

## Cytokine profiles in axial spondyloarthritis

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### Abstract

**Objectives:** Current studies concentrate on the cytokine network and its role in the pathogenesis of spondyloarthritis (SpA). In this study, we analyzed whether the serum cytokine profile (interleukins: IL-10, IL-11, IL-12, IL-15, IL-17, IL-23 and IL-33) correlates with demographic data, clinical manifestations, disease activity and treatment outcome in a group of patients with axial spondyloarthritis.

**Material and methods:** Forty-nine patients with an established diagnosis of axial spondyloarthritis (aSpA) and 19 healthy volunteers as controls were enrolled in the study. Clinical evaluation included patient's medical history, 44 joint count, back pain intensity and global disease activity in the preceding week (VAS), the duration of morning stiffness and blood tests. Disease activity was assessed using BASDAI and ASDAS-CRP. Serum concentration of IL-10, IL-11, IL-12, IL-15, IL-17, IL-23 and IL-33 was determined.

**Results:** In patients with aSpA, elevated serum concentration of IL-10, IL-15, IL-17 and IL-23 was detected. In the aSpA group we detected higher values of serum concentration of IL-23 and IL-33 in the subgroup with anterior uveitis (83.1 ±184.0 pg/ml vs. 14.0 ±17.1 pg/ml,  $p < 0.0001$  and 45.5 ±71.9 pg/ml vs. 18.4 ±14.3 pg/ml,  $p < 0.0001$ , respectively). Additionally, in the subgroup with peripheral arthritis, elevation of serum concentration of IL-12 (249.3 ±246.9 pg/ml vs. 99.9 ±105.9 pg/ml,  $p = 0.0001$ ) was detected. Patients with preradiological SpA had higher serum concentration of IL-17 than patients with established diagnosis of AS (6.37 ±8.50 pg/ml vs. 2.04 ±2.98 pg/ml,  $p = 0.0295$ ). No differences in serum concentration of analyzed cytokines were found between the subgroup with low to moderate disease activity and the subgroup with high to very high disease activity.

**Conclusions:** We report that in aSpA patients, compared to controls, elevated serum concentrations of IL-10, IL-15, IL-17 and IL-23 were observed. Some cytokines may predispose to a more severe course of aSpA.

**Key words:** cytokines, spondyloarthritis, uveitis.

### Introduction

The spondyloarthritides (SpA) are a group of diseases which can be classified either as peripheral SpA or as axial SpA; the latter is characterized by predominant involvement of the spine or sacroiliac joints or both. Axial SpA (aSpA) includes nonradiographic aSpA (nr-aSpA) and ankylosing spondylitis (AS), which is a radiographic form of axial SpA. Both diseases can be considered as two different stages of the same disease. Numerous

studies have shown an overall progression rate from nr-aSpA to AS by about 10–12% over the span of two years. Consequently, the results of the studies confirm the concept of axial SpA as one disease [1, 2]. Extra-articular manifestations of the disease include e.g. enthesitis, uveitis, dactylitis or heart involvement. One of the largest studies analyzing a large panel of biomarkers in AS indicates that higher disease activity and impaired function in AS patients are associated with elevated serum levels of the metalloproteinases MMP-8 and MMP-9,

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hepatocyte growth factor and the chemokine CXCL8 [3]. Current studies concentrate on the cytokine network and its role in the pathogenesis of SpA. Recent data confirmed an important role of the cytokine axis IL-17/IL-23 in the pathogenesis of SpA [4]. Interleukin-23 can upregulate IL-17 production by different cells, e.g.  $\alpha/\beta$  T cells,  $\gamma/\delta$  T cells, mast cells or innate lymphoid cells. IL-17 has strong proinflammatory properties. Elevated levels of IL-17 and IL-23 were found in sera of AS patients when compared to healthy controls [5]. Another relevant cytokine, IL-33, is a member of the IL-1 cytokine family and represents a potential therapeutic target in different diseases of autoimmune origin. In AS, serum levels of IL-33 reflect disease activity. Interleukin-33 and its ST2 receptor mRNA significantly differ between AS patients and controls [6]. Londono et al. [7] also emphasize cytokines as potential biomarkers of prognosis in AS, indicating IL-6, IL-1 $\alpha$  and lipopolysaccharide-binding protein as markers of poor prognosis. Still there are limited data concerning the possible role of other cytokines in the pathogenesis of AS.

In this study, we analyzed whether the serum cytokine profile (IL-10, IL-11, IL-12, IL-15, IL-17, IL-23 and IL-33) correlates with demographic data, clinical manifestations, disease activity, laboratory parameters and treatment outcome in a group of patients with axial spondyloarthritis.

## Material and methods

Forty-nine patients with an established diagnosis of aSpA and 19 healthy volunteers as controls were enrolled in the study. When recruiting participants we used the following exclusion criteria: pregnancy or breastfeeding; clinically significant impairment of hepatic and renal function; alcohol abuse; hepatotropic viral infection; treatment-resistant infection; ongoing history of cancer if no remission was achieved; uncontrolled diabetes. The study protocol was accepted by the Wrocław Medical University Ethic Committee. Written informed consent was obtained from all participants before they entered the study.

Clinical evaluation was based on the patient's medical history, the number of painful and swollen joints (44 joint count), back pain intensity and global disease activity in the preceding week, as assessed by the patient on a 100 mm visual analogue scale (VAS), and the duration of morning stiffness. Blood tests, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) concentration, blood cell count (BCC), and creatinine levels, were performed in a certified commercial laboratory.

Disease activity was assessed using BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) and ASDAS-CRP (Ankylosing Spondylitis Disease Activity Score) [8–10].

Function was assessed using the Bath Ankylosing Spondylitis Functional Index (BASFI), which comprises 10 questions on function and the patient's ability to cope with everyday life [11].

Answers to both BASDAI and BASFI were given on a 10 cm VAS.

The patients were divided into two groups based on the disease activity. One group comprised patients with low and moderate disease activity (ASDAS < 2.1); the other comprised patients with high and very high disease activity (ASDAS  $\geq$  2.1). Moreover, patients with BASDAI < 4 were compared to those with BASDAI  $\geq$  4.

Serum concentration of IL-10, IL-11, IL-12, IL-15, IL-17, IL-23 and IL-33 was determined with commercial ELISA Kits (Diacclone SAS, F-25020 Besanson Cedex, France).

Statistical methods: normality of distribution was tested using the Kolmogorov-Smirnov test. Independent quantitative variables consistent with normal distribution were compared using Student's *t*-test. Results at  $p < 0.05$  were considered statistically significant. All tests were performed using the STATISTICA version 10 software.

## Results

Forty-nine patients (mean age 40.6  $\pm$ 13.4 years, range 24–75 years) fulfilling ASAS (Assessment of SpondyloArthritis international Society) criteria for axial spondyloarthritis (aSpA) [12, 13] and 19 healthy volunteers (mean age 40.4  $\pm$ 10.4 years, range 28–62 years) as a control group were recruited.

Clinical characteristics of aSpA patients are presented in Table I.

In 20% of patients pre-radiological SpA was diagnosed. In 80% of patients the diagnosis of ankylosing spondylitis (AS) was established according to modified New York criteria of AS [14]. Enthesitis was found in 29% of SpA patients, anterior uveitis in 16%, and peripheral arthritis in 49%.

Most of the SpA patients received non-steroidal anti-inflammatory drugs (NSAIDs) (84%), while 26 (53%) were receiving disease-modifying drugs (DMARDs) (21 sulfasalazine, 5 methotrexate) and 9 patients biologics (tumor necrosis factor inhibitors). Thirteen (26%) patients were treated with a stable dose of glucocorticosteroids (GCs).

Serum concentration of IL-10, IL-11, IL-12, IL-15, IL-17, IL-23 and IL-33 in patients with SpA and in the control group was analyzed. In patients with aSpA elevated serum concentration of IL-10, IL-15, IL-17 and IL-23 was detected (Table II).

In the aSpA group we detected higher serum concentration of IL-23 and IL-33 in the subgroup with anterior uveitis (83.1  $\pm$ 184.0 pg/ml vs. 14.0  $\pm$ 17.1 pg/ml,  $p < 0.0001$  and 45.5  $\pm$ 71.9 pg/ml vs. 18.4  $\pm$ 14.3 pg/ml,  $p < 0.0001$ ,

**Table I.** Clinical characteristics of patients with axial SpA

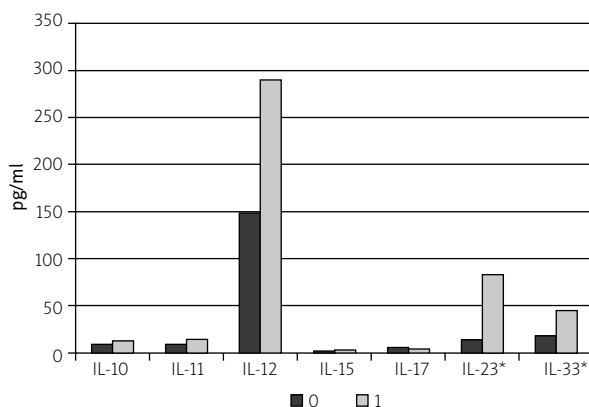
Parameter	Patients with axial SpA (n = 49)
Age [years]*	40.6 ±13 (24–75)
Gender	M 35, F 14
aSpA duration [months]*	59.6 ±71.5 (1–260)
Age of aSpA diagnosis [years]*	35 ±11.2 (17–65)
Age at the onset of aSpA*	29 ±10.7 (12–58)
Delay of diagnosis [years]*	6.5 ±6.9 (0–30)
ESR [mm/h]*	30 ±24 (2–102)
CRP [mg/dl]*	24.6 ±36 (0.5–201)
BASDAI*	4.8 ±2.6 (0.3–9.6)
VAS back pain [mm]*	48 ±30 (1–98)
BASFI*	4.9 ±2.7 (0.1–9.9)
ASDAS – CRP*	3.0 ±1.2 (0.6–5.7)
Disease activity#	
ASDAS < 2.1	8 (16%)
ASDAS > 2.1	41 (84%)
HLA B27 positive (%)	92%

\*Results presented as: mean ± SD (range)

# Results presented as: number (percentage)

n – number of patients; M – males; F – females; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; BASDAI – Bath Ankylosing Spondylitis Disease Activity Index; VAS – visual analogue scale; BASFI – Bath Ankylosing Spondylitis Functional Index; ASDAS – Ankylosing Spondylitis Disease Activity Score; HLA-B27 – human leukocyte antigen B27; aSpA – axial SpA

respectively) (Fig. 1). Additionally, in the subgroup with peripheral arthritis we detected elevation of serum concentration of IL-12 (249.3 ±246.9 pg/ml vs. 99.9 ±105.9 pg/ml,  $p = 0.0001$ ) (Fig. 2). Patients with preradiological



**Fig. 1.** Serum cytokine concentration in axial spondyloarthritis patients with (1) and without (0) anterior uveitis.

\* $p < 0.0001$

**Table II.** Serum concentrations of selected cytokines in patients with aSpA and in control group

Interleukin [pg/ml]	aSpA group (n = 49)	Control group (n = 19)	p-value
IL-10	9.18 ±10.47	1.36 ±0.88	0.0014
IL-11	9.85 ±65.15	3.43 ±5.37	NS
IL-12	173.1 ±201.3	84.6 ±121.9	NS
IL-15	1.96 ±0.66	0.02 ±0.06	0.0000
IL-17	3.84 ±6.66	0.00 ±0.00	0.0176
IL-23	14.27 ±15.96	2.12 ±1.37	0.0012
IL-33	22.42 ±31.92	28.24 ±30.96	NS

Results presented as: mean ± SD

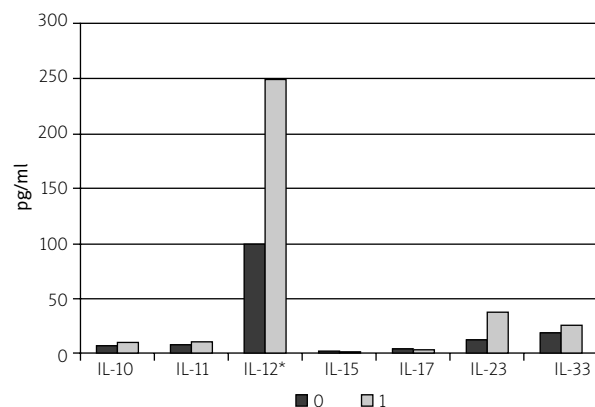
NS – not significant

SpA had higher serum concentration of IL-17 than patients with established diagnosis of AS (6.37 ±8.50 pg/ml vs. 2.04 ±2.98 pg/ml,  $p = 0.0295$ ) (Fig. 3).

No difference in serum concentration of analyzed cytokines was found between the subgroup with low to moderate disease activity and the subgroup with high to very high disease activity. We detected no influence of treatment on the analyzed cytokine profile.

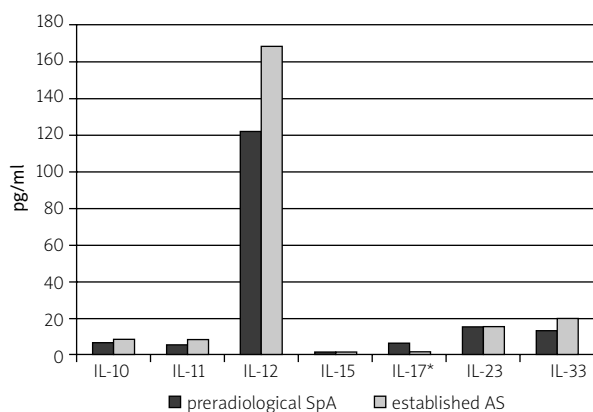
## Discussion

The main result of the present study confirmed the relationship between organ involvement and cytokine profile in aSpA. In our study serum concentrations of IL-23 and IL-33 were significantly higher in the subgroup of patients with aSpA and correlated with susceptibility to the occurrence of eye involvement, manifesting as anterior uveitis. Interleukin-23 seems to be a key mediator in the inflammatory process in axial SpA by inducing production of proinflammatory cytokines including



**Fig. 2.** Serum cytokine concentration in axial spondyloarthritis patients with (1) and without (0) peripheral arthritis.

\* $p = 0.0001$



**Fig. 3.** Serum cytokine concentration in patients with preradiological spondyloarthritis (SpA) and established spondyloarthritis (AS).

\* $p < 0.05$

IL-6, IL-8, IL-17 and TNF- $\alpha$ . Recent studies showed that the IL-23 signaling pathway influences the function of Th17 and Th1 cells in patients with AS [15]. Interleukin-23 is a member of the IL-12 family, being a heterodimeric cytokine and having a common p40 subunit with IL-12. Earlier data also indicate a possible relationship between IL-23 and uveitis. Dong et al. [16] analyzed the association between single-nucleotide polymorphisms (SNPs) of the IL-23 receptor (IL-23R) and AS, and found that in the Chinese population rs17375018 GG of IL-23R was associated with AS concomitant with uveitis. There have been studies examining IL-12/IL-23 inhibitors in experimental autoimmune uveoretinitis that confirm the data [17]. The role of polymorphism of IL-23R (whose ligand is IL-23) in the pathogenesis and clinical manifestation of AS remains unclear [16]. We think that elevated serum concentrations of IL-23 could be considered as a risk factor of organ (e.g. uveitis) involvement in AS.

Our results are consistent with earlier data and confirm the observation that serum levels of IL-23 are higher in SpA patients than in healthy controls [18] and that they do not correlate with disease activity and response to treatment in SpA patients [19, 20]. According to our results, increased levels of IL-33 may predispose to the inflammatory process affecting the vascular membrane of the eye. The data concerning the relationship between IL-33 concentration and uveitis in AS or SpA is limited. It was reported previously by Li et al. [21] that elevated levels of IL-33 were related to eye involvement in AS. Recent data suggest that IL-33 and ST2 may play an important role in experimental autoimmune uveitis and may represent a novel therapeutic option in the treatment of uveitis [22]. Higher concentrations of IL-33 and its receptor ST2 were also observed in patients with peripheral arthritis and correlated with BASDAI

and inflammatory parameters (ESR, CRP) [6, 21]. Serum concentrations of IL-33 were significantly higher in AS patients than in healthy subjects. In the present study we did not confirm this observation. The serum concentration of IL-33 in our aSpA population did not differ significantly between the study group and control group. Moreover, there was no association between IL-33 concentration and any disease activity parameters.

Interleukin-17 is one of the key mediators in the pathogenesis of SpA. It acts as a proinflammatory cytokine by increasing production of other proinflammatory cytokines (IL-6, IL-8) and MMPs, by upregulating the innate immune response and influencing angiogenesis and chemotaxis [23, 24]. We found elevated serum levels of IL-17 in aSpA patients. Interleukin-17 may play a role in the development of enthesitis and peripheral joint involvement [5]; however, we did not find such a relation in our study population. The increased levels of IL-17 and IL-23 in sera of SpA patients support previous data suggesting the role of the IL-17/IL-23 axis in pathogenesis and development of SpA [15]. Higher concentration of IL-17 in preradiological SpA compared to AS patients may suggest its role in active formation of future radiological changes. Recent reports on the effectiveness of blockade of the IL-23/IL-17 axis in treatment of AS confirm the important role of those cytokines in pathogenesis of AS [25].

We observed higher serum concentration of IL-12 in a subgroup with peripheral arthritis. Appel et al. [26] reported that in subchondral bone marrow and fibrous tissue of facet joints in the spine, IL-12 expression is lower than IL-23 expression. Although, as reported by Appel et al., both cytokines are increased in AS patients, our results suggest its predominant role in peripheral changes.

One hypothesis concentrates on the HLA B27-related response leading to the production of IL-23, which is an activator of Th17 T cells (a subtype of CD4+ T cells), which in turn are responsible for enhanced production of IL-17 [27]. It was also proven that macrophages isolated from peripheral blood of AS patients more strongly produce IL-23 (a member of the IL-12 family) in response to lipopolysaccharide [28]. As a result, IL-17 became a promising therapeutic target in treatment of AS (as well as psoriatic arthritis), and antibodies to IL-17 were created – known as secukinumab and ixekizumab [25].

## Conclusions

In conclusion, we report that in axial SpA patients, compared to controls, elevated serum concentrations of IL-10, IL-15, IL-17 and IL-23 were observed; some of the cytokines may predispose to a more severe course of aSpA.

However, the essential limitation of our study was the small subgroups of aSpA patients, especially with low disease activity. Axial spondyloarthritis is a heterogeneous disease, and therefore our results need to be evaluated in larger cohorts.

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