

# IL-8 but not other biomarkers of endothelial damage is associated with disease activity in patients with ankylosing spondylitis without treatment with anti-TNF agents

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**Abstract** The objective of the study was to investigate the association between IL-8 and other biomarkers of endothelial dysfunction (MCP-1, V-CAM, I-CAM) and the disease activity scores in a sample of 54 patients with ankylosing spondylitis (AS) without use of biological agents. Fifty-four AS patients without treatment with anti-TNFs agents between 18 and 80 years old, who met modified New York criteria and at the same time the axial ASAS criteria, were evaluated using an epidemiological questionnaire that included among others clinical data, BASDAI, BASFI, ASQoL, ASDAS and plasma levels of CRP, ESR, MCP-1, IL-8, ICAM-1 and VCAM-1. IL-8 varied in proportion to disease activity rates (BASDAI and ASDAS)  $p < 0.05$ , being strongly correlated with the disease activity. The levels of adhesion molecules I-CAM and VCAM, as described in other studies, were positively correlated with predisposing factors for cardiovascular disease. IL-8 has shown to be strongly correlated with clinical markers of disease activity and inflammatory activity and may be an additional variable to the overall assessment of the activity of the AS.

**Keywords** IL-8 · MCP-1 · VCAM-1 · ICAM-1 · Biomarkers · Endothelial damage · Ankylosing spondylitis

## Introduction

Ankylosing spondylitis (AS) is a chronic autoimmune inflammatory disease, typically with axial joint involvement. Its prevalence is higher among young men, Caucasians and HLA-B27 positives [1, 2]. Inflammatory back pain (IBP) associated with morning stiffness is the main symptom of the disease [1, 3]. Although it does not occur in all patients with AS, extra-articular manifestations are responsible for increased morbidity and mortality. High incidence of cardiovascular mortality in patients with AS has stimulated researchers to the study of possible etiologic or risk factors involved [4]. Han et al. [5] found an increased risk of atherosclerosis by 1.5 times in a cohort of patients with AS compared to the controls.

The cardiovascular risk in autoimmune diseases is multifactorial. Traditional cardiovascular risk factors have been reported to be more prevalent in patients with rheumatic diseases as smoking, hypertension, low high-density lipoprotein (HDL)-cholesterol levels, diabetes and obesity. Furthermore, some inflammation factors are being correlated with accelerated atherogenesis. The C-reactive protein is the well-established factor related with the low-grade inflammation of the vessels [6] but reduced plasma levels of lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) and apoprotein A-I also predispose to coronary heart disease [7]. Specifically in AS, the hypertension [8], low levels of HDL-cholesterol and apoA-I [9] are correlated with increased cardiovascular risk.

Currently, the endothelium is considered the main regulator of vascular homeostasis and pathogenesis of atherosclerosis is closely related to systemic inflammation, autoimmunity, chronic diseases and dyslipidemia [10]. Lipoproteins, activated neutrophil, platelets and other pro-inflammatory factors play an important role in the

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inflammation in autoimmune diseases [11, 12]. Atherosclerosis has been associated with markers of systemic inflammation such as CRP, fibrinogen, and recently, biomarkers such as cytokines, chemokines, adhesion molecules and proteases [13, 14]. Due to biological variability and availability, many of these biomarkers, such as MCP-1, IL-8, V-CAM, I-CAM, have shown contradictory results, which somehow still limits their measurements to clinical research. IL-8, a member of the superfamily of chemokines CXC [15], was found increased in studies approaching the role of cytokines in the atherosclerotic process [16]. Its production is stimulated by the action of TNF alpha, and most of its actions depend on the interaction with neutrophils [17] and monocytes [18]. IL-8 also stimulates proliferation and migration of smooth muscle endothelial cells [19]. MCP-1 (monocyte chemoattractant protein-1) is a molecule belonging to the family of C-C chemokines  $\beta$ , which plays an important role in inflammation because of its power attractor on monocytes. MCP-1 interacts with the CCR2 receptor of these cells stimulating tropism to the inflammatory site [20], and it is secreted by endothelial cells, smooth muscle cells, fibroblasts and immune cells such as T lymphocytes and macrophages located in the stimulus place [21]. The adhesion molecules VCAM-1 and ICAM-1 are stimulated by inflammatory cytokines, expressed in the luminal surface of endothelial cells or soluble in plasma, and mediate the adhesion of various types of leukocytes to the endothelial surface. The objective of this study was to investigate the association between the levels of IL-8 and other biomarkers of endothelial dysfunction such as MCP-1, V-CAM, I-CAM with disease activity scores (BASDAI, ASDAS); the index of quality of life ASQoL, factors related to cardiovascular disease and the functional activity score (BASFI) in a sample of patients with AS.

## Materials and methods

### Study population

Fifty-four patients with AS followed between December 2009 and February 2010 at the Outpatient Spondylarthritis Clinic of Federal University of Paraná (UFPR) were initially recruited for this study and submitted to an informed consent (IC). Inclusion criteria were age between 18 and 80 years, the presence of AS according to the modified New York criteria [22] and also axial ASAS at the same time [23]. The exclusion criteria were the use of anti-TNF agents or other biologics, signs of acute inflammation or infectious disease, the presence of other concomitant autoimmune diseases, the presence of malignancy, liver dysfunction or diabetes. At the recruitment, patients were categorized according to age, sex, weight, height, smoking, diabetes mellitus (DM), hypertension (HBP), cardiovascular

disease, dyslipidemia, cerebrovascular disease, peripheral vascular disease and the use of concomitant medication. No control group was included because the main purpose of the study was to correlate the level of these endothelial markers, mainly IL-8 with specific disease activity scores in AS. All patients were using NSAIDs for more than 1 year, and no one was using methotrexate for axial disease. Two patients were being treated with sulfasalazine, and none of them was taking steroids.

### Measures

After the initial consultation, patients who agreed and signed the IC were submitted to the proposed methodology. The assessment of disease activity and functional status were performed by using protocols BASDAI and BASFI. A questionnaire of quality of life in AS (ASQoL) was also applied. After responding to the questionnaires, all of them answered a clinical and epidemiological survey. Then, patients were examined with a specific focus for clinical signs of AS. After the physical examination, two blood samples were collected from each patient. The tubes with blood samples were stored in a refrigerator at  $-80^{\circ}\text{C}$  and were later transported to the laboratory of the Health Sciences Research Center at the Catholic University of Paraná.

Samples of patients serum were separated by immediately centrifugation (1,500g for 10 min), divided into tubes and stored at  $-80^{\circ}\text{C}$  until the analysis. Plasma levels of MCP-1, IL-8, ICAM-1 and VCAM-1 were measured by ELISA method (enzyme-linked immunosorbent assay) using commercial kits with antibodies (R&D Systems, Minneapolis, Minn., USA). The concentrations of chemokines (pg/ml) and adhesion molecules (ng/ml) were calculated with reference to standard curves performed with the corresponding recombinant molecule. The measurement range of the ELISA system was 31.25–2,000 pg/ml for MCP-1 and IL-8 and 0.03–2 ng/ml for ICAM-1 and VCAM-1. When the values were outside the expected range, the samples were previously diluted. The intra-assay coefficients of variation (CV) for MCP-1, IL-8, ICAM and VCAM-1 were 6.0, 8.0, 7.9 and 8.2 %, respectively, and inter-assay CV 6.2, 7.7, 7.7 and 8.1 %, respectively. There were also assayed in the same blood tests CRP, ESR, total cholesterol, HDL-cholesterol, triglycerides and glucose. ASDAS CRP was calculated only after the release of ultra-sensitive CRP result.

### Statistical analysis

The results obtained in the study were expressed as averages, median, minimum values, maximum values and standard deviation (quantitative variables) or as frequencies and percentages (qualitative variables). All variables were normally distributed according to Shapiro–Wilk test.

To compare the groups regarding quantitative variables, it was considered the nonparametric Mann–Whitney test or the Kruskal–Wallis nonparametric test. To assess the association between two quantitative variables, it was estimated the Spearman correlation coefficient.  $p$  values  $< 0.05$  were considered statistically significant. The data were organized into an Excel spreadsheet and analyzed with the computer program Statistica v.8.0.

## Results

Fifty-four patients were evaluated (09 women and 45 men). The average age was  $43 \pm 11.6$  years. Forty-six patients were regularly taking NSAIDs, and none was using anti-TNF. The sample general characteristics are presented in Table 1. The measurements of the 4 biomarkers were correlated to the indexes of disease activity such as CRP, VAS, ASDAS and BASDAI. There were also correlated with factors related to cardiovascular disease such as HBP, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), waist circumference (WC), total cholesterol (TC), DM, dyslipidemia, history of coronary artery disease (CAD Hx), smoking, alcohol consumption, as well as the functional activity BASFI score.

We observed that the rate of MCP-1 showed an inverse correlation to the measurement of CRP (Spearman correlation coefficients of  $-0.33$  with  $p = 0.018$ ) and factors related to cardiovascular disease, such as HBP ( $p = 0.01$ ) and CAD Hx ( $p = 0.058$ ).

**Table 1** Sample general characteristics

Variable	$n$	Average	Standard deviation
Age	54	43.0	11.6
Time to diagnosis (months)	54	121.5	96.8
MCP-1 (Pg/ml)	54	201.3	124.9
IL-8 (Pg/ml)	54	134.6	331.8
VCAM-1 (Ng/ml)	54	385.5	169.9
ICAM-1 (Ng/ml)	54	79.4	42.2
SBP	54	123.1	20.0
DBP	54	82.5	12.5
BMI	52	25.0	4.2
WC (cm)	54	92.7	9.2
Total cholesterol	52	194.9	31.3
CRP-U <sub>s</sub>	52	2.7	2.9
VAS by med	54	5.6	3.0
ASQoL	52	10.1	5.8
BASFI	54	5.7	3.3
BASDAI	54	4.9	2.5
ASDAS	54	2.4	1.4

**Table 2** Relation between V-CAM, CRP and factors related to cardiovascular disease

Variables	References	$n$	Average	$p$ value
HBP	No	39	353.4	0.018
	Yes	15	468.9	
CRP	$\leq 0.6$	7	331.3	0.511
	$> 0.6$	47	396.6	
Dyslipidemia	No	48	372.5	0.178
	Yes	6	489.2	
CAD Hx	No	48	378.9	0.122
	Yes	6	438.4	
Smoking	No	42	372.3	0.544
	Yes	12	431.8	

**Table 3** Relation between IL-8, BASDAI and ASDAS

Variables	$n$	Spearman correlation coef.	$p$ value
BASDAI	54	0.34	0.015
ASDAS	54	0.35	0.036
CRP	54	0.05	0.728*
VAS back pain	54	0.09	0.539*
ASQoL	54	0.17	0.221*

\* Without statistical relevance

V-CAM was increased in hypertensive patients, but not correlated with CRP or BASDAI (Table 2).

I-CAM was positively correlated with the presence of factors such as HBP, DBP, DBP, dyslipidemia, smoking and CAD Hx, but not with disease activity.

IL-8 varied proportionally with two indices of disease activity analyzed, BASDAI and ASDAS (Table 3).

## Discussion

No control group was included in this study. The main purpose was to correlate the level of these endothelial markers, mainly IL-8 with disease activity in a sample of patients with AS without use of anti-TNFs agents.

Our selected sample did not include patients using anti-TNFs in order to facilitate the analysis of the criteria for inflammatory activity, although most of them were in regular use of NSAIDs. There is considerable evidence that the biomarkers evaluated in our study are very well correlated with endothelial dysfunction in case controlled studies in AS and other rheumatic diseases as well. The link between high disease activity and the onset of cardiovascular disease in patients with ankylosing spondylitis is under investigation.

While employed as a marker of changes and activity in the endothelium, IL-8 has been presented with conflicting

and inconclusive results in the medical literature. While some studies show correlation between patients with angina and increased plasma levels of IL-8 [19], or even high levels of IL-8 in patients who later came to coronary artery disease [20], other studies suggest a protective effect of IL-8, delivering little attraction and little migration of neutrophils to the chemotactic site of atherosclerotic plaques via modulation by VCAM-1 [24].

IL-8 has also been studied in patients with AS, and its levels seem to be elevated when compared with controls, but it has not been compared with specific AS disease activity scores [25, 26].

In this study, IL-8 did not correlate with factors related to cardiovascular disease, SBP, DBP, BMI and TC (descriptive levels: 0.531, 0.585, 0.860, 0.463 and 0.934). IL-8 also did not correlate with BASFI functional activity score ( $p = 0.403$ ). However, levels of IL-8 were positively correlated and statistically significant to ASDAS and BASDAI ( $p$  value = 0.015) and curiously not statistically significant related to CRP (0.728) alone, VAS back pain (0.539) or ASQoL (0,221) (Table 2). Based on our results, we consider that IL-8 may be an additional useful marker of disease activity although it has not had a correlation with acute inflammatory activity when measured only by CRP.

Hwang et al. [27] demonstrated that ICAM-1 and E-selectin are potent biomarkers of atherosclerosis and cardiovascular damage, yet not so strong correlation was found with VCAM-1. A meta-analysis conducted by Malik et al. [28] demonstrated a significant association between levels of ICAM-1 and VCAM-1 and traditional risk factors for coronary disease. Corroborating with the same idea, the prospective study of Haim et al. [29] showed significant association between the concentration of soluble ICAM-1 and increased risk for future coronary events, but did not take into account several other factors. Thus, although it was not our primary objective, our results also reinforce what has been described in the literature, that is, the unquestionable quality of these molecules, particularly ICAM-1, as early markers of cardiovascular lesions.

In this study, we have shown that VCAM-1 is a good marker for HBP, while ICAM-1 is a good marker for DBP, smoking, HBP, dyslipidemia and CAD Hx but do not have association with specific disease activity scores of AS. Finally and the most important, IL-8 proved to be a good marker for disease activity, since it is associated only with BASDAI and ASDAS.

## Conclusion

Our results should be interpreted cautiously, all the observations are cross-sectional and do not provide evidence of causality or directionality. Some limitations

include the small sample size and the lack of analyses between medications used in the treatment and IL-8. On the other hand, we believe that the absence of a control group does not jeopardize our results. At the same time, a single temporary measure of four biomarkers and of three criteria for disease activity was performed to determine correlations between variables. The results of this study demonstrated that IL-8 can be used as a marker of inflammatory activity due to its statistically significant correlation with BASDAI and ASDAS.

**Conflict of interest** None.

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