Asymmetric dimethylarginine serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy

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Abstract

Objectives

This paper aims to determine whether disease activity, systemic inflammation and metabolic syndrome are potential determinants of circulating asymmetric dimethylarginine (ADMA) in ankylosing spondylitis (AS) patients undergoing TNF-α antagonist-infliximab-therapy.

Methods

We investigated ADMA serum concentrations in a series of 30 non-diabetic AS patients without history of cardiovascular (CV) events that were treated with the TNF-α antagonist infliximab, immediately prior to an infliximab infusion. Correlations of ADMA serum levels with disease activity, systemic inflammation and metabolic syndrome were assessed. Also, potential changes in ADMA concentration following an infusion of the anti-TNF-α monoclonal antibody-infliximab were analysed.

Results

A higher concentration of ADMA in men (p=0.012) and patients with hypertension was found (p=0.001). There was also a marginally positive correlation of ADMA serum levels with C-reactive protein levels (p=0.08). Moreover, a significant negative correlation between ADMA levels and total cholesterol and LDL-cholesterol was observed (p= 0.05).
No differences in ADMA levels according to the specific clinical features of the disease were seen. A single infliximab infusion did not lead to significant changes in ADMA serum levels.

Conclusion

In AS patients undergoing periodical treatment with the anti-TNF-α monoclonal antibody-infliximab a link between some features of metabolic syndrome and ADMA concentrations was observed.

Key words

ankylosing spondylitis, atherosclerosis, inflammation, anti-TNF-α antibody-infliximab, ADMA

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterised by predominant axial joint involvement, sacroiliitis and some extra-articular manifestations. Increased cardiovascular (CV) mortality, 1.5–2 times higher than the normal population, has been reported in patients with AS (1, 2), and it is probably due to a process of accelerated atherosclerosis (3). Both endothelial dysfunction and increased common carotid artery intima-media wall thickness have been reported in AS patients (4-6). These findings indicate the presence of subclinical atherosclerosis associated to the disease. The hallmark of endothelial dysfunction, an early step in the atherogenic process, is an impaired nitric oxide-mediated endothelial-dependent vasodilatation (7). Asymmetric dimethylarginine (ADMA), which is the result of the degradation of methylated proteins, is an endogenous inhibitor of the NO synthase. This inhibition triggers a reduction in NO production. This is the reason why ADMA has been proposed as a marker for endothelial dysfunction and a risk factor for CV disease (8, 9).

Traditional CV risk factors such as obesity and its related metabolic syndrome contribute to the increased CV morbidity and mortality observed in AS patients. The chronic pro-inflammatory state present in AS patients is an additional CV risk factor (2). Recently, we have observed that non-diabetic patients with AS on treatment with the anti-TNF-α monoclonal antibody-infliximab, which specifically and with high affinity binds to TNF-α and neutralises this cytokine, experience a rapid and dramatic reduction in the serum insulin levels and a rapid improvement of insulin sensitivity following administration of this drug (10). To further establish potential beneficial effects mediated with the anti-TNF-α blockade on the metabolic syndrome associated to AS, we also studied serum levels of several adipokines in non-diabetic AS patients undergoing infliximab therapy. We found that adiponectin serum levels positively correlated with insulin sensitivity, suggesting that low circulating adiponectin concentrations may be involved in the pathogenesis of the CV disease in AS (11). In assessing visfatin serum levels in the same population, we also disclosed a significant positive correlation of this adipokine with insulin resistance (12). Because of that, we also analysed apelin serum levels, a new adipokine recently involved in CV risk, but we could not find apelin association with disease activity or with metabolic syndrome (13).

Treatment with anti-TNF-α agents has been found to be effective in patients with AS and other spondyloarthropathies (14, 15, 16). As discussed before, a rapid beneficial improvement of insulin sensitivity mediated by infliximab was also observed (10). Therefore, it is plausible to think that TNF-α blockade might account for biological changes that may slow the progression of atherosclerosis in patients with AS. For this reason, an important step forward in our understanding of the effect of anti-TNF-α drugs in AS may be to establish potential changes in adipokines and biomarkers of endothelial cell activation and endothelial dysfunction following the administration of these biologic agents.

Taking these considerations together, in the present study we aimed to establish whether inflammation and/or metabolic syndrome have any influence on circulating ADMA concentrations in non-diabetic AS patients. We also studied possible associations of circulating ADMA concentrations with clinical and demographic characteristics of these patients. Moreover, we investigated whether an infliximab infusion altered circulating ADMA concentrations in a series of non-diabetic AS patients who required this therapy because of disease refractory to non-steroidal anti-inflammatory drugs (NSAIDs).

Patients and methods

Patients

We assessed a series of 30 patients with AS attending hospital outpatient clinics seen over 14 months (Jan. 2009 to March 2010), who fulfilled the modified New York diagnostic criteria for AS (17). They were treated by the same group of rheumatologists and were recruited from the Hospital Xeral-Calde, Lugo, Spain.

For ethical reasons, patients included in the present study were not randomised to a placebo group. The same procedure has been found acceptable and followed in studies on the short term effect of infliximab therapy on the lipid profile, adipokines and biomarkers of endothelial cell activation in patients with rheumatoid arthritis (RA) (18-20).

Patients on treatment with infliximab seen during the period of recruitment with diabetes mellitus or with plasma glucose levels greater than 110 mg/dl were excluded. None of the patients included in the study had hyperthyroidism or renal insufficiency. Also, patients seen during the recruitment period who had experienced CV events, including ischaemic heart disease, heart failure, cerebrovascular accidents or peripheral arterial disease were excluded. Hypertension was diagnosed in patients with a blood pressure of ≥140/90 mmHg and in those taking antihypertensive agents. Obesity was defined if body mass index (BMI) (calculated as weight in kilograms divided by height in squared meters) was greater than 30. In all cases the anti-TNF-α monoclonal antibody-infliximab was prescribed because of active disease. All patients included in the current study had begun treatment with NSAIDs immediately after the disease diagnosis. All of them were still being treated with these drugs at the time of the study. At the time of this study most patients were on treatment with naproxen: 500–1000 mg/d. Although the 2010 updated recommendations facilitate initiation of TNF-α blockers in AS and only ask for 2 NSAIDs with a minimum total treatment period of 4 weeks (21), for the initiation of anti-TNF-α therapy in these series of patients recruited between January 2009 to March 2010, they had to be treated with at least 3 NSAIDs prior to the onset of infliximab.

A clinical index of disease activity (Bath Ankylosing Spondylitis Disease Activity Index- BASDAI- range of 0 to 10) (22) was evaluated in all patients at the time of the study. Clinical information on hip involvement, history of synovitis in other peripheral joints and peripheral enthesitis, history of anterior uveitis, presence of syndesmophytes and HLA-B27 status (typed by cell cytotoxicity) was assessed. Moreover, C-reactive protein (CRP)- by a latex immunoturbidity method, erythrocyte sedimentation rate (ESR)-Westergren, serum glucose, total cholesterol, HDL and LDL cholesterol and triglycerides (fasting overnight determinations) were assessed in all the patients at the time of the study.

The main demographic, clinical and laboratory data of this series of 30 AS patients at the time of the study are shown in Table I. Since at that time all patients were undergoing periodical treatment with the anti-TNF-α monoclonal antibody-infliximab (median duration of periodical treatment with biologic agent: 23 months), the mean BASDAI±standard deviation (SD) was only 2.94±2.11.

The local institutional committee approved anti-TNF-α therapy. Also, patients gave informed consent to participate in this study. Neither this study nor the former studies on the short term effect of infliximab therapy on insulin resistance in AS (10) or adipokines (11, 12) were supported by any pharmaceutical drug company.

Study protocol

In all cases, the drug was given to patients as an intravenous infusion in a saline solution over 120 minutes. All measurements were made in the fasting state. Blood samples were taken at 08:00 hours for determination of the ESR (Westergren), CRP (latex immuno-turbidimetry), lipids (enzymatic colorimetry), plasma glucose and serum insulin (DPC, Dipesa, Los Angeles, CA, USA). As previously described, insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula=(insulin (μU/ml) x glucose (mmol/l)÷22.5[7] (10). A commercial ADMA ELISA kit was used to measure serum ADMA levels (Immundiagnostik, K7860; assay sensitivity=0.04 μmol/l; intra-and interassay coefficients of variation were 6.9% and 9.2%, respectively) (Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. Serum levels of ADMA were measured in samples obtained immediately prior to an infliximab infusion and 120 minutes later. A commercial Apelin-12 Enzyme Immunoassay kit was used to measure serum apelin levels (Phoenix pharmaceuticals, EK-057-23; assay sensitivity=0.06 ng/ml; intra-and interassay coefficients of variation were <10% and <15%, respectively) (Phoenix pharmaceuticals, Burlingame, CA, USA), according to the manufacturer's instructions, immediately prior to an infliximab infusion (13). Commercial ELISA kits (Linco Research, St. Charles, MO, USA) were used to measure total plasma adiponectin (Millipore, EZHADP-61K; assay sensitivity=0.5 ng/ml; intra- and interassay coefficients of variation were 3.3% and 5.5%, respectively), serum resistin (Millipore, EZHR-95K; assay sensitivity=0.16 ng/ml; intra- and interassay coefficients of variation were <5% and <7%, respectively), serum leptin (Millipore, EZHL-80SK; assay sensitivity=0.135 ng/ml ± 2 SD; intra-and interassay coefficients of variation were 3.7% and 4%, respectively), and serum ghrelin levels (Millipore, EZGRT-89K; assay sensitivity=100 pg/ml; intra- and interassay coefficients of variation were 1.32% and 6.62%, respectively), according to the manufacturer's instructions, immediately prior to an infliximab infusion (11, 12). Visfatin serum levels were also determined by commercially available ELISA (Phoenix pharmaceuticals, EK-003-80; assay sensitivity=2.68 ng/ml; intra- and interassay coefficients of variation were <10% and <15%, respectively) (Phoenix pharmaceuticals, Burlingame, CA, USA), according to the manufacturer's instructions, prior to infliximab infusion (12). Angpt-2 serum levels were determined by commercially available ELISA (Abcam, AB99971; assay sensitivity=10 pg/ml; intra-and interassay coefficients of variation were <10% and <12%, respectively) (Human Immunoassay Quantikine, R&D Systems, Cambridge, UK), according to the manufacturer's instructions, prior to an infliximab infusion.

Statistical analyses

Variables were expressed as mean±SD, median (interquartile [IQ] range) or percentages. Correlation between basal ADMA at time 0 with selected continu-

ous variables was performed adjusting by age at the time of the study, sex, and classic cardiovascular risk factors via estimation of the Pearson partial correlation coefficient (r).

The associations between baseline characteristics and serum ADMA concentrations were assessed by the Student's paired *t*-test for categorical variables. Differences in ADMA levels between men and women and patients with hypertension or not were assessed by Mann-Whitney U-test.

ADMA serum levels before (time 0) and postinfusion (time 120) were compared using the paired Student's *t*-test. Two-sided *p*-values ≤0.05 were considered to indicate statistical significance. Analyses were performed using Stata 12/SE (StataCorp, College Station, TX, USA).

Table I. Demographic, clinical and laboratory data at the time of the study in 30 patients with ankylosing spondylitis.

Variable	n (%)
Age (years)	
At the time of study	50.47 ± 14.85
At the time of onset of symptoms	28.23 ± 10.40
Delay to the diagnosis (years) ±SD	11.48 ± 9.01
Men/women	21(70) / 9(30)
Mean disease duration (years) ±SD	21.97 ± 13.16
History of classic cardiovascular risk factors	
Hypertension	12 (40)
Dyslipidemia	11 (36.67)
Obesity (BMI > 30 kg/m2)	3 (10.00)
Current smokers (n=30)	13 (43.33)
Mean blood pressure (mm Hg) ±SD	
Systolic	123.17 ± 18.17
Diastolic	75.67 ± 12.51
Mean body mass index (kg/m^2) ±SD	26.70 ± 3.26
Mean BASDAI±SD	2.94 ± 2.11
Mean VAS spinal pain±SD	31.13 ± 24.23
Hip involvement	6 (20)
Synovitis in other peripheral joints and peripheral enthesitis	11 (36.67)
Anterior uveitis	6 (20.00)
Syndesmophytes, n (%)	10 (33.33)
Mean CRP (mg/l) ±SD*	6.24 ± 8.65
Mean ESR (mm/1st hour) ±SD**	19.00 ± 15.18
Mean cholesterol or triglycerides (mg/dl) ±SD	
Total cholesterol	199.10 ± 30.61
HDL cholesterol	53.17 ± 12.81
LDL cholesterol	126.77 ± 26.54
Triglycerides	93.97 ± 56.70
Mean fasting serum glucose (mg/dl) ±SD	92.77 ± 8.63
HLA-B27 positive (n=27)	20 (74.07)

*Normal value <5 mg/l. **Normal value < 20 mm/1st hour.
BASDAI: Bath ankylosing spondylitis disease activity index; BMI: Body mass index; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HLA: human leukocyte antigen; LDL: low-density lipoprotein; SD: standard deviation; VAS: visual analogue scale.

Results

Relationships of demographic features, inflammation, adiposity and adipokines with circulating ADMA concentration

ADMA concentration did not show significant correlation with age at the onset of symptoms, BMI, and ESR at the time of the study. Nevertheless, a marginally significant positive correlation was observed between ADMA basal serum levels and CRP at the time of the study (p=0.08) (Table II). Also, the few obese patients (n=3) from this series had a marginally significant lower level of ADMA than the remaining AS patients (p=0.062) (Table III). Moreover, higher ADMA levels were also observed in men when compared with women (p=0.012) (Table III). However, no correlation of ADMA with adiponectin, resistin, leptin, visfatin, ghrelin, apelin and Angpt-2 concentrations was observed (Table II).

Relationships of ADMA concentration with metabolic syndrome features other than adiposity

Although no significant correlation between ADMA concentration with systolic or diastolic blood pressure was observed (Table II), ADMA serum levels were significantly increased in AS patients that fulfilled definitions of hypertension (p=0.001) (Table III). No correlation between ADMA levels and HDL-cholesterol, triglycerides and glucose levels was observed (Table II). In keeping with these observations, no significant differences in ADMA concentration were seen when patients were stratified according to the presence or absence of dyslipidemia (Table III). Nevertheless, we observed a significant negative correlation between ADMA levels and total cholesterol and LDL-cholesterol (p=0.05) (Table II). However, no significant correlation between ADMA concentration and insulin resistance (HOMA-IR at the time of the study) and insulin sensitivity (QUICKI at the time of the study) was found (Table II).

Relationships of ADMA concentration with other recorded baseline characteristics

Circulating ADMA concentration did not correlate with disease duration, BASDAI and VAS spinal pain at the time of the study (Table II). Likewise, no difference in ADMA concentration was observed when patients with a history of anterior uveitis, presence of syndesmophytes, hip involvement or synovitis and/or enthesitis in other peripheral joints was compared with the remaining patients who did not exhibit these features (Table III). It was also the case when patients were compared according to HLA-B27 status (Table III).

Changes in ADMA concentration upon infliximab therapy

ADMA serum levels did not change following an infliximab infusion. In this regard, the median (IQ range) values of ADMA were 0.53 (0.47–0.64) μmol/l immediately prior to infliximab infusion (time 0) and 0.47 (0.40-0.58)

Table II. Partial correlation of basal serum ADMA (time 0) with selected continuous variables adjusting for age at the time of the study, sex, and classic cardiovascular risk factors (at the time of the study) in 30 patients with ankylosing spondylitis.

Variable	r	*p*-value
Age at the onset of symptoms	0.150	0.46
Disease duration	-0.180	0.38
BMI	-0.250	0.22
Systolic blood pressure	0.259	0.20
Diastolic blood pressure	0.188	0.36
BASDAI	0.244	0.23
VAS	0.116	0.57
ESR (natural-log-transformed)	0.093	0.65
CRP (natural-log-transformed)	0.348	0.08
Total cholesterol (natural-log-transformed)	-0.393	0.05
HDL cholesterol (natural-log-transformed)	-0.105	0.61
LDL cholesterol (natural-log-transformed)	-0.396	0.05
Triglycerides (natural-log-transformed)	-0.043	0.83
Serum glucose (natural-log-transformed)	0.108	0.60
HOMA-IR	0.081	0.69
QUICKI	-0.160	0.44
Resistin at time 0	-0.162	0.48
Adiponectin at time 0	-0.119	0.57
Leptin at time 0	-0.187	0.37
Visfatin at time 0	0.082	0.69
Ghrelin at time 0	0.086	0.68
Angpt-2 at time 0	-0.139	0.50
Apelin at time 0	-0.067	0.75

BASDAI: Bath ankylosing spondylitis disease activity index; BMI: Body mass index; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein; QUICKI: quantitative insulin sensitivity check index; VAS: visual analogue scale.

Table III. Differences in basal ADMA serum levels (time 0) according to categorical variables.

Variable	Category	ADMA: mean±SD	*p*-value
Sex	Men	0.58 ± 0.16	0.012
	Women	0.46 ± 0.09	
Arterial hypertension	Yes	0.65 ± 0.15	0.001
	No	0.47 ± 0.11	
Dyslipidemia	Yes	0.56 ± 0.16	0.701
	No	0.54 ± 0.15	
Obesity	Yes	0.39 ± 0.20	0.062
	No	0.57 ± 0.14	
Current smoker	Yes	0.56 ± 0.20	0.618
	No	0.54 ± 0.12	
Hip involvement	Yes	0.56 ± 0.11	0.869
	No	0.54 ± 0.17	
Synovitis in other peripheral joints and peripheral enthesitis	Yes	0.58 ± 0.14	0.473
	No	0.53 ± 0.16	
Anterior uveitis	Yes	0.58 ± 0.18	0.584
	No	0.54 ± 0.15	
Syndesmophytes	Yes	0.59 ± 0.16	0.318
	No	0.53 ± 0.15	
HLA-B27	Positive	0.58 ± 0.16	0.223
	Negative	0.46 ± 0.09	

HLA: human leukocyte antigen; SD: standard deviation.

μmol/l at the end of the infusion (time 120 minutes) (p=0.968) (Table IV).

Discussion

ADMA is an endogenous molecule synthesised from L-arginine that inhibits NO synthesis (8). Since NO acts as an anti-atherosclerotic molecule (by inducing vasodilatation and inhibiting aggregation of platelets, adhesion of monocytes and leukocytes to the endothelium, smooth muscle cell proliferation and oxidation of LDL-cholesterol) a reduction in its levels usually triggers endothelial dysfunction (9). ADMA is considered a risk factor for CV disease since increased concentrations of this molecule have been associated with hypertension (23), hypertriglyceridemia (24), hypercholesterolemia (25), diabetes mellitus (26) and insulin insensitivity (27).

In the present study, we found high ADMA levels associated with sex as ADMA concentrations were increased in men. As previously described, ADMA levels were also increased in AS patients with hypertension. However, to our surprise, we found a negative correlation of total cholesterol and LDL-cholesterol with ADMA levels. These results are in contrast with those previously published by Böger *et al*. (25) and other authors that described the presence of higher ADMA levels with patients with hypercholesterolemia. It is possible that the negative correlation between total cholesterol and ADMA serum levels in our study might be the result of the metabolic changes mediated by the prolonged administration of anti-TNF-α therapy. In this regard, it is well known that infliximab treatment suppresses inflammation (28), and that both short-term (29) and long-term (30) treatment with this biologic agent improve endothelial function in patients with chronic inflammatory rheumatic diseases. Paradoxically, long-term treatment with the anti-TNF-α monoclonal-antibody infliximab was associated with an increase in total cholesterol levels (31). In an extremely interesting study performed in patients with RA treated with infliximab for more than 6 months, Popa *et al*. observed a beneficial effect of anti-TNF-α

Table IV. Differences in ADMA serum concentration immediately before (time 0) and after (time 120 minutes) infliximab infusion.

ADMA	Basal (time 0)	Postinfusion (time 120)	*p*-value
Mean ±SD (μmol/l)	0.55±0.15	0.55±0.29	0.968
(Median; IQ range)	0.53; 0.47–0.64	0.47; 0.40–0.58	

IQ: interquartile; SD: standard deviation.

treatment on disease activity. However, these authors also observed an increase in total cholesterol and LDL-cholesterol (32). The same investigators reported that anti-TNF-α infliximab therapy improved paraoxonase-1 (PON-1) activity (an antioxidant enzyme present in HDL) in parallel with a decrease in the inflammatory status (33). The median duration of periodical treatment with biologic therapy in our series of AS patients undergoing infliximab therapy was almost 2 years. Therefore, based on the observations reported by Popa *et al*., it is plausible to think that both total cholesterol and LDL-cholesterol levels may be higher at the time of the present study than when infliximab therapy was initiated. Thus, complex lipidic changes mediated by the influence of prolonged TNF-α blockade may help to explain the inverse correlation between ADMA levels and total cholesterol and LDL-cholesterol observed in our series of AS patients. We also found marginally statistically significant lower levels of ADMA in obese (BMI superior to 30) AS patients. However, we cannot consider this result as relevant since we only had 3 obese patients in our series of patients with AS.

ADMA serum levels have been reported to be increased in certain conditions, such as AS (where its levels also correlated with ESR) (34), RA (35, 36) and coronary artery ectasia (37). When we analysed the effect of a single infusion of infliximab on ADMA concentration in patients undergoing periodical treatment with this biologic agent, we could not observe any significant change. Even though we did not observe a reduction in ADMA levels after an infliximab infusion, it is possible that long-term treatment with this biologic therapy might account for a reduction of ADMA levels, as well as decrease in the inflammatory burden. The missing effect of one single infliximab infusion on the ADMA levels found in our series is most probably due to the fact that the patients had been receiving infliximab for a long time period and, because of that, they were in a state of low disease activity. With respect to this, they showed even a BASDAI lower than 3, which is indicative for a favourable disease activity state (38). In keeping with that, it is possible that the prolonged periodical use of the anti-TNF-α inhibitor agent in our series of AS patients might have reduced the statistical correlation between CRP/disease activity and ADMA levels. In this regard, Sari *et al*. found higher ADMA levels in AS patients when compared to healthy controls (39). However, the levels of ADMA did not differ significantly between AS patients treated with TNF-α blocking agents and healthy subjects (39). In keeping with these observations, Di Franco *et al*. also found higher serum ADMA levels in early RA patients when compared to controls (40). However, after 12 months of anti-TNF-α inhibitor therapy, the mean ADMA level for these patients decreased and it was similar to that observed in the controls (40).

Taken together all these observations, it is possible that a reduction of ADMA levels as well as an increase of cholesterol mediated by the prolonged periodical use of the anti-TNF-α agent-infliximab may explain the negative correlation of ADMA with cholesterol observed in our series. Due to the fact that our study encompassed AS patients undergoing periodical treatment with infliximab, we found low disease activity at the time of the study. In this regard, CRP only showed a marginally significant positive correlation with ADMA levels.

In conclusion, in AS patients undergoing periodical treatment with the anti-TNF-α monoclonal antibody-infliximab a link between some features of metabolic syndrome and ADMA concentrations was observed.

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