

Lack of association between carotid intima-media wall thickness and carotid plaques and markers of endothelial cell activation in rheumatoid arthritis patients undergoing anti-TNF therapy

Miguel A. González-Gay¹, Carlos González-Juanatey²,
José A. Miranda-Fillooy³, María T. García-Unzueta⁴, Javier Llorca⁵

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ABSTRACT

Introduction: To determine the relationship between biomarkers of endothelial cell activation, and carotid artery intima-media wall thickness (IMT) and plaques, two surrogate markers of atherosclerosis, in a series of rheumatoid arthritis (RA) patients undergoing anti-TNF therapy.

Methods: 29 consecutive Spanish patients who fulfilled the 1987 American College of Rheumatology classification criteria for RA, had no history of cardiovascular (CV) disease, and had at least one year of follow-up after disease diagnosis were selected. All patients were undergoing anti-TNF-infliximab therapy because of severe disease refractory to conventional disease modifying antirheumatic drugs. Carotid ultrasonography was performed to determine IMT and carotid plaques. Levels of sICAM-3, sICAM-1, sVCAM-1, sP-selectin and sE-selectin were assessed by ELISA immediately before an infusion of infliximab.

Results: The median disease duration was 14 years. Despite infliximab, no patient experienced a disease remission (DAS28: median 4.17). Only a marginally significant correlation between sVCAM-1 and carotid IMT was observed when both total correlation using Spearman correlation coefficient ($p=0.08$) or partial correlation adjusting for sex, age at the time of study, disease duration, rheumatoid factor, and classic CV risk fac-

tors was performed ($p=0.09$). Also, no association between presence of carotid plaques and levels of biomarkers of endothelial cell activation was observed.

Conclusion: In long-standing RA patients without CV disease undergoing anti-TNF therapy no association between levels of soluble markers of endothelial cell activation and carotid ultrasonography abnormalities was observed. Further studies are needed to establish the best tools to be used in the assessment of CV risk of RA.

Keywords: Rheumatoid arthritis; Carotid intima-media thickness; Carotid plaques; Markers of endothelial cell activation; Adhesion molecules.

INTRODUCTION

Cardiovascular (CV) events constitute the leading cause of death in patients with rheumatoid arthritis (RA)¹. They are the result of progressive vascular impairment due to accelerated atherogenesis². Elevated circulating levels of soluble adhesion molecules are associated with CV risk factors and predict atherosclerosis and CV events in the general population³. Measurement of these markers of endothelial cell activation has also confirmed the presence of endothelial dysfunction in patients with RA⁴.

Carotid intima-media wall thickness (IMT) of the common carotid artery, determined by high resolution B-mode ultrasound of the common carotid artery, is also a useful non-invasive surrogate marker of macrovascular atherosclerosis disease. Increased common carotid artery IMT determined by high resolution B-mode ultrasound was found to be a good indicator of generalized atherosclerosis and coronary artery di-

1. Department of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV, Santander, Spain

2. Cardiology Division, Hospital Xeral-Calde, Lugo, Spain

3. Rheumatology Division, Hospital Xeral-Calde, Lugo, Spain

4. Endocrinology Research Unit, Hospital Universitario Marqués de Valdecilla, IFIMAV, Santander, Spain

5. Department of Epidemiology and Computational Biology, School of Medicine, University of Cantabria, IFIMAV, and CIBER Epidemiología y Salud Pública (CIBERESP), Santander, Spain

sease in non-RA individuals⁵. In this regard, both carotid artery IMT >0.90 mm and the presence of carotid plaques are considered to be expression of sub-clinical organ damage and factors influencing CV prognosis in the general population⁶. Therefore, carotid ultrasonography studies may provide information of atherosclerosis in subclinical stages of the disease in individuals at risk⁶. The IMT corresponds to the width of the vessel intima and media, which consists of endothelium, connective tissue and smooth muscle⁷. Interestingly, a recent meta-analysis confirmed the presence of abnormally increased carotid IMT in RA patients compared with controls⁸. Also, carotid IMT was found to predict the development of CV events in RA patients⁹. Because of that, the presence of abnormally high carotid IMT values should raise clinical suspicions as an alarm sign of the development of CV complications in these patients. In addition, a recent study has shown that the presence of carotid plaques in both internal carotid arteries following carotid ultrasonography nearly quadrupled the incidence of new acute coronary syndromes in patients with RA compared with those in RA patients without carotid plaques¹⁰.

An issue of potential interest in the stratification of the CV risk in patients with RA may be to define the relationship between functional and structural-anatomical- surrogate markers of vascular damage and atherosclerosis. With respect to this, in a recent study we observed that carotid IMT values were unrelated to those obtained following the functional evaluation of the endothelial function by brachial flow-mediated endothelium-dependent vasodilatation (FMD) in a series of 118 patients with RA. Correlation between FMD and carotid IMT was only observed in RA patients with long-standing disease¹¹.

Measurement of markers of endothelial cell activation such as circulating vascular cell adhesion molecule [VCAM]-1, intercellular adhesion molecule [ICAM]-1, and endothelial leukocyte adhesion molecule, also called E-selectin, constitutes an alternative method to assess endothelial function. These biomarkers play a more important role than traditional risk factors in the CV disease of RA^{12,13}. Also, levels of soluble (s) P-selectin have been found to be elevated during acute coronary syndromes, and independently predict risk of future cardiac events in women^{14,15}.

Taking into account these considerations, in the present study we aimed to establish associations between biomarkers of endothelial cell activation and ultra-

sonographically determined common carotid artery IMT and carotid plaques in patients with RA.

METHODS

PATIENTS

We assessed 29 Spanish patients with severe RA refractory to conventional disease modifying antirheumatic drugs (DMARDs). Information on the characteristics of this Caucasian population was previously reported¹⁶. They formed part of an ongoing study on CV disease in RA¹⁷. Each of the 29 RA patients had been switched from traditional DMARDs to anti-TNF- α infliximab treatment because of severe and active disease (Disease Activity Score-28 [DAS28] >5.1)^{16,18}. In all patients, treatment with a DMARD had been initiated when a diagnosis of RA was made. Prior to anti-TNF- α therapy, patients were required to have been treated with at least two DMARDs including chloroquine or hydroxychloroquine, sulphasalazine, gold, methotrexate (at least 15 mg/week), leflunomide, and cyclosporine A (3 mg/kg/day). Infliximab therapy (initial dose of 3 mg/kg) was administered intravenously at 0, 2, and 6 weeks and subsequently every 8 weeks. However, in some patients, because of disease severity, the dose was increased to 5 mg/kg and, if deemed necessary, the interval between infliximab infusions was shortened to 6 weeks. All patients had received treatment with both non-steroidal antiinflammatory agents and low doses of prednisone (generally 5 mg bid) immediately after disease diagnosis. At the time of the study, each patient was on infliximab 3 or 5 mg/kg given at 6 or 8 weekly intervals and methotrexate 15-25 mg weekly with or without chloroquine 250 mg day or hydroxychloroquine 200 mg/day, prednisone 2.5-7.5 mg daily and a non-steroidal antiinflammatory agent (naproxen 500-1000 mg or diclofenac 50-100 mg daily). The blood pressure was below 140/90 mmHg in each patient at the time of the study. However, some patients were required to be on treatment with antihypertensive agents or statins. Patients with diabetes were excluded. Evaluation of carotid IMT and carotid plaques (defined as a distinct protrusion, >1.5 mm, into the vessel lumen) was performed as previously described^{17,19}.

DETERMINATION OF SERUM BIOMARKERS

Determination of soluble intercellular adhesion molecule-3 (sICAM-3), sICAM-1, sVCAM-1, sP-selectin and sE-selectin, was performed by ELISA im-

mediately before infliximab administration as previously reported¹⁶.

STATISTICAL ANALYSIS

Data were shown as mean ± standard deviation (SD) or median and interquartile range (IQR). Correlation between carotid IMT and markers of endothelial cell activation was estimated via Spearman correlation coefficient; partial correlation was obtained in order to control for sex, age at the time of study, disease duration, rheumatoid factor status, and classic CV risk factors (hypertension, dyslipidemia, smoking and obesity since diabetic patients were excluded from the study). The association between adhesion molecules and carotid plaques was tested using the Mann-Whitney U test. Ethical approval and informed consent was obtained.

RESULTS

CLINICAL CHARACTERISTIC OF THE PATIENTS

The recorded variables in the 29 RA patients on periodical treatment with infliximab are shown in **Table I**. Eight patients were on angiotensin-converting-enzyme inhibitors and 4 on statins. Most of them were seropositive women with long disease duration (median 14 years). The median duration of infliximab therapy at the time of the evaluation was 2 years (IQR: 1.5- 2.5 years). However, despite the use of infliximab, prednisone and methotrexate, no patient experienced a disease remission (DAS28 < 2.4)²⁰ as mean (IQ range) was 4.17 (3.27 – 5.06). Long disease duration and disease severity have been shown to be associated with presence of increased carotid IMT and carotid plaques in patients with RA^{11,19}. In keeping with these observations, 12 of the 29 patients had carotid plaques and the median carotid IMT (0.85 mm) was close to the levels associated with increased risk of CV events⁹.

RELATIONSHIPS OF CAROTID IMT AND CAROTID PLAQUES WITH SERUM BIOMARKERS OF ENDOTHELIAL DYSFUNCTION

Table II shows the relationship of carotid IMT and carotid plaques with serum biomarkers of endothelial cell activation in this series of patients with RA. However, no correlation between carotid IMT and serum levels of soluble biomarkers was found. With respect to this, only a marginally significant correlation ($p > 0.05$ and < 0.10) between sVCAM-1 and carotid IMT was

TABLE I. DEMOGRAPHIC, CLINICAL AND ULTRASONOGRAPHY FEATURES OF 29 RA PATIENTS ON PERIODICAL TREATMENT WITH ANTI-TNF- α (INFLIXIMAB) BECAUSE OF DISEASE REFRACTORY TO AT LEAST TWO DMARDS

Central Nervous System	Peripheral Nervous System
Sex: Women	21 (72.4%)
Age at the time of the study (years) (mean ± SD)	55.46 ± 12.5
median (IQ range)	61 (49-68)
Disease duration (years) (mean ± SD)	12.7 ± 7.6
median (IQ range)	14 (5-16)
Extra-articular manifestations	14 (48.3%)
Cortical bone erosions (on plain radiographs of hands and or feet)	27 (93.1%)
Rheumatoid Factor positive	27 (93.1%)
DAS28 (mean ± SD)*	4.26 ± 1.20
median (IQ range)	4.17 (3.27-5.06)
CRP (mg/l)* median (IQ range)	5.5 (3.5-11.7)
ESR (mm/1st hour)* median (IQ range)	25 (16-34)
Carotid IMT (mean ± SD)	0.82 ± 0.19
median (IQ range)	0.85 (0.68-0.99)
Carotid plaques	12 (41.4%)

*At the time of the ultrasonography assessment

observed when both total correlation using Spearman correlation coefficient ($p = 0.08$) or partial correlation adjusting for sex, age at the time of study, disease duration, rheumatoid factor, and classic CV risk factors was performed ($p = 0.009$). Likewise, association was not observed when disease activity measured by DAS28 or CRP at the time of the study was included in the analysis (data not shown).

When serum levels of markers of endothelial cell activation were compared according to presence of absence of carotid plaques only a marginal decrease of sP-selectin was found in the subgroup of patients with carotid plaques.

DISCUSSION

In the present study long-standing RA patients without CV disease undergoing anti-TNF therapy because of severe disease did not exhibit significant association

TABLE II. RELATIONSHIPS OF CAROTID INTIMA-MEDIA WALL THICKNESS (IMT) AND CAROTID PLAQUES WITH SERUM BIOMARKERS OF ENDOTHELIAL CELL ACTIVATION IN A SERIES OF 29 PATIENTS WITH RHEUMATOID ARTHRITIS

Variable	Carotid IMT Total correlation (p value)*	Carotid IMT Partial correlation (p value)**	With Carotid plaques mean± SD	Without Carotid plaques mean± SD	p value
sE-selectin(ng/ml)	0.113 (0.56)	0.121 (0.57)	46± 22	54 ± 29	0.61
sP-selectin(ng/ml)	0.095 (0.62)	-0.153 (0.47)	268± 133	286± 482	0.08
sVCAM-1(ng/ml)	0.334 (0.08)	0.358 (0.09)	1265± 500	1003± 196	0.22
sICAM-3 (ng/ml)	0.004 (0.98)	0.367 (0.08)	62± 21	58± 13	0.93
sICAM-1 (ng/ml)	0.301 (0.11)	0.312 (0.14)	384± 133	334± 84	0.17

*Spearman correlation coefficient

**Partial correlation and p values adjusting for sex, age at the time of study, disease duration, rheumatoid factor, and classic cardiovascular risk factors

between endothelial dysfunction measured by the levels of soluble biomarkers of endothelial cell activation and carotid artery ultrasonography abnormalities. In contrast, in a former study conducted by Wallberg-Jonsson *et al.* in 39 patients with RA, ICAM-1 and E-selectin levels were found to be related to ultrasonographically detected common carotid artery and femoral artery plaque as well as to haemostatic factors of endothelial origin¹³. Dessein *et al.* found an association of VCAM-1 with common carotid artery IMT and carotid plaques in 74 patients with RA⁴. However, these authors did not observe association between carotid ultrasonography results and ICAM-1 or E-selectin concentrations⁴. Regrettably, our data do not support association between any of the biomarkers of endothelial cell activation and carotid IMT or presence of carotid plaques in our series.

Although potential limitations due to the sample size may exist, the lack of association between a single determination of these biomarkers and structural surrogate markers of atherosclerosis supports the need of further investigations to establish the best set of predictors of CV risk in RA. Chronic inflammatory burden is the reason for the development of accelerated atherosclerosis in RA. Therefore, single determinations of biomarkers endothelial cell activation and inflammation may not be good predictors of CV outcome in these patients. A good example of that was the analysis of the results derived from a study that we conducted to establish a potential association between carotid IMT and C-reactive protein (CRP). We observed that the magnitude and chronicity of the in-

flammatory response measured by CRP correlated directly with the presence of atherosclerosis in patients with RA²¹. While no correlation between a single determination of CRP and carotid IMT at the time of the ultrasound assessment was found in 47 patients with RA, a strong correlation was observed between the maximum CRP values and the carotid IMT. Moreover, the distribution of patients in 4 quartiles according to the average CRP values showed significant differences in the carotid IMT. Those RA patients exhibiting the highest mean CRP values had greater carotid IMT²¹.

Another potential factor that may influence the results is the treatment with anti-TNF drugs. In this regard, although progression of carotid IMT has been observed in long-standing RA patients undergoing infliximab therapy¹⁹, a recent study showed that anti-TNF drugs may modify the progression of subclinical atherosclerosis expressed by a reduction in the logical increase of carotid IMT that one can expect in patients with RA as a consequence of a chronic inflammatory response²².

CONCLUSIONS

In conclusion, since no correlation seems to exist between levels of biomarkers of endothelial cell activation and anatomical (structural) damage determined by carotid ultrasonography in long-standing anti-TNF treated RA patients, further studies are needed to establish the best tools to be used in the assessment of the CV risk in patients with this chronic inflammatory disease.

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ABBREVIATIONS

CRP: C-reactive protein, CV: cardiovascular, DMARDs: disease modifying antirheumatic drugs, FMD: flow-mediated endothelium-dependent vasodilatation, ICAM: intercellular adhesion molecule, IMT: intima-media thickness, IQR: interquartile range, RA: rheumatoid arthritis, s: soluble, SD: standard deviation,

COMPETING INTEREST

The authors declare that they have no competing interest.

CORRESPONDENCE TO

Miguel A. González-Gay, MD, PhD
Department of Rheumatology,
Hospital Universitario Marqués de Valdecilla, IFIMAV,
Avenida de Valdecilla s/n, E-39008,
Santander, Spain
E-mail: miguelaggay@hotmail.com

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