

Osteopontin is associated with increased arterial stiffness in Rheumatoid Arthritis

Running head: OPN and arterial stiffness in RA

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Abstract

Rheumatoid arthritis (RA) patients are characterized by increased arterial stiffness, an independent predictor of cardiovascular risk. It has been suggested that osteopontin (OPN), a cytokine involved in RA pathogenesis, might have vascular effects. To study a possible relationship between OPN and arterial stiffness, aortic pulse-wave velocity (PWV) was measured by tonometry in 69 patients, 41 with RA, 28 with systemic sclerosis (SSc) and 18 healthy controls. Plasma OPN levels, oxidative stress markers and ET-1 were assessed.

OPN levels were significantly ($p<0.05$) higher in RA (median 9.93; range 4.36-47.80 ng/ml) than SSc (4.3; 2.1-19.7 ng/ml) and controls (5.2; 4.1-9.4 ng/ml). In RA patients, log-OPN was related to log-C reactive protein ($r=0.30$; $p<0.05$), age ($r=0.38$; $p<0.01$), HAQ ($r=0.58$; $p<0.0001$), and inversely related to total cholesterol ($r=-0.33$; $p<0.05$) and apo A ($r=-0.58$; $p<0.001$), but not to oxidative stress markers and ET-1.

PWV was similar in RA (median 8.1, range 4.7-16.4 m/s) and SSc (median 8.7; range 7.1-13.1 m/s) but significantly ($p<0.01$) greater than controls (median 7.5; range 4.1-10.4 m/s). Aortic PWV was related to log-OPN ($r=0.40$, $p<0.01$) only in RA patients. It was also related to age ($r=0.34$; $p<0.05$), mean blood pressure ($r=0.44$; $p<0.001$) and HAQ ($r=0.48$; $p<0.001$). In multiple regression analysis ($r^2=0.36$), including confounders, log-OPN remained a significant ($p<0.05$) predictor of PWV in RA.

Elevated plasma OPN levels are associated with increased arterial stiffness in RA patients, suggesting that this protein might represent a bridge protein between inflammation and the consequent joint damage and cardiovascular risk in RA patients.

Introduction

Rheumatoid arthritis (RA) is a chronic progressive inflammatory disease, characterized by synovial inflammation and hyperplasia leading to progressive cartilage and bone destruction (1). Patients with RA have a shortened life span and cardiovascular diseases, caused by accelerated atherosclerosis (2), are the most common cause of mortality in these patients (3-7).

Recent studies have shown a possible role of RA as independent risk factor for atherosclerosis, since traditional cardiovascular risk factors could be insufficient to explain the high incidence of cardiovascular events (8,9). In particular, RA with extra-joint involvement, associated with high inflammation, is associated with greater cardiovascular mortality, suggesting a direct role of RA in the pathogenesis of cardiovascular damage (10).

Increased arterial stiffness, which is characteristic of aging for reduction of elastin and increase in collagen (11), can be measured as pulse wave velocity (PWV) (12). It is a vascular parameter of important clinical significance since it has demonstrated to be an independent predictor of cardiovascular events in high risk patients and in the general population (12). Increased arterial stiffness was demonstrated in RA patients at the abdominal aorta (13) and carotid site (14) and as elevated aortic PWV (15). The latter alteration was related to C- reactive protein (CRP) levels, was reversible after anti-TNF therapy (15,16), but it was also associated with risk factors such as age, blood pressure and abdominal obesity (17). Interestingly, aortic PWV was related to para-articular trabecular bone loss at the ultra-distal radius (18).

Osteopontin (OPN) is physiologically a potent inhibitor of mineralization, it prevents ectopic calcium deposits and is a potent inducible inhibitor of vascular calcification (25). On the other hand, this cytokine is expressed in chronic inflammatory and autoimmune diseases (19-24) with pro-inflammatory functions and it has been suggested as a potential mediator of the promotion of joint destruction in RA (26). Enhanced levels of OPN mRNA and protein were found in synovial tissue from RA patients (27). Moreover, OPN deficiency has shown to protect joints against destruction in induced arthritis mice, suppressing articular destruction, chondrocyte apoptosis, and synovial

angiogenesis (28), suggesting a direct role in bone re-adsorption, beyond its effects on inflammation (29). It has been also hypothesized a role for OPN in atherosclerosis, since elevated levels of OPN are associated with the extent of cardiovascular disease, independently of traditional risk factors (30) and with restenosis (31).

Based on the literature reports, the aim of the present study was to investigate the possible relationship between plasma OPN levels with an established vascular parameter of risk such as aortic PWV in RA patients. The interaction with disease activity and severity, inflammatory and oxidative stress markers and common atherosclerotic risk factors were also evaluated.

Plasma OPN levels and aortic PWV will be evaluated (besides a control group), in a group of patients affected by systemic sclerosis (SSc), as non-case group characterized by inflammation, ectopic calcification, vasculites, but without the articular involvement typical of RA patients.

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Materials and Methods

Patients and OPN measurements

41 RA patients (30 F, 11 M; mean age \pm SD: 58.8 \pm 10.3 years) were recruited who fulfilled the American College of Rheumatology (ACR, formerly, the American Rheumatism Association) 1987 revised criteria for RA (32). Duration of disease was 11 \pm 9 years (mean \pm SD). A non-case group (25 F, 3 M; 60.4 \pm 9.4 years) consisting of 28 scleroderma (SSc) patients, and 18 healthy volunteers (controls) (13F, 5M; 54.9 \pm 7.8 years) were also recruited.

Patients were recruited at the Division of Rheumatology (University of Pisa), healthy controls were either friends or neighbours of patients.

Exclusion criteria for patients and controls were: past coronary angina, previous myocardium infarction, cerebral ischemic stroke, hypertensive subject with values of the arterial pressure \geq 140/90 mm Hg or in antihypertensive drug treatment.

Patients with statin and non steroidal anti-inflammatory treatment were excluded.

Patients (RA and SSc) and controls were examined to evaluate the common cardiovascular (CV) risk factors for coronary artery disease such as: positive family history, smoking, diabetes mellitus, dislipidemia.

RA patient assessment included a standard assessment of disease activity and severity, including the following parameters: number of tender (TJC) and swollen joint count (SJC), SW44, General Health Status (GH), DAS28, ESR and CRP for the evaluation of disease activity; modified Health Assessment Questionnaire (HAQ) score (32), joint deformities, extraarticular features, erosions for the evaluation of disease severity.

The protocol was approved by the Local Research Ethical Committee, and written informed consent was obtained from each participant.

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Experimental procedures

Determination of OPN

For plasma OPN measurement, blood samples (3 ml) of RA, SSc patients and healthy controls were collected in EDTA-containing tubes early in the morning. Samples were centrifuged (400xg, 15 min, at 4°C) to remove cells and debris, and stored at -80°C until used. Human Osteopontin TiterZyme[®] Enzyme Immunometric Assay (EIA) kit was used to perform the quantitative determination of plasmatic OPN. The kit utilized a monoclonal antibody to human OPN.

Evaluation of inflammation markers, stress oxidative factors and common atherosclerotic risk

All these parameters were evaluated in RA, SSc patients and controls.

The unspecific markers of inflammation erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were analyzed. Rheumatoid factor (RF) was determined by nephelometry.

Atherosclerotic risk was evaluated by means of the quantitative analysis of total cholesterol, triglycerides, apolipoprotein apoA1, apoB and homocysteine.

Stress oxidative factors were also analyzed: lipid peroxides, malondialdehyde (MDA), the ferric reducing ability of plasma (FRAP) and ET-1. A sensitive enzyme immunoassay (EIA) for human endothelin(1-21) was used (BIOMEDICA GRUPPE). Lipid peroxides were analyzed by thiobarbituric acid reaction (benzie). FRAP was evaluated according to Benzie et al. (33). MDA was evaluated by means of the colorimetric assay for lipid peroxidation (OxisResearch, Biotech[®] LP586[™]).

Vascular function

Blood pressure (BP) was measured three times at 3 minutes interval by an automatic device (OMRON-950 CP) at the dominant arm and calculated as the mean value of the last two measurements. All measurements were performed after an overnight fast, with the subjects in supine position in a quiet, air-conditioned room (22-24°C).

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Arterial tonometry was performed by one trained operator (34) according to the international recommendations (12). A hand held probe was placed on the artery and 10-15 subsequent images were recorded. Aortic pulse wave velocity (PWV) was assessed (SphygmoCor) recording waveforms at the femoral and carotid site sequentially as distance to time ratio. Surface distance between the two recording sites was measured and a simultaneously recorded ECG was used as a reference frame to calculate wave transit time. Coefficient of variation for PWV was 13% (34).

Statistical analysis

Because of skewed distribution of OPN and aortic PWV, these variables were logarithmically transformed, and expressed as median and range.

Data were analyzed using the non parametric Mann-Whitney U test, and multiple regression. Spearman's correlation test was used for correlation analysis. A p value <0.05 was taken as the level of statistical significance. All statistical analyses were performed using SPSS statistical software, Version 9.

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Results

Clinical characteristic of patients with RA, SSc and controls are shown in table 1. The three groups were comparable for age, gender distribution, blood pressure and metabolic profile. CRP levels were greater in RA patients and similar in SSc patients and controls. Disease activity variables of RA patients are showed in table 2.

OPN levels were significantly ($p<0.05$) higher in RA patients (median: 9.93, range 4.36- 47.80 ng/ml) as compared to the other two groups, in which OPN levels were not different (SSc patients: 4.3, range: 2.1-19.7 ng/ml, healthy controls: 5.2; 4.1-9.4 ng/ml) (figure 1).

Patients with RA exhibited significantly ($p<0.01$) increased aortic PWV (median 8.1, range 4.7-16.4 m/s) as compared to controls (median 7.5; range 4.1-10.4 m/s) (figure 1). Aortic PWV was also significantly greater ($p<0.01$) in SSc patients (median 8.7; range 7.1-13.1 m/s) than controls but not different to RA patients (figure 1).

In RA patients, logistic regression showed that OPN levels above the median value was associated with a significant increases risk for having aortic PWV above the median value of 7.6 m/s (odds ratio of 4.3, 95% confidence intervals: 1.09-17.2). Moreover, only in RA patients, log-OPN was related to log-aortic PWV ($r=0.39$, $p<0.01$) (figure 2). In this group, log-OPN was related to log-CRP ($r=0.30$, $p<0.05$), age ($r=0.38$; $p<0.01$), and inversely related to total cholesterol ($r=-0.33$; $p<0.05$) and apo A ($r=-0.58$; $p<0.001$), but not to mean BP. On the other hand, log-aortic PWV was also related to age ($r=0.38$; $p<0.01$), mean BP ($r=0.53$; $p<0.0001$), but not to cholesterol levels or log-CRP.

No correlations were found between log-OPN and oxidative stress markers, the other common atherosclerotic risk (except cholesterol) and ET-1.

Multiple regression analysis ($r^2=0.49$), including possible confounders such as age, mean BP, cholesterol levels and log-CRP, showed that log-OPN remained a significant ($p<0.05$) predictor of log-aortic PWV.

Log-OPN was related to HAQ ($r=0.58$; $p<0.0001$) but not SW44 and DAS28. Aortic PWV was related to HAQ ($r=0.44$; $p<0.01$), while no correlations were found with SW44 and DAS28.

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Discussion

OPN is rapidly emerging as a major player in both physiological and pathological processes throughout the body as is evident from data that has emerged from studies conducted over the past few years. Literature showed that OPN is involved in the evolution and progression of multiple systemic diseases (35), it has been linked to the aetiopathogenesis of breast cancer (36), to osteoporosis and multiple sclerosis (37,38), inflammatory bowel diseases (21), psoriasis (19,24) and lupus erythematosus systemicus (23,39). There are evidences that OPN may be implicated also in RA pathogenesis (22,40).

Our aim was to study a possible relationship between OPN and arterial stiffness, measured by aortic pulse-wave velocity, in RA patients. PWV is considered the gold standard for assessing arterial stiffness and an independent predictor of CV events in high risk patients and in the general population (12).

Results showed that RA patients exhibited higher plasmatic OPN levels (approximately two times) compared to the healthy subjects and to the non-case SSc patients, suggesting a peculiar role for this protein in RA pathogenesis. This is supported by the associations found in RA patients between log-OPN and the clinical parameters HAQ, and log-OPN vs log-PWV.

RA patients exhibited higher aortic PWV compared to controls, according to literature (15) and similar to SSc patients, but the novelty is the association between log-OPN and log-PWV, which resulted significant only in RA patients.

Considering the known properties of OPN as regulator of inflammation and bone destruction in RA (26,27,41) we may suggest that OPN might be a point of contact between inflammation and the consequent articular damage and atherosclerosis in RA. The lower observed OPN levels of non-case and healthy donors led us to speculate that this inflammatory and plaque destabilizing protein is peculiar in RA pathogenesis. This hypothesis is supported by the fact that patients with SSc, which themselves are characterized by inflammation, ectopic calcification, vasculites, but don't have

articular involvement, have similar OPN levels as compared to healthy subjects; moreover we have found that OPN levels correlated with PWV only in RA patients, even if PWV levels were similar in RA and SSc.

In multiple regression analyses, including the possible confounders such as age, mean BP, cholesterol levels and log-CRP, log-OPN remained a significant predictor of log-aortic PWV in RA patients.

Although the RA patients studied were free from overt CV disease, half of them had high levels of total cholesterol. A negative correlation was observed between OPN levels and total cholesterol, and OPN levels and apoA. Also Takemoto et al. (41) observed, as we did, a significant negative correlation between the plasma OPN level and serum total cholesterol concentration.

Literature suggests a role for OPN in RA pathogenesis, enhanced levels of OPN mRNA and proteins were found in synovial tissue from RA patients (26) and OPN deficiency has been showed to protect joints against destruction in induced arthritis mice (28).

Recently, a candidate treatment to suppress OPN expression, based on a novel murine anti-OPN, has been developed, and resulted of great potential in treatment of RA. In fact, administration of this anti-OPN (namely 23C3) to mice with collagen-induced arthritis (CIA) not only strongly suppresses the development of CIA but also decreases the severity of the existing arthritis (42).

Moreover, an OPN promoter polymorphism at -66G and -443C have recently been identified to be associated with carotid artery intima media thickness, which reflects generalized atherosclerosis and is predictive of future vascular events (43,44)

In conclusion our results suggest that patients affected by RA, free from cardiovascular risk factors and overt cardiovascular disease, showed high levels of plasmatic OPN, and that OPN levels correlated with clinical severity index and with a marker of atherosclerosis and CV disease like PWV.

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Moreover, in SSc patients, which are themselves characterized by ectopic calcification, inflammation, but differently by RA patients without bone erosion, the levels of OPN are much lower than RA patients' levels. Besides in these patients OPN is not related to PWV.

We hypothesize an active role of circulating OPN in RA pathogenesis and an unfavourable cardiovascular risk factor in patients with RA. OPN might have a role in perpetuating inflammatory state as a promoter of cardiovascular disease in RA and might characterize the cardiovascular risk together to the classic parameters. Our results suggest this protein might represent a bridge protein between inflammation, the consequent articular damage, atherosclerosis and cardiovascular risk in RA patients.

The authors declare that they have no competing interests.

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Figure legends.

UNCORRECTED PROOF Fig. 1. Box plots show plasma osteopontin (OPN) levels and carotid to femoral pulse wave velocity (PWV) (median and interquartile range), in rheumatoid arthritis (RA), systemic sclerosis (SSc) and controls.

OPN: RA vs SSc: $p < 0.05$, RA vs controls: $p < 0.05$, SSc vs controls: $p = \text{n.s.}$

PWV: RA vs SSc: $p = \text{n.s.}$, RA vs controls: $p < 0.01$, SSc vs controls: $p < 0.01$

Fig. 2. Correlation between log-transformed aortic pulse wave velocity (PWV) and log-transformed osteopontin OPN in RA patients ($r = 0.40$, $p < 0.01$).

Tab. 1. Demographic characteristics of patients with rheumatoid arthritis (RA), Systemic Sclerosis (SSc) and healthy controls (results are mean±SD or median; range).

	RA patients (n=41)	SSc patients (n=28)	Controls (n=18)
Age (years)	57.2±9.9	55.7±9.4	54.9±7.8
Sex (males/females)	30 / 11	25 / 3	13 / 5
Smokers	6	4	6
Body mass index (kg/m ²)	24.9±3.5	25.4±3.0	24.1±2.3
Systolic blood pressure (mmHg)	129.8±17.5	128.5±16.8	126.1±12.1
Diastolic blood pressure (mmHg)	75.4±10.8	73.4±8.7	78.9±8.5
Plasma glucose (mg/dl)	88.7±7.7	89.4±9.1	90.0±8.6
Total cholesterol (mg/dl)	195.0±42.6	201.9±40.5	206.1±37.9
HDL cholesterol (mg/dl)	61.6±18.3	61.2±14.5	63.2±14.2
Triglycerides (mg/dl)	100 (43-241)	105 (41-219)	113 (44-214)
C reactive protein (mg/dl)	1.37 (0.6-7)	0.51 (0.30-2.92)	0.37 (0.30-1.63)

Tab. 2. Disease activity variables of patients with RA (mean±SD).

HAQ (Health Assessment Questionnaire) score	0.94 ± 0.69
DAS 28 (Disease Activity Score)	3.60 ± 1.42
GH (General Health Status)	53.68 ± 21.20
SW44 (swelling joint)	6.65 ± 6.98

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