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Disease activity in patients with rheumatoid arthritis increases serum levels of apolipoprotein C-III

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Abstract

Objective

Rheumatoid arthritis (RA) has been unequivocally associated with an increased burden of accelerated atherosclerosis, which, at least in part, is a consequence of the inflammation present in the disease. Apolipoprotein C-III (ApoC3) is a key molecule in triglycerides metabolism that has been linked to cardiovascular (CV) disease. Our objective was to study how ApoC3 is related to the characteristics of RA, paying special attention to its relationship with the inflammatory activity of the disease.

Methods

Cross-sectional study that included 430 patients with RA. In these patients, data related to the disease, classic CV risk factors, complete lipid profile, and serum ApoC3 levels were evaluated. A multivariable regression analysis was performed to study the relationship of the characteristics of RA with ApoC3.

Results

Abdominal circumference, obesity, type 2 diabetes, and circulating triglycerides were significantly associated with higher ApoC3 serum levels. Furthermore, C-reactive protein and erythrocyte sedimentation rate, as well as the disease activity score -DAS28- were significantly related to a higher circulating ApoC3 after multivariable analysis. Patients included in the moderate or high disease activity groups had higher ApoC3 serum levels compared to those in remission (beta coefficient 1.28 [95% confidence interval 0.16-2.39] mg/dl, p=0.025) when adjusting for confounders. The use of prednisone, disease-modifying anti-rheumatic drugs and anti-tumour necrosis factor therapies was associated with lower values of ApoC3.

Conclusion

The activity of the disease in patients with RA is independently associated with higher serum levels of ApoC3.

Key words

rheumatoid arthritis, disease activity, apolipoprotein C-III

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Introduction

Apolipoprotein C-III (ApoC3) inhibits the hydrolysis of triglycerides by lipoprotein lipase and has multiple proatherogenic effects on the arterial wall, including interference with normal endothelial function (1). Elevated ApoC3 levels are associated with increased triglycerides levels and an increased risk of atherosclerotic cardiovascular disease (CVD). In contrast, lower ApoC3 levels are associated with inferior triglycerides and a reduced risk of CVD (2). The mechanisms through which ApoC3 can contribute to the development of atherosclerosis are believed to be related to the fact that ApoC3 increases arterial inflammation through effects on both peripheral monocytes and endothelial cells, and that it inhibits nitric oxide function in endothelial cells by interfering with insulin signalling, thus causing endothelial dysfunction (3). For this reason, new therapies that inhibit ApoC3 have recently been developed. These new therapies may be a promising strategy in the prevention and treatment of CVD (4).

Rheumatoid arthritis (RA) has been unequivocally associated with an increased burden of accelerated atherosclerosis and CVD (5). This has been attributed, at least in part, to the effect that accompanying inflammation has on traditional CV risk factors, lipid molecules, adipokines, and endothelial cell activation biomarkers (6). It has been described that the lipid pattern is altered in patients with RA (7). This alteration of the lipid profile consists of a decrease in total cholesterol and HDL-cholesterol and LDL-cholesterol, and an elevation of triglycerides in the inflammatory phases of the disease (8,9). These modifications of the lipid pattern in RA has been also attributed to therapies like tocilizumab (10).

Since ApoC3 has been related to CVD in the general population and plays a key role in triglycerides and lipid metabolism, our objective was to study how ApoC3 is associated to the characteristics of RA. Specifically, we intended to analyse its relationship with the biological and clinical activity of the disease.

Material and methods

Study participants

This was a cross-sectional study that included 430 consecutively recruited patients with RA. All of them were 18 years old or older and fulfilled the 2010 ACR/EULAR classification criteria (11). They had been diagnosed by rheumatologists and were periodically followed-up at rheumatology outpatient clinics. For the purpose of inclusion in the present study, the duration of RA disease was required to be ≥1 year. Regardless of the possible use of statins, the patients included in the study should not have medical conditions or receive other pharmacological treatments that could influence lipids and not be taking any other lipid-lowering drugs (fibrates, resins, and niacin). Therefore, patients under statins were not excluded. However, since glucocorticoids are often used in the treatment of RA, patients taking prednisone or an equivalent dose ≤10 mg/day were allowed to participate. Patients 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 like hypothyroidism, heart or respiratory diseases, nephrotic syndrome, as well as evidence of active infection. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and at Hospital Universitario Doctor Negrín (both in Spain), and all subjects provided informed written consent (approval no. 2015_84).

Data collection and laboratory assessments

Individuals included in the study completed a CV risk factors and medications use questionnaire and underwent a physical examination. Body-mass index (the weight in kilograms divided by the square of the height in metres), abdominal circumference, and systolic and diastolic blood pressure were assessed under standardised conditions. Information regarding smoking status, diabetes and hypertension was obtained from the questionnaire. Medical records were reviewed to ascertain specific diagnoses and medications. Dis-

ease activity in patients with RA was measured using the Disease Activity Score (DAS28) in 28 joints (12). For the detection of ApoC3 an ELISA kit was used (Elabscience, USA). No significant cross-reactivity or interference between human ApoC3 and analogues is observed with this kit. Both intra and inter-coefficients of variability are <10% for this assay. Cholesterol, triglycerides, and HDL-cholesterol were measured using the enzymatic colorimetric assay. LDL-cholesterol was calculated using the Friedewald formula. Blood analysis were taken fasting and alcohol consumption was omitted the day before.

Statistical analysis

Demographic and clinical characteristics in patients with RA were described as mean (standard deviation) or percentages for categorical variables. For non-normally distributed continuous variables, data were expressed as median and interquartile range (IQR). Multivariable linear regression analysis, adjusting for confounders, was assessed to analyse the association between disease-related data and ApoC3 serum levels. Confounding variables were selected from demographics and traditional cardiovascular (CV) risk factors if they had a *p*-value lower than 0.20 in the univariable relationship with ApoC3. Besides, any variable that because clinical reasons was considered as confounder was included in the multivariable analysis. Beta coefficients of the linear regression analyses were calculated as non-standardised coefficients. Collinearity in the multivariable regression models was checked through the calculation of the variance inflation factor. All the analyses used a 5% two-sided significance level and were performed using Stata software, v. 17/SE (StataCorp, College Station, TX, USA). *p*-values <0.05 were considered statistically significant.

Results

Demographic and disease-related data

A total of 430 patients with RA were included in this study. Demographic- and disease-related characteristics of the

Table I. Demographics, cardiovascular risk factors, and disease-related data in patients with RA.

	RA (n=430)
Age, years	55 ± 10
Women, n (%)	350 (81)
BMI, kg/m^2	29 ± 15
Abdominal circumference, cm	97 ± 13
Cardiovascular data	
Cardiovascular risk factors, n (%)	
Current smoker	93 (22)
Obesity	137 (32)
Hypertension	148 (34)
Diabetes mellitus	54 (13)
Blood pressure, mm Hg	
Systolic	132 ± 20
Diastolic	81 ± 12
Statins, n (%)	138 (32)
Lipids	-
Total cholesterol, mg/dl	206 ± 38
Triglycerides, mg/dl	147 ± 86
HDL-cholesterol, mg/dl	57 ± 15
LDL-cholesterol, mg/dl	120 ± 34
LDL:HDL-cholesterol ratio	2.27 ± 0.93
Non-HDL cholesterol, mg/dl	149 ± 39
Lipoprotein (a), mg/dl	34 (11-107)
Apolipoprotein A1, mg/dl	174 ± 31
Apolipoprotein B, mg/dl	107 ± 43
Apo B:Apo A1 ratio	0.63 ± 0.24
Apolipoprotein C3, mg/dl	4.8 (2.1-8.7)
Disease-related data	
Disease duration, years	11 ± 9
CRP at time of study, mg/l	2.7 (1.3-6.1)
ESR at time of study, mm/ 1º hour	18 (7-32)
Rheumatoid factor, n (%)	303 (72)
ACPA, n (%)	253 (61)
DAS28-ESR	3.1 ± 1.4
Remission	166 (40)
Low activity	77 (18)
Moderate or high	176 (42)
HAQ	0.750 (0.250-1.250)
Current drugs, n (%)	
Prednisone	156 (36)
Prednisone doses, mg/day	5 (3-5)
NSAIDs	194 (45)
DMARDs	373 (87)
Methotrexate	316 (73)
Leflunomide	94 (22)
Hydroxychloroquine	45 (10)
Salazopyrin	28 (7)
Anti TNF therapy	83 (19)
Tocilizumab	23 (5)
Rituximab	7 (2)
Abatacept	12 (3)
Baricitinib	6 (1)
Tofacitinib	11 (3)
Historical disease-related data	
History of extraarticular manifestations, n (%)	38 (10)
Erosions, n (%)	166 (43)
CRP at time of disease diagnosis, mg/l	6.4 (2.6-17.1)
CRP >3 at time of diagnosis, n (%)	344 (80)
ESR at the time of diagnosis, mm/1º hour	24 (14-38)

Data represent mean ± SD or median (IQR) when data were not normally distributed.
LDL: low-density lipoprotein; HDL: high-density lipoprotein; CRP: C-reactive protein; NSAID: non-steroidal anti-inflammatory drugs; DMARD: disease-modifying anti-rheumatic drug; TNF: tumour necrosis factor; Obesity; ESR: erythrocyte sedimentation rate, BMI: body mass index; DAS28: Disease Activity Score in 28 joints; ACPA: anti-citrullinated protein antibodies; HAQ: Health Assessment Questionnaire.
DAS28-ESR categories are: remission <2.6, low 2.6-3.2, moderate >3.2-5.1, high >5.1

participants are shown in Table I. The mean age was 55±10 years and 81% of the patients were women. Patients had a body mass index and an abdominal circumference of 29±15 kg/m^2 and 97±13 centimetres, respectively. Traditional CV risk factors were common. In this regard, 22% were current smokers, 13% had type 2 diabetes, 32% were considered obese (body mass index equal to or greater than 30 kg / m^2) and 34% had hypertension. Additionally, 32% of the patients were taking statins at the time of the study. Full lipid profile is additionally described in Table I. The mean duration of the disease in this series of patients with RA was 11±9 years. The mean values of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), at the time of the study were 2.7 (IQR 1.3–6.1) mg/l and 18 (IQR 7–32) mm/1st hour, respectively. Seventy-two percent of patients were positive for rheumatoid factor, and 61% for anti-citrullinated protein antibodies (ACPA). Disease activity measured by DAS28-ESR was 3.1±1.4. Regarding this, 40% of the patients met the definitions of remission, and 18% and 42% were in the low, moderate/ high disease activity categories, respectively. Thirty-six percent of the patients were being treated with prednisone and 87% were taking at least one conventional DMARD in any of its types, methotrexate being the most widely used (73%). Nineteen percent of the patients were receiving anti-tumour necrosis factor therapies. The frequency of use of other treatments and historical disease-related data is shown in Table I.

Demographics, cardiovascular risk factors, and disease characteristics relation to apolipoprotein C3

Although age, sex, and body mass index were not associated with ApoC3, abdominal circumference and the presence of obesity and type 2 diabetes were significantly associated with higher serum levels of ApoC3 (Table II). Similarly, statin intake was significantly associated with higher circulating ApoC3. Regarding lipid profile, only triglycerides (beta coefficient -coef.- 0.01 [95% confidence interval (CI) 0.01-0.02] mg/dl, $p<0.001$) and HDL-cholesterol (beta coef. -0.04 [95%CI -0.07 to -0.002] mg/dl, $p=0.033$) were significantly associated with ApoC3 serum levels. All the aforementioned relationships were found in the univariable analysis (Table II).

Many associations were found between RA disease-related data and ApoC3 (Table II). In this sense, both acute phase reactants CRP (beta coef. 0.05 [95%CI 0.01–0.09] mg/dl, $p=0.008$) and ESR (beta coef. 0.03 [95%CI 0.004–0.06] mg/dl, $p=0.022$) were positively associated with increased circulating ApoC3 after fully multivariable analysis that included traditional CV risk factors, statins intake and triglycerides. Similarly, after adjusting, the disease activity score –DAS28-ESR– showed a positive and significant relationship with ApoC3 both when it was considered as continuous (beta coef. 0.43 [95% CI 0.05–0, 80] mg / dl, $p=0.025$) or as an ordinal variable (moderate/ high disease activity *versus* remission, beta coef. 1.28 [95% CI 0.16–2.39] mg / dl, $p=0.025$). It is noteworthy that the use of RA therapies was generally negatively related to ApoC3. In this sense, the dose of prednisone mg/day was associated with lower levels of ApoC3 (beta coef. -0.29 [95%CI -0.49 to -0.09] mg/dl, $p=0.005$), and the use of any conventional DMARD was related to significantly inferior ApoC3 values (beta coef. -1.69 [95%CI -3.16 to -0.21] mg/dl, $p=0.025$). Similar results were found when this analysis was performed individually for each DMARD. In this sense, the current use of leflunomide, salazopyrin, and hydroxychloroquine was associated with significantly lower serum levels of ApoC3 (Table II). A similar trend was observed for the intake of methotrexate and anti-alpha tumour necrosis factor therapies, although, in this case, significance was not reached. Additional information regarding the relationship of disease-related data with ApoC3 is shown in Table II.

Discussion

The present study encompasses the largest series of RA patients in whom ApoC3 has been studied. According to our findings, the clinical activity of the disease, and the acute phase reactants, are associated with higher levels of ApoC3.

Few studies have studied ApoC3 in RA. In this sense, in a report of 152 patients with RA in whom coronary artery calcium was evaluated at the beginning of the study and at year 3, it was found that ApoC3 was increased in those who showed progression of coronary artery calcium score compared to non-progressors (13). In this report, ApoC3 was considered a risk factor for the development of atherosclerosis in patients with RA. In another study that included a series of RA patients treated with etanercept for six months, ApoC3 showed significantly higher levels in non-responders compared to those who met criteria for improvement (14). This is in agreement with our findings, supporting the fact that disease activity is positively related to ApoC3. However, in contrast to these results, in another report, baricitinib was associated with an increase in serum ApoC3 after 12 weeks of treatment (15). In our study, that only included six patients taking baricitinib, we did not find this relationship.

The association of ApoC3 with inflammation has been previously described. A report of 1,455 people showed that those with metabolic syndrome had higher levels of ApoC3 and inflammatory markers like CRP and white blood cell count (16). In that study, mediation analysis showed that ApoC3 partially mediated the effect of inflammation on metabolic syndrome independently of triglycerides (16). Additionally, ApoC3 has been identified as an endogenous mediator that leads to alternative inflammasome activation in human monocytes. It is believed that, unlike classic inflammasome activators, this is due to the fact that ApoC3 induces the release of IL-1β from human monocytes in a dose-dependent manner (17).

It is noteworthy that the relationship between disease activity and ApoC3 found in our study was independent of triglyceride levels. This is of interest since ApoC3 is a key molecule in-

Table II. Demographics, cardiovascular risk factors, and disease-related relation to ApoC3 serum levels.

	Apo C3, mg/dl Beta coef. (95%CI), *p*-value					
	Univariable	*p*	Adj. R2	Multivariable	*p*	Adj. R2
Age, years	0.00 (-0.05-0.05)	0.96	-0.002			
Female, n (%)	-0.70 (-2.01-0.60)	0.29	0.000			
BMI, kg/m^2	0.01 (-0.02-0.04)	0.59	-0.002			
Abdominal circumference, cm	**0.09 (-0.05-0.13)**	**<0.001**	0.046			
Cardiovascular data						
Cardiovascular risk factors, n (%)						
Current smoker	-0.59 (-1.83-0.66)	0.35	0.000			
Obesity	**1.41 (0.32-2.50)**	**0.011**	0.013			
Hypertension	0.56 (-0.51-1.62)	0.30	0.000			
Diabetes mellitus	**3.08 (1.57-5.60)**	**<0.001**	0.035			
Blood pressure, mm Hg						
Systolic	0.01 (-0.01-0.04)	0.26	0.001			
Diastolic	0.00 (-0.04-0.04)	0.98	0.003			
Statins, n (%)	**1.25 (0.17-2.33)**	**0.024**	0.010			
Lipids						
Total cholesterol, mg/dl	0.00 (-0.01-0.01)	0.99	-0.002			
Triglycerides, mg/dl	**0.01 (0.01-0.02)**	**<0.001**	0.035			
HDL-cholesterol, mg/dl	**-0.04 (-0.07- -0.002)**	**0.033**	0.009			
LDL-cholesterol, mg/dl	-0.01 (-0.02-0.01)	0.31	0.000			
LDL:HDL cholesterol ratio	0.29 (-0.27-0.84)	0.32	0.000			
Non-HDL cholesterol, mg/dl	0.01 (-0.01-0.02)	0.39	-0.001			
Lipoprotein (a), mg/dl	0.00 (-0.01-0.01)	0.67	-0.002			
Apolipoprotein A1, mg/dl	-0.01 (-0.03-0.002)	0.092	0.004			
Apolipoprotein B, mg/dl	0.00 (-0.01-0.01)	0.90	-0.002			
Apo B:Apo A ratio	0.98 (-1.19-3.15)	0.38	-0.001			
Disease-related data						
Disease duration, years	-0.04 (-0.10-0.02)	0.15	0.003	-0.01 (-0.10-0.01)	0.14	0.090
CRP at time of study, mg/l	**0.02 (0.01-0.09)**	**0.012**	0.013	**0.05 (0.01-0.09)**	**0.008**	0.097
ESR at time of study, mm/ 1º hour	**0.03 (0.02-0.07)**	**0.001**	0.026	**0.03 (0.004-0.06)**	**0.022**	0.125
Rheumatoid factor, n (%)	-0.69 (-1.84-0.45)	0.24	0.001			
ACPA, n (%)	0.29 (-0.78-1.36)	0.60	-0.002			
DAS28-ESR	**0.52 (0.14-0.90)**	**0.007**	0.015	**0.43 (0.05-0.80)**	**0.025**	0.095
Remission	ref.			ref.		
Low activity	-0.02 (-1.45-1.42)	0.98	0.015	0.29 (-1.14-1.72)	0.69	0.094
Moderate and high	**1.48 (0.36-2.60)**	**0.010**		**1.28 (0.16-2.39)**	**0.025**	
HAQ	**-0.83 (-1.57- -0.09)**	**0.028**	0.009	**-1.04 (-1.77- -0.32)**	**0.005**	0.097
Current drugs, n (%)						
Prednisone	-0.28 (-1.34-0.79)	0.61	-0.002			
Prednisone doses, mg/day	**-0.32 (-0.51- -0.12)**	**0.002**	0.059	**-0.29 (-0.49- -0.09)**	**0.005**	0.078
NSAIDs	-0.43 (-1.46-0.59)	0.41	-0.001			
DMARDs	**-1.76 (-3.27- -0.25)**	**0.023**	0.010	**-1.69 (-3.16- -0.21)**	**0.025**	0.093
Methotrexate	-0.92 (-2.08-0.24)	0.12	0.004	-0.78 (-1.91-0.36)	0.18	0.085
Leflunomide	**-1.57 (-2.78- -0.33)**	**0.013**	0.012	**-1,57 (-2.79- -0.34)**	**0.012**	0.095
Hydroxychloroquine	**-4.52 (-6.10- -2.93)**	**<0.001**	0.068	**-4.88 (-6.46- -3.40)**	**<0.001**	0.159
Salazopyrin	**-4.69 (-6.68- 2.70)**	**<0.001**	0.047	**-4.75 (-6.78- -2.71)**	**<0.001**	0.127
Anti TNF therapy	-1.21 (-2.49-0.08)	0.066	0.006	-1.20 (-2.51-0.10)	0.069	0.089
Tocilizumab	-0.70 (-2.93-1.53)	0.54	-0.002			
Rituximab	1.52 (-2.45-5.48)	0.45	-0.001			
Abatacept	0.77 (-2.28-3.82)	0.62	-0.002			
Baricitinib	-2.45 (-6.72-1.83)	0.26	0.001			
Tofacitinib	**-3.74 (-6.90- -0.58)**	**0.021**	0.011	**-3.25 (-6.31- -0.19)**	**0.038**	0.091
Historical disease-related data						
History of extraarticular manifestations, n (%)	-0.40 (-2.25-1.45)	0.67	-0.002			
Erosions, n (%)	**-1.92 (-2.97- -0.87)**	**<0.001**	0.031	**-1.47 (-2.52- -0.43)**	**0.006**	0.106
CRP at time of diagnosis, mg/l	-0.01 (-0.04-0.01)	0.33	-0.001			
CRP >3 at time of diagnosis, n (%)	-0.02 (-1.31-1.27)	0.97	-0.002			
ESR at time of diagnosis, mm/1º hour	**0.04 (0.01-0.07)**	**0.004**	0.022	**0.03 (0.01-0.06)**	**0.019**	0.135

Beta coefficients in the linear regression analysis consider ApoC3 as the dependent variable.
Adjusted R2 are shown for the regression analysis. Multivariable analysis is adjusted for abdominal circumference, obesity, diabetes mellitus, statins intake and serum levels of triglycerides, apolipoprotein A1 and HDL cholesterol. For binary variables beta coefficients express the effect of the presence of that characteristic against it absence.
LDL: low-density lipoprotein; HDL: high-density lipoprotein; CRP: C reactive protein; NSAID: non-steroidal anti-inflammatory drugs; DMARD: disease-modifying anti-rheumatic drug; TNF: tumour necrosis factor; Obesity; ESR: erythrocyte sedimentation rate; BMI: body mass index; HAQ: Health Assessment Questionnaire; DAS28: Disease Activity Score in 28 joints; ACPA: Anti-citrullinated protein antibodies.
DAS28-ESR categories are: remission <2.6, low 2.6-3.2, moderate >3.2-5.1, high >5.1.

volved in the metabolism of triglycerides, and the latter are also modified in inflammatory states (16). In this sense, in our work, the association between disease activity and ApoC3 remained significant after multivariable adjustment that included triglycerides.

Several therapies used in the treatment of RA were associated with lower serum ApoC3 levels in our study. This was the case for prednisone, conventional DMARDs, and anti-TNF therapies (although statistical significance was not achieved in this case). We believe that patients receiving these therapies have lower disease activity and, for this reason, also express lower circulating levels of ApoC3. However, due to the cross-sectional design of our work, the exact effect of RA therapies on circulating ApoC3 will need to be elucidated in the future. Similarly, while disease activity was positively related to ApoC3, HAQ and erosions were negatively related to it. This may be considered contradictory. However, RA-related data like erosions and HAQ probably express an accumulation of damage over time that does not have to be correlated with the disease activity at a given moment.

Statins have been described to exert an inhibition over ApoC3 (18). Despite this, in our work we found a positive relation of statins on ApoC3 serum levels. We believe that statins were used, probably, in those patients with a worse lipid profile, that, in turn, may have higher level of ApoC3. We understand that for this reason, and because or study has a cross-sectional design, we have not found a negative influence of statins on circulating ApoC3.

Our study has certain clinical consequences. First, since ApoC3 has been associated with the risk of developing CV events in the future, and because disease activity in RA patients has also been related to these events (19), ApoC3 could be a marker that predicts the development of such events. And second, the assessment of ApoC3 in these patients could be a serum marker of the convenience of using certain CV disease prevention strategies, either with the use of drugs such as statins, as well as through the performance of techniques for the detection of subclinical atheromatosis.

We recognise that the cross-sectional nature of our study may be a limitation. Moreover, we acknowledge that it would have been of interest to recruit controls to study if ApoC3 levels differed between them and patients. However, our goal was to study the relationship between disease-related features and this molecule. Future studies will have to elucidate this aspect. We also acknowledge that we did not record the number of patients that were ex-smokers so the impact of previous smoking cannot be analysed in the current work. Besides, some analyses like the relation of tofacitinib intake to ApoC3 must be taken with caution since patients under this drug were few. However, our study has a number of strengths. The most important was the inclusion of a large series of RA patients homogeneously evaluated by experienced rheumatologists. This has allowed a full multivariable analysis to be performed, thus reducing the possibility of biases.

In conclusion, the activity of the disease in patients with RA is independently and positively associated with ApoC3.

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