Proprotein convertase subtilisin/kexin type 9 in rheumatoid arthritis

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Abstract

Objective

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease that regulates cholesterol metabolism through low-density lipoprotein receptor degradation and that has been linked with cardiovascular risk. The purpose of the present study was to examine whether PCSK9 levels are related to both abnormalities in the lipid profile and the severe atherosclerosis that occur in rheumatoid arthritis (RA) patients.

Methods

Cross-sectional study that encompassed 520 individuals; 326 patients with RA and 194 age- and sex-matched controls. PCSK9 and lipoproteins serum concentrations, standard lipid profile and carotid intima-media thickness (cIMT) and carotid plaques were assessed in patients and controls. A multivariable analysis, adjusted for standard cardiovascular risk factors, was performed to evaluate the influence of PCSK9 on RA related dyslipidaemia and subclinical carotid atherosclerosis.

Results

After adjusting for classical cardiovascular risk factors, lipid profile and statins, RA patients showed lower PCSK9 serum concentrations than controls (beta coefficient -45 95%CI [-53, -38] ng/ml, p=0.00). PCSK9 was associated with both cIMT and the presence of carotid plaques in RA patients. However, this association was lost after adjusting for classical cardiovascular risk factors.

Conclusion

PCSK9 is down-regulated in patients with RA.

Key words

PCSK9, rheumatoid arthritis, carotid intima media thickness, carotid plaques, lipids

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Introduction

Several reports indicate that rheumatoid arthritis (RA) is a proatherogenic disease associated with increased cardiovascular mortality (1, 2). A high inflammatory burden in patients with RA appears to be the key driver of this increased cardiovascular risk (3). There is growing evidence suggesting that an excessive inflammatory burden is responsible, at least partially, for the 'lipid paradox' in RA in which cholesterol, an important cardiovascular risk factor in the general population, is inversely related to cardiovascular risk in patients with untreated RA (4, 5). In contrast, suppression of RA-associated inflammation leads to elevation of lipid values, which also coincides with a reduction of cardiovascular events (6, 7). A similar inverse relationship has also been observed with other medical conditions associated with a proinflammatory state-like sepsis, cancer, immediate post-myocardial infarctions, and post-surgery settings (8). The mechanisms by which the inflammatory process in RA can lead to these lipids changes are still far from being fully understood (9, 10).

Proprotein convertase subtilisin kexin 9 (PCSK9), a serine protease, plays an important role in low-density lipoprotein (LDL) metabolism. PCSK9, which is synthesised primarily in the liver, enters the circulation, where it binds to hepatic LDL receptors and targets them for degradation (11). This process reduces the capacity of the liver to bind and remove LDL-cholesterol and results in increased LDL-cholesterol levels (12). Consistent evidence from pre-clinical studies indicates that modulation of PCSK9 activity may have potential positive effects on coronary heart disease (13, 14). The reduced incidence of cardiovascular events in patients bearing PCSK9 loss-of-function mutations supported this concept and provided a strong rationale for the development of molecules capable of inhibiting PCSK9 function (15). In this sense, blocking the interaction between PCSK9 and LDL receptors by the use of a fully human monoclonal antibody that binds PCSK9 has been found to lower LDL-cholesterol levels in patients with hypercholesterolaemia (16) and to reduce the rate of cardiovascular events (17, 18).

To the best of our knowledge, there is little information on the role of PCSK9 in RA vis-à-vis inflammation dyslipidaemia. For this reason, we conducted a study to assess whether PCSK9 is associated with the changes that inflammation and the disease exert over the lipid profile of RA patients. We additionally aimed to establish if PCSK9 is linked to severe atherosclerosis based on carotid ultrasounds in individuals with RA.

Materials and methods

Study participants

This was a cross-sectional study that included 520 individuals, 326 patients with RA and 194 age- and sex matched controls. All RA patients were 18 years old or older and fulfilled the 2010 ACR/EULAR diagnostic criteria (19). They had been diagnosed by rheumatologists and were periodically followed at rheumatology outpatient clinics. For the purpose of inclusion in the present study, RA disease duration was required to be ≥1 year. Although anti-tumor necrosis factor-alpha (TNF) treatment has been associated with changes in lipid profiles (20), RA patients undergoing TNF-alpha antagonist therapy were not excluded in the present study. None of the patients included in this series received anti-IL6 receptor monoclonal antibody (tocilizumab) or any other type of biologic agents different from anti-TNF-alpha blockers. The control group consisted of patients recruited from the Spanish Camargo Cohort (21, 22). This cohort was set up between February 2006 and February 2011, and individuals included in this cohort have been followed ever since. The aim of this cohort was to evaluate the prevalence and incidence of metabolic bone diseases and mineral metabolism disorders. Controls included in the current study were age- and sex-matched subjects without any known condition or drug treatment that could influence lipids and were not taking any lipid-lowering medications other than statins. None of the controls was receiving glucocorticoids. However, since they are often used in the

management of RA, patients taking prednisone, or an equivalent dose ≤10 mg/day, were not excluded. As previously mentioned, both patients and controls under statins treatment were allowed to participate in the study. Patients and controls were excluded if they had a history of myocardial infarction, angina, stroke, a glomerular filtration rate <60 ml/min/1.73 m^2, a history of cancer, or any other chronic disease, or evidence of active infection. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and at Hospital Universitario Marqués de Valdecilla (both in Spain), and all subjects provided informed written consent.

Data collection

Surveys in RA patients and controls were performed in the same way. Subjects completed a cardiovascular risk factor and medication use questionnaire and underwent a physical examination to determine their anthropometrics and blood pressure. Medical records were reviewed to ascertain specific diagnoses and medications. In patients with RA, disease activity was measured using the Disease Activity Score (DAS28) in 28 joints (23), while disease disability was determined using the Health Assessment Questionnaire (HAQ) (24). Clinical Disease Activity Index (CDAI) (25) and Simple Disease Activity Index (SDAI) (26) scores for RA disease activity were performed as previously described.

Lipids and PCSK9 assessments

Fasting serum samples were collected and frozen at -80°C until analysis of circulating lipids. Human PCSK9 was measured using an ELISA kit (Cell Biolabs Inc., San Diego, CA, USA). Intra- and inter-assay coefficients of variation were 4% and 8%, respectively. Cholesterol, triglycerides, and high-density lipoprotein (HDL)-cholesterol were measured using the enzymatic colorimetric assay (Roche). Cholesterol ranged from 0.08 to 20.7 mmol/l (intra-assay coefficient of variation 0.3%); triglycerides ranged from 4 to 1.000 mg/dl (intra-assay coefficient of variation 1.8%); and HDL-cholesterol ranged from 3 to 120 mg/dl (intra-assay variation coefficient 0.9%). LDL-cholesterol was calculated using the Friedewald formula (27). Standard techniques were used to measure plasma C-reactive protein (CRP) and the Westergren erythrocyte sedimentation rate (ESR).

Table I. Demographic data of controls and patients.

	Controls (n=194)	Patients (n=326)	*p*
Female, n (%)	134 (70)	233 (72)	0.75
Age, years	62 ± 9	62 ± 13	0.81
Weight, kg	74 ± 15	74 ± 15	0.61
Height, cm	161 ± 8	161 ± 9	0.86
BMI, mg/cm^2	28 ± 5	28 ± 5	0.51
Abdominal circumference, cm	93 ± 14	98 ± 13	**0.00**
Systolic pressure, mmHg	133 ± 15	138 ± 20	**0.00**
Diastolic pressure, mmHg	84 ± 41	80 ± 11	0.08
Comorbidities			
Hypertension, n (%)	62 (33)	166 (51)	**0.00**
Diabetes, n (%)	10 (5)	61 (19)	**0.00**
Dyslipidaemia, n (%)	39 (20)	148 (46)	**0.00**
Currently smoking, n (%)	42 (22)	29 (16)	0.17
Antihypertensive treatment, n (%)	36 (19)	167 (52)	**0.00**
Statins, n (%)	21 (11)	126 (39)	**0.00**
Laboratory data			
ESR, mm/1st h	-	25 (12-40)	
CRP, mg/l	1.0 (1.0-4.0)	3.5 (1.6-6.9)	**0.00**
Glucose, mg/dl	90 (83-97)	88 (80-101)	0.46
Cholesterol, mg/dl	223 ± 36	202 ± 37	**0.00**
Triglycerides, mg/dl	95 (71-129)	111 (82-158)	**0.00**
LDL-cholesterol, mg/dl	139 ± 34	118 ± 32	**0.00**
HDL-cholesterol, mg/dl	63 ± 17	57 ± 16	**0.00**
LDL:HDL-cholesterol ratio	2.36 ± 0.85	2.24 ± 0.95	0.15
Non-HDL-cholesterol, mg/dl	159 ± 36	144 ± 37	**0.00**
Atherogenic index	3.74 ± 1.00	3.79 ± 1.62	0.61
PCSK9, ng/ml	174 ± 25	141 ± 44	**0.00**
RA-related data			
Disease duration, years		6.5 (2.7-12.7)	
DAS28		3.48 ± 1.31	
DAS28-CRP		3.35 ± 1.22	
HAQ		0.750 (0.375-1.125)	
SDAI		11 (6-19)	
CDAI		30 (9-92)	
Rheumatoid factor, n (%)		188 (59)	
ACPA, n (%)		161 (52)	
Current prednisone, n (%)		156 (48)	
Prednisone, mg/day		0 (0-5)	
Current DMARD		284 (87)	
Methotrexate, n (%)		243 (75)	
Leflunomide, n (%)		44 (14)	
Anti-TNF-apha therapy, n (%)		53 (16)	

Data represent number (%), mean ± standard deviation or median (interquartile range).
BMI: body mass index; ACPA: anti-cyclic citrullinated peptide antibody; DAS28: Disease Activity Score 28; HDL: high-density lipoprotein; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; CDAI: Clinical Disease Activity Index, SDAI: Simple Disease Activity Index; CRP: C-reactive protein; PCSK9: Proteinase K-like serine protease; HAQ: Health Assessment Questionnaire.

Carotid ultrasound assessment

A carotid ultrasound examination was used to assess carotid intima-media wall thickness (cIMT) in the common carotid artery and to detect focal plaques in the extracranial carotid tree in patients with RA. A commercially available scanner, Mylab 70, Esaote (Genoa, Italy) equipped with a 7–12 MHz linear transducer and an automated software guided radiofrequency technique—Quality Intima Media Thickness in real-time (QIMT, Esaote, Maastricht, Holland)—was used for this purpose (28-32). Based on the Mannheim consensus, plaque criteria in the accessible extracranial carotid tree (common carotid artery, bulb and

Table II. Multivariate analysis of the differences in PCSK9 and lipid profile in patients and controls.

	β coefficient (95% CI), *p*-value				
	Model 0	Model #1	Model #2	Model #3	Model #4
	Univariate model	Adjusted for sex, age, BMI and waist circumference	Model #1 plus hypertension, diabetes, dyslipidaemia and smoking	Model #1, #2 plus statins	Model #1, #2, #3 plus total cholesterol, triglycerides, LDL and HDL cholesterol
Controls (n=194) vs. RA patients (n=326)					
PCSK9, ng/ml	**-33 (-40, -27), <0.001**	**-36 (-43, -29), <0.001**	**- 41 (-48, -34), <0.001**	**-43 (-50, -36), <0.001**	**-45 (-53, -38), <0.001**
Lipid profile					
Cholesterol, mg/dl	**-21 (-28, -15), <0.001**	**-18 (-27, -12), <0.001**	**-18 (-28, -10), <0.001**	**-16 (-25, -7), 0.001**	
Triglycerides, mg/dl	**27 (13, 40), <0.001**	**36 (20, 51), <0.001**	**43 (27, 61), <0.001**	**42 (25, 60), <0.001**	
LDL-cholesterol, mg/dl	**-21 (-27, -15), <0.001**	**-22 (-29, -14), <0.001**	**-19 (-27, -12), <0.001**	**-16 (-24, -8), <0.001**	
HDL-cholesterol, mg/dl	**-6 (-9, -3), <0.001**	**-8 (-11, -4), <0.001**	**-9 (-13, -5), <0.001**	**-9 (-12, -5), <0.001**	
LDL:HDL-cholesterol ratio	-0.12 (-0.39, 0.05), 0.152	-0.04 (-0.24, 0.15), 0.659	0.05 (-0.17, 0.27), 0.665	0.13 (-0.09, 0.36), 0.231	
Non-HDL-cholesterol, mg/dl	**-15 (-22, -8), <0.001**	**-12 (-20, -4), 0.003**	**-10 (-19, -1), 0.028**	-7 (-16, 2), 0.130	
Atherogenic index	0.06 (-0.16, 0.29), 0.607	0.22 (-0.04, 0.48), 0.101	**0.34 (0.04, 0.63), 0.024**	**0.42 (0.12, 0.72), 0.006**	

p<0.05 are depicted in bold. PCSK9: proteinase K-like serine protease; HDL: high-density lipoprotein; LDL: low-density lipoprotein; RA: rheumatoid arthritis.

internal carotid artery) were defined as follows: a focal protrusion in the lumen measuring at least cIMT >1.5 mm; a protrusion at least 50 % greater than the surrounding cIMT; or arterial lumen encroaching >0.5 mm (33).

Statistical analysis

On the basis of previously published findings (34), we assumed a normal PCSK9 serum level of 160±30 ng/ml in controls and a difference of 5% in patients. Proceeding with these assumptions, by using a 1:0.6 relation, and according to a Student *t*-test with a level of 0.05 and a b level of 0.20, we estimated that we would need to enroll 278 patients and 185 controls. Demographic and clinical characteristics shown in Table I were compared between RA patients and controls using χ^2 tests for categorical variables or Student *t*-tests for continuous variables (data expressed as mean ± standard deviation- SD). For non-continuous variables, either a Mann-Whitney U-test was performed or a logarithmic transformation was made, and data were expressed as a median and interquartile range (IQR). To investigate the differences in PCSK9 serum levels between RA patients and controls, we constructed five models: an unadjusted model (Model 0) for the univariate difference in PCSK9 between patients and controls; Model 1 for the differences between patients and controls adjusted for sex, age, body mass index and waist circumference; Model 2 for the analysis of Model 1 plus hypertension, diabetes, dyslipidaemia and smoking; Model 3 for the analysis of Model 1 and 2 plus statins; and Model 4 for the analysis of the three previous models plus total cholesterol, triglycerides, and LDL-cholesterol and HDL-cholesterol. The relationship between PCSK9 serum concentrations and demographic variables, comorbidity and RA-related data was studied through univariate lineal regression. The differences in cIMT and the presence of carotid plaque according to percentiles of PCSK9 levels were assessed using both multivariable lineal and logistic regressions adjusting for age, sex and cardiovascular risk factors. All the analyses used a 5% two-sided significance level and were performed using SPSS software, v. 21 (IBM, Chicago, IL, USA). A *p*-value <0.05 was considered statistically significant.

Results

Demographic, analytical and disease-related data

A total of 520 age- and sex-matched participants, 326 patients with RA and 194 controls, with a mean ± SD age of 62±9 years and 62±13 years (*p*=0.81), respectively, were included in this study. Demographic and disease-related characteristics of the participants are shown in Table I. There were no differences between patients and controls with regard to body mass index. However abdominal circumference and the presence of hypertension, dyslipidaemia, or diabetes were more common in patients with RA. Similarly, statins intake was more frequently observed in RA patients when compared to controls (39% *vs*. 11%, *p*=0.00). RA patients had moderately active disease as shown by DAS28 (3.48±1.31) and displayed a median HAQ of 0.750 (IQR 0.375–1.125). Almost half of them (48%) were taking prednisone (median current dose 0 [IQR 0–5] mg/day). As expected, CRP values were statistically significantly higher in patients compared to controls. One hundred and eighty eight (59%) patients were found to be positive for rheumatoid factor, 284 (87%) were on disease-modifying anti-rheumatic drugs and 53 (16%) were receiving anti-TNF-alpha therapy.

Differences in lipid profiles and PCSK9 serum levels between patients and controls

RA patients displayed lower levels of lipid metabolism molecules (Table I). This was the case for total cholesterol (202±37 *vs*. 223±36 mg/dl, *p*=0.00), LDL-cholesterol (118 ± 32 *vs*. 139±34 mg/dl, *p*=0.00), HDL-cholesterol (57±16 *vs*. 63±17 mg/dl, *p*=0.00), and non-HDL-cholesterol (144±37 *vs*. 159±36 mg/dl, *p*=0.00). In contrast, triglycerides were higher in RA patients than in controls (95 [71, 129] *vs*. 111 95%CI [82, 158] mg/dl, *p*=0.00). Although the atherogenic index was

Table III. Relationship of PCSK9 with RA-related data.

	PCSK9, ng/ml Beta coefficient (95% CI), *p*-value	
	Controls (n=194)	RA (n=326)
Demographics		
Male	-2 (-10, 6), 0.59	**-14 (-25, -4), 0.01**
Age, years	**-0.7 (-1.2, -0.1), 0.02**	**0.3 (-0.1, 0.7), 0.11**
BMI, mg/cm^2	-0.2 (-0.9, 0.6), 0.63	**1.9 (1.0, 2.9), 0.00**
Abdominal circumference, cm	0.00 (-0.27, 0.27), 0.98	**0.63 (0.25, 1.02), 0.00**
Hypertension	-2 (-10, 5), 0.55	**23 (13, 32), 0.00**
Diabetes	-6 (-21,10), 0.46	-2 (-13, 11), 0.81
Dyslipidaemia	**9 (0, 18), 0.045**	**22 (12, 31), 0.00**
Currently smoking	2 (-7, 10), 0.67	5 (-9, 20), 0.45
Statins	9 (-3, 20), 0.13	**24 (14, 34), 0.00**
Lipid profile		
Cholesterol x10, mg/dl	**1.1 (0.2, 2.1), 0.02**	1.1 (-0.2, 2.4), 0.11
Triglycerides x10, mg/dl	**0.9 (0.2, 1.6), 0.01**	**1.1 (0.5, 1.6), 0.00**
LDL cholesterol x10, mg/dl	0.8 (-0.2, 1.9), 0.12	0.0 (-1.4, 1.6), 0.91
HDL cholesterol x10, mg/dl	1.0 (-1.1, 3.2), 0.36	-0.8 (-3.7, 2.2), 0.59
LDL:HDL cholesterol ratio	1.1 (-3.3, 5.6), 0.61	1.4 (-3.7, 6.6), 0.58
Non-HDL cholesterol x10, mg/dl	**1.2 (0.2, 2.3), 0.02**	**1.3 (0.0, 2.6), 0.05**
Atherogenic index	1.6 (-2.1, 5.4), 0.40	2.8 (-0.8, 6.5), 0.13
Disease-related data		
ESR, mm/1st h	-	-0.6 (-0.3, 0.2), 0.53
CRP, mg/l	0.3 (-0.4, 0.9), 0.40	0.2 (-0.1, 0.4), 0.30
Disease duration, years		0.5 (-0.1, 1.0), 0.09
DAS28-ESR		3 (-1, 7), 0.13
DAS28-CRP		**4 (0, 9), 0.04**
HAQ		**11 (3, 20), 0.01**
SDAI		0.2 (-0.1, 0.5), 0.29
CDAI		0.0 (-0.0, 0.1), 0.73
Rheumatoid factor		-2 (-12, 9), 0.77
ACPA		-7 (-17, 2), 0.14
Current prednisone		0 (-9, 10), 0.95
Prednisone, mg/day		**1.85 (0, 4), 0.03**
Current DMARD		-3 (-18, 11), 0.65
Methotrexate		-2 (-13, 9), 0.73
Leflunomide		**19 (5, 33), 0.01**
Anti-TNF-alpha therapy		10 (-3, 23), 0.14

PCSK9 is considered the dependent variable.
p<0.05 are depicted in bold. RA: rheumatoid arthritis; PCSK9: proteinase K-like serine protease; BMI: body mass index; ACPA: anti-cyclic citrullinated peptide antibody; DAS28: Disease Activity Score 28; HDL: high-density lipoprotein; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; CDAI: Clinical Disease Activity Index, SDAI: Simple Disease Activity Index; CRP: C-reactive protein; PCSK9: Proteinase K-like serine protease; HAQ: Health Assessment Questionnaire.

higher in patients than in controls, the differences were not statistically significant (Table I).

RA patients showed lower PCSK9 serum concentrations than controls (141±44 *vs*. 174±25 μg/ml, *p*=0.00) (Table II shows the multivariable analysis of the differences in PCSK9 between controls and patients). The difference remained statistically significant after adjusting for demographic data, comorbidity, cardiovascular risk factors and statins intake. Additionally, RA patients still displayed decreased PCSK9 serum concentrations when PCSK9 differences between RA patients and controls were also adjusted for total cholesterol, triglycerides, LDL-cholesterol and HDL- cholesterol (beta coefficient -45 95%CI [-53, -38] μg/ml, *p*=0.00).

Relationship of PCSK9 with comorbidity and lipid levels in patients and controls and with RA-related data

Patients and controls with dyslipidaemia showed higher PCSK9 serum concentrations. Statin intake was associated with higher levels of PCSK9 (beta coef. 24 [14, 34] ng/ml, *p*=0.00) in RA patients. This association tended to be similar in controls (beta coef. 9 [-3, 20] ng/ml, *p*=0.13), although statistical significance was not reached. Male gender was found to negatively correlate with PCSK9 in patients (beta coef. -14 [-25, -4], *p*=0.01) but not in controls. Similarly, age, body mass index, abdominal circumference, and hypertension were positively associated with PCSK9 in RA patients. With regards to lipid profiles, although LDL-cholesterol did not show any relation with PCSK9, total cholesterol, triglycerides and non-HDL cholesterol were positive associated, to statistically significant degree, with PCSK9 serum levels in both controls and patients (Table III).

Regarding RA-related data, neither ESR nor CRP was associated with serum concentrations of PCSK9. However, DAS28-CRP (beta coef. 4 [0, 9] ng/ml, *p*=0.04) and HAQ (beta coef. 11 [3, 20] ng/ml, *p*=0.01) were positively associated with higher levels of PCSK9. Likewise, prednisone dose (beta coef. 2 [0, 4] ng/ml, *p*=0.03) and leflunomide treatment (beta coef. 19 [5, 22] ng/ml, *p*=0.00) were found to be associated with higher PCSK9 serum concentrations.

Relationship of PCSK9 with carotid ultrasound results in RA patients

RA patient PCSK9 levels in the third tertile had higher cIMT values (0.739 [0.660, 0.850] mm) when compared with the remaining patients (0.690 [0.600, 0.790] mm, *p*=0.02) as well as a higher frequency of carotid plaques (69% *vs*. 53%, *p*=0.02). However, this difference was lost when the results were adjusted for age, gender and classical cardiovascular risk factors (Table IV).

Discussion

Monoclonal antibodies that inhibit PCSK9 – evolocumab and alirocumab – have recently emerged as a new class of drugs that very effectively lowers LDL-cholesterol levels (17, 18). These drugs have also been shown to reduce the rate of cardiovascular events (35). To the best of our knowledge, the present study assesses for first time PCSK9 levels in a large series of RA patients. It is worth noting that patients with RA had lower serum concentrations of PCSK9 than matched controls. This was independent of the expected decrease in LDL-cholesterol levels in relation to RA disease activity.

Table IV. Relationship of PCSK9 with cIMT and carotid plaques.

	PCSK9 (tertiles)			*p*-value	
	1	2	3	Unadjusted	Adjusted
cIMT, mm	0.690 (0.600-0.790)	0.734 (0.635-0.852)	0.739 (0.660-0.850)	0.02	0.82
Carotid plaque	53%	56%	69%	0.03	0.80

p-values denotes [#]3 tertile *vs*. [#]1. PCSK9: proteinase K-like serine protease. cIMT: carotid intima media thickness (expressed as median and interquartile range). Adjusted *p*-value for age, gender, diabetes, hypertension, dyslipidaemia and smoking.

Dyslipidaemia is commonly observed in patients with active RA, with lower total cholesterol levels, and with lower levels of HDL-cholesterol and LDL-cholesterol (36). In this regard, the differences observed in our study between RA patients and controls are in accordance with the current knowledge in this area. Remarkably, PCSK9 serum concentrations were lower in RA patients after adjusting for comorbidity, statins intake and LDL cholesterol levels. This finding is of potential relevance since one could initially believe that reduced PCSK9 levels in RA patients might stem from decreases in LDL-cholesterol levels related to disease activity. Nevertheless, PCSK9 and plasma LDL-cholesterol correlation in the general population is modest and variations in plasma PCSK9 concentrations only explained 7% of the variability in plasma LDL cholesterol (37). In our study, we did not find any correlation between LDL cholesterol and PCSK9 levels in patients or controls. In an attempt to explain the findings previously described, we sought to establish whether disease activity or other features of RA may be related to PCSK9 levels. In this regard, DAS28 showed a mild positive correlation with PCSK9 concentrations. However, ESR and CRP were not associated with PCSK9 levels. At present we cannot explain these findings. It is plausible to postulate that patients with higher disease activity could have increased PCSK9 levels. However, we did not find any correlation between LDL cholesterol and disease activity scores. Interestingly, prednisone intake was also positively associated with PCSK9. This finding could partially explain the effect that glucocorticoids exert on the lipid profiles of patients with RA (38).

We observed a positive association between statin intake and PCSK9 in our cohort of RA patients. This has already been described in the general population (34). Furthermore, treatment with high-dose statins has been found to reduce plasma levels of LDL cholesterol, which in turn may lead to an increase in levels of circulating PCSK9 (39). The reason statins exert this paradoxical effect may be due to an upregulation of LDL-receptor and PCSK9 gene expression mediated by the elevation of SREBP-2 (sterol regulatory element-binding protein-2) activity (40).

In keeping with the current knowledge on the relationship between PCSK9 and cardiovascular risk (14), we found a positive association of PCSK9 with cIMT and the presence of carotid plaques in patients with RA. Nevertheless, this association was lost after adjusting for classical cardiovascular risk factors. This finding supports the complex relationship of lipids with atherosclerosis in RA. Since PCSK9 was down-regulated in RA patients, while levels of PCSK9 remained positively correlated with cIMT or carotid plaques, we believe that PCSK9 maintains a proatherogenic role in chronic inflammatory diseases. Our findings are in accordance with a recent report suggesting that high HDL cholesterol confers a protective effect in RA patients similar to that observed in the general population and that there is a non-linear relationship between LDL cholesterol and cardiovascular disease risk in RA patients (41).

In conclusion, PCSK9 is down-regulated in RA patients and this does not appear to be the result of the decrease in low-density lipoprotein cholesterol observed in RA patients with active disease. Our findings provide additional evidence supporting the complex relationship between dyslipidaemia and cardiovascular risk in patients with RA.

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