Correlation between two biomarkers of atherosclerosis, osteopontin and angiopoietin-2, in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy

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Abstract

Objective

To determine whether circulating osteopontin (OPN) levels in patients with ankylosing spondylitis (AS) undergoing TNF-α antagonist-infliximab-therapy are increased compared with controls and to establish whether disease activity, systemic inflammation, metabolic syndrome, adipokines and biomarkers of atherosclerosis are potential determinants of circulating OPN levels in these patients.

Methods

We assessed OPN serum concentrations in a series of 30 non-diabetic AS patients without cardiovascular disease undergoing TNF-α antagonist-infliximab therapy and 48 matched controls. OPN levels were measured immediately before and after an infliximab infusion, at time 0 and at time 120 minutes respectively. Correlations of OPN serum levels with clinical features, disease activity, systemic inflammation, metabolic syndrome and several biomarkers of atherosclerosis were assessed. Potential changes in OPN concentration following an infusion of anti-TNF-α monoclonal antibody-infliximab were also analysed.

Results

At the time of the study AS patients undergoing anti-TNF-α therapy had low disease activity (mean BASDAI 2.94) and they showed similar OPN serum levels to healthy controls. No differences in OPN levels according to the specific clinical features of the disease were seen. Also, no correlation between OPN concentration and insulin resistance and adipokines was observed. However, a positive correlation between OPN and angiopoietin-2 (Angpt-2) serum levels was found (r=0.397; p=0.04). In addition, a single infliximab infusion led to a marginal statistically significant reduction in OPN levels (24112.19±14608.73 pg/ml at time 0 versus 21806.62±11390.83 pg/ml at time 120'; p=0.05).

Conclusion

OPN and Angpt-2 serum levels are correlated in non-diabetic AS patients undergoing TNF-α antagonist therapy.

Key words

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

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease associated with a higher cardiovascular (CV) mortality than the normal population (1, 2), probably due to a process of accelerated atherosclerosis (3).

Traditional CV risk factors such as obesity and its related metabolic syndrome, as well as inflammation, contribute to the increased CV morbidity and mortality observed in AS patients (2). Osteopontin (OPN) is a phosphorylated glycoprotein which is synthesised by different cell types such as osteoclasts, osteoblasts, chondrocytes and different cells of the immune system (macrophages, T cells and dendritic cells) (4, 5). This protein is involved in many physiological and pathological processes, which include cell adhesion, migration, angiogenesis, differentiation, regulation of apoptosis, inflammation and tumor metastasis (5, 6). OPN levels have been found to be increased in different pathologies including rheumatoid arthritis (RA), atherosclerosis, multiple sclerosis, obesity and cancer (7).

Since OPN levels have been found associated with the presence and extent of coronary heart disease, it is plausible to think that OPN may play a role in the mechanisms associated with the development of atherosclerosis (8, 9). In this setting, it is possible that this protein with pleiotropic functions may serve both as a CV and AS disease marker.

Interestingly, treatment with anti-TNF-α agents has been found to be effective in patients with AS and other spondyloarthropathies (10-12). Additionally, it is possible that TNF-α blockade might account for biological changes that may slow the progression of atherosclerosis in patients with AS. Hence, the establishment of potential correlations among adipokines and biomarkers of endothelial cell activation, endothelial dysfunction and inflammation following the administration of anti-TNF-α drugs in AS patients may improve our understanding of the effect of these biologic agents in this condition.

Our group has studied the effect of the treatment of a series of non-diabetic AS patients with anti-TNF-α monoclonal antibody-infliximab on features of metabolic syndrome, in order to study the possible link between chronic inflammation and metabolic syndrome. We observed that infliximab treatment reduced serum insulin levels and improved insulin sensitivity (13). Next, to further establish potential beneficial effects of the use of anti-TNF-α therapy on the metabolic syndrome associated to AS, we 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 (14). When we assessed visfatin serum levels in the same population, we also disclosed a significant positive correlation of this adipokine with insulin resistance (15). We also analysed apelin serum levels, an adipokine involved in CV risk, but we could not find an association with disease activity or with metabolic syndrome (16). Interestingly, ghrelin serum levels also showed a correlation with insulin resistance (17).

Finally, we measured the effect of infliximab therapy on biomarkers of endothelial activation. With respect to this, in our series asymmetric dimethylarginine (ADMA) serum levels were associated with some features of metabolic syndrome such as sex and hypertension (18). More importantly, anti-TNF-α infliximab infusion in these AS patients led to a dramatic reduction angiopoietin-2 (Angpt-2), a marker of endothelial cell activation that is involved in angiogenesis and makes the endothelium responsive to inflammatory cytokines (19).

Taking these considerations together, in the present study we aimed to determine whether circulating OPN levels in patients with AS undergoing TNF-α antagonist-infliximab-therapy are increased compared with controls. We also studied possible associations of circulating OPN concentrations with clinical and demographic characteristics of these patients as well as with disease activity and inflammation. Moreover, we investigated potential correlations between OPN and metabolic

syndrome, adipokines and biomarkers of endothelial activation and atherosclerosis. Finally, we studied whether an infliximab infusion altered circulating OPN 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 (January 2009 to March 2010), who fulfilled the modified New York diagnostic criteria for AS (20). They were treated by the same group of rheumatologists and were recruited from the Hospital Xeral-Calde, Lugo, Spain. For the comparative analysis with AS patients we used 48 controls matched for age, sex, ethnicity and traditional CV risk factors, without history of CV events.

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 adipokines and biomarkers of endothelial cell activation in patients with RA (21-23).

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 (24), 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 therapy. A clinical index of disease activity (Bath Ankylosing Spondylitis Disease Activity Index- BASDAI - range of 0 to 10) (25) 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 mono-

Table I. Demographic, clinical and laboratory data of 30 patients with ankylosing spondylitis.

Variable	n (%)
Mean age (years) ± SD	
At the time of study	50.4 ± 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	13 (43.33)
Mean blood pressure (mm Hg) ± SD*	
Systolic	123.17 ± 18.17
Diastolic	75.67 ± 12.51
Mean body mass index (kg/m2) ± SD	26.70 ± 3.26
Mean BASDAI ± SD*	2.94 ± 2.11
Mean VAS spinal pain ± SD*	3.1 ± 2.4
Hip involvement	6 (20)
Synovitis in other peripheral joints and peripheral enthesitis	11 (36.67)
Anterior uveitis	6 (20.00)
Syndesmophytes	10 (33.33)
Mean CRP (mg/l) ± SD**	
At the time of disease diagnosis	24.01 ± 33.43
At the time of study	6.24 ± 8.65
Mean ESR (mm/1st hour) ± SD***	
At the time of disease diagnosis	30.10 ± 28.23
At the time of study	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)

*At the time of the study. **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.

clonal antibody-infliximab (median duration of periodical treatment with this 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 (13), adipokines (14-16) or biomarkers of endothelial cell activation, endothelial dysfunction and inflammation (18, 19) 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 0800 hours for determination of the erythrocyte sedimentation rate [ESR] (Westergren), C-reactive protein [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)÷22.57 (13). A commercial ELISA kit was used to measure serum OPN levels (Abcam, ab100618; assay sensitivity=50 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. Serum levels of OPN were measured in samples obtained immediately prior to an infliximab infusion and 120 minutes later at the end of the infusion. Commercial ELISA kits were used to measure serum resistin, adiponectin, leptin, visfatin, apelin, ghrelin, ADMA and Angpt-2 levels immediately prior to and after an infliximab infusion (at time 120 minutes) as previously described (14-19).

Statistical analyses

Variables were expressed as mean ± SD or percentages. Correlation between basal OPN at time 0 with selected continuous variables was performed adjusting for 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 OPN concentrations were assessed by the Student's paired *t*-test for categorical variables. Differences in OPN levels between men and women and patients with hypertension or not were assessed by Mann-Whitney U-test.

OPN serum levels before (at time 0) and postinfusion (at time 120 minutes) were compared using the paired Student *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).

Table II. Partial correlation of serum Osteopontin prior to infliximab infusion (at time 0) with selected continuous variables adjusting by age at the time of the study, sex, and classic cardiovascular risk factors in 30 patients with ankylosing spondylitis.

Variable	Osteopontin (time 0)	
	r	*p*
Age at the onset of symptoms	0.300	0.13
Disease duration*	-0.148	0.46
BMI*	0.092	0.65
Systolic blood pressure*	-0.242	0.22
Diastolic blood pressure*	-0.306	0.12
BASDAI*	-0.014	0.94
VAS*	0.011	0.96
ESR* (natural-log-transformed)	-0.054	0.79
CRP* (natural-log-transformed)	-0.005	0.98
ESR** (natural-log-transformed)	-0.024	0.91
CRP** (natural-log-transformed)	-0.099	0.62
Total cholesterol* (natural-log-transformed)	0.066	0.74
HDL cholesterol* (natural-log-transformed)	-0.176	0.38
LDL cholesterol* (natural-log-transformed)	0.174	0.39
Triglycerides* (natural-log-transformed)	-0.089	0.66
Serum glucose* (natural-log-transformed)	0.120	0.55
HOMA-IR at time 0*	-0.067	0.74
QUICKI at time 0*	0.010	0.96
Resistin at time 0	0.107	0.64
Adiponectin at time 0	0.026	0.90
Leptin at time 0	-0.141	0.49
Visfatin at time 0	-0.171	0.40
Angpt-2 at time 0	0.397	0.04
Apelin at time 0	-0.269	0.17
ADMA at time 0	-0.146	0.48
Ghrelin at time 0	0.226	0.27

*At the time of the study; **At the time of disease diagnosis.
ADMA: Asymetric dymethilarginine; Angpt-2: Angiopoietin 2; 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.

Results

Differences in osteopontin serum levels between AS patients and controls

OPN serum concentrations found in our series of AS patients was similar to those observed in healthy controls (AS patients: mean±SD: 24112.19±14608.73 pg/ml; healthy controls: mean±SD: 21255.21±8033.97 pg/ml; *p*=0.269).

Relationships of osteopontin concentration with disease activity and clinical features

Circulating OPN concentration did not correlate with disease duration, BASDAI or VAS spinal pain at the time of the study (Table II). Likewise, no difference in OPN concentration was observed when patients with a history of anterior uveitis, presence of syndesmophytes, hip involvement or synovitis in other peripheral joints and peripheral

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

Variable	Category	Osteopontin: Mean ± SD (pg/ml)	*p*
Sex	Men	23934.06 ± 16480.74	0.921
	Women	24527.84 ± 9.711.596	
Arterial hypertension	Yes	22851.73 ± 17398.22	0.707
	No	24952.49 ± 12896.84	
Dyslipidemia	Yes	23353.72 ± 15251.33	0.833
	No	24551.31 ± 14630.66	
Obesity	Yes	34176.44 ± 30295.75	0.214
	No	22993.94 ± 12427.86	
Current smoker	Yes	22680.41 ± 11497.21	0.647
	No	25207.08 ± 16874.12	
Hip involvement	Yes	31229.69 ± 23030.14	0.187
	No	22332.82 ± 11716.14	
Synovitis in other peripheral joints and	Yes	24193.91 ± 18301.35	0.982
peripheral enthesitis	No	24064.88 ± 12559.92	
Anterior uveitis	Yes	20231.79 ± 11165.05	0.477
	No	25082.29 ± 15397.37	
Syndesmophytes	Yes	27583.98 ± 18758.06	0.367
	No	22376.3 ± 12229.07	
HLA-B27	Positive	24844.63 ± 15193.28	0.880
	Negative	23821.29 ± 15625.09	

HLA: human leukocyte antigen; SD: standard deviation.

enthesitis were compared with the remaining patients who did not exhibit these characteristics (Table III). It was also the case when patients were compared according to HLA-B27 status (Table III).

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

OPN serum levels did not show significant correlation with age at the onset of symptoms, BMI, CRP and ESR at the time of the study or at the time of disease diagnosis (Table II). Neither did we find any association with resistin, adiponectin, leptin, visfatin, apelin, and ghrelin serum levels (Table II). Also, significant differences in OPN serum levels were not observed when AS patients were stratified according to sex (Table III).

Relationships of osteopontin concentration with metabolic syndrome features other than adiposity

We did not observe any statistically significant correlation between OPN serum levels with systolic or diastolic blood pressure, total cholesterol, HDL- and LDL-cholesterol, triglycerides, serum glucose levels, insulin sensitivity (QUICKI) or insulin resistance (HOMA-IR) (Table II). Besides, no significant differences in OPN concentration were seen when patients were stratified according to the presence or absence of arterial hypertension, dyslipidemia or obesity (Table III).

Relationship of osteopontin serum levels with biomarkers of endothelial cell activation and atherosclerosis

No correlation between OPN and ADMA serum levels were observed. Nevertheless, we found a positive correlation between OPN and Angpt-2 serum levels (r=0.397; *p*=0.04) (Table II).

Changes in osteopontin concentration upon infliximab therapy

Following an infliximab infusion, a marginally significant reduction of OPN serum levels was observed. In this regard, the mean ± SD values of OPN decreased from 24112.19±14608.73 pg/ml immediately prior to infliximab infusion (at time 0) to 21806.62±11390.83 pg/ml at the end of the infusion (at time 120 minutes) (*p*=0.05).

Discussion

OPN is a proinflammatory mediator implicated in both physiological and pathological situations (5, 6). In a cross-sectional study performed in 2008, Choi *et al*. found that OPN levels were increased in AS when compared to healthy controls (4). In contrast, in our study we did not observe significant differences in the levels of OPN between AS patients and controls. We believe that these different results may be explained by the long duration of treatment with anti-TNF-α therapy that our AS patients had received at the time of the study. With respect to this, it is possible that prolonged anti-TNF-α may lead to reduction of the inflammatory burden and consequently to a decrease of OPN levels. In this regard, OPN concentrations observed in our study in both AS patients and healthy controls were very similar to those observed in the control group reported by Choi *et al*. (4).

OPN levels have previously been associated with CV disease (26, 27). This mediator enhances the inflammatory process that occurs in vascular diseases (28). Due to its proinflammatory nature, increased expression of this mediator has been postulated to promote the development of atherosclerotic lesions (7). More specifically, OPN has been suggested to promote arterial decalcification (29). Results from animal models support this hypothesis (30, 31). Therefore, it has been proposed that high concentrations of OPN may promote atherosclerotic plaque instability (27). In addition, OPN has also been reported to be chemotactic for inflammatory cells such as macrophages, which consequently produce matrix-degrading enzymes enhancing further plaque rupture (27).

In the present study we found a positive correlation between OPN and Angpt-2 serum levels. Angpt-2 is a marker of endothelial cell activation, involved in angiogenesis, which makes the endothelium responsive to inflammatory cytokines (32). Angpt-2 serum levels have been found increased in RA patients with CV disease when compared

with those without CV disease (32). In this regard, we have recently reported that Angpt-2 serum levels correlate with disease severity, early onset and CV disease in RA patients (33). Consequently, the correlation between OPN and Angpt-2 serum levels observed in our study is in agreement with the suggested role of these two molecules in the development of the atherosclerotic disease, reinforcing the idea of their potential use as biomarkers to predict CV risk in patients with AS.

Finally, in a previous work we observed that a single infusion of the anti-TNF-α monoclonal antibody- infliximab led to a significant reduction in Angpt-2 serum levels in our series of AS patients (19). Likewise, a single infusion of infliximab also yielded a decrease in OPN serum levels in AS patients. This is in accordance with the proinflammatory role proposed for these biomarkers of endothelial cell activation and atherosclerosis and the beneficial effect against the development of CV disease mediated by anti-TNF-α therapy.

In conclusion, in our series of non-diabetic AS patients undergoing periodical anti-TNF-α monoclonal antibody-infliximab therapy and low disease activity, we observed a correlation between OPN and Angpt-2 serum levels.

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