Insulin resistance is an independent risk factor for atherosclerosis in rheumatoid arthritis

GIOVANNI LA MONTAGNA, FEDERICO CACCIAPUOTI, ROSARIO BUONO, DANIELA MANZELLA,
GIANNA ANGELA MENNILLO, ALESSANDRO ARCIELLO, GABRIELE VALENTINI, GIUSEPPE PAOLISSO

Abstract

The objective of this study was to investigate the relationship between insulin resistance (IR) and subclinical atherosclerosis in patients with rheumatoid arthritis (RA).

Carotid artery intima media thickness (IMT), using ultrasound evaluation, and other clinical and laboratory variables were investigated in 45 RA outpatients and in 48 controls with soft tissue disorders. IR was assayed by homeostasis model assessment (HOMA2) and metabolic syndrome by National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) criteria.

Insulin resistance, as defined by HOMA2-IR>1, was seen in 40 (88.9%) RA patients and in three (6.2%) controls (p<0.001). No significant difference was detected in the prevalence of metabolic syndrome. The median IMT was greater in RA patients (0.76 mm; interquartile range [IQR] 0.65, 0.85) than in the controls (0.66 mm; IQR 0.60, 0.72) (p<0.001). Dividing the RA patients according to the cut-off IMT value (0.72 mm), a difference was detected in both systolic (p=0.04) and diastolic blood pressure (p=0.02), disease activity score (DAS28) (p=0.008), HOMA2-IR (p<0.001) and cumulative oral steroid dose (p=0.001). Moreover, the frequency of cases with increased IMT was higher in glucocorticoid users than in non-users (21/23 vs. 9/22, respectively) (p<0.001). Spearman’s rho correlation showed a significant positive relationship between IMT and HOMA2-IR (p<0.001). Multivariate stepwise analysis selected HOMA2-IR plus diastolic BP plus glucocorticoid exposure as the best predictive model for subclinical atherosclerosis (R²=0.577, F=24, p<0.001).

In conclusion, this study showed a significantly higher prevalence of IR in RA patients and pointed out a significant association between IR and subclinical atherosclerosis. This relationship may be driven primarily by exposure to steroid therapy.


Key words: insulin resistance, intima-media thickness, rheumatoid arthritis, subclinical atherosclerosis.

Introduction

Rheumatoid arthritis (RA) has long been known to be associated with increased mortality,1 which is mostly attributable to an increased prevalence of atherosclerosis, particularly ischaemic heart disease.2,3

The pathogenesis of accelerated atherosclerosis in RA has not yet been clearly defined.4 Traditional risk factors, RA-related inflammation and drug-related mechanisms are thought to play a role.5,6 In addition, a number of other risk factors have been suggested, including increased levels of lipoprotein (a),7 hypercoagulability,8,9 hyperhomocysteinaemia10 and apolipoprotein A1-dependent reduced cholesterol esterification.11

It has been hypothesised that insulin resistance (IR) may have a role in promoting atherosclerosis in RA.12,13 Impaired insulin sensitivity has been shown to be an independent cardiovascular risk factor in individuals with14 and without15 diabetes. Despite this evidence, the association between IR and atherosclerosis remains under debate, even though a recent study suggested a relationship with accelerated atherogenesis in active RA.16 In addition, the role of glucocorticoid therapy, which is commonly used in RA, in this relationship is unclear.17,18

This study was designed to investigate the relationship between IR and subclinical atherosclerosis.

Materials and methods

Patients and controls
Forty-five patients with RA and 48 controls with soft tissue...
disorders (myofascial pain, carpal tunnel syndrome or scapulo-humeral periarthritis), consecutively admitted to the outpatient clinic of the Rheumatology Unit of the Second University of Naples, were enrolled in the study. All the RA patients satisfied the American Rheumatism Association criteria for classification of the disease. They were being treated with classical disease-modifying antirheumatic drugs (DMARDs) (i.e. 43 with methotrexate, 24 with hydroxychloroquine sulphate, 11 with leflunomide, 10 with ciclosporine and five with sulphasalazine); 23 were also using low doses of steroids (i.e. prednisone equivalent < 7.5 mg/day); 30 were taking anti-tumour necrosis factor (TNF) alpha agents (7 infliximab; 19 etanercept; four adalimumab). Control subjects were being treated with acetylsalicylic acid or non-steroidal anti-inflammatory drugs (NSAIDs), as appropriate. None of the patients had overt diabetes, defined as either fasting basal glycaemia (>126 mg/dL, 6.93 mmol/L) or use of antidiabetic drugs. No patient was being treated with lipid-lowering drugs, such as statins or fibrates. Seven out of all RA patients with hypertension were using angiotensin-converting enzyme (ACE)-inhibitors. All subjects gave their informed consent. The study design was approved by the local ethical committee.

Methods
Both patients and controls were investigated for their demographic characteristics, namely sex, age and menopausal status. Previous cardiovascular events (stable or unstable angina, myocardial infarction, stroke) were elicited using patient recall and confirmed by history of medical advice.

Subclinical atherosclerosis was investigated in RA patients and in control subjects using carotid ultrasound evaluation. Briefly, individuals in the study population were investigated in the supine position, with the head turned away slightly from the sonographer. The common carotid arteries were carefully examined for wall changes in all subjects, obtaining different longitudinal and transverse views with high-resolution B mode ultrasound (ATL-5000 HDI) using a linear array 7.5 MHZ probe. A region about 1.5 cm proximal to the carotid bifurcation was identified, and the intima media thickness (IMT) of the far wall was evaluated as the distance between the luminal-intimal interface and the medial-adventitial interface. One transverse and two longitudinal measurements of IMT were obtained from 10 contiguous sites at 1 mm intervals, and the average of the 10 measurements was used for the analysis. All ultrasound measurements were performed by a trained sonographer who was unaware of subject characteristics.

Subclinical atherosclerosis was defined as a mean IMT > 75th percentile of the IMT values in the controls since the IMT distribution was skewed (0.20±0.34).

Traditional cardiovascular risk factors
Overweight or obesity was determined by calculating body mass index (BMI, kg/m²). Arterial blood pressure (BP) was evaluated three times, on the first two occasions with the subject in the sitting position, one minute apart, on the third with the patient in the supine position after four minutes; the mean value of both systolic and diastolic arterial pressure was considered. Hypertension was defined according to JNC VII criteria. Plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were evaluated in blood samples taken the morning after a 14-hour fast, using standard enzymatic methods. Serum insulin was detected according to chemiluminescent microparticle immunoassay (Abbott, US). Smoking status was assessed by dividing patients and controls into three groups: those who had never smoked, previous smokers and current smokers.

Insulin resistance (IR) and metabolic syndrome
IR was evaluated by the homeostasis model assessment (HOMA) (calculator available from www.OCDEM.ox.ac.uk), which is based on fasting plasma glucose and serum insulin concentrations. The output of the HOMA2 model was calibrated to give a beta-cell fraction of 100% and an IR of 1 as normal. Therefore, values were considered abnormal when HOMA2-IR was > 1.

Metabolic syndrome (MetS) was defined according to the updated third report of the National Cholesterol Education Program’s Adult Treatment Panel (NCEP-ATP III) criteria by the presence of at least three out of five items: waist circumference (>102 cm, women > 88 cm); triglycerides > 150 mg/dL (1.69 mmol/L) or drug treatment for elevated triglycerides; HDL-cholesterol levels (men < 40 mg/dL, 1.03 mmol/L; women < 50 mg/dL, 1.29 mmol/L) or drug treatment for reduced HDL-cholesterol; elevated blood pressure (> 150 mmHg systolic blood pressure or > 85 mmHg diastolic blood pressure or drug treatment for hypertension); elevated fasting glucose ≥ 100 mg/dL (5.5 mmol/L) or drug treatment for hyperglycaemia.

Disease features
RA patients were investigated for the following aspects: disease duration, rheumatoid factor status as evaluated by enzyme-linked immunosorbent assay (ELISA), disease activity (DAS 28), calculated using the erythrocyte sedimentation rate (ESR), tender joint count (TJC, 28 joints), swollen joint count (SJC, 28 joints), and the patient’s assessment of global wellbeing (100 mm visual analogue scale); disability as evaluated by the Italian version of the health assessment questionnaire (HAQ) disability index (DI).

Computation of cumulative glucocorticoid dose
For each RA patient, pharmacy and medical records were reviewed. Only oral steroids had been prescribed. The cumulative glucocorticoid dose was calculated by multiplying the current daily dose by the effective number of days for which the patients had received steroids since they were first prescribed.

Statistical analysis
Data were expressed as mean ± SD, unless indicated otherwise. Comparisons among groups were analysed by non-parametric tests. Correlations were investigated by univariate analysis and multivariate linear regression models, when appropriate. All analyses were performed using the SPSS software package. P values of less than 0.05 were considered significant.
Table 1. Anthropometric and traditional risk factors of rheumatoid arthritis (RA) patients and controls

<table>
<thead>
<tr>
<th>RA patients</th>
<th>Control subjects</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=45</td>
<td>n=48</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>53.8±11.6</td>
<td>51.8±11.6</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>42 (93.3)</td>
<td>45 (93.7)</td>
</tr>
<tr>
<td>Menopausal status, n (%)</td>
<td>27 (60)</td>
<td>28 (58.3)</td>
</tr>
<tr>
<td>Previous cardiovascular events</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66.6±11.3</td>
<td>68.6±13.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0±4.4</td>
<td>26.3±4.7</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>125±12</td>
<td>127±10.8</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>81±6.4</td>
<td>80±6.1</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>231±58</td>
<td>218±38</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>40.5±11.3</td>
<td>41.2±15</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>144±52</td>
<td>136±34.6</td>
</tr>
<tr>
<td>Current steroid therapy, n (%)</td>
<td>23 (51.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

Key: BMI = body mass index; BP = blood pressure; HDL = high-density lipoprotein; Values are mean ± standard deviation unless otherwise indicated; * = Mann-Whitney U test, or Fisher Exact test when appropriate; † = To convert triglycerides from mg/dL to mmol/L, multiply by 0.01129; ‡ = To convert triglycerides from mg/dL to mmol/L, multiply by 0.02586.

Table 2. Prevalence of traditional cardiovascular risk factors in rheumatoid arthritis (RA) patients and controls

<table>
<thead>
<tr>
<th>RA patients</th>
<th>Control subjects</th>
<th>p***</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=45</td>
<td>n=48</td>
<td></td>
</tr>
<tr>
<td>Overweight/obesity</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td>(BMI ≥ 25), n (%)</td>
<td>64 (20.4)</td>
<td>69 (21.3)</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>18 (42.2)</td>
<td>6 (13.5)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>29 (64.4)</td>
<td>30 (62.5)</td>
</tr>
<tr>
<td>(≥ 200 mg/dL), n (%)</td>
<td>41 (88.8)</td>
<td>32 (66.6)</td>
</tr>
<tr>
<td>Reduced HDL-cholesterol, n (%)</td>
<td>18 (39.5)</td>
<td>18 (37.5)</td>
</tr>
<tr>
<td>Hypertriglyceridaemia</td>
<td>16 (35.6)</td>
<td>16 (35.7)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>10 (22.2)</td>
<td>13 (27)</td>
</tr>
</tbody>
</table>

Key: HDL = high-density lipoprotein; BMI = body mass index; * = WHO criteria; ** = seven out of 10 RA patients used angiotensin-converting enzyme (ACE)-inhibitors; † = No patient was taking lipid-lowering therapy; *** = Fisher’s exact test.

Results

Table 1 shows anthropometric and traditional atherosclerotic risk factors for the 45 RA patients and 48 controls. There were no significant differences between patients and controls.

Table 2 shows the prevalence of traditional cardiovascu-

lar risk factors in RA patients and controls. Again, no differences were detected.

Table 3 shows the prevalence of IR and MetS in RA patients and controls. A significant difference was found in HOMA2-IR values (p<0.001) between patients and controls, whereas no difference emerged in the prevalence of MetS in one or two of its factors. In addition, no significant difference was found in the frequency of either MetS or abnormal values of HOMA2-IR in RA steroid users as compared to non-users. Mean IMT was 0.75±0.11 mm in the 45 RA patients, ranging from 0.56 to 0.99 mm; median IMT was 0.76 mm (interquartile range [IQR] 0.65, 0.85). In the 48 control subjects, mean IMT was 0.65±0.10 mm, ranging from 0.48 to 0.88 mm, and median IMT was 0.66 mm (IQR 0.60, 0.72) (p<0.001). With the 75th percentile value in the controls at 0.72 mm, we chose to subdivide RA patients into two groups, those with a mean IMT < 0.88 mm, and median IMT was 0.65±0.10 mm, ranging from 0.48 to 0.88 mm, and median IMT was 0.66 mm (IQR 0.60, 0.72) (p<0.001). With the 75th percentile value in the controls at 0.72 mm, we chose to subdivide RA patients into two groups, those with a mean IMT < 0.88 mm, and median IMT was 0.65±0.10 mm, ranging from 0.48 to 0.88 mm, and median IMT was 0.66 mm (IQR 0.60, 0.72) (p<0.001).

Table 4 shows the anthropometric features, traditional cardiovascular risk factors, HOMA2-IR values, disease features and steroid exposure in the two groups. Significant differences were detected in both systolic (p=0.04) and diastolic BP (p=0.02), DAS28 (p=0.008), HOMA2-IR (p<0.001), current daily dose of steroids (p=0.001) and cumulative steroid dose (p=0.001).

Notably, the prevalence of cases with increased mean IMT was significantly higher in patients using glucocorticoids (21/23 cases) than in non-users (9/22 cases) (p<0.001), as were the values of HOMA2-IR (1.76–95% CI 1.538 to 1.993 vs. 1.45–95%CI 1.257 to 1.643, respectively). However, no significant difference emerged when we analysed the prevalence of MetS in steroid users (12/23 cases, 52.2%) compared to non-users (12/22 cases, 54.5%) and controls (22/48 cases, 45.8%).

Table 5 shows the IMT and HOMA2-IR in RA patients arranged by DAS28 scores. Remission (defined as DAS28 ≤ 2.4) was observed in six out of 45 (13.3%) cases; low disease activity (≤ 3.6) was found in 10 (22.2%), moderate
activity (≤ 5.5) in 21 (46.7%) and high disease activity (> 5.5) in eight cases (17.8%). Both mean IMT and HOMA2-IR were found to parallel disease activity. In addition, a significantly lower DAS28 (3.7 ± 1.27; 95% CI 3.14 to 4.26) was found in RA patients unexposed to steroids compared to steroid users (4.8 ± 1.6; 95% CI 3.08 to 5.48). Furthermore, dividing the patients according to a cut-off for moderate to high disease activity (DAS28 > 3.6) or low disease activity (DAS28 ≤ 3.6), a significant difference emerged either in cumulative steroid dose (4.6 g; 95% CI 3.14 to 5.55, respectively) or daily dose (3.8 mg; 95% CI 2.43 to 5.15 vs. 2.34 mg; 95% CI 0.556 to 4.13, respectively).

Univariate analysis
Linear Spearman’s rho correlation showed a significant positive relationship between IMT and both systolic (rs=0.392, p=0.008), diastolic BP (rs=0.511, p<0.001), current steroid therapy (0.595, p<0.001), cumulative glucocorticoid dose (rs=0.58; p=0.001) and current daily glucocorticoid dose (rs=0.544, p=0.001). When the effect of other factors, namely hypertension, smoking, current steroid daily dose and cumulative steroid dose, was excluded from the regression analysis, the relationship between IMT and HOMA2-IR remained significant (r=0.46; p=0.002). In addition, a significant correlation was found between IMT and definite MetS (r=0.353, p=0.017) or number of MetS features (r=0.479, p=0.001). A significant correlation was also found between HOMA2-IR and DAS28 (rs=0.549, p<0.001) or current daily glucocorticoid dose (rs=0.372, p=0.012). Even when the effect of DAS28 and that of previously listed factors was excluded, the relationship between IMT and HOMA2-IR remained significant (r=0.37; p=0.002). Furthermore, a trend was observed between HOMA2-IR values and definite MetS (p=0.062), number of MetS components (p=0.059) or higher cumulative glucocorticoid dose (p=0.08). No correlation was found between IMT, HAQ-DI and other parameters, i.e. BMI, waist circumference, menopausal status, smoking status, disease duration, lipid profile and use of DMARDs or anti-TNF alpha agents.

Multivariate analysis
To establish the best model to predict subclinical atherosclerosis (i.e. IMT > 0.72 mm), multivariable linear regression analysis was performed choosing the variables that were significantly correlated by univariate analysis with IMT. Thus, the dependent variable was the IMT and the independent variables were respectively: both systolic and diastolic BP, HOMA2-IR, definite MetS and number of their components, and steroid exposure. The stepwise procedure selected HOMA2-IR plus diastolic BP and steroid exposure as the best predictors (R2c=0.577, F=21, p<0.001). In this model R2c for HOMA2-IR was 0.324 (F=22.1 p<0.001). Since with the relatively small cohort of RA patients examined, controlling for more than one confounder in regression analysis could constitute overfitting,22 we constructed other models. In the first, we included HOMA2-IR, both definite MetS and number of its components, both current daily and cumulative steroid dose as independent variables, and the stepwise procedure selected HOMA2-IR and daily dose of steroid as the best predictors (R2c=0.483, F=14.7, p<0.001). Then we included HOMA2-IR, MetS and steroid daily dose as independent variables, and the procedure selected HOMA2-IR plus MetS plus steroid daily dose as the best predictors (R2c=0.479; F=14.5 p<0.001). Finally, we included HOMA2-IR and DAS28 and the former was the better predictor (R2c=0.269; F=17.2, p<0.001).

Table 4. Comparison of RA patients with an increased and normal mean carotid IMT

<table>
<thead>
<tr>
<th>Disease activity state</th>
<th>Cases</th>
<th>IMT ≤ 0.72 mm</th>
<th>IMT &gt; 0.72 mm</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission, DAS28 &lt; 3.6</td>
<td>22</td>
<td>0.62±0.08</td>
<td>1.60±0.37</td>
<td>0.001</td>
</tr>
<tr>
<td>Moderate disease, DAS28 &lt; 5.5</td>
<td>21</td>
<td>0.61±0.09</td>
<td>1.43±0.42</td>
<td>0.001</td>
</tr>
<tr>
<td>Low disease, DAS28 &lt; 3.6</td>
<td>10</td>
<td>0.62±0.13</td>
<td>1.37±0.19</td>
<td>0.001</td>
</tr>
<tr>
<td>High disease activity, DAS28 &gt; 5.5</td>
<td>8</td>
<td>0.64±0.10</td>
<td>2.14±0.49</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 5. Behaviour of carotid IMT and HOMA2-IR in RA patients divided according to cut-off values for different disease activity (DAS28) states

<table>
<thead>
<tr>
<th>Disease activity state</th>
<th>Cases</th>
<th>IMT</th>
<th>HOMA2-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission, DAS28 &lt; 3.6</td>
<td>22</td>
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<td>8</td>
<td>0.64±0.10</td>
<td>2.14±0.49</td>
</tr>
</tbody>
</table>

Key: IMT = intima media thickness; BMI = body mass index; BP = blood pressure; ESR = erythrocyte sedimentation rate; DAS28 = disease activity score; RA = rheumatoid arthritis; HOMA = homeostasis model assessment; Values are mean ± standard deviation; * = Mann-Whitney U test

Table 6. Comparison of RA patients with an increased and normal mean carotid IMT

<table>
<thead>
<tr>
<th>Disease activity state</th>
<th>Cases</th>
<th>IMT ≤ 0.72 mm</th>
<th>IMT &gt; 0.72 mm</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.37±0.19</td>
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</tr>
<tr>
<td>High disease activity, DAS28 &gt; 5.5</td>
<td>8</td>
<td>0.64±0.10</td>
<td>2.14±0.49</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Discussion
Accelerated atherosclerosis is a well defined feature of rheumatoid arthritis. The pathogenesis of this condition is not clearly known, but cardiovascular risk factors, drugs and disease activity are all thought to play a role. Among traditional cardiovascular risk factors, most authors have pointed out low levels of HDL-cholesterol. However, we did not find this association, either in a previous paper by our team or in the present study. Instead, we found a significant association with BP, confirming the results obtained by Wallberg-Jonsson et al. The discrepancy in results is likely to depend on differences among the cohorts of patients investigated, including differences in their nutritional habits. Nevertheless, traditional risk factors clearly play a role in the pathogenesis of atherosclerosis in RA and must be investigated when evaluating the individual RA patient.

Other metabolic risk factors are thought to be involved, such as raised levels of lipoprotein (a) and homocysteine. As far as we know, the role of insulin resistance has not been extensively investigated in RA patients. Conflicting results have been found regarding the relationship between IR and subclinical atherosclerosis, and regarding glucocorticoid use. The present data confirm our previous finding of an increased prevalence of insulin resistance in RA compared to controls (p<0.001), and indicate a significant correlation between HOMA2-IR and increased IMT, both in simple linear regression and in stepwise multiple regression. With the latter, IMT was independently related to HOMA2-IR, with steroid exposure, MetS, number of its components and atherothrombotic BP acting as other contributors. On the whole, our data show a relationship between subclinical atherosclerosis, insulin resistance and glucocorticoid use that is in agreement with the findings of other authors.

In conclusion, we have confirmed IR in a significantly high proportion of RA patients and indicated a significant association between IR and subclinical atherosclerosis.

In our cohort of RA patients, the frequent use of low-dose steroids in active disease seems to indicate that IR can be driven primarily by steroids. On the other hand, we cannot exclude a role for systemic inflammation in the development of insulin resistance. Other studies on larger series of patients are needed to define the true weight of each of these factors on IR development.

Conflict of interest declaration
None declared.

References
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