCSK9 concentrations at different stages of initiation, as well as the progression of early atherosclerotic changes in relation to hs-CRP in clinical models.

This study aimed to determine the PCSK9 concentrations at various stages of subclinical atherosclerosis, as well as the relationship between the PCSK9 concentrations and quantifiable values of vascular changes and laboratory inflammatory parameters. This study aimed to describe the importance of PCSK9 in various phases of atherogenesis as well.

2 Methods

In this prospective study that was conducted between November 2015 and January 2017, general practitioners randomly selected 300 patients without manifested cardiovascular (CV) diseases (asymptomatic patients), who were examined in the Cardiology and General Medicine outpatient clinics of the Faculty of Medicine, UPJŠ Košice (1st Department of Internal Medicine; outpatient clinics of Medicomp Košice). In this study, the following inclusion criteria were specified: SCORE ≤ 5%, total cholesterol below 8 mmol/L and age between 18 and 60 years. All patients who volunteered for this research study agreed by their own free will and signed the informed consent approved by the Local Ethics Committee of the Faculty of Medicine of Pavol Jozef Šafárik University by the date and number of 14/7/2015; 10N/2015. From this study, we excluded those patients with one or more of the exclusion criteria: patients undergoing a lipid-lowering therapy or patients not meeting the minimum period of three months of discontinuation of therapy, lipid profile outside the inclusion criteria and secondary cause of dyslipidaemia, manifested CV diseases (such as ischaemic heart diseases, etc.), patients with diabetes mellitus, acute

infection or chronic inflammatory diseases, significant dietary or lifestyle changes in the period shorter than six months based on the protocol of our parallel study [14].

The selected group of 159 patients underwent basic history-taking, physical examination and blood sampling for laboratory examination. Subsequently, they were divided into three groups, based on the ultrasonographic values of cIMT and the presence of plaque detected during the carotid arteries' ultrasound.

- Normal cIMT ($cIMT_{<75‰}$): patients with the cIMT values below 75‰ for the age and sex of the patients.
- Subclinical atherosclerosis group ($cIMT_{>75‰}$): patients with the cIMT values above 75‰ for the age and sex of the patients, but without the plaque presence (defined below) during the carotid arteries' ultrasound.
- Asymptomatic plaque group (plaque): the group with asymptomatic atherosclerosis with plaque formation. Carotid plaque was defined as a local thickening of the cIMT of >50% compared to the surrounding vessel wall, an IMT >1.5 mm or a local thickening >0.5 mm [15].

Lipid (LDL, high-density lipoprotein [HDL], total cholesterol, etc.) and non-lipid (aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyltransferase [GMT], alkaline phosphatase [ALP]) parameters were recorded using standard laboratory assays on an automatic analyser Daytona (RANDOX). The circulating levels of PCSK9 were quantified in plasma by the ELISA kit (Abcam, Human PCSK9 ELISA Kit) and measured on a Daytona analyser (RANDOX) by a spectrophotometric method at 450 nm. The detection of high-sensitivity C-reactive protein concentrations was carried out in plasma by an immunoturbidimetric method using a standardized Daytona Analyser (Randox, UK) by High-Sensitivity CRP kit at 570 nm. Values were calibrated with High-Sensitivity CRP Standards 2–3 (Randox).

Subclinical vascular changes were detected by an ultrasound measurement of the carotid artery intima-media thickness (cIMT) and by the Echo-tracking method, as described before [14]. Patients were further classified into the study groups according to the values of cIMT. By the Echo-tracking method, the following parameters were further analysed: β (parameter of stiffness); AI (augmentation index) and PWV (pulse wave velocity).

3 Statistical analysis

Statistical analysis was performed using SPSS version 20.0 for Windows (IBM Corp. 2011. Published SPSS, version 20.0 ARMONK, NY: IBM Corp.). The values of each parameter were expressed as the mean ± SD. Univariate and multivariate linear regression analyses were performed to assess the correlations between the plasma levels of PCSK9 and subclinical vascular changes and biochemical parameters. Changes in quantitative results (PCSK9, vascular changes) in the study groups were determined by one-way ANOVA with a multiple comparison Tukey–Kramer *post hoc* test. The value of $p < 0.05$ was considered statistically significant.

4 Results

The primary characteristics of the entire study population and follow-up groups are given in the table (Table 1). The average age of the study population was 38.72 ± 14.15 years and 98 women and 59 men were enrolled. There were no significant differences in baseline parameters such as age, male and female proportion in the study groups.

Significantly higher weight and BMI were detected in the groups with more advanced subclinical vascular changes (both $p < 0.001$) in comparison with $cIMT_{<75‰}$, respectively, in the plaque group vs $cIMT_{>75‰}$. Values of LDL were significantly increased in the plaque group vs $cIMT_{<75‰}$ ($p = 0.03$) as well as $cIMT_{>75‰}$ ($p = 0.05$). HDL values were significantly reduced in the plaque group compared to $cIMT_{<75‰}$ and $cIMT_{>75‰}$ (both $p < 0.001$). Significantly higher values of systolic blood pressure, as well as diastolic blood pressure, were detected in most of the groups with advanced vascular profile in comparison with earlier stages of atherosclerosis (Table 1).

Significantly higher PCSK9 concentrations (Figure 1) were detected in the plaque group compared to $cIMT_{<75‰}$ ($p = 0.003$) and $cIMT_{>75‰}$ compared to $cIMT_{<75‰}$ ($p = 0.03$). No significant increase was observed ($p = 0.54$) between the plaque group and $cIMT_{>75‰}$. Significantly higher concentrations of hsCRP (Figure 2) were detected in the plaque group vs $cIMT_{>75‰}$ ($p < 0.001$) as well as in $cIMT_{<75‰}$ ($p < 0.001$). However, hsCRP values were not significantly increased in the $cIMT_{>75‰}$ compared to the non-atherosclerotic change group ($p = 0.97$).

Values of cIMT (Table 2) were significantly increased in the $cIMT_{<75‰}$ group compared to $cIMT_{>75‰}$ ($p < 0.001$), in the $cIMT_{>75‰}$ group compared to the plaque group ($p < 0.001$) and in the plaque group compared to $cIMT_{<75‰}$ ($p < 0.001$). The AI was significantly increased in $cIMT_{<75‰}$ compared to $cIMT_{>75‰}$ ($p < 0.001$), in $cIMT_{>75‰}$ compared to the plaque group ($p = 0.003$) and in the plaque vs $cIMT_{<75‰}$ ($p < 0.001$). The stiffness index β was significantly increased

Table 1: Baseline characteristics of subjects

	$cIMT_{<75‰}$	$cIMT_{<75‰}$ vs $cIMT_{>75‰}$	$cIMT_{>75‰}$	Plaque vs $cIMT_{>75‰}$	Plaque	Plaque vs $cIMT_{<75‰}$
Male/female	26/40	*ns*	18/36	ns.	15/22	ns.
Age	35.41 ± 12.34	*ns (0.06)*	40.55 ± 10.9	ns. (*0.06*)	41.12 ± 13.81	ns. (*0.97*)
Weight (kg)	59.32 ± 13.34	*<0.001*	74.81 ± 8.15	<0.001	96.74 ± 11.9	<0.001
BMI (kg/m^2)	26.69 ± 6.39	*<0.001*	30.72 ± 6.49	<0.001	36.18 ± 5.13	<0.001
Total cholesterol (mmol/L)	5.23 ± 1.27	*0.91*	5.31 ± 0.86	0.12	5.76 ± 0.98	0.04
LDL-C (mmol/L)	3.72 ± 0.94	*0.99*	3.73 ± 0.8	0.05	4.19 ± 1.01	0.03
HDL-C (mmol/L)	1.3 ± 0.26	*0.06*	1.4 ± 0.27	<0.001	0.93 ± 0.14	<0.001
TG (mmol/L)	1.03 ± 0.94	*0.05*	1.33 ± 0.51	0.85	1.41 ± 0.24	0.02
sTK (mmHg)	112.1 ± 17.61	*<0.001*	129.11 ± 15.98	0.05	137.45 ± 15.32	<0.001
dTK (mmHg)	68.84 ± 11.92	*<0.001*	83.18 ± 15.07	0.32	87.28 ± 13.09	<0.001

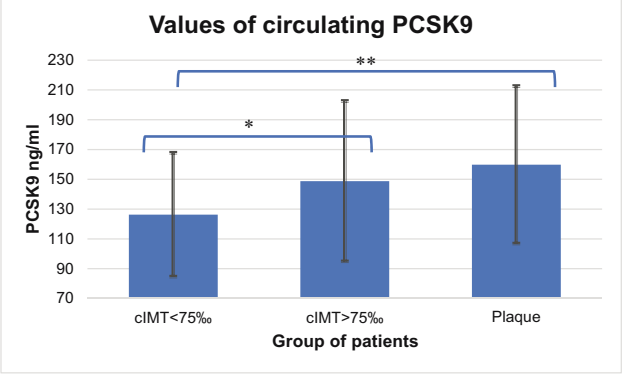

Figure 1: Values of circulating PCSK9 in different groups of patients according to the vascular changes (*p < 0.05; **p < 0.01; ***p < 0.001).

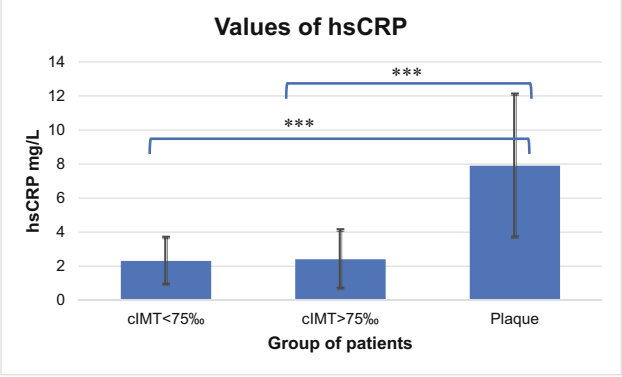

Figure 2: Values of circulating hsCRP in different groups of patients according to the vascular changes (*p< 0.05; **p < 0.01; ***p < 0.001).

in $cIMT_{<75‰}$ compared to $cIMT_{>75‰}$ (p < 0.001), in the $cIMT_{>75‰}$ compared to plaque (p < 0.001) and in the plaque group vs $cIMT_{<75‰}$ (p < 0.001). The PWV was significantly increased in $cIMT_{<75‰}$ compared to $cIMT_{>75‰}$ (p < 0.001), in $cIMT_{>75‰}$ compared to the plaque group (p < 0.001) and in the plaque group vs $cIMT_{<75‰}$ (p < 0.001).

In this study, a significant linear correlation of PCSK9 concentrations and several biochemical parameters was observed after the data analysis (selected parameters listed in Table 3). Between the PCSK9 concentrations and haematological parameter – a number of monocytes (r = 0.19; p = 0.014) as well as the lipid panel: LDL concentrations (r = 0.38; p < 0.001) and LPA (r = 0.17; p = 0.036), a significant linear correlation was also found with insulin concentrations (r = 0.16; p = 0.041). In addition to biochemical parameters, the relationship between the PCSK9 levels and vascular changes was also analysed. Correlations between the PCSK9 concentrations and subclinical vascular changes are shown in Table 4.

The PCSK9 concentrations correlated positively with cIMT (r = 0.37; p < 0.001), AI (r = 0.36; p < 0.001), β (r = 0.38; p < 0.001) and PWV (r = 0.38; 0.45, p < 0.001). In addition, multivariate regression analysis was performed to calculate the effect of various parameters such as gender, age, LDL and PCSK9 concentrations on vascular changes. Multivariate regression analysis showed that the PCSK9 concentrations were a significant positive predictor of increased cIMT, β and PWV (Table 5).

In addition to the evaluation of the entire study population, we also performed analyses in individual study groups. The results for each group are shown in Table 6. It was found that a significant correlation of vascular changes and PCSK9 concentrations was present only in the $cIMT_{<75‰}$ group, whereas the other groups' values were, except the PWV in $cIMT_{>75‰}$, not significant.

Table 2: Vascular changes in study groups

	$cIMT_{<75‰}$	$cIMT_{<75‰}$ vs $cIMT_{>75‰}$	$cIMT_{>75‰}$	$cIMT_{>75‰}$ vs Plaque	Plaque	Plaque vs $cIMT_{<75‰}$
cIMT (mm)	0.437 ± 0.05	<0.001	0.646 ± 0.156	<0.001	0.892 ± 0.169	<0.001
AI (%)	3.82 ± 7.16	<0.001	16.09 ± 10.32	0.003	23.19 ± 13.02	<0.001
β (stiffness)	5.19 ± 1.61	<0.001	8.06 ± 2.47	<0.001	9.76 ± 1.85	<0.001
PWV (m/s)	4.95 ± 0.89	<0.001	6.41 ± 1.1	<0.001	8.18 ± 1.08	<0.001

Table 3: Correlations between the selected laboratory parameters and PCSK9 concentrations

Parameters	PCSK9	
	R	*p* value
hsCRP	0.02	0.774
monocytes	0.19	0.014
HDL	0.04	0.607
LDL	0.38	<0.001
TG	0.41	<0.001
LPA	0.17	0.036
Insulin	0.16	0.041

Table 4: Correlations between the subclinical vascular changes and PCSK9 concentrations

Parameters	PCSK9	
	R	*p* value
cIMT	0.37	<0.001
AI	0.36	<0.001
β	0.38	<0.001
PWV	0.45	<0.001

5 Discussion

Many studies emphasize that the interaction between various risk factors such as dyslipidaemia and hypertension forms the foundation for developing cardiovascular complications. The same factors, which play a role in the onset of atherosclerosis, ultimately lead to the progression of cardiovascular disease as a whole [16]. The progression and stability of an already formed plaque are significantly decreased by inflammation and its mediators [10]. Studies have shown that the PCSK9 expression is strongly stimulated by infection, respectively, by inflammation itself. The inhibition of PCSK9 has been shown to decrease the expression of pro-inflammatory genes [17,18]. However, clinical trials following the effect of PCSK9 inhibition on inflammatory changes are unclear. Preclinical studies examining the association between inflammation and PCSK9 have demonstrated that PCSK9 levels are closely correlated with the inflammatory response [19]. These studies describe the strong effect of PCSK9 on the induction of inflammation as well as on plaque stability. The recent Glagov study [10] has reported significant reductions in LDL and total cholesterol levels after administration of evolocumab. In the active arm of this study, a significant reduction in plaque volume was also noted. This effect on atherosclerotic changes was probably independent of inflammation since hsCRP values did not change significantly in the arm with evolocumab treatment.

This study aimed to determine the serum PCSK9 concentrations at different degrees of subclinical atherosclerosis and then to compare them with the subclinical inflammation measured by the hsCRP. In our previous study [14], we found that there was a significant correlation between the PCSK9 concentrations and elevated BMI values. This study also indicated that the weight gain was related to elevated circulating levels of PCSK9. However, when factors other than PCSK9 were included in the calculations, especially lipid parameters, the significant linear correlation/significant effect of BMI on vascular changes decreased. The positive correlation of PCSK9 with both cIMT and BMI highlights that PCSK9 levels could be one of the mediators of cardiometabolic changes in obesity. It is widely accepted that lipid metabolism, especially of LDL particles, determines the progression of atherosclerotic changes. When serum levels fluctuate, functional changes accelerate the formation of atherosclerosis [20]. PCSK9 is responsible for the regulation of lipoprotein metabolism, but it may also have non-lipid effects such as its role in liver regeneration and steatosis, as are currently being studied. In our study, PCSK9 values were identified as being correlated with HDL, LDL and LPA. PCSK9 was also found to be associated with insulin concentrations, correlating with the results of previous studies [21].

To determine the degree of atherosclerotic changes, we chose to quantify the thickness of the intima-media of the carotid artery. Numerous studies have shown that the cIMT has a prognostic value for subsequent cardiovascular events such as MI and stroke. It can

Table 5: Multivariable regression analysis of various parameter effects on vascular changes

	cIMT		Beta		AI		PWV	
	β	*p* value	*β*	*p* value	*β*	*p* value	*β*	*p* value
Sex	−0.3	0.25	−0.12	0.57	−3.26	0.03	0.01	0.6
Age	0.001	0.387	0.13	<0.001	0.4	<0.001	0.05	<0.001
PCSK9	7.26×10^{-4}	0.016	0.01	0.01	0.03	0.13	0.007	<0.001
LDL	0.026	0.17	0.24	0.37	4.92	<0.001	0.1	0.45
TG	0.053	0.05	−0.29	0.47	−1.49	0.35	−0.01	0.96

also be used as a predictive value in determining the cardiovascular morbidity and mortality [22]. Crucially, this study found that levels of PCSK9 concentrations were significantly increased in the subclinical vascular change group, compared to the group with normal vascular findings. However, no significant increase in PCSK9 values was found between the subclinical group and the already detected plaque group. The cIMT thickness values increase with age, and its physiological values are also determined based on sex and the measurement side [23]. Because of these factors, a multivariate analysis was performed to analyse the impact of PCSK9 together with age, gender, as well as LDL on cIMT values. The analysis revealed a significant effect of age, sex and PCSK9 levels on cIMT values and therefore assumed that PCSK9 levels may act as a significant mediator of vascular changes side by side from LDL-C. We also assume the possible role of PCSK9 in early atherosclerotic changes as well as in the development of cardiometabolic changes, as described in the previous study [10]. Other studies have shown complementary results, observing increases in PCSK9 levels in patients with metabolic syndrome and type 2 diabetes. In these studies, PCSK9 levels correlated with the finding of atherosclerosis and degree of insulin resistance [24].

Table 6: Relationship between the subclinical vascular changes and PCSK9 concentrations in different groups of patients according to the severity of vascular changes

Parameter	PCSK9 in $cIMT_{<75‰}$		PCSK9 in $cIMT_{>75‰}$		PCSK9 in Plaque group	
	R	*p* value	*R*	*p* value	*R*	*p* value
cIMT	0.35	0.004	0.14	0.297	0.17	0.304
AI	0.43	<0.001	0.08	0.583	0.04	0.851
β	0.45	<0.001	0.15	0.277	0.08	0.674
PWV	0.37	0.002	0.29	0.028	0.22	0.184

However, the results of our study after re-analysing the data but separately in the groups with different vascular changes showed a correlation between the PCSK9 and cIMT only in the patient group with values of cIMT below 75‰, thus only in the patient group still in the reference values. No significant correlation between cIMT and PCSK9 was found in the groups with more advanced vascular changes. Based on these results, we assume that PCSK9 plays an essential role in the initiation of atherosclerotic lesions and during the later stages, the effect is essential but not linear, and the PCSK9 pathways act as one of the risk factors.

Several studies have shown an association between the elevated levels of PCSK9 with acute coronary syndrome [25] and polytrauma [26]. A recent study found that the future risks of cardiovascular events could be demonstrated by monitoring the circulating levels of PCSK9. Increased levels of PCSK9 were found to be associated with a higher frequency of cardiovascular events during the following 15-year study period. Even after adjusting the LDL cholesterol levels and associated risk factors, this predisposition persisted [27]. In pathological conditions such as acute coronary syndrome and sepsis, elevated levels of PCSK9 are found [28].

Significantly elevated PCSK9 values were detected in groups with atherosclerotic changes, either plaque or $cIMT_{>75‰}$ compared to the healthy group. However, PCSK9 values correlated with vascular changes, despite a significant increase only in the healthy group. This finding suggests that PCSK9 is essential to the overall initiation of atherosclerotic changes, but its concentrations no longer change linearly with the grade of vascular changes, whether there is only subclinical atherosclerosis or plaque. A critical marker and mediator of the inflammatory response is hsCRP, which is a marker of subclinical atherosclerotic changes [29]. hsCRP also plays an essential role in atherothrombosis in plaque rupture. Statins are currently the best known and most commonly used drugs in primary and

secondary prevention of cardiovascular events. Their effect is also crucial in terms of pleiotropic effects in reducing vascular wall inflammation. The JUPITER [30] study with rosuvastatin focused not only on the effect of this statin but also on the confirmation that inflammation plays a vital role in atherosclerotic changes. This study has proven that an essential marker of these changes is hsCRP, which predicts an increased cardiovascular risk independently from LDL-C concentrations. On the other hand, several studies have revealed that the inhibition of PCSK9 does not have a significant hsCRP-lowering effect [10,12].

In previous studies, plasma levels of PCSK9 have been associated with obstructive coronary lesions in patients [31–33]. Elevated levels are also seen in cases of subclinical coronary atherosclerosis. The relationship between PCSK9 and coronary atherosclerosis may be influenced by numerous factors. The clinical presentation develops from atherosclerosis as seen in acute coronary syndromes to chronic atherosclerotic changes, which may indicate a possible change in the underlying mechanisms and prevent the use of association studies that allow results to be extended from high-risk groups to lower risk populations [34].

Clinical, genetic and experimental evidence linked PCSK9 with metabolic syndrome [35]. In the study of Caselli et al., a robust link was found between the lower PCSK9 levels and HDL cholesterol levels, independent of statin use [36]. Lower PCSK9 levels and lower levels of adiponectin, which further emphasize the link between PCSK9 and a specific metabolic phenotype, were also associated [36]. Identification of PCSK9 in the atherosclerotic plaque suggests a possible local effect on smooth muscle cells (SMCs), macrophages and endothelial cells [37]. PCSK9 theoretically targets the plaque via two distinct strategies: hematogenously (binding to lipoproteins) or can be synthesized within the arterial wall from SMCs [38]. Macrophages, which are present in the atherosclerotic plaque, function to scavenge lipoprotein particles. They eventually however become foam cells contributing to a local release of inflammatory molecules and factors that further promote lipoprotein retention and extracellular matrix degradation. Both local cytokines and oxidized LDL have a significant influence on macrophage polarization [39] and it has been shown that PCSK9 is also capable of regulating the inflammatory properties of macrophages [40].

In our study, we determined the inflammatory changes by the values of hsCRP in different groups, according to the severity of subclinical vascular changes. The levels of hsCRP were significantly increased in the group with plaque versus the subclinical group and also in comparison with the healthy cIMT group. These results were complementary to the results of PCSK9, whose values were significantly increased in the subclinical group versus healthy and plaque groups versus healthy but not between the subclinical and plaque group. Interestingly, no significant correlation was found between the concentrations of hsCRP and PCSK9. According to these results, we assume that PCSK9 could play a crucial role in the initiation of atherosclerotic changes, but later with the progression of lesion, inflammatory and degenerative pathways significantly co-participate, independently of the lipid metabolism.

6 Conclusion

A significant increase in PCSK9 levels was found in patients with subclinical atherosclerotic changes, compared to groups with normal cIMT values. Surprisingly, no significant increase in PCSK9 values was observed between the early stages in comparison with the plaque group. PCSK9 concentrations correlated significantly with vascular change values; however, after re-analysing the data for each study group, this significant correlation persisted only in the group with normal cIMT values. Based on the results, we suggest that PCSK9 levels significantly affect the initiation of atherosclerotic lesions, but after the initiation of lesions, there are other important factors (inflammation, cytokines, etc.) that regulate the progression of atherosclerosis. These findings highlight the characteristics of atherosclerosis behind lipid metabolism and emphasize the need to address the management of patients comprehensively to reduce their cardiovascular risk.

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