Endothelin-1 serum levels in women with Rheumatoid Arthritis

Alvarez-Cienfuegos A[1], Cantero-Nieto L[2], García-Gómez JA[3], Ríos-Fernández R[4], Martin J[5], González-Gay MA[6], Ortego-Centeno N[4]

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ABSTRACT

Objective: The purpose of this study was to evaluate serum Endothelin-1(ET-1) levels in female Rheumatoid Arthritis (RA) patients compared with healthy controls, examine possible associations between ET-1 with different characteristic of the disease and investigate possible associations between ET-1 with surrogate markers of cardiovascular disease (CVD).

Methods: This cross-sectional study was performed in Vega-Baja Hospital, Orihuela (Spain) from November 2016 to May 2018. Sixty-three women with RA and sixty-five age and sex healthy controls were included in this study. Serum ET-1 was analyzed using ELISA.

Results: Serum levels of ET-1 in RA female patients were higher than those in healthy controls (p 0.001). Serum levels of ET-1 were positively associated with N-terminal pro-brain natriuretic peptide (NT-proBNP) ($r = 0.27$, $p < 0.05$) and with C-reactive protein (CRP) ($r = 0.36$, $p < 0.05$). ET-1 levels in women with RA were higher in smokers. Prednisone use was associated with lower ET-1 levels. No association with carotid intima media thickness was found.

Conclusions: we observed the presence of higher levels of serum ET-1 in RA women patients compared with healthy controls. These increased levels of ET-1 are associated with inflammation and smoking and reduced by prednisone intake.

Keywords: Endothelin-1; Rheumatoid Arthritis; CRP; NT-proBNP; Prednisone.

1. Department of Rheumatology, Hospital Vega-Baja, Alicante, Spain
2. Department of Emergency, Hospital Santa Ana, Granada, Spain;
3. Infectious Diseases Unit, Hospital General de Elche, Alicante, Spain
4. Systemic Autoimmune Diseases Unit, Hospital Universitario San Cecilio, Granada, Spain
5. Institute of Parasitology and Biomedicine López-Neyra, IPBLN-CSIC, Granada, Spain
6. Epidemiology, Genetics and Atherosclerosis Research Group on Systemic Inflammatory Diseases, Rheumatology Division, IDIVAL, Santander, Spain

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic multisystem disease with an estimated occurrence of 1 to 2 percent of the world population[1]. RA patients have an increased risk of cardiovascular (CV) events with high morbidity and mortality as a result of rapid atherosclerosis[2].

Interestingly, RA and atherosclerosis are both chronic inflammatory diseases sharing inflammatory biomarkers as well as a similar cellular activation pattern. The development of CV disease in RA patients has been associated with inflammation and autoimmunity. Considering the previously mentioned incidence of CV events in patients with RA, the identification of high risk RA individuals that may benefit from treatment, should be an important step in order to prevent overt CV disease. In this regard, in asymptomatic RA patients, several non-invasive surrogate markers have demonstrated the presence of subclinical atherosclerosis[3,4]. However, information on serological biomarkers of CVD in patients with RA is limited[5].

Endothelial dysfunction itself is a process that involves genetic characteristics, cardiovascular risk factors, and inflammation[6]. Endothelin-1 (ET-1)[7] which is mainly secreted by endothelial cells is a potent endogenous vasoconstrictor. It acts through two different types of receptors: ETA and ETB. ET-1 contributes to the development of inflammatory processes in the vascular wall, increasing superoxide anion production and cytokine secretion. It has been found to be associated with the activation of transcription factors such as nuclear factor (NF)- B and also the expression of proinflammatory cytokines[8]. In turn, these transcription factors and proinflammatory cytokines stimulate ET-1 production[9]. Bellisai *et al.*[10] report that ET-1 increases the synthesis of TNF-α in macrophages and monocytes, which enhances the inflammatory response by stimulating the chemotaxis and phagocytosis of macrophages, monocytes and neutrophils. In different

types of cells, increased production of reactive oxygen species (ROS) occurs via the NF- B, cyclooxygenase (COX) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent pathways[11-13].

The objectives of this study were to analyze ET-1 in a cohort of women with RA, examine possible associations between ET-1 with different characteristic of the disease and investigate possible associations between ET-1 with surrogate markers of CVD.

MATERIALS AND METHODS

PATIENT SELECTION

The study was performed in Vega-Baja Hospital, Orihuela (Spain) from November 2016 to May 2018. We prospectively enrolled 63 consecutive women patients affected by RA and 65 healthy women who served as controls. All patients included in this study had normal serum creatinine (Cr) levels, and met the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for RA[14]. Individuals with prevalent cardiovascular disease were excluded.

At the clinic visit, participants completed questionnaires about their lifestyle characteristics, medical history, and current medication used. Informed consent was obtained for all subjects, and the study was approved by the Research Ethics Committee of Hospital Universitario de Elche in Alicante, Spain, date: 22/11/2016, protocol number: pi35/2016 and conducted in accordance with the guidelines in the Declaration of Helsinki.

CARDIOVASCULAR ASSESMENT

Disease severity was scored through disease activity score of 28 joints and joint damage was evaluated based on the Steinbrocker radiographic criteria (I-IV). Current smokers were defined as those who reported having smoked ≥1 cigarette per day regularly during the year preceding the examination. Waist circumference, weight, and height were measured; and body mass index (BMI) was calculated as weight (kg)/height (m^2). Blood pressure (BP) was measured twice in the left arm of the seated subject with a mercury column sphygmomanometer. The average of the 2 readings was used as the examination BP, and hypertension was defined as self-reported antihypertensive medication use, or a systolic BP ≥140 mm Hg, or a diastolic BP ≥90 mm Hg. Type 2 diabetes mellitus (T2DM) was defined by self-reported use of insulin, or oral hypoglycemic medications, or a fasting glucose level ≥126 mg/dl. Kidney function was assessed using the estimated glomerular filtration rate (eGFR) calculated by the CKD-Epi study equation[15].

The CV risk was assessed using the Modified Systemic Coronary Risk Evaluation (mSCORE). The mSCORE was calculated using validated risk tables for both low and high risk populations[16]. For this study, the low risk table was used since Spain has been classified as a low risk country for CVD. Carotid intima-media thickness (c-IMT) was measured by performing carotid ultrasound examination in the common carotid artery and the detection of focal plaques in the extracranial carotid tree by manual technique using a commercially available scanner equipped with 7–12 MHz linear transducer as the patient was lying in the supine position with the neck rotated to the opposite side of examination as previously reported[17]. Carotid plaques were counted in each territory and defined as no plaque, unilateral plaque or bilateral plaques[17]. Values of cIMT greater than 0.9 mm were considered abnormal (cIMT thickening) and plaques were defined if the cIMT was greater than 1.5 mm[18]. In our study, ankle-arm index was evaluated using a BIDOP model ES-100V3 vascular screening system (Hadeco, Inc, Kawasaki, Japan).

LABORATORY MEASUREMENTS

In all the cases, a fasting blood sample was taken in the morning, and was stored at -70°C until the assays were performed.

The sera were tested for creatinine, CRP, NT-ProBNP and ET-1. Creatinine was determined by Jaffe method (Siemens Healthcare Diagnostic Inc. NY, USA). CRP was measured by turbidimetric immunoassay (Siemens Healthcare Diagnostic Inc. NY, USA). NT-proBNP was quantified in heparinised plasma using a solid-phase two-site chemiluminescent immunometric assay (Biomérieux, France). Serum ET-1 (Elabscience, USA) was measured by ELISA according to the manufacturer's recommendations. Anti-citrullinated protein antibodies (ACPAs) were detected using a second-generation ELISA (ACPAs) kit (ORGENTEC Diagnostika GmbH. Mainz, Germany) while IgM RF was determined as part of routine analysis by turbidimetric assay (Siemens Healthcare Diagnostic Inc. NY, USA) according to the manufacturers' instructions. Fasting plasma glucose was measured in fresh specimens with a hexokinase reagent kit (Siemens Health-

care Diagnostic Inc. NY, USA). Total cholesterol and triglyceride levels were determined by fully enzymatic techniques. High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein B (apoB)–containing lipoproteins with magnesium sulfate and dextran sulfate. Low-density lipoprotein (LDL) was calculated using the Friedewald formula. All other routine serum biochemical parameters were measured at the Department of Clinical Chemistry, Vega-Baja Hospital.

STATISTICAL ANALYSIS

Data were analyzed by statistical software SPSS 18 (Chicago, IL, USA) and with the program R version Rx64.3.5.0 (Vienna, Austria), using independent samples *t*-test, Mann-Whitney U test, and Chi-square test when appropriate. Spearman's coefficient and Pearson's correlation were calculated as suitable to determine the correlation between the bio-chemical parameters. *P*-values of less than 0.05 were considered statistically significant. The quantitative data were shown as mean ± standard deviation (SD) and median (Q1–Q3) as suitable. To test if we can admit that the distribution is normal we use the Shapiro-Wilk test. Linear regression was used to examine the cross-sectional associations of plasma ET-1 concentrations with CRP and NT-proBNP.

RESULTS

CHARACTERISTICS OF THE STUDY SUBJECTS

The main features of the 63 women with RA and 65 controls included in this study are shown in Table I. The mean age (SD) of the patients was 53 ± 8 years. The majority were Caucasian (90.5%). The mean disease duration was 8.5 ± 5.8 years. The mean disease activity score in 28 joints (DAS28) according to the erythrocyte sedimentation rate (ESR) indicated low disease activity 3.0 ± 1.3. The mean health assessment questionnaire (HAQ) was 0.75 ± 0.67. The mean Steinbrocker s stage was 2.75 ± 1.17 and the mean Steinbrocker s class 1.87 ± 0.69. At the time of the study 32 (50.7%) patients were receiving biologic agents (9 etanercept, 9 certolizumab pegol, 7 tocilizumab, 6 adalimumab and 1 rituximab). Most patients (73%) had received or were undergoing methotrexate therapy (mean weekly dose 11.5 ± 4.8 mg in patients on methotrexate) and 16 patients took prednisone with a median daily dose of 6.5 ± 3.5 mg.

In addition, a total of 65 healthy women were included in our study as controls; mean age (SD) 52 ± 9 years. Most of them were also Caucasian (98.3%).

LABORATORY RESULTS

Laboratory tests of the patients and healthy controls included in the present study are shown in Tables I and II.

Forty-six (73.0%) and 45 (71.4%) of the 63 women with RA were positive for rheumatoid factor and ACPAs, respectively. As expected, laboratory markers of inflammation found at the time of the study were higher in women with RA than in controls (Table I). In this regard, the mean CRP in RA patients was 0.6 ± 0.8 mg/dl versus 0.2 ± 0.1 mg/dl in controls (p <0.001). Likewise, the mean ESR in the group of RA patients was 23.9 ± 15.8 mm/1st hour versus 11.3 ± 10.2 mm/1st hour in controls (p <0.001) (Table I). Patients with RA had lower uric acid levels than controls (3.9 ± 1.3 versus 4.6 ± 1.3 mg/dl; p= 0.002). However, NT-proBNP levels were higher in patients with RA (79.8 ± 54.8 versus 59.7± 38.4 pg/ml in controls; p= 0.01).

Interestingly, the serum ET-1 concentrations were significantly higher in the RA patients than those in the control group: [28.9 (0-50) vs. 21.7 (0-50), pg/ml; p 0.001] (Figure 1).

CARDIOVASCULAR DISEASE RISK FACTORS

As shown in Table I, patients had a mean BMI of 26.6±5.6 kg/m^2, waist circumference of 103.8± 13.2 cm, ankle-arm index of 1.1±0.1, cIMT of 0.7±0.1 mm, mSCORE 2.0 ± 2.3. Fourteen (22%) of them had a smoking history.

Healthy controls had a mean BMI of 25.9 ± 4.3 kg/m^2, waist circumference of 83.1 ± 13.4 cm, ankle-arm index of 1.2 ± 0.2, cIMT of 0.6 ± 0.2 mm, mSCORE 1.8 ± 2.5. Fourteen (21.5%) of them had a smoking history.

RELATIONSHIP BETWEEN ET-1 LEVELS AND CARDIOVASCULAR RISK FACTORS OR DISEASE FEATURES IN PATIENTS WITH RHEUMATOID ARTHRITIS

Table III shows the correlation coefficients between ET-1 and other markers in patients with RA. Levels of ET-1 were significantly correlated with smoking (p= 0.020). ET-1 levels also showed a statistically significant positive correlation with CRP (r = 0.36, p= 0.004) and with NT-proBNP (r = 0.27, p= 0.036). In contrast, an inverse correlation between prednisone intake and ET-1 levels (p= 0.034). However, there was no correlation of ET-1 levels with age, BMI, ankle-arm index, cIMT,

TABLE I. CHARACTERISTICS OF WOMEN WITH RHEUMATOID ARTHRITIS AND HEALTHY CONTROLS

	RA	HC	
	mean ± SD	mean ± SD	p-value
Age, years	53.1 ± 8.3	52.7 ± 9.7	0.80
Height, cm	160.8 ± 6.2	169.9 ± 7.1	<0.001
Body weight, kg	68.7 ± 14.5	67.2 ± 12	0.52
Body mass index, kg/m^2	26.5 ± 5.6	25.9 ± 4.3	0.49
Waist circumference	103.8 ± 13.1	83.1 ± 13.4	<0.001
Ankle-arm index	1.1 ± 0.1	1.2 ± 0.2	<0.001
cIMT mm	0.7 ± 0.1	0.6 ± 0.2	<0.001
mSCORE	2 ± 2.3	1.8 ± 2.5	0.63
Duration of RA, years	8.5 ± 5.8	–	–
DAS28-ESR	3 ± 1.3	–	–
HAQ	0.75 ± 0.67	–	–
Steinbrocker's stage	2.75 ± 1.17	–	–
Steinbrocker's class	1.87 ± 0.69	–	–
Smoking, n (%)	14 (22.2)	14 (21.5)	0.47
Hypertension, n (%)	10 (15.8)	11 (16.9)	0.87
Diabetes mellitus, n (%)	3 (4.7)	2 (3)	0.62
Dyslipidemia, n (%)	13 (20.6)	14 (21.5)	0.57
Prednisone, mg/day	6.5 ± 3.5	–	–
Methotrexate, mg/week	11.5 ± 4.8	–	–
Biologic agent use, n (%)	32 (50.7)	–	–
RF positive, n (%)	46 (73)	–	–
ACPAs positive, n (%)	45 (71.4)	–	–
CRP, mg/dl	0.6 ± 0.8	0.2 ± 0.1	<0.001
ESR, mm/h	23.9 ± 15.8	11.3 ± 10.2	<0.001
Serum creatinine, mg/dl	0.58 ± 0.11	0.7 ± 0.2	<0.001
eGFR, ml/min	108.7 ± 28.7	99 ± 13.2	0.01

SD: standard deviation, RA: rheumatoid arthritis, HC: healthy control, DAS: disease activity score, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, eGFR: estimated glomerular filtration rate, RF: Rheumatoid Factor, ACPAs: Anti-citrullinated protein antibodies, HAQ: Health Assessment Questionnaire, cIMT: Carotid intima-media thickness, mSCORE: Modified Systemic Coronary Risk Evaluation.

TABLE II. SERUM ET-1 AND STUDY PARAMETERS OF RA PATIENTS AND HEALTHY CONTROLS

	RA	HC	
	mean ± SD	mean ± SD	p-value
ET-1, pg/ml	28.9 ± 12.6	21.7 ± 11.7	<0.001
Cholesterol, mg/dl	212.7 ± 41	211.8 ± 37.3	0.89
LDL-C, mg/dl	120.1 ± 29.2	129.7 ± 30.7	0.06
HDL-C, mg/dl	69.9 ± 19.4	63.3 ± 13.1	0.02
Triglycerides, mg/dl	112.9 ± 55.6	107.7 ± 53.9	0.59
Uric acid, mg/dl	3.9 ± 1.3	4.6 ± 1.3	0.002
NT-proBNP, pg/ml	79.8 ± 54.8	59.7 ± 38.4	0.01
Fe, mg/dl	77.7 ± 28.9	85.6 ± 32.3	0.14

SD: standard deviation, RA: rheumatoid arthritis, HC: healthy control, ET-1: endothelin 1, LDL: low density lipoprotein, HDL: high density lipoprotein, Fe: iron, NT-proBNP: prohormone brain natriuretic peptide.

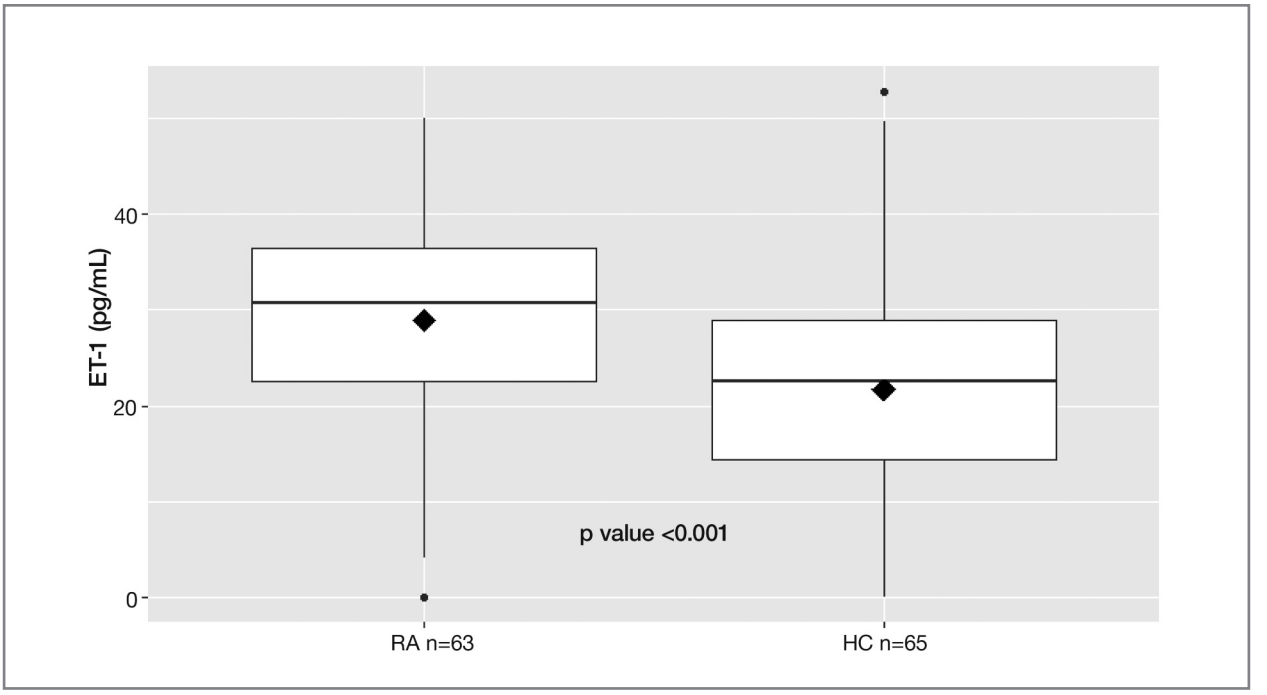

FIGURE 1. Median serum concentration of Endothelin-1 (ET-1) in Rheumatoid Arthritis patients and healthy controls. 2 tailed Mann Whitney U test for unpaired sample. HC: healthy controls; RA: Rheumatoid arthritis.

disease duration, disease activity or ACPA or RF status (Table III).

In the linear regression model, higher log10 ET-1 concentrations were associated with higher CRP [= 0.024 95% CI (0.041, 0.008); p= 0.004] and NT-proBNP [= 1.173 95% CI (2.246, 0.098); p=0.032].

DISCUSSION

RA is an inflammatory, systemic, autoimmune, chronic disease of unknown cause, characterized by physical disability, progressive destruction of the joints and increased mortality, mainly due to CVD[19,20].

ET-1 might play an important role in inflammatory processes and vasculopathy in connective tissue diseases (CTD) as a potent physiological vasoconstrictor, released after activation and/or damage of endothelial cells[21]. In various autoimmune diseases such as systemic lupus erythematosus (SLE)[22], systemic sclerosis (SSc)[23] or RA[24], increased plasma ET-1 levels have been found. Moreover, elevated ET-1 serum levels have been implicated in the pathophysiology of both vascular and fibrotic manifestations in SSc[23]. Increasing evidence suggests a potential central role of endothelial dysfunction in RA pathogenesis[25-27], specifically in patients with high inflammatory activity[28]. Furthermore, some other studies suggest that chronic inflammation in the course of RA leads to endothelial function impairment, regardless of the disease activity[29]. Either by the proliferation of new blood vessels or by over expression of inflammatory mediators, the endothelial cells play a key role in the systemic disease process and further internal organ damage[26,30].

In our study, we found significantly elevated plasma ET-1 levels in women with RA compared with healthy controls. Clinical studies also reported elevated plasma levels of ET-1 in patients with RA[31-33].

ET-1 can stimulate the production of pro-inflammatory cytokines such as interleukin-6, and CRP is induced by IL-6 during inflammation. In keeping with that, we observed a statistically significant positive correlation between ET-1 levels and CRP levels.

Different studies have evaluated the relationship between ET-1 and CRP. Plasma ET-1 levels are found elevated and correlated with CRP in patients with inflammatory pathologies such as acute ischemic stroke[34], exacerbations of chronic obstructive pulmonary disease[35], and acute myocardial infarction treated with direct coronary angioplasty[36].

TABLE III. CORRELATIONS BETWEEN ET-1 AND STUDY PARAMETERS IN RHEUMATOID ARTHRITIS PATIENTS.

	Correlation coefficient	p
Age	0.05	0.704
Height	-0.13	0.324
Body weight	-0.07	0.604
Body mass index	-0.05	0.688
Waist circumference	-0.05	0.697
Smoking	0.29	0.020
Hypertension	-0.13	0.306
Ankle-arm index	-0.22	0.089
cIMT	-0.12	0.359
Carotid plaques	-0.10	0.438
mSCORE	0.02	0.862
Disease duration of rheumatoid arthritis	-0.12	0.339
DAS28-ESR	-0.14	0.282
HAQ	-0.06	0.629
Methotrexate dose	0.18	0.343
Prednisone dose	-0.47	0.034
Biological agent use	0.04	0.972
RF	0.00	0.987
ACPAs	0.10	0.435
C-reactive protein	0.36	0.004
Erythrocyte sedimentation rate	0.19	0.149
Fibrinogen	0.16	0.213
Serum creatinine	0.00	0.998
Serum urea	0.00	0.976
eGFR	0.07	0.064
Cholesterol, mg/dl	0.09	0.474
LDL-C, mg/dl	0.16	0.216
HDL-C, mg/dl	-0.04	0.751
Triglycerides, mg/dl	0.00	0.976
Uric acid, mg/dl	-0.13	0.302
NT-proBNP, pg/ml	0.27	0.036
Fe, mg/dl	0.21	0.102

cIMT: Carotid intimal medial thickness, m-SCORE: modified systematic coronary risk evaluation, DAS: disease activity score, HAQ: Health Assessment Questionnaire , RF: rheumatoid factor, ACPAs: Anti-citrullinated protein antibodies, eGFR: estimated glomerular filtration rate, LDL: low density lipoprotein, HDL: high density lipoprotein; Fe: iron

Calderon *et al.*[37] observed that dexamethasone and triamcinolone acetonide down-regulate the production and synthesis of ET-1 by a transformed human pulmonary epithelial cell line under stimulated or basal conditions. In line with these findings, we disclosed a negative correlation between ET-1 levels and the use of prednisone. With respect to this, patients from our series who were on treatment with prednisone had lower levels of ET-1.

A close relationship exists between the natriuretic peptides of the cardiovascular system and ET-1. Different studies have shown ET-1 to be a potent stimulator of the synthesis and release of these peptides in cardiac tissues[38,39]. It has also been suggested that part of the vasodilatory action of these natriuretic peptides might be due to a reduction in the basal production of ET-1[40,41]. The hypothesis has been advanced that contributing to the regulation of vascular tone, a feedback mechanism exists between them[42,43]. Interestingly, we disclosed a significant positive correlation between ET-1 levels and NT-proBNP levels in our patients with RA, which was independent of age.

liwi ska-Mosso *et al.*[44] found that tobacco smoking has a direct effect on the endothelium, leading to an increased level of ET-1. Bossard *et al.*[45] observed a significant correlation of ET-1 with smoking. In agreement with these observations, we also found a significant association between ET-1 levels and smoking status in patients with RA.

In our study we could not confirm a correlation between disease activity, disease duration or RF/ACPA seropositivity with ET-1 levels. It was also the case for cIMT or traditional CV risk factors.

There are several limitations in our study that should be considered. First, this study was a cross-sectional analysis that reflected the status of a population in a particular period. The cross-sectional design of this study does not allow drawing causal inferences. This study focused only on RA women; therefore, the findings of this study cannot be generalized to men with RA. However, it has a number of strengths derived from the monocentric design of the study with the inclusion of consecutive RA patients homogeneously evaluated and the careful analysis of data performed by a dedicated physician.

CONCLUSIONS

We observed the presence of higher levels of serum ET-1 in RA women compared with healthy controls. These increased levels of ET-1 are associated with inflammation and smoking and reduced by prednisone intake.

CORRESPONDENCE TO
Antonio Alvarez-Cienfuegos
Department of Rheumatology, Hospital Vega-Baja
Crta. Orihuela-Almoradi, S/N
03314 Orihuela (Alicante), Spain
E-mail: antonioalvarezdc@gmail.com

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