Serum cathepsin S and cystatin C: relationship to subclinical carotid atherosclerosis in rheumatoid arthritis

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Abstract

Objective

To assess whether serum cathepsin S and cystatin C, two novel markers of cardiovascular disease risk, are associated with subclinical carotid atherosclerosis in patients with rheumatoid arthritis (RA).

Methods

Serum cystatin C and cathepsin S levels, carotid intima-media thickness (cIMT) and carotid plaques were assessed in a cross-sectional study involving 178 RA patients.

Results

An association between disease activity scores with higher levels of cystatin C, but not with cathepsin S, was found. Cystatin C levels were also associated with cIMT in the patient subgroup included in the higher quartile of cIMT (OR 1.31, 95%CI [1.00–1.72], p=0.04) after adjusting for traditional cardiovascular risk factors, age and sex. An association between serum cystatin C levels and carotid plaques was also found in the univariate analysis (OR 1.37, 95%CI [1.06–1.76], p=0.02). However, this significant association was lost after adjusting for traditional cardiovascular risk factors and age. Cathepsin S was not associated with cIMT or carotid plaques.

Conclusion

High cystatin C serum levels identify a subgroup of RA patients with a high risk of subclinical atherosclerotic disease.

Key words

rheumatoid arthritis, cystatin C, cathepsin S, cardiovascular risk, carotid intima-media thickness.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with increased cardiovascular risks (1). Traditional cardiovascular disease risk factors do not fully explain this situation (2). In this regard, subclinical atherosclerosis has been observed in patients with RA, even in those without traditional cardiovascular risk factors (3). As observed in the general population, carotid intima-media wall thickness (cIMT) has been found to predict the development of cardiovascular events in patients with RA (4).

Cathepsin S is a cysteine protease involved in major histocompatibility complex class II antigen presentation and in intracellular and extracellular proteolysis. A causal interplay between cathepsin S activity and inflammatory activity has been posited and previous studies have reported higher circulating levels of cathepsin S in patients with RA (5-7). Moreover, experimental studies suggest that cathepsin S contributes to the development and vulnerability of advanced carotid plaques (8). In addition, cathepsin S has been associated with increased cardiovascular mortality risk in the elderly (9). Cathepsin S activity is regulated at the cellular level by its endogenous inhibitor cystatin C (10). The serum level of cystatin C provides a precise test of kidney function and has been found to be a strong predictor of risk of death and cardiovascular events in the elderly (11, 12). An imbalance between the expression of cysteine cathepsins and their endogenous inhibitor cystatin C has been shown in human atherosclerotic lesions (13).

Although some studies addressing the relationship of cystatin C with atherosclerosis in patients with RA have been conducted (14), the potential association of cathepsin S with subclinical atherosclerosis in RA has not been reported. Since stratification of the cardiovascular risk to identify patients with RA at risk of disease is an issue of major interest (15), in the present study we aimed to establish if these two new biomarkers of cardiovascular disease risk could be linked to subclinical atherosclerosis in individuals with RA.

Material and methods

Study participants

One hundred and seventy-eight patients with RA were recruited for a cross-sectional study. Patients included in the study were 18 years or older and had been diagnosed with RA by a rheumatologist. All fulfilled the 2010 ACR/EULAR classification criteria (16). For inclusion in the present study, RA disease duration had to be at least 1 year. Exclusion criteria included a history of myocardial infarction, angina, stroke, a glomerular filtration rate <60 ml/min/1.73 m^2, a history of cancer or evidence of infection. Because corticosteroids are often used in the management of RA, patients taking prednisone were not excluded. The study protocol was approved by the institutional review committee from Hospital Universitario de Canarias (Spain), and all subjects provided written informed consent.

Data collection

The subjects completed a cardiovascular risk factor and medication use questionnaire and underwent a physical examination. Weight, height, body-mass index, waist-to-hip ratio and systolic and diastolic blood pressure (measured with the participant in a supine position) were assessed under standardised conditions. Diabetes mellitus was defined as a fasting plasma glucose level of 7.0 mmol per liter (126 mg per deciliter) or more, or the use of oral hypoglycemic agents or insulin. Information regarding smoking status (current smoker *versus* non-smoker), dyslipidemia and hypertension was obtained from the questionnaire. Disease activity was measured using the Disease Activity Score (DAS28) in 28 joints (17), whereas disease disability was determined using the Health Assessment Questionnaire (HAQ) (18). Clinical Disease Activity Index (CDAI) (19) and Simple Disease Activity Index (SDAI) (20) scores for RA disease activity were performed as previously described. A Systematic Coronary Risk Evaluation (SCORE) was calculated to determine the 10-year risk of fatal cardiovascular death in a population with low cardiovascular disease risk (which applies to the Spanish population) (21).

Table I. Demographics, laboratory data and disease-related characteristics of 178 patients with RA.

Age, years	55 ± 11
Female, n (%)	149 (79)
Body mass index, kg/m^2	28 ± 5
Waist circumference, cm	97 ± 13
Hip circumference, cm	106 ± 11
Waist-to-hip ratio	0.92 ± 0.78
Cardiovascular risk factors	
Smoking, n (%)	29 (16)
Hypertension, n (%)	64 (35)
Dyslipidaemia, n (%)	79 (32)
Diabetes, n (%)	27 (15)
SCORE risk assessment, %	0.96 (0.26-2.69)
Disease-related data	
Disease duration, years	7 (4-15)
ACPA, n (%)	98 (58)
Rheumatoid factor, n (%)	119 (70)
Erosions, n (%)	60 (37)
Extraarticular manifestations, n (%)	18 (10)
DAS 28-ESR	3.74 ± 1.91
DAS 28-CRP	2.92 ± 0.98
SDAI	14 (21-8)
CDAI	80 ± 47
HAQ	0.750 (0.375-1.125)
Current prednisone, n (%)	62 (35)
NSAIDs. n (%)	78 (44)
DMARDS, n (%)	153 (86)
Methotrexate, n (%)	135 (76)
Biologic drugs, n (%)	39 (22)
Anti-TNF alpha drugs, n (%)	23 (13)
Analytical data	
CRP, mg/l	3.2 (1.6-6.2)
ESR, mm/1st hour	35 ± 22
Glucose, mg/dL	85 (78-94)
Triglycerides, mg/dL	129 (90-109)
HDL-cholesterol, mg/dL	55 ± 16
LDL-cholesterol, mg/dL	120 ± 33
Total cholesterol, mg/dL	205 ± 38
Apolipoprotein A1, mg/dL	170 ± 28
Apolipoprotein B, mg/dL	105 ± 25
ApoA : ApoB ratio	0.64 ± 0.20
Atherogenic index	4.0 ± 1.5
Cathepsin S, micrg/L	26.54 ± 13.76
Cystatin, mgr/L	1.87 ± 0.85
Carotid assessment	
cIMT, mm	0.670 ± 0.143
Unilateral plaque, n (%)	66 (37)
Bilateral plaques, n (%)	41 (23)

Data are expressed as mean (± standard deviation) or median (interquartile range).
Dichotomous variables are expressed as n and percentage. DAS28: Disease Activity Score.
ACPA: anti-citrullinated peptide/protein antibody; DMARD: disease-modifying anti-rheumatic drug; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; SDAI: Simple Disease Activity Index; CDAI: Clinical Disease Activity Index; cIMT: carotid intima-media thickness; HDL-C: high-density cholesterol lipoprotein; LDL-C: low-density cholesterol lipoprotein.

This was then adapted for RA patients by introducing a 1.5 multiplier factor when at least 2 of the following 3 criteria were met: disease duration >10 years, rheumatoid factor or anti-citrullinated protein antibodies (ACPA) positivity, and the presence of certain extra-articular manifestations (1).

Carotid ultrasound assessment

A Carotid ultrasound examination was used to assess cIMT in the common carotid artery and to detect focal plaques in the extracranial carotid tree. 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 (22-26). Using Mannheim consensus criteria, plaque criteria in the accessible extracranial carotid tree (common carotid artery, bulb and internal carotid artery) were 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 (27). For this study carotid plaques were defined as present or absent. Since a cIMT value greater than or equal to 75th percentile represents a high risk of cardiovascular disease, and values in the 50th percentile are considered average and indicative of unchanged cardiovascular disease risk, these two percentiles (adjusted for sex and age) were reported (27).

Cathepsin S and cystatin C serum level determination

Serum levels of cathepsin S were measured by an enzyme-linked immunosorbent assay (human cathepsin S [total] DY1183; R&D Systems, Minneapolis, Minnesota, USA). Serum levels of cystatin C were measured by an enzyme-linked immunosorbent assay (DY1196; R&D Systems, Minneapolis, Minnesota, USA). The intra-assay coefficient of variation was <3% for both assays. Standard techniques were used to measure plasma glucose, C-reactive protein (CRP), the Westergren erythrocyte sedimentation rate (ESR), and serum lipids.

Statistical analyses

Logarithmic transformation was performed to achieve a normal distribution for skewed variables. Continuous variables were expressed as mean ± standard deviation (SD); for non-continuous variables, data were expressed as a median (interquartile range-IQR). Univariate regression analysis was utilised to study the relationship between cathepsin S and cystatin C serum levels and cardiovascular risk factors and disease-related data. To investigate the relationship of these two molecules with cIMT and carotid plaques, we constructed three models: an unadjusted model for the univariate analysis of the carotid results; model 1 for the analysis adjusted for smoking, dyslipidemia, hypertension and diabetes; and model 2, which was comprised of model 1 plus age and sex.

All the analyses used a 5% two-sided significance level and they were performed using SPSS software, version 21 (IBM, Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

Results

Characteristics of the participants

A total of 178 RA patients with a mean ± SD age of 55±11 years were included in this study. The demographic and disease-related characteristics of the participants are shown in Table I. Regarding cardiovascular risk factors, 35% percent of the patients had hypertension, 32% dyslipidemia and 15% diabetes. Disease duration was 7 (IQR 4-15) years, 58% were positive for anti-citrullinated protein antibodies and 70% were rheumatoid factor positive. Patients had moderate-active disease as shown by DAS28 (3.74±1.91). One third (35%) were taking prednisone, 86% were under disease-modifying antirheumatic drugs and 22% were taking anti-TNF-alpha or other biologic therapies. The mean cIMT was 0.670±0.143 mm, and 66 patients (37%) had carotid plaques in the carotid ultrasound assessment.

Association of cystatin C and cathepsin S with demographic, cardiovascular risk factors and RA-related data

Both cystatin C and cathepsin S were significantly associated with hyper-

Table II. Cystatin C and cathepsin S association with disease-related data and lipid profile.

	beta coef. 95% CI *log* Cystatin C, mg/l	*p*	beta coef. 95% CI *log* Cathepsin S, µg/l	*p*
Cystatin C, mgr/l	**0.05 (-0.01-0.10)**	**0.08**	-	
Cathepsin S, mcgr/l	-		**0.01 (0.00-0.01)**	**0.04**
Demography and cardiovascular risk factors				
Sex, male	0.15 (-0.04-0.34)	0.13	**-0.28 (-0.44-0.11)**	**0.00**
Age, years	**0.02 (0.01-0.02)**	**0.00**	0.02 (-0.00-0.01)	0.56
Hypertension	**0.20 (0.04-0.37)**	**0.02**	**0.18 (0.04-0.32)**	**0.01**
Diabetes	**0.24 (0.02-0.47)**	**0.03**	**0.21 (0.02-0.40)**	**0.03**
Dyslipidaemia	0.09 (-0.07-0.26)	0.26	0.04 (-0.10-0.18)	0.55
Current smoking	-0.03 (-0.25-0.19)	0.79	0.15 (-0.04-0.34)	0.13
Lipid profile				
Cholesterol, mg/dl x 10	0.01 (-0.01-0.03)	0.35	0.01 (-0.01-0.03)	0.22
Triglycerides, mg/dl x 10	**0.01 (0.00-0.02)**	**0.00**	**0.01 (0.00-0.02)**	**0.02**
LDL, mg/dl x 10	0.01 (-0.02-0.03)	0.73	0.00 (-0.02-0.02)	0.81
HDL, mg/dl x10	**-0.05 (-0.10-0.00**	**0.06**	-0.01 (-0.05-0.04)	0.78
Apo A, mg/dl x10	0.00 (-0.03-0.02)	0.74	-0.01 (-0.03-0.02)	0.56
Apo B, mg/dl x10	0.02 (-0.01-0.05)	0.18	0.02 (-0.01-0.05)	0.15
Lipoprotein A, mg/dl x 10	0.01 (0.00-0.02)	0.15	0.00 (-0.01-0.01)	0.99
Atherogenic index	**0.06 (0.01-0.11)**	**0.01**	0.03 (-0.02-0.08)	0.19
Apo B : Apo A ratio	0.29 (-0.10-0.68)	0.15	0.28 (-0.07-0.63)	0.12
Disease-related data				
ESR, mm/1st hour	**0.00 (0.00-0.01)**	**0.02**	0.00 (-0.00-0.00)	0.71
CRP, mg/l	0.00 (-0.00-0.01)	0.90	0.00 (-0.00-0.00)	0.95
Disease duration, years	0.00 (-0.00-0.01)	0.41	**0.01 (0.00-0.02)**	**0.02**
ACPA	-0.08 (-0.25-0.09)	0.33	0.03 (-0.16-0.17)	0.71
Rheumatoid factor	-0.07 (-0.25-0.11)	0.44	0.08 (-0.07-0.23)	0.30
Prednisone use	0.06 (-0.11-0.23)	0.48	-0.05 (-0.20-0.09)	0.49
Prednisone, mg/day	0.01 (-0.02-0.03)	0.59	0.01 (-0.02-0.03)	0.65
DMARDs treatment	0.07 (-0.15-0.30)	0.53	0.06 (-0.14-0.26)	0.56
Anti TNF-alpha treatment	**-0.20 (-0.44-0.04)**	**0.09**	0.09 (-0.12-0.30)	0.38

ACPA: anticitrullinated peptide antibody; TNF: tumour necrosis factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DMARD: disease-modifying anti-rheumatic drug; LDL: low density lipoprotein; HDL: high density lipoprotein; Apo: apolipoprotein. *p*-values <0.10 are depicted in bold.

tension and diabetes. Cathepsin S was inversely related with male gender, whereas cystatin was strongly associated with age. Triglycerides positively correlated with both molecules. Regarding RA-related data, cathepsin S was associated with disease duration and cystatin C was positively related with higher ESR levels. However, neither positive rheumatoid factor nor prednisone use was associated with cystatin C or cathepsin S. A trend for lower levels of cystatin C was observed in patients undergoing anti-TNF-alpha therapy (*log* beta coef. -0.20 (-0.44-0.04), *p*=0.09) (Table II).

Table III shows the relationship of cystatin C and cathepsin S with disease activity scores. Higher cystatin C standard deviation values were associated with higher values of DAS28-ESR, DAS28-PCR and CDAI. When this analysis was performed with cathepsin S, no relationship was found between this molecule and disease activity scores.

Relationship of cystatin C and cathepsin S with SCORE, cIMT and carotid plaques

Cystatin C was statistically related with SCORE as well as with cIMT after adjusting for traditional cardiovascular risk factors like dyslipidemia, diabetes, smoking and hypertension. However, when an adjustment for age was made, statistical significance was lost. Nevertheless, when patients were stratified according to cIMT percentiles, an association of cystatin C levels with cIMT was observed in patients included in the higher quartile of cIMT (OR 1.31, 95% CI [1.00–1.72], *p*=0.04) after adjusting for traditional cardiovascular risk factors, age and sex. An association between serum cystatin C levels and carotid plaques was found in the univariate analysis (OR 1.37, 95%CI [1.06–1.76], *p*=0.02). However, this significant association was lost after adjusting for traditional cardiovascular risk factors and age (Table IV).

No significant association between cathepsin S levels and cIMT or carotid plaque was found.

Discussion

Cathepsin S is probably one of the most potent mammalian elastases and cystatin C is a cysteine protease inhibitor produced by nearly all human cells. Clinical studies have revealed higher serum cathepsin S levels in patients with coronary artery stenosis than in healthy controls, suggesting that this protein is a useful predictor of cardiovascular disease. The increase of cystatin C observed in patients with cardiovascular disease may reflect an attempt to counterbalance the potentially damaging increase in elastolytic activity (28). This imbalance may have an important role in pathological vascular remodeling (13). Our findings suggest that cystatin C may also be implicated in the development of subclinical atherosclerosis in RA. However, based on our data, this does not seem to be the case for cathepsin S.

We found that both molecules are associated with traditional cardiovascular risk factors. In this regard, both cathepsin S and cystatin C were positively associated with diabetes and hypertension, while cystatin C was strongly related to age. Our findings in RA are in keeping with those observed in non-rheumatic individuals and indicate that these two molecules are linked with traditional cardiovascular risk factors and global mortality (9, 12).

It is worth noting that cystatin C was associated with ESR whereas cathepsin S was related to disease duration in our cohort of patients with RA. Although not previously reported, we observed a trend for reduced levels of cystatin C, but not of cathepsin S, in RA patients undergoing anti-TNF-alpha therapy. This finding reinforces our contention that cystatin C is associated with systemic inflammation.

Cystatin C concentrations have been

Table III. Cystatin C and cathepsin S in relation with disease activity scores.

	DAS28		DAS28-PC3R		SDAI		CDAI	
Cystatin C								
-2 SD	2.50 (1.83-3.14)	-	1.94 (1.49-2.37)	-	6 (5-11)	-	37 (30-81)	-
-1 SD	3.78 (2.71-4.57)	**0.02**	2.88 (2.32-3.67)	**0.02**	15 (8-21)	0.11	82 (40-119)	**0.03**
+1 SD	3.60 (2.72-4.60)	**0.02**	2.87 (2.03-3.56)	**0.04**	14 (8-18)	0.13	90 (40-112)	**0.02**
+2 SD	3.90 (3.52-4.60)	**0.01**	2.93 (2.37-3.45)	**0.02**	15 (10-24)	0.11	82 (62-100)	**0.01**
Cathepsin S								
-2 SD	3.58 (3.00-4.24)	-	2.99 (2.39-3.54)	-	12 (9-16)	-	86 (70-104)	-
-1 SD	3.67 (2.81-4.68)	0.78	2.78 (2.25-3.59)	0.97	15 (8-22)	0.77	71 (33-115)	0.59
+1 SD	3.38 (2.39-4.30)	0.73	2.55 (1.96-3.28)	0.53	11 (6-17)	0.56	82 (22-105)	0.80
+2 SD	4.27 (3.10-4.70)	0.59	3.35 (2.49-3.91)	0.67	14 (9-24)	0.86	97 (72-111)	0.55

p-values are referred to the difference of each standard deviation (SD) with -2 SD, which is considered the reference category.
DAS28: Disease Activity Score; CRP: C-reactive protein; SDAI: Simplified Disease Activity Index; CDAI: Clinical Disease Activity Index.
p-values <0.05 are depicted in bold.

Table IV. Cystatin C and cathepsin S in relation with SCORE, CMIT and carotid plaque in rheumatoid arthritis patients.

	SCORE risk	log cIMT, mm	cIMT ≥ 50th percentile*	cIMT ≥75th percentile*	Carotid plaques
	beta coef 95%(CI), *p*		OR (95% CI), *p*		
Cystatin C, mg/l					
Unadjusted	**0.33 (0.10-0.55), 0.01**	**0.04 (0.01-0.06), 0.02**	1.16 (0.92-1.60), 0.21	1.27 (0.99-1.63), 0.06	**1.37 (1.06-1.76), 0.02**
Model 1		**0.03 (0.00-0.06), 0.04**	1.14 (0.89-1.46), 0.31	**1.31 (1.00-1.72), 0.04**	**1.28 (0.99-1.67), 0.06**
Model 2		0.00 (-0.02-0.03), 0.78			0.99 (0.75-1.31), 0.96
Cathepsin S, µg/l					
Unadjusted	-0.02 (-0.05-0.00), 0.09	0.00 (-0.00-0.00), 0.77	0.98 (0.95-1.01), 0.23	1.00 (0.97-1.03), 0.97	1.00 (0.98-1.03), 0.86
Model 1		-0.00 (-0.01-0.00), 0.29	0.97 (0.94-1.00), 0.08	1.00 (0.97-1.04), 0.92	1.00 (0.97-1.02), 0.80
Model 2		-0.00 (-0.00-0.00), 0.64			1.00 (0.98-1.03), 0.81

cIMT: carotid intima-media thikness*; SCORE: Systematic Coronary Risk Evaluation score. Model 1: Adjusted for smoking, dyslipidaemia, hypertension and diabetes. Model 2: Adjusted for model 1 plus age and sex. *p*-values <0.05 are depicted in bold.

found to be higher in patients with RA than in controls (29). Cystatin C was found to be associated with ESR and disease activity scores. Cystatin C levels were also linked to coronary artery calcium score, another useful surrogate marker of subclinical atherosclerosis (29). However, the association was lost when it was adjusted for traditional cardiovascular risk factors. In our study we assessed these correlations using cIMT and the presence of carotid plaques as markers of subclinical atherosclerosis. A carotid ultrasound seems to possess certain potential advantages compared to the coronary artery calcium score. In this regard, in non-rheumatic individuals, as well as in patients with RA, cIMT has proven useful for stratifying cardiovascular risk. In patients with RA, the carotid ultrasound assessment to determine the presence of carotid plaques has been shown useful in the stratification of cardiovascular risk in patients with RA (30). This is true even in those patients included in the category of low risk based on the cardiovascular risk charts (31).

This is especially true in younger women and men where coronary artery calcium scores may have limited discriminatory power due to the high frequency of a zero coronary calcium score in both sexes (32, 33). We believe this to be a possible explanation for the association of cystatin C with cIMT that we observed in our series of RA patients after adjusting for traditional cardiovascular risk factors and age.

Likewise, we found that cystatin C was independently associated with cIMT, but not with carotid plaques. This is not unusual in this kind of study. It is known that the clinical usefulness of cIMT measurement and plaque detection is linked to the patient's pretest cardiovascular disease risk, which is altered by the relative risk based on the presence of cardiovascular comorbidity.

We do not have an explanation for the lack of association between cathepsin S and subclinical carotid atherosclerosis in our series of RA patients. In contrast to cystatin C, cathepsin S had no association with RA disease activity scores. In this regard, even in the univariate analysis, cathepsin S showed no link to subclinical atherosclerosis. The lack of any relationship between cathepsin S and disease activity is in accordance with a prior report showing that although plasma levels of cathepsin S were significantly higher in patients with RA compared to healthy controls, there was no association with DAS28 (7). To the best of our knowledge, our study constitutes the first attempt to determine the relationship of cathepsin S with subclinical atherosclerosis in patients with RA.

We are aware of the potential limitations of our study. First, we did not select a control group because these molecules have been extensively studied in the general population. Another limitation may be the cross-sectional nature of our study and the absence of a replication cohort. Therefore, large-scale studies

in other cohorts of patients with RA are needed to validate our findings.

In conclusion, in our study we found an independent association of cystatin C with cIMT, but not with carotid plaques and a lack of association of cathepsin S with these two subrogates of subclinical atherosclerosis. Further research is needed to establish whether cathepsin S and cystatin C assessment, in addition with other biomarkers (34, 35), can be used to assess the risk of cardiovascular disease patients with RA.

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