High-grade C-Reactive Protein Elevation Correlates with Accelerated Atherogenesis in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Patients with rheumatoid arthritis (RA) are at greater risk of developing cardiovascular events compared with individuals without RA. Increased risk for cardiovascular disease in these patients is a consequence of atherosclerosis. Case-control studies have shown that increased intima-media thickness (IMT) of the common carotid artery is an indicator of generalized atherosclerosis. Some investigators have suggested that the development of atherosclerosis in RA may be related to the magnitude and chronicity of the systemic inflammation. We examined the relationship between carotid IMT to C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), which are the most commonly assessed markers of inflammatory response in patients with RA.

Methods. Retrospective review of CRP and ESR values in 47 patients with longterm actively treated (at least 5 years) RA without clinically evident atherosclerosis or its complications, who had been studied for carotid IMT with high resolution B-mode ultrasound.

Results. No correlation between ESR and carotid IMT was observed. However, a correlation was found between the maximum CRP values and the carotid IMT ($p = 0.009$). The distribution of patients in 4 quartiles according to the average CRP values showed significant differences in the carotid IMT ($p = 0.03$). Those exhibiting the highest mean CRP values (quartile 4) had greater carotid IMT. There was no correlation between CRP at the time of disease diagnosis or at the time of the ultrasound study and the carotid IMT.

Conclusion. Our study confirms that the magnitude and chronicity of the inflammatory response measured by CRP correlates directly with the presence of atherosclerosis in patients with RA. (J Rheumatol 2005;32:1219–23)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
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Patients with rheumatoid arthritis (RA) are at greater risk of developing cardiovascular events compared with individuals without this disease[1]. Cardiovascular disease is also the major cause of excessive mortality in these patients[2]. The increased risk for cardiovascular disease in patients with RA is a consequence of atherosclerosis[3].

Case-control studies have shown that increased intima-media thickness (IMT) of the common carotid artery is an indicator of generalized atherosclerosis[4,5]. It may precede the development of cardiovascular events by many years. Thus, determination of the carotid IMT using ultrasound techniques provides useful early information about atherosclerosis in subclinical stages of the disease in individuals at risk[6,7].

Japanese and Korean investigators have described an increased carotid IMT in unselected patients with RA compared with ethnically matched controls[8,9]. We recently confirmed a high frequency of macrovascular disease, in the form of increased carotid IMT, in white patients with longterm, actively treated RA without history of atherosclerosis or its complications[10].

Del Rincon, *et al* observed a significant linear trend for increased carotid artery IMT associated with increasing erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in both patients with RA and healthy controls[11]. These investigators found a strong correlation between carotid IMT and a determination of ESR and CRP performed at the time of the carotid ultrasound study. However, RA is a chronic inflammatory disease, and because of this some investigators have suggested that the development of

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atherosclerosis in RA patients may be related to the magnitude and chronicity of the systemic inflammation[12]. According to these investigators a chronically high inflammatory response in RA patients compared with healthy controls would promote an increased risk of cardiovascular complications[12]. To investigate this issue, we retrospectively reviewed the ESR and CRP data on a series of RA patients with no clinically evident cardiovascular disease[10]. The relationship of carotid IMT to these markers of inflammatory response was assessed.

MATERIALS AND METHODS

Patients. Clinical information about this series of patients without clinically evident cardiovascular disease or its complications has been described[10]. All patients were recruited from Hospital Xeral-Calde, Lugo, Spain. They fulfilled the 1987 American College of Rheumatology classification criteria for RA[13] and were treated by the same group of rheumatologists (MAG-G and CG-P). The cohort constituted a series of patients attending hospital outpatient clinics seen over a period of 1 month (May 2001). At the time of the ultrasound carotid study only 10 of the 47 patients had clinical criteria of active disease (Disease Activity Score-28 > 3.2)[14].

Since our objective was to examine whether the presence of subclinical morphologic atherosclerotic changes in RA patients who had been treated chronically was associated with the magnitude and severity of the inflammatory response, only those patients treated for at least 5 years (range of followup 5–14 yrs) and currently being treated with one or more disease modifying antirheumatic drugs (DMARD) at the time of the study were included in the analysis. Patients had to be nonsmokers or ex-smokers for at least 5 years. Also, RA patients seen during the period of recruitment were excluded if they had hypertension (systolic blood pressure > 150 mm Hg and/or diastolic blood pressure > 90 mm Hg), diabetes mellitus (fasting overnight venous plasma glucose concentration in all patients was < 110 mg/dl) or renal insufficiency, had had cardiovascular or cerebrovascular events, had evidence of cardiovascular disease, or used estrogens or drugs affecting the cardiovascular system. During the period of recruitment only 47 patients fulfilled the inclusion criteria described above. All had been treated with nonsteroidal antiinflammatory drugs (indomethacin 100 mg/day, naproxen 500–1000 mg/day, or diclofenac 100–150 mg/day) and all but one had received treatment with low doses of prednisone immediately after disease diagnosis. Most patients had started treatment with prednisone 5 mg bid. All have been treated and were in treatment with one or more DMARD, including chloroquine, sulfasalazine, gold, methotrexate (MTX; median dose 15 mg/week), and cyclosporin A (median dose 3 mg/kg/day). Treatment with DMARD was initiated as soon as a diagnosis of RA was made. When the ultrasonographic study was undertaken, 46 patients were under treatment with MTX, generally in combination with chloroquine. Ten were undergoing combined therapy with MTX and cyclosporin A. Five patients who had been under treatment with MTX plus cyclosporin A were switched to treatment with MTX plus anti-tumor necrosis factor-α monoclonal antibody (infliximab) because of the severity of the disease.

Study protocol. Patients were assessed for carotid IMT (millimeters) as described[10]. IMT and plaques were measured in the right common carotid artery. The study was performed using high resolution B-mode ultrasound (Hewlett Packard SONOS 5500) with a 10 MHz linear transducer. Patients were examined in supine position with the neck extended and the chin turned contralateral to the side being examined. Sonography scanning involved examination of the right common carotid artery in longitudinal and transverse planes, and then focused on the interfaces required to measure IMT as well as on any area of focal plaque. Plaque was defined as a distinct protrusion > 1.5 mm into the vessel lumen. Measurement of IMT was assessed in the common carotid artery at 3 points on the far wall of common carotid artery, 1 cm downstream from the carotid bifurcation[10]. IMT was calculated as the average of measurement during 3 cardiac cycles at end-diastole. Measurements of IMT were performed by 2 cardiologists (CG-J, AT). In all cases the cardiologists were blinded to subjects' clinical information and both agreed on the results. Reproducibility of IMT measurements was evaluated in 10 patients and 10 controls within 1 week of the first examination. The correlation coefficient for IMT was 0.986[10].

To quantify the degree of inflammation over time in each patient, CRP by nephelometry (Dade-Behring analyzer, according to the manufacturer's protocol)[15] and Westergren ESR values were retrospectively evaluated at different times during the disease (at least 2 determinations per year from the diagnosis of the disease).

In RA patients, CRP values > 5 mg/l and ESR values > 20 mm/h were considered elevated. As described[10], CRP and ESR levels were also assessed at the time of the carotid ultrasound examination.

The following variables were studied for each patient: maximum CRP and ESR; average CRP and ESR (mean for each patient); current CRP and ESR (at time of ultrasound); CRP and ESR at time of disease diagnosis; proportion of CRP values > 5 mg/l (proportion of CRP values > 5 mg/l divided by total number of CRP determinations in each patient); proportion of CRP values > 10 mg/l (proportion of CRP values > 10 mg/l divided by total number of CRP determinations in each patient). To assess potential correlation between CRP and carotid IMT, patients were stratified in 4 quartiles according to the average and the maximum CRP values.

Statistical analysis. Equality of mean carotid IMT between patients with normal and abnormal CRP and ESR were tested by Student t test. Relationships between carotid IMT and ESR and CRP levels were tested by the Pearson correlation coefficient and the partial correlation coefficient; previously, variables were tested for normality and log-transformed if necessary. When stratifying CRP values in quartiles, its association with carotid IMT was tested using one-way analysis of variance (ANOVA); p values for trend were estimated by the nonparametric method[16]. All statistical tests were performed with the Stata 8/SE package (Stata Corp., College Station, TX, USA).

RESULTS

Correlation between CRP or ESR and carotid IMT. No correlation between ESR and carotid IMT was observed in these series of patients with longterm actively treated RA (Table 1). However, although there was no correlation between serum CRP at the time of disease diagnosis and the carotid IMT, a strong correlation between the maximum CRP values and the carotid IMT was observed (p = 0.009; Table 1). Correlation between the magnitude of the inflam-

Table 1. Correlation coefficient between CRP, ESR, and carotid IMT.

Variable	Correlation Coefficient	p
Maximum CRP, mg/l*	0.379	0.009
Average CRP, mg/l*	0.326	0.025
Current CRP, mg/l*	0.257	0.081
Proportion of CRP > 5 mg/l	0.270	0.066
Proportion of CRP > 10 mg/l	0.316	0.031
CRP at disease diagnosis, mg/l*	0.158	0.313
Maximum ESR, mm/h*	0.092	0.540
Average ESR/h*	0.080	0.595
Current ESR/h*	–0.008	0.958
ESR/h at disease diagnosis*	–0.142	0.342

* Variables with logarithmic transformation to obtain normality.

matory response expressed in CRP values and the carotid IMT was also supported by the statistically significant association between the proportion of CRP values > 10 mg/l in each patient and the carotid IMT (p = 0.031; Table 1). In addition, the distribution of patients in 4 quartiles according to average CRP values revealed statistically significant differences in the carotid IMT (Figure 1). With respect to this, those exhibiting the highest mean CRP values [quartile 4 (Q4): average CRP > 15 mg/l] had greater carotid IMT. The average carotid IMT by CRP quartile was the following: quartile 1 (Q1): average CRP < 6 mg/l and carotid IMT 0.73 mm; quartile 2 (Q2): average CRP 6–9 mg/l and carotid IMT 0.72 mm; quartile 3 (Q3): average CRP 9–15 mg/l and carotid IMT 0.78 mm; Q4: average CRP > 15 mg/l and carotid IMT 0.90 mm (p ANOVA = 0.03; p for trend = 0.01). Figure 2 shows a similar trend when patients were stratified in 4 quartiles according to the maximum CRP values (p ANOVA = 0.06; p for trend = 0.02) (boundaries for the maximum CRP values for quartiles were Q1 20, Q2 32, Q3 54, and Q4 > 54 mg/l). However, as described[10], there was no correlation between CRP levels at the time of the carotid ultrasound study (current CRP) and the carotid IMT (Table 1).

Correlation between CRP and ESR. The results described above may suggest a discordant value of CRP and ESR in

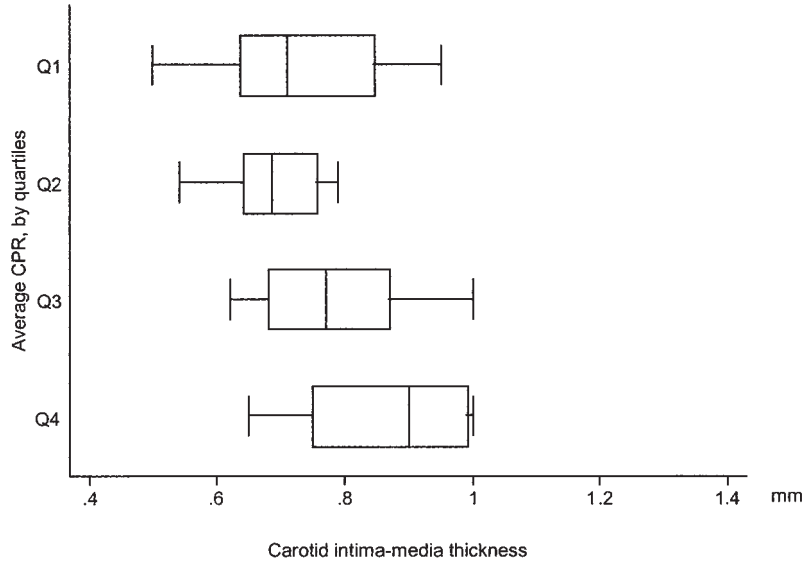

Figure 1. Association between the average CRP values and the carotid IMT. Patients with greater average serum CRP values had greater carotid IMT. *p ANOVA = 0.03.

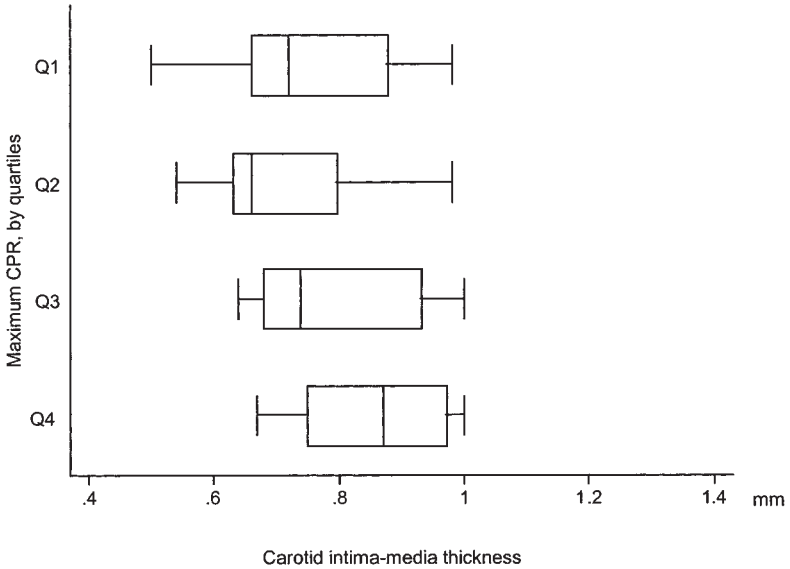

Figure 2. Association between the maximum CRP values and the carotid IMT. Patients with greater maximum serum CRP values had greater carotid IMT. *p ANOVA = 0.06.

the assessment of atherosclerosis in a longitudinal study of RA. However, to our surprise, when we assessed the direct correlation between the 2 parameters, we found a strong concordance between the maximum CRP and ESR ($r = 0.452$, $p = 0.001$) and the average CRP and ESR ($r = 0.336$, $p = 0.021$). This was also the case when we analyzed these data at the time in which the carotid ultrasound study was performed ($r = 0.376$, $p = 0.041$) and, in a lower degree, at the time of diagnosis of RA ($r = 0.261$, $p = 0.091$).

The finding of direct concordance between CRP and ESR parameters but discordance between CRP and ESR in terms of disease severity, expressed by a correlation between CRP levels but not ESR and atherosclerosis (carotid IMT), seems to be a paradox. To investigate this, we performed a partial correlation between CRP or ESR and carotid IMT. Table 2 shows that, as described in Table 1, the maximum CRP was the only variable remaining significantly associated with carotid IMT after adjustment for age and sex ($p = 0.028$). Also, as described in Table 1, the average CRP remained marginally significant ($p = 0.069$) after adjusting for age and sex. However, both current CRP and CRP at the time of disease diagnosis were not associated with the carotid IMT when they were adjusted for age and sex (Table 2).

Carotid IMT in patients with normal or abnormal CRP and ESR. Mean values of carotid IMT were compared between patients with normal and abnormal CRP and ESR; except for maximum CRP, because no patient had normal maximum CRP values (i.e., maximum CRP < 5 mg/l). All these comparisons were nonsignificant (data not shown).

DISCUSSION

Atherosclerosis and RA share similar inflammatory mechanisms that include involvement of cytokines such as tumor necrosis factor-α and interleukin 6 (IL-6)[17]. Inflammatory mechanisms implicated in the development of synovial lesions in RA might also involve the vessel walls and promote the development of atherosclerotic lesions in patients with RA.

Some authors have suggested that in RA, cytokines released into systemic circulation would alter distant tissues, including vascular endothelium, leading to a wide spectrum of proatherogenic changes[12]. We recently described in a cross-sectional study that RA patients without clinically evident cardiovascular disease had increased carotid IMT compared with healthy matched controls[10]. There was no correlation between carotid IMT and cumulative dose of prednisone, HDL and LDL cholesterol, and systolic and diastolic blood pressure measurements at the time of that study[10]. Similarly, carotid IMT was not associated with sex, previous history of smoking, or rheumatoid factor status[10]. However, carotid IMT in RA was independently associated with disease severity. In this regard, RA patients who exhibited extraarticular manifestations had a statistically significantly greater carotid IMT than the remaining patients (0.843 ± 0.187 mm in patients with extraarticular manifestations compared with 0.740 ± 0.136 mm in the others; $p = 0.03$)[10]. In addition, when carotid plaques, which constitute the morphological expression of severe macrovascular atherosclerotic disease, were assessed, we found that RA patients with high morphological evidence of atherosclerosis also had significantly longer disease duration and more extraarticular manifestations than those without plaques[10]. Thus, these data indicate that both the duration and the severity of the disease account for a higher incidence of atherosclerosis in RA.

Table 2. Partial correlation coefficient between CRP, ESR, and carotid IMT.

Variable	Partial Correlation Coefficient*	p
Maximum CRP, mg/l	0.327	0.028
Maximum ESR, mm/h	–0.051	0.741
Average CRP, mg/l	0.274	0.069
Average ESR, mm/h	0.016	0.915
Current CRP, mg/l	0.130	0.509
Current ESR, mm/h	–0.058	0.704
CRP at diagnosis, mg/l	0.057	0.722
ESR at diagnosis, mm/h	–0.250	0.098

* Adjusted by age at diagnosis and sex.

Recent studies have indicated that the serum CRP level may be a good predictor of cardiovascular disease, independently of the serum lipid profile[18]. Higher levels of CRP were associated with poorer outcome for patients with unstable angina[19]. However, in contrast to del Rincon, *et al*[11] but in accord with reports on Japanese and Korean patients with RA[8,9], in our cross-sectional study we found no correlation between serum CRP levels at the time of the carotid ultrasound study and the carotid IMT[10].

Nagata-Sakurai, *et al* have recently described that serum CRP levels correlated with the increase of carotid IMT in RA patients on whom a second measurement of carotid IMT was performed 18–36 months after an initial measurement[20]. These investigators also found an association with other inflammatory markers such as ESR[20]. We assessed CRP and ESR levels measured in patients with longterm actively treated RA. We observed that in these patients the maximum and the average serum CRP levels, but not the ESR, were associated with the development of atherosclerotic disease. According to our findings, longitudinal evaluation of serum CRP levels would be useful in predicting the development of atherosclerotic disease. In accord with our results, Dutch investigators also confirmed that in longitudinal studies CRP was more valuable as a predictor of radiographic damage[21,22].

There are some discrepancies between our study and that by Nagata-Sakurai, *et al*[20]; although assessment of inflammation expressed by CRP and ESR levels was much longer in our series, potential limitations due to the small sample

size and the retrospective design of our study may exist. The small sample size in our series constitutes a major limitation to detect a significant correlation between the carotid IMT and the ESR. Therefore, our finding of a nonsignificant correlation between IMT and ESR may be a false negative result.

However, as described[23], the discordance between CRP and ESR as markers of disease severity may be because ESR is an indirect measure of inflammation, being accounted for primarily by the concentrations of fibrinogen, but also to immunoglobulins, rheumatoid factor, and other factors such as sex, age and hemoglobin. In contrast, CRP is a direct measure of the inflammatory process in RA. Thus, values of CRP are the direct result of increased levels of proinflammatory cytokines, in particular IL-6. With respect to this, Wolfe reported that in RA patients serum CRP levels were more correlated with disease activity, grip strength, joint count, and Health Assessment Questionnaire disability index than ESR[23].

In summary, atherosclerosis and RA share similar inflammatory mechanisms that include involvement of cytokines. Our study confirms that in patients with longterm actively treated RA without clinically evident cardiovascular disease, the magnitude and chronicity of the inflammatory response measured by longitudinal evaluation of CRP levels, rather than by a single determination of this inflammatory marker, correlate directly with the presence of atherosclerosis. This observation emphasizes the importance of active therapy in RA patients to reduce the severity of the systemic inflammatory response and, subsequently, the risk of cardiovascular complications associated with atherosclerosis in this disease.

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