

# Serum sCTLA-4 levels and clinical manifestations in ankylosing spondylitis patients

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## Abstract

**Objective:** T cell abnormal activation is thought to have a main role in the etiology of ankylosing spondylitis (AS). While cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) is suppressing the immune system, in previous studies serum soluble CTLA-4 (sCTLA-4) was detected at high amounts in autoimmune disorders. We sought to evaluate the association between soluble CTLA-4 in serum and disease activity in AS patients.

**Methods:** Thirty-eight patients with AS, 28 rheumatoid arthritis (RA) patients, and 27 disease-free controls were enrolled to the study. The levels of sCTLA-4 were determined for each participant using an enzyme-linked immunosorbent assay. The erythrocyte sedimentation rate (ESR), C-reactive peptide, and demographic characteristics were documented. The data were analyzed by using relevant statistical methods.

**Results:** In comparison with RA patients and controls, patients with AS showed high sCTLA-4 levels ( $p < 0.001$ ). The sCTLA-4 levels did not correlate with the severity of the disease in AS patients ( $p = 0.370$ ). The ESR levels and Bath Ankylosing Spondylitis Disease Activity Index were correlated in AS patients ( $p = 0.012$ ).

**Conclusion:** We evaluated the association between the disease severity of AS and sCTLA-4. Although, the correlation was not shown, sCTLA-4 was highest in the AS group. Further studies with larger samples should be completed to attain a better understanding of the AS etiology.

**Keywords:** Ankylosing spondylitis, sCTLA-4, clinical manifestations



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## Introduction

Ankylosing spondylitis (AS) is a type of inflammatory rheumatic disorder and a prototype of spondyloarthritis, characterized by axial skeleton and sacroiliac joint involvement. In the beginning of the disease, CD4<sup>+</sup> and CD8<sup>+</sup> T cells, macrophages, and elevated amounts of tumor necrosis factor alpha (TNF- $\alpha$ ) were detected in the sacroiliac joint. Two signals were needed by T cell for activation. In addition to the first antigenic signal presented via the major histocompatibility complex, a costimulatory signal is required. The costimulatory signal is produced by cooperation between CD28 attached to T cell and B7 molecules on antigen-presenting cells (1-3). The cytotoxic T lymphocyte-associated molecule-4 (CTLA-4, also termed CD152) on activated T cells connects to CD86 and CD80, and some of its inhibitory effects may be modulated by SH2-containing phosphatase (3, 4).

CTLA-4 attached to the cell surface is increased by activation of T-cell and a CD28-ligand association. CTLA-4 negatively regulates T-cell functions (5, 6). Because of CTLA-4-ligand interactions, early T-cell activation, regulatory T cells, and cell-cycle progression are suppressed, and IL-2 production, cyclin production, and T-cell receptor signal transduction are reduced (7-10).

Recent studies have shown increased levels of soluble CTLA-4 (sCTLA-4) in thyroiditis, myasthenia gravis, and systemic lupus erythematosus (SLE) patients (11-13). Toussiot et al. (1) reported a statistically significant increase in serum CTLA-4 levels in patients with spondyloarthritis. Furthermore, there was correlation between the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the levels of C-reactive peptide (CRP) and sCTLA-4 (1).

We aimed to determine whether levels of serum sCTLA-4 were indicative of clinical severity in AS patients, and to compare levels of serum sCTLA-4 between groups of participants.

## Methods

Thirty-eight participants diagnosed with AS, 28 patients diagnosed with rheumatoid arthritis (RA), and 27 disease-free controls were involved in this study. Written informed consent was collected from each participant. The Ethics Committee and Institutional Review Board approved the study. The patient and control groups' demographic and laboratory data such as sex, age, medical history, physical examination results, CRP and erythrocyte sedimentation rate (ESR) were collected. The BASDAI was used as a measure of disease activity. To measure the serum sCTLA-4 levels, we examined peripheral blood by using an enzyme-linked immunosorbent assay (ELISA), applying the CTLA-4 (Human) ELISA commercial kit (Abnova, Taipei City, Taiwan). Operations were performed with a fully automated ELISA Triturus device.

The Statistical Package for Social Sciences for Windows software, version 19 (SPSS Inc.; Chicago, IL, USA), was used for statistical analysis. The Kolmogorov-Smirnov test was used for quantitative data with a normal distribution. The independent t-test and Mann-Whitney U test were applied to make comparisons between the independent groups. A one-way analysis of variance and the Kruskal-Wallis test were applied to make comparisons between multiple groups. The Bonferroni-corrected Mann-Whitney U test and Fisher's least significant difference test were applied for post-hoc analysis. Pearson's chi-square and Fisher's exact tests were applied for the comparison of categorical data. The categorical data were expressed as percentages and numbers of patients (n). The data were examined at the confidence level of 95%, and p-values <0.05 were accepted as significant.

## Results

The demographic characteristics of participant groups are shown in Table 1. Up to quarter percent (n=10) of all AS patients received indomethacin, while the remaining patients were treated with naproxen, diclofenac, and meloxicam. Only a few patients were treated with sulfasalazine (n=5) and methotrexate (n=2). Participants who were receiving or had consumed anti-TNF- $\alpha$  agents were removed from the study due to possible confounding factor effects. The levels of serum sCTLA-4 in the AS group were higher than those in the disease-free control and RA groups (p<0.001). There was no significant difference between healthy control and RA patients (p>0.05). The serum sCTLA-4 levels and p values of the different groups are shown in Table 2.

The correlations between age (r=-0.382, p=0.001), disease duration (r=0.06, p=0.949),

**Table 1.** Demographic characteristics of the patients and control group

	Ankylosing spondylitis (n=38)	Rheumatoid arthritis (n=28)	Control group (n=27)
Male (n/%)	24 (63.2%)	14 (36.8%)	5 (17.9%)
Female (n/%)	23 (82.1%)	2 (7.4%)	25 (92.6%)
Age (years)	39.37 $\pm$ 12.11	58,32 $\pm$ 13,88	54.89 $\pm$ 12.94

**Table 2.** Serum sCTLA-4 levels in patients with AS and RA and in the control group

	Ankylosing spondylitis	Rheumatoid arthritis	Control group	p
sCTLA-4 Median	0.22	0.04	0.04	<0.001
IQR*	0.09	0.01	0.03	
p (AS-RA)<0.001; p (AS-Control)<0.001; p (RA-Control)>0.05				

Kruskal-Wallis test (post-hoc test: Bonferroni-corrected Mann-Whitney U Test,  $\alpha = 0.017$ ) sCTLA-4: soluble Cytotoxic T-Lymphocyte Associated Antigen-4; AS: ankylosing spondylitis; RA: rheumatoid arthritis; IQR: interquartile rang

**Table 3.** Demographic, biochemical, and clinical correlations of AS patients

		ESR	CRP	CTLA-4	Disease Duration	BASDAI†
Age	r	0.203	-0.012	-0.382	0.160	0.001
	p	0.005	0.866	0.001	0.065	1.000
ESR	r		0.310	-0.114	0.017	0.309
	p		0.001	0.129	0.846	0.012
CRP	r			0.136	0.128	0.730
	p			0.067	0.138	0.001
CTLA-4	r				0.006	0.115
	p				0.949	0.370
Disease Duration	r					0.192
	p					0.123

ESR: erythrocyte sedimentation rate; CRP: C-reactive peptide; sCTLA-4: soluble cytotoxic T-lymphocyte associated antigen-4; BASDAI: bath ankylosing spondylitis disease activity index

BASDAI (r=0.115, p=0.370), CRP (r=0.136, p=0.067), ESR (r=-0.114, p=0.129) and serum sCTLA-4 levels in AS patients are shown in Table-3. CRP and ESR levels were correlated with BASDAI scores.

## Discussion

Although the etiology of AS is unknown, several studies suggest T-cell autoreactivity, genetic and molecular associations, and infections contribute to its progression (14). Defects in coinhibitory molecules may cause autoimmune disorders. The sCTLA-4 molecule and various other costimulatory signal molecules may have a role in the pathogenesis of AS and correlate with clinical findings (15). This hypothesis was initially supported by the demonstration of lymphocyte hyperactivation in CTLA-4-deficient mice. It was reported that CTLA-4-deficient mice died by one month of age because of lymphocytic system disorder and multi-organ lymphocytic infiltration (16, 17).

Previous studies indicate that levels of sCTLA-4 are high in most autoimmune originated disorders. The sCTLA-4 is considered as double-edged molecule which means ability of both activation and inhibition effect on T-cell. Toussiot et al. (1) reported correlations between levels of serum sCTLA-4, intracellular CTLA-4, CTLA-4 mRNA, lymphocytes, and clinical characteristics of the disease in 165 participants with spondyloarthritis (SpA) taking immunosuppressive drugs. The findings were compared with participants with 71 RA and 88 disease-free controls. Higher sCTLA-4 levels were detected in patients with AS compared with RA and disease-free controls, and the differences were statistically significant. There was a positive correlation between BASDAI and sCTLA-4. So, serum sCTLA-4 will be used as a biomarker to measure activity of the disease in AS. Although high levels of sCTLA-4 are detected in patients with autoimmune disease. The plasticity of sCTLA-4 may cause different outcomes in clinical severity of the disease (18).

We have detected higher levels of sCTLA-4 in AS patients than in RA and control groups ( $p=0.001$ ), but no correlation between clinical severity of disease and sCTLA-4 levels ( $r=0.115$ ,  $p=0.115$ ).

Serum sCTLA-4 levels of the AS group were higher than those in the healthy control and RA groups ( $p<0.001$ ). There was no significant difference between the healthy control and RA groups ( $p>0.05$ ). The mean duration of the disease was 19 months in our study, which is less compared with Toussirot's study (9.2 years) (1). Different results may be due to CTLA-4 polymorphism or genetic variations. CTLA-4 gene allele polymorphism and cytokine genes that control inflammation can be variable in patients with AS (2). The differences may be due to the use of a different ELISA kit and equipment (Abnova, Taipei City, Taiwan) or different immunosuppressive therapies. Also, all of the AS patients received non-steroidal anti-inflammatory drugs and 7 of them had an immunosuppressive therapy (sulfasalazine or methotrexate). In our study, the patients were receiving one or more disease-modifying anti-rheumatic drugs. The patients using anti-TNF therapy were excluded. This use of drugs by patients with AS is because of their suppressive effects on disease activity; however, the effects of drugs on sCTLA-4 levels are unknown.

There was not a statistically significant difference between the serum sCTLA-4 levels in the 28 patients with RA compared to the healthy group ( $p>0.05$ ). A significant positive correlation has been reported between Disease Activity Score (DAS-28) and sCTLA-4 levels in RA patients (19). Furthermore, immunosuppressive drugs such as leflunomide may reduce the levels of serum sCTLA-4 and other costimulatory molecules. In our study, low sCTLA-4 levels in patients with RA could be associated with genetic variation or immunosuppressive therapy. 24 of the RA patients used methotrexate, 4 of the RA patients received leflunomide, and 26 of them received combined immunosuppressive therapy (methotrexate+sulfasalazine, methotrexate+hydroxychloroquine, or methotrexate+sulfasalazine+hydroxychloroquine).

The small numbers of patients and controls are major limitations of the study. We investigated the serum sCTLA-4 levels in the pathogenesis of AS and established a significant difference in the levels of serum sCTLA-4 between patients with AS, RA, and a disease-free control group. Earlier diagnosis is substantial for patients with

AS and laboratory tests with high specificity for disease detection are needed. In our study, although higher sCTLA-4 levels were found in AS patients than in the control groups, there was no correlation between clinical severity of disease and serum sCTLA-4 levels. An increase in CTLA-4 levels may be involved in the pathogenesis of AS. The lack of correlation between CTLA-4 levels and disease activity may exhibit the complex mechanism of the disease, and the existence of other possible inflammatory molecules related to disease activity that we couldn't examine. Further studies are required to evaluate the potential role of serum sCTLA-4 in terms of early diagnosis of AS and its impact on the severity of the disease.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of Adnan Menderes University.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

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