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ORIGINAL ARTICLE

Accelerated atherosclerosis in pre-menopausal female patients with rheumatoid arthritis

A. Pahor · R. Hojs · M. Gorenjak · B. Rozman

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Abstract Increased mortality due to cardiovascular disease in rheumatoid arthritis (RA) patients was reported. Using B-mode ultrasonography we compared intima-media thickness (IMT) and plaque occurrence (indicators of asymptomatic atherosclerosis) in the carotid arteries in 70 pre-menopausal, female RA patients and 40 controls. Correlations with different risk factors were evaluated. The IMT values were higher in RA patients (0.59 mm vs. 0.47 mm, $P < 0.0001$) and they had more plaques ($P = 0.023$). In RA patients higher levels of sensitive CRP ($P < 0.0001$), ICAM ($P < 0.0001$), VCAM ($P < 0.0001$), IL-2 ($P < 0.001$), IL-6 ($P = 0.009$) and TNF-alfa ($P < 0.01$) were found. A correlation between IMT and triglycerides ($P = 0.018$) and a negative correlation between IMT and HDL cholesterol ($P = 0.037$) were found. With multiple regression analysis the association between IMT and sensitive CRP ($P = 0.027$) and presence of plaques and apolipoprotein B ($P = 0.028$) was established. The results indicate that even pre-menopausal, female RA patients had accelerated atherosclerosis. Chronic systemic inflammation may play an important role in atherogenesis.

Keywords Rheumatoid arthritis · Atherosclerosis · Inflammation

A. Pahor (✉)
Department of Rheumatology,
Clinical Department of Internal Medicine,
University Hospital Maribor, Ljubljanska 5, 2000 Maribor,
Slovenia
e-mail: artur.pahor@sb-mb.si

R. Hojs
Department of Nephrology, Clinical Department of Internal Medicine, University Hospital Maribor, Maribor, Slovenia

M. Gorenjak
Department of Clinical Chemistry,
University Hospital Maribor, Maribor, Slovenia

B. Rozman
Clinical Department of Rheumatology,
University Clinical Centre, Ljubljana, Slovenia

Introduction

Rheumatoid arthritis (RA) is a chronic disease associated with systemic inflammation. Patients with RA have a shorter life expectancy as compared to the general population [1]. Several studies report an increased mortality due to cardiovascular disease in RA patients as compared to the general population [2–4]. However, the mechanisms underlying the increase of cardiovascular disease in RA patients are not yet elucidated. The immune dysfunction unique to RA results in a chronic inflammatory state, which may have implications on the atherogenesis seen in these patients [5]. Pathophysiologic observations led to the conclusion that atherosclerosis is an inflammatory disease [6]. There is evidence supporting the hypothesis that atherosclerosis shares many similarities with other inflammatory autoimmune diseases, particularly RA. For example activation of inflammatory cells (macrophages and mast cells), local expression of adhesion molecules (ICAM, VCAM), activated T cells and neoangiogenesis have been described in both diseases [7].

It is important that we have methods for non-invasive detection of atherosclerosis. They involve blood tests, vascular ultrasonography, magnetic resonance imaging and electron-beam computed tomography [8]. Non-invasive ultrasonography has been used to detect early signs of atherosclerosis. The test is safe, quick; the results are reproducible and correlate with major cardiovascular risk factors, as well as with the extent of coronary atherosclerosis. Studies have demonstrated that a higher carotid intima-media thickness (IMT) predicts a higher likelihood of subsequent cardiovascular events in high-risk persons [8–10]. There is just one study in which increased carotid IMT and the presence of plaques were associated with markers of systemic inflammation in RA patients [11]. In this study mostly old male and female RA patients were included.

The aim of our study was to confirm accelerated atherosclerosis even in pre-menopausal female RA patients and elucidate importance of traditional and some non-traditional risk factors for atherosclerosis in these patients connected with mediators of immune inflammation.

Patients and methods

Patients

We studied 70 pre-menopausal, non-diabetic, non-hypertonic female RA patients (mean age 42.04 years, SD ± 5.53, mean duration of disease 114.64 months, SD ± 75.08). They were recruited in a consecutive manner from the outpatients unit of Rheumatology Department in Teaching Hospital, Maribor, Slovenia in the year 2003. Positive rheumatoid factor (mean 154.68 IU/ml, SD ± 224.3) was found in 72.9% of patients. On DMARDs were 81.4% of patients and 62.9% were on a low dose of corticosteroids (less then 10 mg of prednisolone per day). RA patients were diagnosed according to the 1987 revised criteria of the American College of Rheumatology [12]. We also included 40 healthy female control subjects (mean age 41.65 years, SD ± 5.43) selected from among hospital personnel. Written informed consents were obtained from all the study participants. This study has been performed in accordance with the principles of the Declaration of Helsinki.

Ultrasonographic examination

Ultrasonographic scanning of the carotid artery was performed on RA patients and healthy controls. It was done with high-resolution echo colour Doppler ultrasonography with a multifrequency 5–10 MHz linear probe, ATL HDI 3000 machine (Advanced Technology Laboratories, High Definition Imaging; Bothel, Washington, USA). All the subjects were placed in the supine position with their necks slightly hyperextended and rotated away from the imaging transducer. Several images were captured in real time on the cineloop frame grabber, and the three most clearly visible ones were used for measurements. Images were displayed with a constant fourfold magnification. Both the carotid artery systems were scanned. We investigated the IMT in the common carotid arteries and the plaque occurrence in the common carotid arteries, in the area of bifurcation, and in the proximal part of the internal carotid arteries. As in the other studies, IMT was defined as the distance between the leading edge of the lumen-intimal interface and the leading edge of the media adventitia interface of the far wall [13–18]. The IMT was measured in a plaque-free section. Three digitised still images from the same section of the artery were measured and the average value was calculated. The mean value was also calculated from the left and the right carotid arteries. All measurements were performed by the same examiner who was unaware of the clinical characteristics of the subjects.

Other tests

Sensitive CRP, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured by an Olympus AU 640 analyser; lipoprotein (a) [Lp(a)], apolipoprotein A-I and B by nephelometer BN (Behring, Dade), homocysteine by analyser Axsym (Abbott), interleukin (IL) 2, IL 6, tumour necrosis factor (TNF alfa) by analyser Immulite (DPC) and adhesion molecules (ICAM-1, VCAM-1) by ELISA (R&D Systems GmbH).

Statistical analysis

Values were expressed as the mean ± SD unless indicated otherwise. Student′s t test was used to compare mean values between two groups. Chi-square test was used to evaluate differences in distribution. Correlation tests were performed to assess correlation between IMT and risk factors for atherosclerosis as well as IMT and mediators of immune inflammation (Pearson or Spearman correlation coefficients were used where appropriate). Multiple regression analysis was performed to assess associations between IMT and various clinical and laboratory factors. *P* values less then 0.05 were considered significant.

Results

In RA patients the IMT values were higher than in controls and the difference was highly significant (Fig. 1). Plaques were present in 13% of RA patients. No plaques were found in controls. RA patients had higher values of sensitive CRP than controls and the difference was highly significant. We found no other differences between RA patients and controls. Data of the well-defined group of RA patients and controls are presented in Table 1.

A highly, statistically significant difference between RA patients and controls in mediators of autoimmune inflammation was found and is presented in Table 2.

We found statistically positive correlation between IMT and triglycerides ($R = 0.283$; $P = 0.018$) and statistically negative correlation between IMT and HDL cholesterol ($R = -0.25$; $P = 0.037$). There was no statistically significant correlation between IMT and homocysteine, apolipoprotein A and B, lipoprotein (a), haemoglobin, total cholesterol, LDL cholesterol, mediators of autoimmune inflammation (shown in Table 2) as well in use of corticosteroids, DMARDs (including methotrexate) and smoking.

With multiple regression analysis there was a relationship between IMT and age ($P = 0.004$), between IMT and sensitive CRP ($P = 0.027$) and between the presence of plaques and apolipoprotein B ($P = 0.028$).

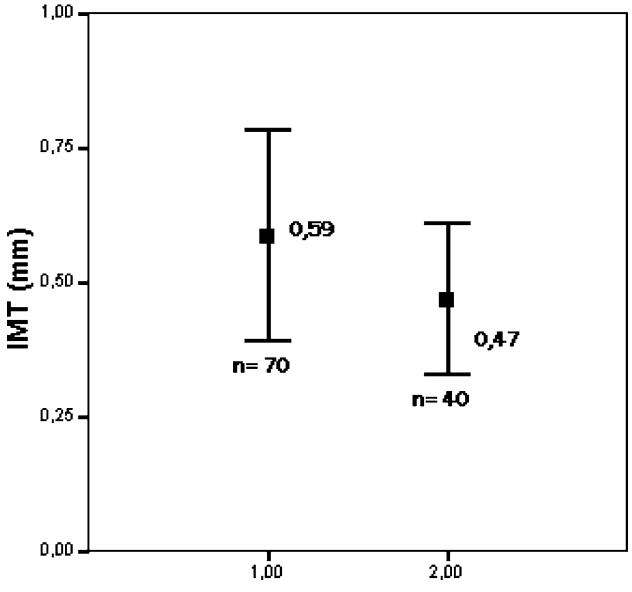

Fig. 1 Values of intima-media thickness were higher in female patients with rheumatoid arthritis ($P < 0.0001$). Error bars show mean ± 2.0 SD

Table 1 The demographics of RA patients and control group emphasising the difference in IMT, parameters of inflammation but not in lipid profile

	RA patients ($N = 70$)	CONTROLS ($N = 40$)	P
	Mean ± SD	Mean ± SD	
AGE (years)	42.04 ± 5.53	41.65 ± 5.43	NS
SMOKING (%)	18.57	20.00	NS
Sen CRP (mg/l)	10.67 ± 15.7	1.8 ± 2.42	**<0.0001**
HB (g/l)	125 ± 12.84	126.9 ± 9.64	NS
IMT-CC (mm)	0.586 ± 0.097	0.479 ± 0.07	**<0.0001**
PLAQUES (%)	12.9	0	**<0.023**
CHOL (mmol/l)	5.38 ± 1.05	5.417 ± 0.868	NS
TRIG (mmol/L)	1.527 ± 0.87	1.54 ± 1.44	NS
HDL (mmol/l)	1.5 ± 0.39	1.53 ± 0.34	NS
LDL (mmol/l)	3.21 ± 0.77	3.28 ± 0.82	NS
APO A (g/l)	1.58 ± 0.32	1.69 ± 0.42	NS
APO B (g/l)	1.018 ± 0.29	1.04 ± 0.25	NS
HOMOC (mmol/l)	10.68 ± 3.5	10.45 ± 3.13	NS
Lp (a) (g/l)	0.337 ± 0.96	0.148 ± 0.17	NS

IMT intima media thickness, *CHOL* total cholesterol, *TRIG* triglycerides, *APO* A, B apolipoprotein A, B, *Sen CRP* sensitive CRP; *Lp(a)* = lipoprotein(a); *HOMOC* homocysteine, *HB* haemoglobin, *HDL, LDL* = HDL, LDL cholesterol, *NS* not statistically significant

Table 2 Mediators of autoimmune inflammation in RA patients and controls

	RA patients ($N = 70$)	CONTROLS ($N = 40$)	P
	Mean ± SD	Mean ± SD	
IL-2 (IU)	587.04 ± 349.9	325.37 ± 126.43	**<0.001**
IL-6 (IU)	7.76 ± 7.59	5.08 ± 0.51	**<0.0001**
TNF-alfa (IU)	9.17 ± 19.06	4.04 ± 0.19	**<0.01**
ICAM 1(IU)	298.14 ± 101.6	253.75 ± 32.79	**<0.0001**
VCAM 1(IU)	646.43 ± 150.5	199.75 ± 60.87	**<0.0001**

IL interleukin, *TNF* tumour necrosis factor, *ICAM, VCAM* adhesion molecules

Discussion

In our study we used high-resolution B-mode ultrasound measurements of the common carotid arteries IMT. This is a widely used non-invasive method to assess atherosclerosis. Histologic studies demonstrate a close correlation between carotid and coronary atherosclerosis, and the ultrasonographic measurements correlate with the histologic measurements of carotid artery IMT [11]. Carotid artery IMT is associated with cardiovascular risk factors and atherosclerosis [19]. It is a strong predictor of myocardial infarction and stroke [9, 20]. The technique is reliable when performed by a single examiner, as was done in this study.

We demonstrated that IMT of common carotid arteries, which is an indicator of asymptomatic atherosclerosis [21] was statistically, significantly higher in RA patients as compared to the female healthy controls. This has been already reported, but RA cohorts in those studies were not homogenous as in our study [11, 22]. Involved RA patients in those studies were significantly older and included also male patients. In our study we included only pre-menopausal, non-diabetic, normotensive female RA patients. They did not differ in risk factors for atherosclerosis including lipid profile and smoking from the controls. These findings strongly suggest that RA itself is an independent risk factor for early atherosclerosis presented with arterial wall thickening.

Among various markers of inflammation the CRP was demonstrated as a powerful predictor of cardiovascular diseases independent of serum lipid levels [23] and can be causally involved in the pathophysiology of atherosclerosis [24]. CRP is produced by the liver in response to interleukin-6 [25]. It can be found within early atheromatous lesions in conjunction with terminal complement components. Suggesting its pathogenic role in atheromatosis [26] we were able to demonstrate the relationship between CRP, assessed by sensitive method, and arterial wall thickness of common carotid arteries in our RA group. In previous studies no significant association between CRP and IMT was detected [22] or the results came from the studies of elderly population of RA patients [11], so this is the first study which demonstrates the association between sensitive CRP and arterial wall thickness in pre-menopausal, female, normotensive, non-diabetic RA patients.

We also found a negative correlation between IMT of common carotid arteries and HDL cholesterol and a positive correlation between IMT and triglycerides. Therefore we cannot completely exclude the additional role of traditional risk factors for atherosclerosis even in pre-menopausal, normotensive, non-diabetic RA patients. We did not find elevated homocysteine levels in RA patients as has been shown in some other studies [27, 28].

RA treatment is an important issue concerning the affliction of an arterial wall. Some studies indicated that corticosteroid treatment is associated with an increased prevalence of cardiovascular disease and mortality [29, 30]. Methotrexate, as well, can contribute to increased cardiovascular mortality in RA patients [31]. On contrary, in our study we failed to detect any association between IMT of common carotid arteries and the use of coricosteroids either between common carotid IMT and the use of any DMARDs including methotrexate.

In conclusion, even pre-menopausal, female RA patients without diabetes and hypertension exhibited higher common carotid artery IMT as compared to the healthy female controls. Common carotid IMT was associated with sensitive CRP. These findings strongly suggest that RA itself is an important (if even not independent) risk factor for early atherosclerosis demonstrated by IMT and plaque formation.

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