Risk Factors Associated with Different Stages of Atherosclerosis in Colombian Patients with Rheumatoid Arthritis

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Objectives: Rheumatoid arthritis (RA) is associated with an increased prevalence of cardiovascular disease (CVD). Since atherosclerosis development is a gradual process of damage inside the artery wall, and the phenotype–genotype correlation of complex diseases may vary depending on ethnicity, we sought to investigate the influence of clinical features, routine inflammatory markers, and the genetic component of RA on different stages of atherosclerosis in northwestern Colombian patients with RA.

Methods: A group of 140 patients with RA were enrolled in this study. All patients underwent a noninvasive evaluation of endothelial function by flow-mediated vasodilation (FMV) and an assessment of carotid intima-media thickness (IMT) by high-resolution B-mode ultrasonography. The patients were classified into 3 categories: endothelial dysfunction (FMV <5%), increased IMT (0.91-1.29 mm), and plaque (IMT >1.30 mm). The risk of being in each category was assessed by investigating traditional and nontraditional cardiovascular risk factors. For each stage of atherosclerosis development, we searched for nontraditional risk factors that were significantly associated with the stage after adjusting for traditional risk factors and current age.

Results: Rheumatoid factor seropositivity was significantly associated with endothelial dysfunction (adjusted odds ratio, AOR = 3.0). A duration of RA >10 years (AOR = 29.0) and being a carrier of an HLA-DRB1 shared epitope allele (AOR = 4.8) were associated with atherosclerotic plaque. No association of extra-articular manifestations, anticyclic citrullinated peptide (anti-CCP3) antibodies, and tumor necrosis factor −308 polymorphism with CVD was found.

Conclusions: Our results reveal the presence of RA-related risk factors for CVD which act independently of traditional risk factors. These factors can be used by clinicians to predict CVD in RA patients, and this data should assist in the development of public health policies in our population for the improvement of patient outcomes.

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Ischemic heart disease secondary to atherosclerosis is the most prevalent cause of death associated with cardiovascular disease (CVD) in patients with rheumatoid arthritis (RA) (1-3). Traditional risk factors such as dyslipidemia, smoking, increased waist circumference, and old age (4-6), and nontraditional risk factors such as extra-articular manifestations, swollen joint count, autoantibody levels, C-reactive protein (CRP), and other inflammatory markers have been implicated in accelerated atherosclerosis in these patients (7-10). Atherosclerosis is a process of gradual inflammation inside the artery wall. It begins with a change in the endothelium phenotype, followed by artery wall thickening, and finally, by the appearance of atherosclerotic plaque (11-13). Carotid intima-media thickness (IMT) is a measurement that is used to detect changes in the thickness of the artery wall secondary to the development of atherosclerosis (14). There is strong evidence supporting an increased carotid IMT in patients with RA (15-17). Increased carotid artery IMT has also been reported in long-term treated RA patients without clinically evident CVD or cardiovascular risk factors (18). Therefore, it is important to investigate the risk factors associated with different stages of atherosclerosis development since knowledge of these factors may help to improve prevention and treatment of CVD in RA patients. In addition, since RA patients may have impaired endothelial function before entering an early stage of atherosclerosis (19,20), the evaluation of endothelial function through flow-mediated vasodilation (FMV) in the brachial artery may be helpful in detecting initial pathological changes in the artery wall and, therefore, in assessing early cardiovascular risk in these patients (21-23).

The mechanisms and risk factors influencing atherosclerotic plaque formation in patients with autoimmune diseases, such as RA, are not fully understood (24-26). A genetic feature that has been consistently associated with RA is the presence of *HLA-DRB1* alleles, which also have been implicated in RA disease severity and atherosclerosis development (27-30). Some tumor necrosis factor-α (*TNF*) gene polymorphisms have been associated with susceptibility to RA (31,32). However, it is not clear whether these polymorphisms are also associated with vascular damage in RA patients. The *TNF* gene influences body weight homeostasis as well as insulin resistance, diabetes mellitus, lipid levels, hypertension, coagulation, endothelial damage, and inflammation (33-36).

Controversy exists as to whether *HLA-DRB1* polymorphisms affect susceptibility to RA, or affect disease severity and progression (30,37,38). Moreover, the fact that more than 1 autoimmune disease may coexist in a single patient or in the same family supports the hypothesis of additional susceptibility genes (39,40). Interestingly, 1 specific autoimmune disease (ie, RA) may be present in various members of a nuclear family, the so-called familial autoimmune disease, and different autoimmune diseases may occur in the relatives of a patient with RA (ie, familial autoimmunity) (41). These facts reinforce the polygenic character of RA (42).

Since the phenotype and genotype of complex diseases may vary depending on ethnicity, we sought to investigate the potential contribution of clinical RA features, routine inflammatory markers, and the genetic component to the development of atherosclerosis in northwestern Colombian patients with RA, controlling for potential confounders such traditional CVD risk factors (43,44). Special attention was given to the different stages of atherosclerosis development. Since in practice the clinician may prefer to assess only 1 or a few inflammatory markers and/or genetic factors to predict CVD risk, we searched for those variables that may, by themselves and independently of traditional CVD risk factors, be predictors of increased atherosclerosis risk. This may be a more sensible approach than searching for predictors that can be used only when the clinician has access to a large number of other nontraditional predictors. An attempt was also made to answer the question of whether or not the presence of familial autoimmunity confers additional susceptibility to atherosclerosis in RA patients.

METHODS

Study Population

Consecutive RA patients attending the Clinical Immunology and Rheumatology Unit at the "Clínica Universitaria Bolivariana-Corporación para Investigaciones Biológicas" at Medellín, and fulfilling the American College of Rheumatology classification criteria were included (45). This study was undertaken between 2006 and 2007 and conducted in compliance with the 1993 Act 008430 by the Ministry of Health of the Republic of Colombia. The institutional review board of the "Corporación para Investigaciones Biológicas" approved the study design.

Age at RA onset was defined as the age at which patients began to suffer from pain, morning stiffness, and inflammation of hand and/or foot joints in a symmetrical fashion. Familial autoimmunity was defined as the presence of any diagnosed autoimmune disease in another member of the patient's nuclear family (46). Autoimmune hypothyroidism was diagnosed when there was a thyroid-stimulating hormone level >5.0 IU, or evidence of thyroid hormone replacement because of primary hypothyroidism together with the presence of antithyroid peroxidase

or antithyroglobulin antibodies. Patients with a history of acute myocardial infarction or stroke were not included in the sample to avoid an overestimation bias, because such patients could have high IMT values and low FMV. The patients underwent a detailed, clinical global examination that included an assessment of disease activity based on Disease Activity Score 28 (DAS 28) (47), and an overall assessment of quality of life based on the Multidimensional Health Assessment Questionnaire (48). Patients with a DAS 28 score >3.2 were considered to have active disease (49). Relevant laboratory variables associated with RA activity were tested: erythrocyte sedimentation rate (ESR), hemoglobin levels, white blood cell count, platelet count, and serum high sensitive CRP levels. Rheumatoid factor (RF) was measured by turbidimetry (Beckman, Brea, CA); titers >40 U/mL were considered positive. Third-generation anticyclic citrullinated peptide (anti-CCP3) antibodies were measured by an enzyme-linked immunosorbent assay kit (QUANTA-Lite, INOVA, San Diego, CA) according to the manufacturer's protocol; titers >60 U were considered positive (50). Serum levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL), and glucose were determined by using standard methods (Enzyme Assay Kit, Roche Diagnostics, Indianapolis, IN).

TNF and *HLA-DRB1* Genotyping

Genomic DNA was extracted from 10 mL of an EDTA-anticoagulated blood sample using the standard salting out technique. Genotyping for the *TNF* single nucleotide polymorphism (SNP) at –308 position was performed by a polymerase chain reaction as previously described (51). This SNP was investigated because some studies suggest that it may influence the risk of developing RA in Latin-Americans (including Colombians) (30). *HLA-DRB1* typing was done by reverse dot-blot hybridization of the polymerase chain reaction products (Inno-LiPA assay, Innogenetics, Ghent, Belgium) as described elsewhere (52). Genetic susceptibility to RA is associated with certain *HLA-DRB1* alleles encoding a similar sequence motif called the "shared epitope" (SE), which spans amino acid positions 70 to 74 in the third diversity region of the outermost domain of the HLA-DRB1 molecule (53). This SE is encoded mainly by the *HLA-DRB1* *0101, *0102, *0401, *0404, *0405, *0408, *0409, *0410, *1402 and *1001 alleles. Also, *HLA-DRB1* *0103, *0402, *1102, *1103, *1301, *1302 and *1304 alleles encode the DERAA sequence at the same positions, which has been shown to confer protection against RA development and severity (30,54-57). Hence, *HLA-DRB1* status was categorized according to the presence or absence of the SE and DERAA motifs.

Cardiovascular Risk Factors Assessment

Patients were assessed for traditional CVD risk factors including current age (>45 years for men and >55 years for women) (58-61), hypertension (defined as having a blood pressure >140/90 mm Hg or using antihypertensive medication) (62), diabetes mellitus [defined as having a fasting plasma glucose level >7 mmol/L (126 mg/dL) or taking antidiabetic medication at the time of the assessment] (63). A diagnosis of dyslipidemia was given when hypercholesterolemia (having a fasting plasma total cholesterol >200 mg/dL), low high-density lipoprotein cholesterol (<40 mg/dL) (58,64), hypertriglyceridemia (>150 mg/dL), or elevated LDL cholesterol (>100 mg/dL) was present. Current hemoglobin <12 g/dL established a diagnosis of anemia. The fact of being smoker or having been a smoker (ever smoking) and history of premature coronary heart disease (CHD) in first-degree relatives were also assessed in all patients as previously described (65). Systolic and diastolic blood pressures were measured twice with at least 15 minutes between measurements and the averages were recorded. Patients were clinically evaluated for the presence of CVD including heart and peripheral vascular disease (66). We estimated CHD risk using the Framingham Heart Study prediction score sheets. Separate score sheets were used for men and women. The Framingham score provides an estimate of total CVD risk over the course of 10 years (67).

Anthropometric Measurements

A body mass index (BMI) >30 kg/m^2 was considered abnormal (68). The patients were assessed for the presence of the metabolic syndrome by using the definition from the *National Cholesterol Education Program Adult Treatment Panel III* (69). Abnormal values of waist circumference (≥102 cm for men, ≥88 cm for women) and waist-to-hip ratio (WHR; >0.9 for men, >0.85 for women) were considered indicators of abdominal obesity, which is the most prevalent manifestation of metabolic syndrome, a marker of dysfunctional adipose tissue and is also associated with increased risks of type 2 diabetes and CVD (70). Waist circumference was measured around the narrowest point between ribs and hips after exhaling when viewed from the front. Hip circumference was measured at the point of maximum extension of the buttocks when viewed from the side (71). Abnormal WHR values are consistent with *National Cholesterol Education Program Adult Treatment Panel III* and World Health Organization definitions (72). The above measurements were used to find the most reliable predictors of vascular involvement in patients with RA.

Assessment of Carotid Intima-Media Thickness

Ultrasound measurements of IMT of the common carotid artery were performed by an expert (LFG) who was

blind to patients' clinical records. High-resolution, 2-dimensional images were obtained using an ultrasound machine (Agilent-Hewlett-Packard, Santa Clara, CA). A 7.5-MHz linear-array transducer with an axial resolution of 0.15 mm and a penetration depth from 1.0 to 5.0 cm was used (73). The patient rested on the examination table for 15 minutes before the initial carotid ultrasound scan. The measurement was applied to the far wall of the right and left carotid arteries. Following Salonen and Salonen (74), a B-mode screening method was used with electronic calipers within 10 mm proximal to the common carotid bifurcation in a temperature-controlled room (22°C to 24°C) (75). Ultrasound images were recorded on videotape (Sony MD 385). IMT was measured at the site of greatest thickness and at 2 additional points: 1 cm upstream and 1 cm downstream from this site. The average of these 3 values was computed. In keeping with previous reports, patients with carotid IMT less than 0.60 mm were considered to be free of atherosclerosis. On the other hand, since carotid IMT greater than 0.90 mm is included among the definitions of subclinical organ damage (76), we established 3 different categories: carotid IMT values less than 0.90 mm; those between 0.91 and 1.29 considered as having severe subclinical atherosclerosis (SSA); and plaques if values were equal to or greater than 1.30 mm. The reproducibility of the IMT was evaluated by taking 2 measurements 1 month apart on 12 volunteers, obtaining an intraclass correlation of 0.98.

Noninvasive Evaluation of Endothelial Function

To detect endothelial dysfunction, brachial FMV was assessed by noninvasive ultrasound. The assessment was made by the same examiner who performed the ultrasound measurements of IMT. The brachial artery diameter was measured on B-mode imaging using a high-resolution (7.5 MHz) linear array transducer. During the diastolic phase, a basal diameter was measured from the anterior to posterior interface between the media and adventitia ("m line") at a fixed distance (77). A pneumatic tourniquet placed around the forearm distal to the target artery was inflated to a pressure of 250 mm Hg and inflation was held for 5 minutes. Increased flow was then induced by sudden cuff deflation. Twenty seconds later, the brachial artery diameter was measured. Fifteen minutes later, another resting scan was recorded to confirm vessel recovery. This method detects diameter changes of 0.1 to 0.2 mm (78). The diameter change caused by FMV was expressed as the percentage change in diameter relative to the basal diameter. A percentage change $\geq$5% after the reactive hyperemia indicated a reactive normal artery (79). For patients under current antihypertensive therapy, the therapy was discontinued a few days before evaluating endothelial function.

Statistical Analysis

Age at RA onset, disease duration, and current age were categorized into groups determined by quartiles. Endothelial dysfunction, increased IMT (SSA), and the presence of carotid plaque were used as dependent variables. First, univariate analyses searching for the association between traditional CVD risk factors and endothelial dysfunction were investigated using χ^2 tests or Fisher's exact tests when the factors were dichotomous, or t-tests for mean differences when the factors were continuous. Similar univariate analyses were performed for the nontraditional risk factors. Next, for each nontraditional risk factor that was significantly associated with endothelial dysfunction in univariate analyses, a multivariate logistic regression model that included endothelial dysfunction as the dependent variable was fit. As independent variables, the model included the nontraditional variable, the traditional variables that were significantly associated with endothelial dysfunction in univariate analyses, and current age. (Current age was controlled for since it was considered a potential confounding factor.) Analogous analyses were made by using increased IMT (SSA) or the presence of atherosclerotic plaque in place of endothelial dysfunction. P values <0.05 were considered significant. Odds ratios (ORs) that measured the effect size of risk factors on endothelial dysfunction, IMT (SSA), or the presence of atherosclerotic plaque were computed together with their 95% confidence intervals (CIs). The adequacy of logistic models was assessed using the Hosmer–Lemeshow goodness-of-fit test. All reported logistic models fit well. Statistical analyses were performed by using the Statistical Package for the Social Sciences (SPSS, v.15, Chicago, IL).

RESULTS

One hundred forty patients were included. Their general findings related to RA are described in Table 1. Patients were between 26 and 78 years old and all patients belonged to the same ethnic group. The most prevalent allele was *HLA-DRB1*0404*, which was present in 23% (30/132) of the patients. Expected SE-amino acid sequences were available in 132 patients and distributed as follows: QKRAA (DRB1*0401), 2% (3/132); QRRAA (DRB1*0404,*0405, *0408, and *1402), 42% (55/132); and RRRAA (DRB1*0101,*0102,*1001), 4% (5/132). The protective sequence, DERAA, was found in 10% (13/132) of the patients. All patients were receiving a standard therapeutic regimen consisting of methotrexate ($<$20 mg/wk) plus regular folic acid supplementation (7 mg/wk), prednisone ($<$10 mg/d), chloroquine (250 mg/d), and sulfasalazine (1.5 g/d). Twenty-one percent (30/140) of the patients were receiving biological therapy.

The prevalence of endothelial dysfunction, increased IMT (SSA), and the presence of atherosclerotic plaque were 31% (44/140), 54% (75/140), and 7% (10/140), respectively. This suggests that FMV and IMT reflect distinct and independent stages in the complexity of atherosclerosis de-

Table 1 Clinical Characteristics of 140 Patients with Rheumatoid Arthritis

	Mean ± SD
Age at RA onset	40.5 ± 12.3
Current age (y)	54.2 ± 11.3
Duration of arthritis (y)	13.8 ± 8.5
Swollen joints count	5.7 ± 5.9
HAQ	1.7 ± 0.7
Patient global assessment (VAS)	4.6 ± 2.9
Physician global assessment (VAS)	4.1 ± 2.8
DAS 28	4.4 ± 1.4
Current RF antibody titers	198.8 ± 326.1
Current anti-CCP antibody titers	260.7 ± 181.1
Hemoglobin (mg/dL)	13 ± 1.5
Total leukocyte count ($\times 10^3/\mu L$)	8 ± 2.3
Polymorphonuclear cell count ($\times 10^3/\mu L$)	5.1 ± 1.9
Lymphocytes count ($\times 10^3/\mu L$)	2.3 ± 0.8
ESR (mm/h)	38.9 ± 25.1
CRP (mg/L)	5.9 ± 15
	Percentage/Numbers
Female	84 (117/140)
Anemia	28 (39/140)
EAM	43 (60/140)
Family history of autoimmunity[a]	21 (29/140)
Autoimmune thyroid disease[a]	22 (31/140)
RF (+)	63 (85/134)
Anti-CCP antibody (+)	78 (73/94)
Methotrexate	94 (131/140)
Steroid therapy	94 (131/140)
Chloroquine	61 (86/140)
TNF inhibitor	21 (30/140)
HLA-DRB1 SE	46 (60/132)
TNF SNP−308 GG	58 (76/132)
TNF SNP−308 AG	26 (34/132)
TNF SNP−308 AA	1 (2/132)
Presence of SE (single copy)	37 (49/132)
Presence of SE in both alleles	8 (11/132)

RA, rheumatoid arthritis; RF, rheumatoid factor; Anti-CCP, anti-cyclic citrullinated peptide antibodies; SE, shared epitope; EAM, extra-articular manifestations; HAQ, Health Assessment Questionnaire score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS 28, disease activity score 28; VAS, visual analog scale; TNF, tumor necrosis factor alpha; *TNF* SNP−308, single nucleotide polymorphism at position −308 in gene promoter.
[a]See Patients and Methods for definition.

velopment (79). Overall, 36% (51/140) of the patients had a 10-year risk of a cardiovascular event that was <5%; 27% (38/140) had a risk of 5 to 10%; 24% (34/140) had a risk of 11 to 15%; and 12% (17/140) had a 10-year risk >15%. Additional cardiovascular findings are shown in Table 2.

Univariate Analyses of Traditional Risk Factors for Cardiovascular Disease

Having elevated triglycerides and elevated LDL cholesterol levels were both significantly associated with endothelial dysfunction (Table 3). Abnormal waist circumference, abnormal WHR, and a Framingham risk score >5% were significantly associated with increased IMT (SSA). Ever smoking was significantly associated with the presence of atherosclerotic plaque.

Table 2 Cardiovascular Characteristics of 140 Patients with Rheumatoid Arthritis

Characteristics	Mean ± SD
BMI (kg/m^2)	25.5 ± 4.7
Waist circumference[a]	90.7 ± 12.7
WHR[a]	0.9 ± 0.2
Systolic blood pressure (mm Hg)	130.7 ± 16.6
Diastolic blood pressure (mm Hg)	82.2 ± 10.4
Total cholesterol (mg/dL)	204.1 ± 41.6
HDL cholesterol (mg/dL)	52.6 ± 13.4
Triglycerides (mg/dL)	141.9 ± 62.3
LDL cholesterol (mg/dL)	123.4 ± 36.1
Creatinine (mg/dL)	0.8 ± 0.2
Glycemia	90.8 ± 27.8
Ten-year risk of a cardiovascular event[a]	7.9 ± 6.3
Brachial diameter change (%)	9.3 ± 8.2
IMT (mm)	0.91 ± 0.24
	Percentage/Numbers
Metabolic syndrome[a]	44 (61/140)
Diabetes[a]	4 (6/140)
Ever smoking	44 (61/140)
First-degree relative with CHD[a]	16 (22/140)
Current hypertension	41 (57/140)
Dyslipidemia[a]	35 (49/140)
Current antihypertensive therapy	32 (45/140)
Current lipid-lowering therapy	13 (18/139)
History of hormone replacement therapy	7 (10/140)
Physical inactivity	85 (119/140)
FMV (change <5%)[a]	31 (44/140)
Increased IMT (severe subclinical atherosclerosis)[a]	54 (75/140)
Presence of atherosclerotic plaque[a]	7 (10/140)
Abnormal waist circumference[a]	56 (78/140)
Abnormal WHR[a]	83 (116/140)
Abnormal BMI[a]	16 (23/140)
Cholesterol levels >200 mg/dL	56 (79/140)
Low HDL cholesterol[a]	15 (21/140)
LDL cholesterol >100 mg/dL	76 (106/140)
Triglycerides ≥150 mg/dL	37 (52/140)
Abnormal fasting glucose levels[a]	17 (24/140)
CRP >5 mg/L	70 (89/128)

BMI, body mass index; WHR, waist-to-hip ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FMV, flow mediated vasodilation; CHD, coronary heart disease; IMT, intima-media wall thickness; CRP, C-reactive protein.
[a]See Patients and Methods for definition.

Univariate Analyses of Nontraditional Risk Factors

Having a positive RF was significantly associated with endothelial dysfunction (Table 4). In univariate analyses,

Table 3 Traditional Risk Factors for Cardiovascular Disease Significantly Associated with Endothelial Dysfunction, Increased Intima-Media Thickness, and Atherosclerotic Plaque in RA Patients According to Univariate Analyses[a]

	Endothelial Dysfunction[b] OR (95% CI)	Increased IMT (SSA)[c,d] OR (95% CI)	Presence of Atherosclerotic Plaque OR (95% CI)
Abnormal waist circumference[e]	—	2.6 (1.3 to 5.3)	—
Abnormal WHR[e]	—	3.4 (1.3 to 8.9)	—
Triglycerides ≥150 mg/dL	2.5 (1.2 to 5.3)	—	—
LDL cholesterol >100 mg/dL	2.6 (1.0 to 6.9)	—	—
Ever smoking	—	—	32.4 (1.9 to 565.7)[f]
Ten-year risk of a cardiovascular event (≥5%)[e]	—	4.4 (2.0 to 9.1)	—

RA, rheumatoid arthritis; OR, odds ratio; CI, confidence interval; IMT, increased intima-media thickness; WHR, waist-to-hip ratio; SSA, severe subclinical atherosclerosis.

Only odds ratios that were significant at a 0.05 level of significance are reported.

[a]Hypertension, having a first-degree relative with CHD, hormone replacement therapy, diabetes, body mass index, total cholesterol, HDL cholesterol, creatinine, and abnormal glycemia were not significantly associated with endothelial dysfunction, IMT (SSA), or presence of atherosclerotic plaque.

[b]A diagnosis of current dyslipidemia was significantly associated with endothelial dysfunction [OR = 3.0; 95% CI (1.4 to 6.2)].

[c]A diagnosis of current dyslipidemia was significantly associated with IMT (SSA) [OR = 2.1; 95% CI (1.0 to 4.3)].

[d]A diagnosis of a metabolic syndrome was significantly associated with increased IMT (SSA) [OR = 3.1; 95% CI (1.5 to 6.2)].

[e]See Patients and Methods for definition.

[f]None of the never smokers had atherosclerotic plaque.

having low hemoglobin levels was a significantly protective factor for endothelial dysfunction. Late RA onset was significantly associated with both increased IMT (SSA) and the presence of atherosclerotic plaque. An arthritis duration ≥10 years, having a familial autoimmunity, and being a carrier of 1 HLA SE allele were significantly associated with the presence of atherosclerotic plaque.

Adjusted Effects of Nontraditional Risk Factors on the Different Stages of Atherosclerosis Development

In a logistic regression analysis that adjusted for current age and potentially confounding traditional risk factors (having triglycerides ≥150 mg/dL and LDL cholesterol >100 mg/dL), RF seropositivity was significantly associated with an increased risk of endothelial dysfunction (OR = 3.0, $P = 0.02$, Table 5). In a similar analysis, having low hemoglobin levels was not significantly associated with endothelial dysfunction ($P = 0.9$). Even though a late RA onset was significantly associated with increased IMT (SSA) in univariate analyses, the association did not remain significant after adjusting for current age, abnormal WHR, and having a Framingham risk score ≥5% ($P = 0.5$, Table 5).

After adjusting for potential confounders (current age and ever smoking), a late RA onset ($P = 0.01$, OR = 12.8), a duration of disease ≥10 years ($P = 0.003$, OR = 29.0), and being a carrier of a single copy of HLA-DRB1 SE ($P = 0.045$, OR = 4.8) were significantly associated with an increased risk of atherosclerotic plaque (Table 5). Familial autoimmunity was not significantly associated with having atherosclerotic plaque ($P = 0.3$).

DISCUSSION

This study describes the prevalence of endothelial dysfunction and atherosclerosis in Latin-American patients with RA for the first time. The prevalences of diabetes and abdominal obesity in these RA patients were comparable

Table 4 Nontraditional Risk Factors Significantly Associated with Endothelial Dysfunction, Increased IMT, and Atherosclerotic Plaque in RA Patients According to Univariate Analyses

	Endothelial Dysfunction OR (95% CI)	Increased IMT (SSA) OR (95% CI)	Presence of Atherosclerotic Plaque OR (95% CI)
Age at RA onset >51 years	—	3.8 (1.4 to 10.2)	23.4 (4.6 to 118.6)
Duration of arthritis (>10 y)	—	—	6.0 (1.2 to 29.4)
RF (>40 IU)	2.4 (1.0 to 5.4)	—	—
Current hemoglobin <12 g/dL	0.31 (0.12 to 0.82)	—	—
Familial autoimmunity[a]	—	—	4.4 (1.2 to 16.5)
SE (single copy)	—	—	4.5 (1.1 to 18.3)

OR, odds ratio; CI, confidence interval; RA, rheumatoid arthritis; IMT, increased intima-media thickness; SSA, severe subclinical atherosclerosis; RF, rheumatoid factor; SE, shared epitope.

Only odds ratios that were significant at a 0.05 level of significance are reported.

[a]See Patients and Methods for definition.

Table 5 Adjusted Odds Ratios for the nontraditional Variables that Were Significantly Associated with Endothelial Dysfunction, Increased IMT (Severe Subclinical Atherosclerosis), and Atherosclerotic Plaque in Univariate Analyses

	AOR[a]	95% CI	*P* Value
Endothelial dysfunction			
Positive RF >40 IU	3.0[b]	(1.2 to 7.5)	0.02
Current hemoglobin <12 g/dL	1.1[b]	(0.40 to 3.1)	0.9
Increased IMT (SSA)			
Age at RA onset (>51 y)	1.5[c]	(0.49 to 4.8)	0.5
Presence of atherosclerotic plaque			
Age at RA onset (>51 y)	12.8[d]	(1.7 to 93.8)	0.01
Duration of arthritis (>10 years)	29.0[d]	(3.1 to 270.3)	0.003
Familial autoimmunity	2.2[d]	(0.50 to 9.5)	0.3
SE (single copy)	4.8[d]	(0.98 to 23.8)	0.04

OR, adjusted odds ratio; CI, confidence interval; RA, rheumatoid arthritis; IMT, increased intima-media thickness; SSA, severe subclinical atherosclerosis; RF, rheumatoid factor; SE, shared epitope.

[a]The adjustment was performed through logistic regression analyses that controlled for current age and the traditional variables that were significantly associated with the dependent variables in univariate analyses.

[b]The OR and its corresponding CI were adjusted for current age and the traditional variables that were significantly associated with endothelial dysfunction in univariate analyses (triglycerides ≥150 mg/dL and LDL cholesterol ×100 mg/dL).

[c]The OR and its corresponding CI were adjusted for current age and the traditional variables that were significantly associated with IMT (SSA) in univariate analyses [abnormal waist-to-hip ratio (WHR) and 10-year risk of a cardiovascular event according to the Framingham Risk Score (≥5%)]. Since abnormal WHR had a higher OR than waist circumference, the former was included in the logistic model.

[d]The OR and its corresponding CI were adjusted for current age and the traditional variable that was significantly associated with presence of atherosclerotic plaque in univariate analyses, ever smoking.

with those in healthy but younger Colombian volunteers (80). By contrast, hypercholesterolemia (56% versus 17%; OR = 6.15; 95% CI, 3.86-9.81) and smoking (44% versus 7%; OR = 10.72; 95% CI, 5.91-19.43) prevalences were significantly higher in the RA patients than in the healthy population (80).

An advantage of this study was that we analyzed patients at 3 different stages in the development of atherosclerosis: endothelial dysfunction, increased IMT (SSA), and the presence of atherosclerotic plaque. The evaluation of these stages allows a better description of the risk factors associated with atherosclerosis development in RA patients. However, this study represents a cross-sectional view of an ongoing process.

Our results confirm that endothelial dysfunction, which is considered an early stage of atherosclerosis, is associated with dyslipidemia, especially hypertriglyceridemia and elevated LDL cholesterol levels, both considered atherogenic risk factors (81-83).

Of the nontraditional risk factors, RF seropositivity was significantly associated with endothelial dysfunction after adjusting for hypertriglyceridemia, elevated LDL cholesterol levels, and current age. The odds of endothelial dysfunction in patients with a positive RF were 3 times higher than the odds in patients with a negative RF, suggesting a strong association that is independent of traditional CVD risk factors. A recent study suggested that a positive RF is an independent risk factor for ischemic heart disease even in the general population (84). However, there is no biological explanation supporting an ethiopathogenic effect of RF on endothelial dysfunction. The association of a positive RF with endothelial dysfunction, however, may reflect a high degree of inflammation rather than an RF causal effect on atherosclerosis development (85,86).

We found that an increased IMT was related to late RA onset (>51 years) and abnormal abdominal adiposity but only in the univariate analyses. After adjusting for current age and a 10-year risk of a cardiovascular event ≥5% through a logistic regression model, these associations disappeared. This suggests that other risk factors that have not been studied could be associated with an increased IMT in our population. Nevertheless, measuring waist circumference instead of BMI alone is advisable, because individuals with selective excess of intra-abdominal or visceral adipose tissue are at a substantially higher risk of becoming insulin resistant and of developing metabolic syndrome. Those measurements can also be useful for the clinician to determine the CVD risk associated in patients with RA (87,88).

A Framingham risk score >5%, which measures the total CVD risk over the course of 10 years, was associated with increased IMT in univariate analysis (OR = 4.4), suggesting that it may be a good predictor of atherosclerosis development in RA patients. This finding demonstrates that clinical score predictors of total CVD risk in the general population as well as in RA patients may be useful tools for following up patients and designing early interventions (89).

After adjusting for current age and ever smoking, we found that both late RA onset (>51 years) and disease duration of more than 10 years were associated with development of atherosclerotic plaque. The finding that long disease duration was associated with the presence of atherosclerotic plaque confirms that RA is a risk factor for blood vessel deterioration that may act independently of traditional cardiovascular risk factors (90). In a previous study conducted in Spain, investigators also observed significantly longer disease duration in RA patients with plaque when compared with those RA patients without plaque (18). In this elegant study, in which RA patients lacked clinically evident CVD or classic cardiovascular risk factors, age and disease duration at the time of the ultrasonographic assessment were the best predictive factors of the development of severe morphologic expression of atherosclerotic disease (presence of carotid plaques) (18).

Ever smoking was associated with the presence of atherosclerotic plaque in univariate analyses; this association was apparently strong but disappeared after adjusting for nontraditional risk factors. A family history of autoimmune diseases was not associated with the presence of atherosclerotic plaque after adjusting for current age and ever smoking.

After adjusting for current age and ever smoking, we found that the *HLA-DRB1* SE was associated with a 5-fold increased risk of having atherosclerotic plaque. *HLA-DRB1* SE alleles influence the risk of developing RA in Colombians (91) as well as atherosclerosis in these patients. In keeping with these observations, a recent population-based study disclosed that in Northwestern Spanish patients with *RA HLA-DRB1*04* SE alleles and in particular *HLA-DRB1*0404* increased the risk of cardiovascular death (92). In our study, after adjusting for having a single SE allele, there was no significant association between ever smoking and the presence of atherosclerotic plaque. In other populations, smoking appears to be an important risk factor that increases IMT and accelerates atherosclerosis in RA patients (93-95).

In the present study, inflammatory markers such as CRP and ESR were not associated with any stage of atherosclerosis development in RA. However, CRP is a good indicator for inflammation as well as a predictive risk factor of CHD in healthy people (96,97). In patients with autoimmune diseases, the interpretation of high levels of inflammatory markers is confounded by the correlation with systemic inflammation (98,99). Prospective epidemiological studies have found associations between an increased risk of vascular deterioration and increased basal levels of cytokines such as IL-6, cell adhesion molecules such as soluble intercellular adhesion molecule 1 (ICAM-1) and P-selectin (100,101), and of downstream acute-phase reactants such as CRP, fibrinogen, and serum amyloid A protein (102). Other studies suggest that the strength and chronicity of the inflammatory response measured by CRP levels correlates directly with the presence of atherosclerosis in patients with RA (103,104). Moreover, a significantly increased risk of CV death was also associated with chronic inflammation determined by CRP and ESR (92).

No association was found between the *TNF* SNP −308 and the 3 stages of atherosclerosis. Previous studies found that the *TNF−308GG* genotype was protective against autoimmunity and that the *TNF−308A* allele was associated with an increased risk of RA (51). Neither the most prevalent *TNF* genotype (GG) nor the less prevalent (GA) correlated with atherosclerosis in this study. This suggests that *TNF* polymorphism is not a cardiovascular risk factor in our population of RA patients.

CVD are complex genetic diseases that cluster in families (105-109). There is evidence indicating the possibility of susceptibility genetic variants shared by RA and CVD (110,111) as well as specific susceptibility loci for coronary artery disease that are independent of susceptibility loci for RA (112). Specific loci have been significantly associated with an abnormal BMI, hypertension, and elevated LDL cholesterol levels (112). Studies of patients with a strong family history of coronary artery disease confirmed that cardiovascular diseases cluster in families just as autoimmune diseases do (113,114). In contrast to other reports (115), the current study did not find a significant association between a history of premature cardiovascular heart disease in patients' first-degree relatives and any stage of atherosclerosis development. Further identification of genetic loci associated with RA and its complications (ie, CVD) is warranted and should be translated into prediction of disease, understanding of disease physiopathological mechanisms, and early interventions to prevent premature coronary disease in RA patients.

Although the lack of prospective follow-up and inclusion of patients with longstanding disease could be considered as shortcomings of this study, our results indicate the presence of RA-related risk factors for CVD in our population, which act independently of traditional risk factors. We confirmed the influence of HLA-DRB1-SE on the risk of developing atherosclerotic plaque in RA. Prevention and treatment of CVD are essential not only to reduce mortality risk in RA but also to reduce the economic burden of the disease. In fact, CVD is the most important determinant of high costs associated with hospitalizations of patients with RA (116). These data point to the need for new public health policies to improve patient outcomes.

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