Atherosclerosis in Rheumatoid Arthritis Versus Diabetes
A Comparative Study

Kimon S. Stamatelopoulos, George D. Kitas, Christos M. Papamichael, Elda Chryssohoou, Katerina Kyrkou, George Georgiopoulos, Athanassios Protogerou, Vasileios F. Panoulas, Aamer Sandoo, Nikolaos Tentolouris, Myron Mavrikakis, Petros P. Sfikakis

Objective—The extent to which atherosclerosis is accelerated in chronic inflammatory diseases is not established. We compared preclinical atherosclerosis in rheumatoid arthritis with diabetes mellitus, a known coronary heart disease equivalent.

Methods and Results—Endothelial function, arterial stiffness, carotid intima-media thickness, and analysis of atheromatous plaques were examined in 84 rheumatoid arthritis patients without cardiovascular disease versus healthy controls matched for age, sex, and traditional cardiovascular disease risk factors, as well as in 48 diabetes patients matched for age, sex, and disease duration with 48 rheumatoid arthritis patients. Rheumatoid arthritis duration associated with arterial stiffening, whereas disease activity associated with carotid plaque vulnerability. All markers of preclinical atherosclerosis were significantly worse in rheumatoid arthritis compared to controls, whereas they did not differ in comparison to diabetes despite a worse cardiovascular risk factor profile in diabetics. Both diseases were associated independently with increased intima-media thickness; rheumatoid arthritis, but not diabetes, was independently associated with endothelial dysfunction.

Conclusions—Preclinical atherosclerosis appears to be of equal frequency and severity in rheumatoid arthritis and diabetes of similar duration with differential impact of traditional risk factors and systemic inflammation. Cardiovascular disease risk factors in rheumatoid arthritis may need to be targeted as aggressively as in diabetes. (Arterioscler Thromb Vasc Biol. 2009;29:1702-1708.)

Key Words: preclinical atherosclerosis ■ rheumatoid arthritis ■ diabetes mellitus ■ inflammation

Rheumatoid arthritis (RA) is characterized by excessive cardiovascular morbidity and mortality. The reasons remain unclear; although there is evidence for a significant cardiovascular disease (CVD) risk factor burden, such as hypertension, dyslipidemia, obesity, and insulin resistance, epidemiological studies suggest that this accounts only for part of the excess CVD mortality. Thus, mechanisms mediating CVD in RA may be specific to, or augmented in, this disease entity. Interest has focused on the impact of “high-grade” systemic inflammation and immune system activation, which through early and enhanced effects on vascular function and morphology may lead to accelerated atherosclerosis.

Several studies have examined the presence of preclinical atherosclerosis in RA, using noninvasive validated assessments of vascular function and morphology. Most, but not all, demonstrate abnormal endothelial function, arterial stiffness, and carotid intima-media thickness (IMT) in RA patients compared to healthy controls. Some of these abnormalities may be reversible. Very few, if any, studies have addressed the degree to which classical CVD risk factors and systemic inflammation each contribute to vascular abnormalities in RA. Moreover, very few studies have used multiple methods of assessment at different levels of the vascular tree (ie, conduit arteries and microvessels); this is important because each method may reflect different aspects of the atherosclerotic process. Flow-mediated dilatation (FMD) reflects conduit artery endothelial function, an early stage of atherosclerosis greatly affected by short-term stimuli with a high potential of reversibility. Pulse wave velocity (PWV) reflects arterial stiffening representing both functional and structural deterioration of the vascular wall, whereas IMT reflects chronic vascular thickening attributable to atherosclerosis or smooth muscle hypertrophy. The presence of nonocclusive atheromatous plaques represents even more advanced stages of atherosclerosis, whereas plaque echor-
nicity provides information on their vulnerability to rupture. Although these markers are validated in the general population and some disease groups, whether such assessments are good surrogates of future events specifically in patients with RA remains unclear.3,11

The archetypal disease carrying excessive risk for future CVD events is diabetes mellitus (DM).18 Diabetic patients without a prior history of myocardial infarction (MI) have the same risk for future events as nondiabetic patients who have had a prior MI.19 Excessive CVD risk in DM is thought to be attributable to accelerated atherosclerosis resulting predominantly from classical CVD risk factor burden; systemic, but low grade, inflammation may also be of importance.20 Consequently, DM is considered a coronary heart disease (CHD) equivalent in terms of risk for future CVD events and is managed using aggressive secondary prevention strategies and targets, which have already resulted in survival benefits.21

We have previously suggested that the magnitude of CVD morbidity and mortality in RA may be analogous to that of DM1 and that screening and management strategies used in DM may be beneficial in RA. This is supported by recent studies suggesting that RA and DM have very similar CVD outcomes.22 However, the 2 conditions may share some (e.g., classical CVD risk) but not all (e.g., high versus low grade inflammation) mechanisms. Studies comparing the severity and rate of progression of functional and morphological vascular abnormalities, and the relative contribution of classical CVD risk and inflammation in the two conditions, are lacking.

The aims of the present study were: (1) to assess preclinical atherosclerosis in RA compared to normal controls, using 4 distinct methods; (2) to examine whether vascular abnormalities are of equal frequency and severity in RA and a known CHD equivalent such as DM; (3) to consider whether there is differential contribution of classical CVD risk factors and systemic inflammation in the 2 conditions.

Methods

The study has been subject to Institutional Body Review, and all subjects provided informed consent according to the Declaration of Helsinki.

To accomplish the first aim, vascular studies were performed in 84 consecutively recruited RA patients (meeting the 1987 revised ACR criteria) and 84 controls (hospital personnel or patients’ “buddies”) matched (1:1) for age, sex, and for self-reported classical CVD risk factors. RA patients with known CVD, DM (diagnosed by a physician, on antidiabetic medication, or fasting plasma glucose >126 mg/dl), uncontrolled hypothyroidism, chronic renal failure, malignancy, and patients on nonsteroidal antiinflammatory drugs (NSAIDs) were excluded.

For the second and third aims, 48 patients with type II DM without concomitant chronic inflammatory disease or any of the exclusion criteria described above were matched 1:1 with 48 of the 84 studied RA patients (and their respective 48 healthy controls) only for age, sex, current smoking status, and disease (DM or RA) duration, but not for other CVD risk factors.

Studies were performed in a fixed order: IMT, PWV, FMD, blood sampling, anthropometric assessments. All subjects abstained from any food or drink except for water and all medications for 12 hours before the study. RA patients were also assessed for disease activity (remission versus active disease). Remission was defined as fulfilling 5 or more of the following criteria for at least 2 months: duration of morning stiffness <15 minutes; no fatigue, joint pain, joint tenderness/pain on motion, soft tissue swelling; erythrocyte sedimentation rate <30 mm/h for females or <20 mm/h for males. Disease stage and functional class were assessed as described.23 Biochemical parameters, including high-sensitivity C-reactive protein (hs-CRP), were measured by standard methods.

Vascular Studies

Endothelial function was evaluated by ultrasound measures of endothelium-dependent FMD using a 14.0 MHz multi-frequency linear array probe attached to a high-resolution ultrasound machine (Vivid 7 Pro, GE Healthcare), as described in detail elsewhere16: intraobserver coefficient of variation (IOCV) was 11.6%. PWV, an established index of aortic stiffness,24 was calculated from measurements of pulse transit time and the distance traveled between the common carotid artery and the common femoral artery with a validated noninvasive device (Complior, Artech Medical); IOCV was 1.7%. IMT was measured using the same equipment as for FMD, as previously described.25 The average of the maximal IMT from all 6 carotid segments (defined as mean IMT) from each carotid segment separately were all used in the analyses. Carotid IMT was measured at 3 paired segments: in the right and left common carotid artery (defined as the segment 1 cm proximal to carotid dilation), carotid bulb (defined as the segment between the carotid dilation and carotid flow divider), and internal carotid artery (defined as 1 cm long arterial segment distal to the flow divider). In each segment 3 measurements of the maximal IMT (the image was captured 3 times and measured each time) in the far wall were averaged, after excluding plaque thickness (gated R-wave). IMT adjacent to plaque shoulders was measured at a site free of any discrete plaques; that is a site with a clearly seen double echogenic line and without plaque irregularities. The average of the maximal IMT from all 6 carotid segments (defined as mean carotid IMT) was used in the analyses. IMT measurements were performed manually (IOCV of 10.6%). IOCV was defined as the standard deviation of the difference between paired values divided by the overall mean. Measurements for the calculations of IOCV were obtained in a random patient sample by 2 repeated measurements of each variable in 2 consecutive days performed by the same experienced operator. FMD and IMT measurements were performed by the same operator, whereas PWV was performed by a different operator. All operators were blinded to the medical history of the patients.

After detailed interrogation of each of the 6 segments to identify a carotid plaque, the optimal image showing maximum plaque size with the greatest encroachment into lumen was digitally stored. Plaques were defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness of >1.5 mm.26 The relative echogenicity of the atheromatous plaques, a marker of plaque instability associated with future cardiovascular events27–28 was also assessed by Artery Measurement Software (AMS), an automated piece of software for dedicated analysis of gray scale median (GSM) as previously described.29 Analyses were performed by a single experienced operator blinded to the history of the participants. The measurements of plaque-GSM were repeated in 18 random subjects from all groups with a mean difference for plaque-GSM of 1.8 (95% CI −1.8 to +5.5).

Statistical Analysis

Statistical analysis was performed using SPSS14 (SPSS Inc). Variables were tested for normality by the Kolmogorov-Smirnov test. Means and standard deviations (SD) or medians and 25th to 75th percentile values (interquartile range [IQR]) were calculated for continuous variables (normally and not normally distributed, respectively) and proportions for categorical variables. Because RA were matched 1:1 to healthy controls for age, sex, and risk factors, paired t test and Wilcoxon tests for normally and nonnormally distributed parameters, respectively, were used to compare vascular parameters in this analysis. On the other hand, for DM versus RA analysis,
Results

Vascular Function and Morphology in RA Compared to Healthy Controls

Mean disease duration for the 84 RA patients was 7.86 (6.75) years, with 52 (61.9%) of them being RF positive; 43 (51.2%) were in remission, the remaining 41 (48.8%) had active RA; 26 (31%) had early, 40 (47.6%) moderate, 16 (19%) severe, and 2 (2.4%) terminal RA; 39 (46.4%) were in functional class 1, 29 (34.5%) in class 2, 13 (15.5%) in class 3, and 3 (3.6%) in class 4. No difference was observed in demographic characteristics, CVD risk factors, and medication use. Similar analyses were performed to evaluate differences between RA and DM, after additional adjustment for CVD risk factors and antihypertensive medication that differed between these patients.

Table 1. Vascular Markers of Preclinical Atherosclerosis in RA Patients Without CVD (Mean Age 59 Years, Range 20 to 79, 82% Women, Hypertension in 30%, Dislipidemia in 77%, 30% Smokers) vs Healthy Controls Matched 1:1 for Age, Sex, and Classical CVD Risk Factors

<table>
<thead>
<tr>
<th>Endothelial function</th>
<th>Controls (n=84)</th>
<th>RA (n=84)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (%)</td>
<td>4.52±2.1</td>
<td>3.06±1.7</td>
<td>&lt;0.001 (&lt;0.001)†</td>
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<tr>
<td>Arterial stiffness</td>
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<tr>
<td>PWV (carotid-femoral) (m/sec)</td>
<td>8.74±1.8</td>
<td>10.02±3.3</td>
<td>0.004 (0.074)†</td>
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<tr>
<td>Intima media thickness</td>
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<tr>
<td>Mean carotid IMT (mm)</td>
<td>0.649±0.147</td>
<td>0.753±0.151</td>
<td>&lt;0.001 (&lt;0.001)†</td>
</tr>
<tr>
<td>Atheromatous plaques</td>
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</tr>
<tr>
<td>Carotid plaque, n (%)</td>
<td>8 (9.5)</td>
<td>40 (47.6)</td>
<td>&lt;0.001 (&lt;0.001)†</td>
</tr>
<tr>
<td>Carotid plaque-GSM‡</td>
<td>55.1 (10.4)</td>
<td>60.2 (17.5)</td>
<td>0.634 (0.852)†</td>
</tr>
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</table>

RA indicates rheumatoid arthritis; CVD, cardiovascular disease; FMD, flow-mediated dilatation; IMT, intima media thickness; GSM, gray scale median.

Vascular Function and Morphology in RA Subgroups

Subanalysis within the RA group demonstrated no significant differences between remission and active disease in classical CVD risk factors or any of the vascular measurements (data not shown) apart from plaque-GSM, which was significantly lower in patients with active disease (53.3±14.4 versus 65.4±19.9, P=0.047 for active versus remission), suggesting a more vulnerable state. There were no differences according to functional class (data not shown).

Patients with RA duration in the highest quartile (≥11 years, n=13) had higher PWV compared to those in the lowest quartile (≤3 years, n=15; 11.5 versus 8.7 m/s, P=0.050). Although there were no differences in traditional cardiovascular risk factors between these 2 subgroups, hsCRP was higher in patients with longer disease duration (7.85 versus 3.28 mg/L, P=0.025). In multivariate linear regression, RA duration was an independent determinant of PWV (P=0.047 for disease duration, beta=0.210, model R²=0.483, P=0.001) in a model including age (beta=0.574, P<0.001, using age squared because of the known nonlinear relationship with PWV) and hsCRP (beta=0.105, P=0.315). Also, being in the highest quartile of disease duration was independently associated with higher PWV (P=0.026, beta=0.423, model R²=0.392, P=0.007) in a similar model including age (squared, beta=0.451, P=0.010) and hsCRP (beta=-0.085, P=0.640).

Vascular Function and Morphology in RA Compared to DM and Healthy Controls

DM patients had the worst CVD risk profile in terms of hypertension, BMI, HDL cholesterol, and CHD family history (Table 2), yet there were no significant differences between the 48 RA and 48 DM in any of the vascular assessments (FMD, PWV, IMT) including prevalence of atheromatous plaques and plaque-GSM. As expected, significant
differences in virtually all vascular assessments were observed between DM and healthy controls (Table 3). Linear and logistic regression, as appropriate, revealed that even after adjustment for CVD risk factors and medication that were significantly different between RA and DM, there were still no significant differences between the 2 groups in FMD (beta = 0.030, P = 0.852), PWV (beta = 1.49, P = 0.268), IMT (beta = -0.008, P = 0.867), carotid plaques (ORM vs. RA = 2.56, 95% CI: 0.59 to 11.19, P = 0.211), or carotid plaque-GSM (beta = -7.13, P = 0.678).

Impact of Traditional CVD Risk Factors Versus Inflammation on Vascular Assessments

Stepwise analysis was performed to assess whether the differences observed in vascular markers between control subjects and RA or DM were mediated via traditional or nontraditional risk factors. The presence of RA was independently associated with decreased FMD, whereas DM appeared to exert its deleterious effect on FMD via traditional risk factors (Table 4). In contrast, DM but not RA was associated with increased PWV independently of traditional risk factors. Finally, both RA and DM were associated independently with increased IMT and higher prevalence of plaques.

Discussion

The present study confirmed a higher prevalence and severity of preclinical atheromatosis (assessed by FMD, PWV, IMT, and presence of carotid plaques) in RA patients compared to healthy controls. It also produced the novel findings that: (1) RA activity associates with carotid plaque instability, whereas RA duration associates with arterial stiffening; (2) the degree of abnormalities of all markers of preclinical atherosclerosis in RA was similar to that of DM of equal duration; (3) presence of RA and DM per se appear to have a differential impact on endothelial function and arterial stiffening.

This is the first study to use multiple methods, including GSM analysis, to globally assess vascular function and structure in RA and compare it not only with well-matched healthy controls but also with patients with a known CHD equivalent such as DM. Because of the number of RA patients in remission (51%), the mean CRP levels were relatively low, thus vascular deterioration may have been
underestimated. In this study, the 21 RA patients treated with anti-TNF agents, who by definition should have at least moderate or severe RA before biological therapy, had comparable vascular markers (FMD, IMT, PWV, and plaque prevalence) to the remaining patients (data not shown). Prospective characterization of recruited subjects has minimized missing values and classification bias, whereas corrections for multiple testing increase the probability that the associations found are true. Unlike other studies, patients on NSAIDs were excluded to avoid an additional important confounder. However, despite the relatively large number of patients, sample size and power calculations were not performed a priori. Although GSM analysis has been shown to demonstrate that the vascular abnormalities detected are surrogates of CVD outcome in RA population.

Our results concur with previous studies demonstrating evidence of vascular impairment in RA patients compared to healthy controls at multiple levels of atherosclerosis.12–14 The RA group studied here was representative of patients seen in routine clinical practice in Western countries, and comparable in terms of demographic and RA characteristics to patients included in previous studies. In our study, worse functional (FMD) and structural (IMT) arterial indices associated with RA independently of traditional CVD risk factors, suggesting a relationship with RA-specific pathology. Higher prevalence of nonobstructing atheromatous plaques, reflecting more advanced atherosclerosis in RA versus healthy controls, was also found, confirming the results of another study.12 The prevalence of plaques in the control group may seem low (9.5%) as compared to the results of the large scale EVA study in an apparently healthy population aged between 59 to

<table>
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<tr>
<th>Table 3. Vascular Markers of Preclinical Atherosclerosis in RA vs DM Patients</th>
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<td>Carotid plaques, n (%)</td>
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<td>Carotid plaque-GSM§</td>
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RA indicates rheumatoid arthritis; FMD, flow mediated dilatation; IMT, intima media thickness; GSM, grey scale median.
*Global P value represents the P value showing the overall effect of being in a different group (RA-DM or control) by ANOVA.
†Numbers in parentheses indicate SD except otherwise noted.
‡In parentheses, P values adjusted for total cholesterol, glucose, BMI, CRP, ACE-I/ARB, and statin use by linear or logistic regression where appropriate.
§Because of the small number of plaques in controls (n=2), Student t test was used to compare RA vs DM only.

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<th>Table 4. Influence of the Presence of RA and DM on Vascular Markers of Preclinical Atherosclerosis</th>
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<tr>
<td>Mean Carotid IMT (mm)</td>
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<td>Carotid Plaque-GSM§</td>
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**RA vs controls**
- Age, sex: −1.410, <0.001
- Model 1: −1.542, <0.001
- Model 2: −1.449, <0.001
- Model 3: −1.484, <0.001

**DM vs controls**
- Age, sex: −1.182, 0.006
- Model 1: −1.038, 0.075
- Model 2: −0.912, 0.176
- Model 3: −0.736, 0.272

Model 1: Age, gender, SBP, BMI, cholesterol, HDL, triglycerides, glucose, ever smoker.
Model 2: Model 1 + CRP, ferritin.
Model 3: Model 2 + ACE-I.
in RA, suggesting that RA-specific mechanisms are involved. In contrast, arterial stiffening (as reflected by PWV), a distinct but parallel process to atherosclerosis, appeared to be mediated mainly by traditional CVD risk factors, particularly hypertension in RA, but not in DM. Finally, in later stages of atherosclerosis (as reflected by structural changes in IMT), traditional CVD risk factors were not sufficient to explain the abnormalities, either in DM or in RA. These results imply that aggressive CVD risk factor management may be “sufficient” in DM but not in RA early on, again emphasizing the need for sustained control of systemic inflammation in RA. This would reconcile with the evidence that usage of some disease-modifying antirheumatic agents, including anti-TNF biologics, appears to improve overall and cardiovascular outcomes in RA.

In conclusion, the findings of the present study support the concept that RA is a novel cardiovascular risk factor with severity comparable to that of DM, and underscores the necessity to focus on primary prevention for CVD through aggressive identification and management of traditional risk factors, as well as effective sustained control of systemic inflammation. The impact of such strategies needs to be assessed prospectively in future studies designed specifically for the purpose.

Disclosures

None.

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