Low levels of inflammation and the absence of subclinical atherosclerosis in rheumatoid arthritis

SALVATORE SANTO SIGNORELLI[1], SAVERIO CANDIDO[2], ROSSELLA SALEMI[2], VALERIO FIORE[1], MARCO MANGIAFICO[1] and MASSIMO LIBRA[2]

[1]Department of Clinical and Experimental Medicine, Medical Angiology Unit, Garibaldi Hospital, University of Catania, Catania I-95123; [2]Department of Biomedical and Biotechnological Sciences, Section of General & Clinical Pathology and Oncology, University of Catania, Catania I-95123, Italy

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Abstract. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease. Patients with RA have an increased risk for the development of cardiovascular diseases, however, the pathophysiological mechanisms of arterial complications in RA remain to be fully elucidated. Understanding the early markers of vascular damage may aid in preventing cardiovascular complications in patients with RA. The current study investigated this by recruiting 30 patients with RA and 30 healthy subjects. Intima-media thickness (IMT) was used to detect the presence of atherosclerotic disease and was measured in the carotid and femoral arteries. Tumor necrosis factor α, interleukin-6 (IL-6), IL-8, IL-10 and matrix metalloproteinase-2 were measured as markers of inflammation. An IMT ≥0.9 mm was observed in 7/30 patients with RA, however, no significant differences between patients with RA and the controls were observed in the inflammatory markers analyzed. Of note, these results indicated that the appropriate management of RA may have affected the inflammatory status of these patients and consequently may have impacted upon subclinical atherosclerosis.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects ~1% of the general population, and is associated with a high rate of morbidity or mortality due to cardiovascular diseases (CVDs) (1-7). Mortality associated with CVD is 35-50% higher in patients with RA compared with the general population (4). Previous studies have focused on markers of subclinical atherosclerosis, however, the results appear controversial (8-11). Furthermore, the association between RA and CVD complications was investigated by flow mediated vasodilation, pulse wave velocity and the measurement of intima-media thickness (IMT) using ultrasound (US) (12-16). Understanding the early markers of vascular damage may prevent cardiovascular complications in patients with RA. Therefore, the current study analyzed the plasma levels of known inflammatory biomarkers in addition to measuring the IMT of the carotid and femoral arteries.

Patients and methods

A total of 30 patients who were diagnosed with RA according to the American Rheumatology Association guidelines (16) and treated at the Rheumatology Unit of the Garibaldi Hospital (Catania, Italy) were recruited for the current study (Table I). All patients were treated with disease modifying anti-rheumatic drugs (DMARDs) or with tumor necrosis factor α (TNF-α) blockade drugs following failure with DMARDs. Patients included in the present study were defined as ''clinically stable patients'', with a low pain grade, articular motility and no ankylosing spondylitis. A total of 30 voluntary healthy subjects were enrolled as controls. The present study was approved by the Ethics Committee of Garibaldi Hospital (Catania, Italy) and all patients and volunteers provided informed consent to participate in the study.

IMT measurement. US examination of the carotid and the femoral arteries was conducted in all study participants. The IMT was measured in the common carotid arteries, 1 cm below the arterial bifurcation, and in the origin of the common femoral arteries of the lower limbs. The results of the US examinations were stored prior to an additional evaluation, conducted blind by a different physician. An IMT value of ≥0.9 mm was considered as a marker for cardiovascular risk and for atherosclerotic plaque formation in the carotid and/or in the common femoral arteries. A MyLab™ 70 XVision US system, (Esaote SpA, Genoa, Italy), equipped with a 7 mHz linear probe (Esaote SpA), was used to perform the US measurements.

Correspondence to: Professor Salvatore Santo Signorelli, Department of Clinical and Experimental Medicine, Medical Angiology Unit, Garibaldi Hospital, University of Catania, Piazza Santa Maria di Gesù, 7, Catania I-95123, Italy
E-mail: ssignore@unict.it

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Table I. Clinical characteristics of patients with rheumatoid arthritis.

Clinical characteristic	Value
Age (years)[a]	55.6±5.3
Disease duration (years)[a]	6.5±2.1
Gender	
Male (n)	8
Female (n)	23
Dyslipidemia	
Yes (n)	7
No (n)	23
Arterial hypertension	
Yes (n)	12
No (n)	18
Intima-media thickness (carotid artery)	
≥0.9 mm (n)	7
<0.9 mm (n)	23

[a]Mean ± standard deviation; n, number of patients.

Table II. Median concentrations of inflammatory biomarkers in patients with RA and controls.

Biomarker	Patients with RA, n=30	Controls, n=30	P-value
TNF-α (pg/ml)	0.45 (0.35-1)	0.37 (0.26-0.68)	NS
IL-6 (pg/ml)	0.85 (0.59-1.62)	0.88 (0.55-1.51)	NS
MMP-2 (ng/ml)	275.00 (244.21-296.17)	236.36 (219.34-284.62)	NS
IL-10 (pg/ml)	0.78 (0.78-0.78)	0.80 (0.78-0.91)	NS
IL-8 (pg/ml)	14.22 (10.32-16.6)	12.67 (10.02-21.36)	NS

Parentheses contain the 25th and 75th percentile range values. P-values were calculated using the Mann-Whitney U test. RA, rheumatoid arthritis; TNF-α, tumor necrosis factor α; IL, interleukin; MMP-2, matrix metalloproteinase-2; NS, non significant.

Inflammatory markers. Measurement of the inflammatory markers was conducted at the Department of Biomedical and Biotechnological Sciences of the University of Catania (Catania, Italy). Blood was collected from each patient and the healthy controls, and was drawn into pyrogen-free blood collection tubes (BD Vacutainer Plus plastic citrate tube and BD Vacutainer rapid serum tube; Becton Dickinson, Franklin Lakes, NJ, USA), to obtain plasma and serum. Citrated platelet-poor plasma was produced using two centrifuge steps: 5 min at 1,000 x g and 10 min at 2,000 x g at room temperature. Multiple aliquots of serum and plasma were stored at -80˚C prior to analysis. IL-6 (cat. no. HS600B), IL-8 (cat. no. HS800), IL-10 (cat. no. HS100C), TNF-α (cat. no. DTA00C) and MMP-2 (cat. no. MMP200) were measured by enzyme-linked immunosorbent assays (R&D Systems Europe, Ltd., Abingdon, UK). All assay procedures were conducted according to the manufacturer's instructions. Control specimens were analyzed simultaneously on each plate for each marker.

Statistical analysis. Quantitative variables are presented as the median with the 25th and 75th percentiles. The IMT mean values were compared by the Mann-Whitney U test. To evaluate the correlation of IMT with age and disease duration, Spearman's test was used. Statistical analysis was performed using the The R Project for Statistical Computing (version 3.0.3; https://www.r-project.org/). $P<0.05$ was considered to indicate a statistically significant difference.

Results

IMT measurement. A carotid IMT value of ≥0.9 mm was observed in 7/30 patients with RA (23%). However, no patients exhibited an IMT of ≥0.9 mm in the femoral arteries. IMT was correlated with age ($R=0.6$, $P<0.001$) and duration of the disease ($R=0.4$, $P<0.005$) in the RA group (data not shown).

Inflammatory biomarkers. Table II presents the mean values of TNF-α, IL-6, IL-8, IL-10 and MMP-2, analyzed in patients with RA compared with those in the control group. No significant differences between patients with RA and the controls were observed for all the markers analyzed ($P>0.05$; Table II).

Discussion

CVD is a common complication for patients with RA, with patient outcomes affected by the high morbidity and mortality associated with CVDs. The chronic low-level inflammation observed in patients with RA may explain the rate of CVD (17-19). High levels of anti-cyclic citrullinated peptide antibodies have been observed in patients with RA and may contribute to the development of CVD (20). High plasma levels of pro-inflammatory cytokines including IL-1, IL-6 and TNF-α in addition to elevation of C-reactive protein (CRP), are observed in patients with RA, and are suggested to serve a crucial role in the association between RA and atherosclerosis (21). It has been demonstrated that high plasma levels of CRP are associated with increased cardiovascular risk, however, to date the potential and additive effects mediated by this protein on the conventional risk factors for CVD (i.e. obesity, hypertension, hyperlipidemia) remain to be fully understood (22). A prospective analysis demonstrated that a positive test for rheumatic factors, active articular damage and subcutaneous nodules, in addition to further clinical indicators, were useful in the assessment of cardiovascular risk, and their combination with certain conventional factors (such as age and gender) improved the risk prediction for CVDs (23). A genetic study demonstrated the capability of an IL-6 polymorphism to increase IL-6 levels (24) with this polymorphism associated with higher levels of acute phase reactive proteins (such as CRP and fibrinogen) and, consequently, with an increased risk of CVDs. The association between polymorphisms and subclinical atherosclerosis in patients with RA was demonstrated in a previous study in which the TT genotype of the ZC3HC1 rs11556924 polymorphism was observed to be associated with greater IMT values (25). However, the evidence is conflicting, with a study investigating Toclizumab, an anti-TNF-α drug, observing an immunological profile similar to the IL-6 receptor polymorphism, indicating that the association between inflammation and conventional risk factors for CVDs is unclear (26).

The early and aggressive treatment with anti-inflammatory drugs is suggested by treatment guidelines for patients with RA, and aims to reduce clinical symptoms (pain, functional limitations, regional inflammation, ankylosis and ankylosing spondylitis) and additionally, to reduce the risk and occurrence of CVD (26). Treatment with DMARDs has been demonstrated to have clinical and preventive beneficial effects. The combination of methotrexate, hydroxychloroquine and sulfalazine is recommended in patients with high and/or moderate disease, and in addition, these drugs were able to influence the CVDs risk in patients with RA (27,28). Therapy with anti-TNF-α drugs has been suggested for patients with RA that failed to exhibit improvements using DMARDs (26). The aim of current treatment for RA is to reduce clinical symptoms of RA and CVD risk (29-32). However, arterial remodeling, as the first step in the atherosclerotic process, leading progressively to atherosclerotic plaque generation, has been demonstrated in patients with RA (33). A close association between carotid arterial remodeling and the progression of arterial damage has been previously demonstrated in patients with RA with a long disease duration (34). However, additional studies have indicated maladaptive outward remodeling of the carotid artery alone in patients with RA (35). Previous studies have not indicated any differences between patients with RA and healthy subjects with regards to the carotid IMT (33,36,37). The IMT is considered as an effective and useful marker to indicate the arterial process or to suspect large arterial involvement (13-15). In addition, the IMT of the carotid artery has been suggested to explain the risk and the morbidity rate for CVDs in the patients with RA who are without additional risk factors, including diabetes, dyslipidemia and hypertension (30).

The current study focused on the arterial remodeling process of the carotid and femoral arteries as indicated by the IMT in patients with RA diagnosed with a moderate disease duration (6.5±2.1 years), in addition to measuring the plasma levels of inflammatory biomarkers. The results of the current study demonstrated that only a small proportion of the patients with RA (7/30) exhibited abnormal carotid IMT values (≥0.9 mm), with none of the patients ≥0.9 mm in the femoral arteries. Median plasma levels of the inflammatory markers in the patients with RA were not significantly different when compared with those measured in the controls subjects. Overall, these results suggest that appropriate management of RA reduces the inflammatory status of patients and consequently may affect subclinical atherosclerosis. Of note, all enrolled patients were treated with standard therapy including DMARDs and anti-TNF-α. The long-term treatment with these compounds in patients with RA has demonstrated clinical efficacy, further supported by the US examination of the carotid and femoral arteries, as only a small proportion of patients with RA exhibited arterial remodeling.

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