Health, Sports & Rehabilitation Medicine
Vol. 24, no. 3, July-September 2023, 132–140

IL-17, IL-23 and disease activity in a cohort of ankylosing spondylitis patients

Laura-Ioana Crînguș[1], Alina Elena Ciobanu[2], Daniela Ciobanu[3], Andrei Adrian Tica[1]
[1] *Department of Pharmacology, University of Medicine and Pharmacy of Craiova, Romania*
[2] *University of Medicine and Pharmacy of Craiova, Romania*
[3] *Department of Internal Medicine, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

Background. The identification of serum biomarkers associated with the degree of ankylosing spondylitis (AS) can give us a more accurate picture of the evolution, prognosis and future quality of life of these patients.

Aims. The aim of the study was to compare, evaluate and correlate changes in the serum titer of the most important cytokines, interleukin-17 (IL-17), IL-23, tumor necrosis factor (TNF-α) and IL-6 with a role in the pathogenesis of AS, with the disease activity score and inflammation markers.

Methods. We included in the study 21 patients diagnosed with AS, hospitalized consecutively, according to the Assessment of SpondyloArthritis international Society (ASAS) criteria, who were evaluated in the Rheumatology Clinic, Emergency County Clinical Hospital of Craiova. For the comparative analysis, we included a control group (group C) consisting of 20 subjects with no history of spondylarthritis or autoimmune inflammatory diseases.

Results. For both studied interleukins, IL-17 and IL-23, we obtained significantly higher concentrations in the serum of patients with AS compared to group C. We found that the serum levels of IL-17, IL-23 and IL-6 increased directly proportional to the severity of AS activity, with the highest concentrations in patients with High activity, unlike TNF-α whose serum levels have increased inversely proportional to SA activity, with the highest concentrations in patients with Moderate disease activity. We identified the presence of correlations between AS activity and serum concentrations of the studied interleukins, IL-17 and IL-23 andwe observed that serum levels of IL-17 correlated much better with disease indices used to assess this entity, ASDAS and BASFI, respectively.

Conclusions. Due to the almost 100% specificities obtained for IL-17 and IL-23, we can consider that they can be a diagnostic alternative to the already known cytokines, TNF-α and IL-6 and other scores such as ASDAS, BASDAI and BASFI, which evaluate AS activity. We can also use these cytokines with determined threshold values to differentiate patients with High activity from those with Moderate activity.

Keywords: ankylosing spondylitis, Interleukin-17, Interleukin-23, disease activity.

Introduction

Ankylosing spondylitis (AS) is a chronic, autoimmune rheumatic condition with multiple etiopathogenetic pathways, following the interaction between the genetic background and environmental factors. Currently, the factors known to be related to AS are the genetic background, immune reaction, microbial infection, endocrine abnormalities (Zhu et al., 2019).

It is a condition characterized by entheseal involvement, bone neoformation, with axial and peripheral involvement. The degree of disease activity is dependent on the level of inflammatory status, with reduced axial and/or peripheral mobility, and multiple extra-articular involvement. Numerous recent publications have emphasized the importance of the contribution of genetic factors and immune-mediated processes, by studying the genetic profile underlying cytokine receptors, transcription factors or the alteration of the antigen presentation process (Ranganathan et al., 2017).

The intestinal microbiota has a major role in the pathogenic process, without being of significant importance in terms of the therapeutic management of the disease (Sieper & Poddubnyy, 2020). Although many data have been published regarding the genetic, cellular and molecular pathways underlying the pathogenesis of AS, therapeutic options are still open to new molecules targeting each of these pathways (Ranganathan et al., 2017).

It is well known that tumor necrosis factor (TNF)

Received: 2023, August 25; *Accepted for publication:* 2023, September 10
Address for correspondence: University of Medicine and Pharmacy of Craiova. No 2-4, Petru Rareş Str. Craiova, PC 200349, Romania
E-mail: elena.ciobanu210@gmail.com
Corresponding author: Alina Elena Ciobanu; elena.ciobanu210@gmail.com
https://doi.org/10.26659/pm3.2023.24.3.132

plays a central role in the pathogenesis of the disease, along with interleukin-17 (IL-17) and interleukin-23 (IL-23). Furthermore, it has been shown that numerous autoimmune diseases, including AS, psoriatic arthritis, rheumatoid arthritis or Crohn's disease, can be triggered by the activation of the IL-17/IL-23 pathway (Yin et al., 2020; Gravallese & Schett, 2018). Ankylosing spondylitis also has a genetic predisposition, inherited through the HLA-B27 antigen (Pedersen & Maksymowych, 2019).

In addition to the HLA-B27 antigen, other major histocompatibility complex (MCH) genes have been directly associated with AS (HLA-B60, HLA-B61, HLA-B39), as well as multiple non-CMH genes (ERAP-1, ERAP-2), lekuyl-cystenyl-aminopeptidase (LNPEP), genes of the IL-23 pathway, or genes associated with CD8 T cells (van Gaalen et al., 2013; Reveille et al., 2019; Burton et al., 2007; Evans et al., 2011).

Rheumatoid arthritis, psoriasis, like AS, are regular pathological processes through T helper 1 (Th1) cell subpopulations, interferon-γ (IFN-γ) and IL-2; recent data have emphasized the major role of Th 17 and IL-17 cells in the pathological processes related to the onset of chronic rheumatic diseases (Cauli et al., 2015). The concept that AS is an IL-17 dependent condition has been clarified by genetic and immunological studies (van Gaalen et al., 2013; Reveille et al., 2019; Burton et al., 2007; Evans et al., 2011; Cauli et al., 2015).

The relevance of the IL-23/IL-17 pathway was first discovered in 2007 through a genome-wide association study (GWAS) that revealed a single nucleotide polymorphism (SNP) of the IL-23 receptor. T helper 17 lymphocyte differentiation can be triggered by IL-23, TGF-β and IL-1β, as well as other cytokines. Then the differentiated immune cells generate IL-17A, IL-17F, IL-22, IL-26 and CCL 20 (Zhu et al., 2019). Affecting this pathway is also relevant for other diseases such as psoriasis, inflammatory bowel disease, rheumatoid arthritis and axial spondyloarthritis (Zhu et al., 2019).

The involvement of the innate immune system was revealed due to some studies that showed an increased serum level of IL-23 and IL-17 as well as the presence of IL-17 positive cells located on the articular surfaces of patients with AS (Zhu et al., 2019).

Our study is a continuation of the publications found in the specialized literature, involving the major etiopathogenic pathways noticed in patients with spondylarthritis, and correlating the changes in the serum titer of the most important cytokines determined in the patients' serum, with the disease activity score and markers of inflammation.

An important factor to consider regarding the cytokine profile is the difficulty of an accurate measurement of serum cytokine levels. Due to the short lifetime of cytokines and the level that varies depending on their location in the body, measurement of cytokines proves to be very difficult (Keller et al., 2003).

The aim of the current study is to compare, evaluate and correlate changes in the serum titer of the most important cytokines (IL-17, IL-23, TNF-α, IL-6) with a role in the pathogenesis of ankylosing spondylitis, with the disease activity score and inflammation markers. The final objective was to identify certain variables that can be integrated in obtaining an algorithm with a diagnostic and prognostic role.

Hypothesis

Our hypothesis is that the serological status contributes significantly to the inflammatory process in AS. We aim to verify this hypothesis by dosing some pro-inflammatory cytokines. Also, as a secondary hypothesis, we proposed to identify serological markers that would be able to identify people at risk for AS, be specific to the disease, be able to have a prognostic value in the stratification into various phenotypes, be useful in monitoring AS activity and of the response to the administered therapy and last but not least to have a prognostic value of relapses.

Material and methods

For the implementation of the study, permission was requested and obtained from the Academic and Scientific Ethics and Deontology Commission of the University of Medicine and Pharmacy in Craiova (No. 40/18.02.2019). The study strictly followed the General Data Protection and Privacy Regulation (GDPR) and the Declaration of Helsinki 1975/2008.

Research protocol

a) Period and place of the research

This study evaluated data from patients who were diagnosed with AS in the Rheumatology Clinic, Craiova Emergency County Clinical Hospital, between October 2019 and October 2022.

b) Subjects and groups

We included in the study 21 patients diagnosed with AS (the AS group), hospitalized consecutively, according to the Assessment of SpondyloArthritis international Society (ASAS) criteria (Heijde et al., 2016), who were evaluated anamnestically, through clinical, biological and imaging evaluation, within the Rheumatology Clinic, Emergency County Clinical Hospital of Craiova.

For the comparative analysis, we included a control group (group C) consisting of 20 subjects with no history of spondylarthritis or autoimmune inflammatory diseases, with general characteristics compatible with the control group. Exclusion criteria were the presence of organic diseases, metabolic abnormalities or other autoimmune conditions.

For each patient, the initial assessment included: contact information, demographic information, personal pathological history, clinical manifestations, laboratory tests, disease activity scores, complications (anemia, infections, pulmonary involvement, etc.), drug classes used, and the option current therapeutic.

To evaluate the activity of the disease, we used as methods, the Ankylosing Spondylitis Disease Activity Score with C-reactive protein (ASDAS-CRP) score (Lukas et al., 2009; Machado et al., 2011; (1) based on the CRP value : remission (ASDAS CRP < 1.3), medium/low disease activity (1.3 < ASDAS CRP ≤ 2.1), increased/moderate activity (2.1 < ASDAS CRP ≤ 3.5), severe/high activity (ASDAS CRP > 3.5).

The Bath Ankylosing Spondylitis Functional Index (BASFI) (Calin et al., 1994) and the Bath Ankylosing

Spondylitis Disease Activity Index (BASDAI) (Garrett et al., 1994) were used to assess the functional status of the disease.

c) Applied tests

Both the SA group and the control group had biological samples collected, which consisted of 5 ml venous blood. These samples were collected using Becton Dickinson vacutainers that did not contain coagulant.

After collection, the blood was centrifuged at a speed of 3,000 x g for 10 minutes, following the standard procedure and ensuring that the clot was separated from the rest of the sample as soon as possible after collection. The separated serum was then distributed into several cryotubes, stored in the freezer at temperatures of at least -20°C and even -80°C, depending on the time the samples were processed. Each cryotube for each patient was labeled with identification data.

For each dosed target parameter, one cryotube was used, thus avoiding freeze-thaw cycles that can denature the proteins and provide us with erroneous values.

The samples of AS patients were processed in the Immunology Laboratory of the University of Medicine and Pharmacy Craiova.

Research kits were provided by Invitrogen, Thermo Fisher Scientific, Inc., having the following characteristics: IL-17 (catalogue BMS2037-2; detection range: 15.6-1000.0pg/mL; analytical sensitivity: <3.3 pg/mL); IL-23 (catalog BMS2023-3; detection range: 8.23–6000.0 pg/mL; analytical sensitivity: 6.0 pg/mL); TNF-α (catalog BMS223-4; detection range: 7.8–500.0 pg/mL; analytical sensitivity: 2.3 pg/mL; Control Low Lot#185277000, range: 20.0–100.0 pg/mL; Control High Lot#185278000, range: 350.0–700.0 pg/mL); IL-6 (catalog BMS213–2; detection range: 1.56–100.00 pg/mL; analytical sensitivity: 0.92 pg/mL; Control Low Lot#229983–000, range: 3.0–10.0 pg/mL; Control High Lot# 229984–000, range: 50.0–150.0 pg/mL).

For the serum determination of IL-17, IL-23, TNF-α and IL-6 concentrations, the Enzyme Linked Immunosorbent Assay (ELISA) technique was used. ELISA is run on 96-well polystyrene plates and is useful for the detection and quantification of specific proteins in a complex mixture.

d) Statistical processing

The data collected from the patients' medical records were organized and processed using Microsoft Excel. The Trial Version of GraphPad Prism 5 (San Diego, CA, USA) was used to perform statistical analysis of these data.

The data obtained for statistical processing were tested for normality, using the Shapiro-Wilk test, or the D'Agostino & Pearson omnibus normality test. Thus, parameters with normal distribution will be found as mean ± standard deviation (SD), and parameters that did not meet this test will be found as medians along with the interquartile range (CI, 95%).

Continuous variables were compared using the Mann-Whitney test due to the small number of patients included. Statistical correlations between different types of data were assessed using Pearson's correlation coefficient. Significance was assigned to p values ≤ 0.05.

To determine the diagnostic accuracy of serological markers, we used the receiver-operator characteristic (ROC) curve, expressed as the area under the ROC curve (AUC), accompanied by the threshold value, the confidence interval (CI) of 95%, their sensitivity (Sn), specificity (Sp), respectively the Youden index (Sn + Sp - 1). For the Youden index, a value of 1 indicates that there are no false positives or false negatives, meaning the test is perfect. The index gives equal weight to false positives and false negatives, so that all tests with the same index value give the same proportion of total misclassified results.

Results

Demographics and clinical characteristics of the study subjects

The descriptive analysis of patients diagnosed with AS admitted to the Rheumatology Clinic, of the Emergency County Clinical Hospital of Craiova, between October 2019 and October 2022, established that of the 21 patients investigated and diagnosed with AS, male patients predominated (19 patients, 90.74 %).

Depending on the area of origin, residents from the urban residence constituted the majority, representing 85.71% of the participants (18 patients), while those from the rural residence represented approximately 13.29%, highly statistically significant differences (p<0.0001).

Regarding age, the variable had a normal distribution, with an average value at the time of diagnosis of 40.48±16.52. No outliers or older individuals were observed in the AS study groups, female group and male group, respectively (Table I).

Table I

Demographic and clinical characteristics of the patients.

Parameter		AS group (n=21)
Gender (Male/Female)		19/2
Residence (Urban/Rural)		18/3
Age (mean±SD)		40.48±16.52
Average duration of the disease		6.71±3.22
	Male	5.50±3.54
	Female	11±1.41
Smoking (Yes/No)		11/10
Diabetes (Yes/No)		8/13
Body mass index (BMI) (kg/m^{2}sc)		27.44±6.35
	overweight	8 (38.09%)
	obesity	6 (28.57%)
	normal weight	7
Form of the disease		
	axial form	15
	peripheral damage	6

Assessment of the activity level of patients with AS, using the ASDAS, BASDAI and BASFI activity scores

After evaluating the level of disease activity, using the ASDAS, BASDAI activity scores and the BASFI functional index respectively, we observed significant differences between the patient groups (Table II).

The majority of patients, 14 patients (66.67%), presented a moderate level (M group) of disease activity based on the ASDAS score (2.95 ± 0.34), while one third (6 patients, 28.57%) presented an ASDAS score (4.05 ± 0.31) indicating high disease activity (H group), and

one patient (4.76%) was in remission (R), ASDAS score <1.3. For an eloquent statistical significance, the patient in remission was excluded from the statistical processing of the data. Comparing the average values of ASDAS, we observed significantly higher values (p<0.0001) in patients who presented a high *vs* moderate form of activity (Fig. 1).

For the BASDAI score, average values were recorded, corresponding to a moderate activity (5.27 ± 0.69), respectively a high activity at a cut-off of 7.92 ± 0.58, significantly higher values (p<0.0001). For the BASFI functional score, the following average values were observed, corresponding to moderate activity (cut-off, 5.35 ± 0.66), respectively high activity at a cut-off of 7.75 ± 0.69, significantly higher values (p<0.0001).

Table II

Means of ASDAS, BASDAI and BASFI activity scores of AS.

Parameter	M group (N=14)	H group (N=6)	p-Value
ASDAS (mean±SD)	2.95 ± 0.34	4.05 ± 0.31	<0.0001
BASDAI (mean±SD)	5.27 ± 0.69	7.92 ± 0.58	<0.0001
BASFI (mean±SD)	5.35 ± 0.66	7.75 ± 0.69	<0.0001

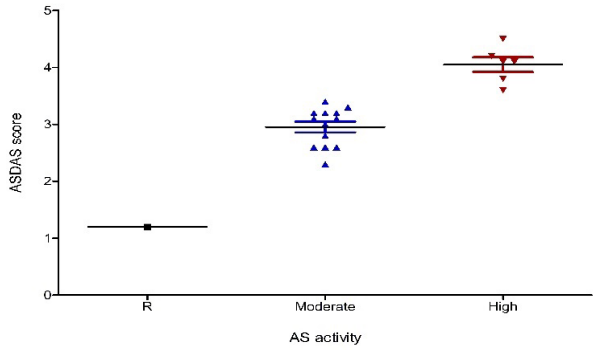

Fig. 1 – The distribution of the number of patients according to the activity of the disease, based on the ASDAS score.

Concentrations of Serum IL-17, IL-23, TNF-α, and IL-6

We included in the cytokinetic status study only the 20 patients diagnosed with AS, who had an moderate ASDAS score (M group) and respectively those who had a high ASDAS score (H group).

All investigated cytokines showed a normal distribution of their values, so they will be found in our presentation as mean ± SD.

A first objective of the serological study was the dosage of the analyzed serological markers in the serum of the included patients. Thus we obtained for both studied interleukins, IL-17 (48.23 ± 6.92 pg/mL) and IL-23 (348.40 ± 71.56 pg/mL), significantly higher concentrations in the serum of patients with AS compared to group C (4.22 ± 0.61 pg /mL, p< 0.0001 and respectively 30.35 ± 6.19 pg/mL, p< 0.0001) (Table III; Fig. 2; Fig. 3).

Also, TNF-α and IL-6, serological markers, used to quantify the body's inflammatory response, had significantly higher serum levels in patients with AS compared to group C (TNF-α, 19.30 ± 6.28 *vs* 1.68 ± 0.54 pg/mL, respectively IL-6, 22.93 ± 6.44 *vs* 1.98 ± 0.57 pg/mL, highly statistically significant differences) (Table III; Fig. 4; Fig. 5).

Table III

Serum biomarkers concentrations for AS patients.

Parameter (mean ± SD)	AS group	C group	p-Value
IL-17 (pg/mL)	48.23 ± 6.92	4.22 ± 0.61	*
IL-23 (pg/mL)	348.40 ± 71.56	30.35 ± 6.19	*
TNF-α (pg/mL)	19.30 ± 6.28	1.68 ± 0.54	*
IL-6 (ng/mL)	22.93 ± 6.44	1.98 ± 0.57	*
CRP (mg/mL)	21.62 ± 6.84	1.96 ± 0.62	*

*: p < .0001, high statistical significance

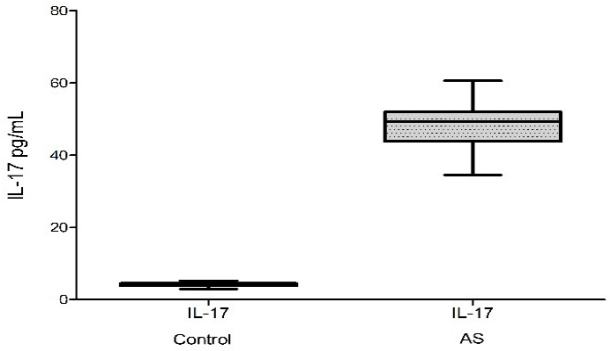

Fig. 2 – IL-17 concentrations in the serum of patients with AS *vs* C.

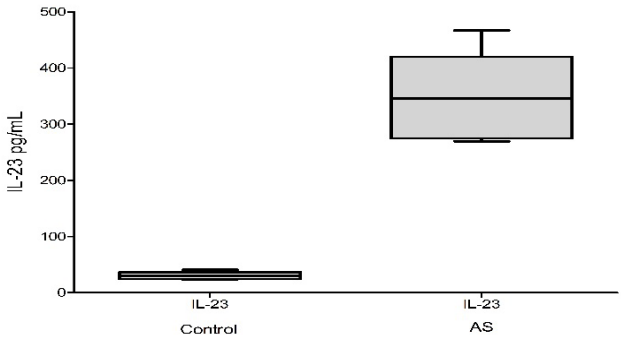

Fig. 3 – IL-23 concentrations in the serum of patients with AS *vs* C.

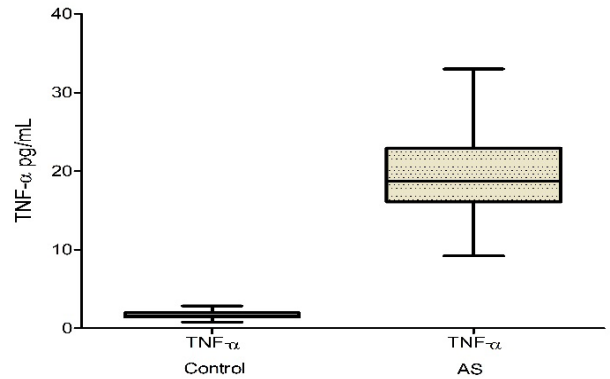

Fig. 4 – TNF-α concentrations in the serum of patients with AS *vs* C.

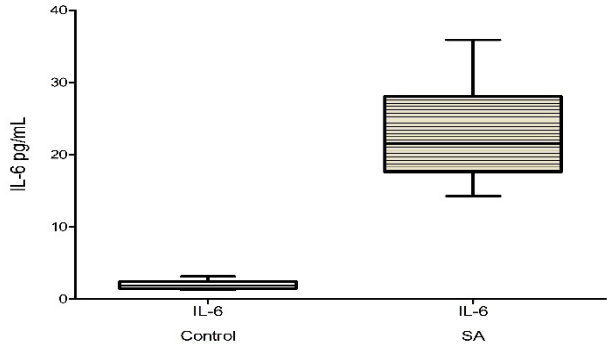

Fig. 5 – IL-6 concentrations in the serum of patients with AS *vs* C.

Analysis of the clinical utility of IL-17 and IL-23 by identifying possible associations between the dynamics of target parameters and different clinical forms of the disease

The second objective of the cytokine status study was to identify associations between target cytokines and disease activity.

We compared the mean values of serum cytokines, for the M group compared to the C group, obtaining: IL-17 (47.52 ± 7.53 *vs* 4.22 ± 0.61 pg/mL, p < 0.0001), IL-23 (309.90 ± 44.29 *vs* 30.35 ± 6.19 pg/mL, p < 0.0001), TNF-α (20.22 ± 6.71 *vs* 1.68 ± 0.54 pg/mL, p < 0.0001), IL-6 (22.69 ± 6.97 *vs* 1.98 ± 0.57 pg/mL, p < 0.0001), respectively IL-6 (22.69 ± 6.97 *vs* 1.96 ± 0.62 pg/ml, p < 0.0001) (Table IV).

Regarding the H group compared to group C, we obtained: IL-17 (49.90 ± 4.47 *vs* 4.22 ± 0.61 pg/mL, p < 0.0001), IL-23 (438.20 ± 23.03 *vs* 30.35 ± 6.19 pg/mL , p < 0.0001), TNF-α (17.14 ± 4.98 *vs* 1.68 ± 0.54 pg/mL, p < 0.0001), IL-6 (23.49 ± 5.52 *vs* 1.98 ± 0.57 pg/mL, p < 0.0001), respectively IL-6 (23.49 ± 5.52 *vs* 1.96 ± 0.62 pg/ml, p < 0.0001) (Table IV).

We also found that serum levels of IL-17, IL-23 and IL-6 increased in accordance with SA activity, so that patients with High activity had the highest serum levels (Figures 6 and 7). Unlike the investigated interleukins, in the case of TNF-α we observed that serum levels had an evolution inversely proportional to the severity of AS, patients with Moderate disease activity presenting the highest serum levels.

Statistically highly significant differences were observed only between IL-23 serum levels in patients from H group compared to M group (p= 0.0009).

No statistical differences were recorded between IL-17 concentrations in the serum of patients from H group compared to M group (p= 0.665), between IL-6 concentrations in the serum of patients from H group compared to M group (p= 0.262) and respectively between TNF-α concentrations in the serum of patients from H group compared to M group (p= 0.796).

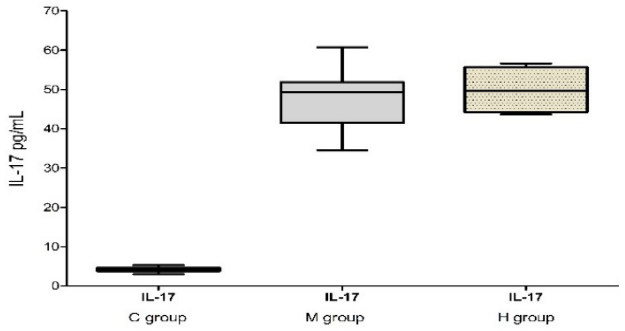

Fig. 6 – IL-17 concentrations in the serum of patients in different stages of clinical activity of SA *vs* C.

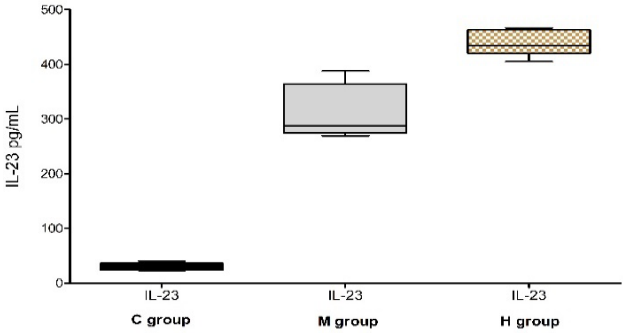

Fig. 7 – IL-23 concentrations in the serum of patients in different stages of clinical activity of SA *vs* C.

Analysis of the clinical utility of IL-17 and IL-23 by establishing correlations between serological markers and the severity of AS activity

The study of the cytokine status of patients diagnosed with AS also aimed at establishing the correlations between the target parameters (IL-17, IL-23, TNF-α, IL-6 and CRP), as well as the correlations between the target parameters and the ASDAS, BASDAI and BASFI, which evaluates the severity of disease activity.

Table IV

Serum levels of parameters in SA groups vs C group.

Parameter (mean ± SD)	M group	H group	C group	p-Value
IL-17 (pg/mL)	47.52 ± 7.53	49.90 ± 4.47	4.22 ± 0.61	*
IL-23 (pg/mL)	309.90 ± 44.29	438.20 ± 23.03	30.35 ± 6.19	*
TNF-α (pg/mL)	20.22 ± 6.71	17.14 ± 4.98	1.68 ± 0.54	*
IL-6 (ng/mL)	22.69 ± 6.97	23.49 ± 5.52	1.98 ± 0.57	*
CRP (mg/mL)	26.68 ± 9.92	19.45 ± 3.70	1.96 ± 0.62	*

*: p < 0.0001, high statistical significance.

Table V
The correlations between IL-17 and IL-23 concentrations and the other investigated cytokines, TNF-α, IL-6, and CRP, as well ASDAS, BASDAI and BASFI scores.

Markers	ASDAS	BASDAI	BASFI	IL-17	IL-23	TNF-α	IL-6	CRP
ASDAS		r = 0.902 p = 0.00001 *	r = 0.813 p = 0.00001 *	r = 0.423 p = 0.046 *	r = 0.315 p = 0.039 *	r = 0.039 p = 0.870	r = 0.213 p = 0.036 *	r = -0.333 p = 0.151
BASDAI			r = 0.887 p = 0.00001 *	r = 0.328 p = 0.158	r = 0.321 p = 0.167	r = 0.093 p = 0.695	r = 0.211 p = 0.373	r = -0.349 p = 0.132
BASFI				r = 0.339 p = 0.043 *	r = 0.117 p = 0.624	r = 0.094 p = 0.692	r = 0.130 p = 0.585	r = -0.267 p = 0.254
IL-17					r = 0.071 p = 0.766	r = -0.130 p = 0.586	r = -0.174 p = 0.463	r = -0.205 p = 0.386
IL-23						r = -0.175 p = 0.459	r = -0.118 p = 0.621	r = -0.352 p = 0.128
TNF-α							r = -0.020 p = 0.934	r = -0.227 p = 0.335
IL-6								r = 0.052 p = 0.828

Table VI
Diagnostic performance of the investigated parameters.

Parameter	AUC	Threshold Values	Sensitivity %	Specificity %	Youden index	p-Value
IL-17	0.995	19.91	100.00	99.50	0.995	< 0.0001
IL-23	0.980	155.10	100.00	98.00	0.980	< 0.0001
TNF-α	1.000	6.052	100.00	100.00	1.000	< 0.0001
IL-6	1.000	8.673	100.00	100.00	1.000	< 0.0001
ASDAS	0.975	1.75	100.00	95.00	0.950	< 0.0001
BASDAI	0.975	2.65	100.00	95.00	0.950	< 0.0001
BASFI	0.975	2.75	100.00	95.00	0.950	< 0.0001

The correlations established between IL-17 and IL-23 concentrations and the other investigated cytokines, TNF-α, IL-6, and CRP, as well as ASDAS, BASDAI and BASFI scores are highlighted in Table V. In our study, we obtained the presence of correlations between SA activity and serum concentrations of the studied interleukins, IL-17 and IL-23.

We observed that IL-17 serum levels correlated much better with disease indices and joint functionality, ASDAS and BASFI, respectively (positive, moderate, statistically significant correlations; rho = 0.423, p = 0.046 and rho = 0.339, p = 0.043) compared to IL-23. In the case of IL-23, serum concentrations only correlated with ASDAS, a correlation also observed for IL-6.

Diagnostic performance of IL-17 and IL-23

Analyzing the area under the ROC curve (AUC) obtained for the target parameters (Table VI), we noticed that the diagnosis of patients with Moderate forms of those with High activity forms of AS is best done by TNF-α and IL-6 which have a maximum accuracy (100%) to correctly distinguish these patients, and a sensitivity and specificity of 100%.

For the target interleukins in the study, we recorded a diagnostic performance close to 100%: for IL-17 a performance of 99.50%, slightly better compared to IL-23, which recorded a performance of 98.00%.

Due to the specificity of 99.50% and 98.00%, respectively, the two target cytokines in our study IL-17 and IL-23 can be considered as a diagnostic or additional alternative to the cytokines, TNF-α and IL-6 and other scores such as ASDAS, BASDAI and BASFI, which assesses the severity of AS activity.

Discussion

The primary objective of our study was to determine whether proinflammatory cytokines and serum titers are correlated, and to what extent, with disease activity, as well as to establish a diagnostic and prognostic algorithm with statistically significant impact in current clinical practice.

For both studied interleukins, IL-17 and IL-23, we obtained significantly higher concentrations in the serum of patients with AS compared to group C; we found that the serum levels of IL-17, IL-23 and IL-6 increased directly proportional to the severity of AS activity, with the highest concentrations in patients with High activity, unlike TNF-α whose serum levels have increased inversely proportional to SA activity, with the highest concentrations in patients with Moderate disease activity.

We identified the presence of correlations between SA activity and serum concentrations of the studied interleukins, IL-17 and IL-23; we observed that serum levels of IL-17 correlated much better with disease indices used to assess this entity, ASDAS and BASFI, respectively.

The results obtained in our study are consistent with those reported in other specialized studies.

IL-17, a subset of the CD4+ cell population, was originally described in 2005, and has a role in immunity against bacterial and fungal infections as well as a major physiological effect. Differentiation of Th17 cells is regulated by a number of cytokines, IL-1β, IL-6, TGF-β and, especially, IL-23. Th17 cells release a series of cytokines, represented by IL-17-A, IL-17F, IL-22, IFN-γ, or granulocyte-macrophage colony-stimulating factor (GM-CSF) (Cauli et al., 2015; Ruiz de Morales et al., 2020). In patients with AS, increased serum levels of IL-17 have been identified in serum, joint, or synovial fluid. It has been proven that it represents a major pathway in the pathogenesis of the disease, which constitutes an essential therapeutic target for these patients. Within the group included in the study, we obtained statistically significantly different values compared to the control group.

Numerous studies have been carried out in order to elucidate the role of IL-17: it is either the starting point or the target of other cytokines, in the pathogenesis of AS (Wendling et al., 2007; Mei et al., 2011). It has been published that IL-17A producing cells are frequently found in AS patients (Lau et al., 2017). Despite the pro-inflammatory effect, IL-17A and IL-17F do not have the most potent inflammatory action, but the effect is the result of the recruitment of immune cells, as well as the synergistic effect with other pro-inflammatory cytokines, TNF, IL-1β, IFN-γ, GM-CSF and IL-22 (Jethwa & Bowness, 2016).

Blocking IL-17A has been shown to be effective in the treatment of patients with active AS, and data on the IL-17/IL-23 pathway show that it plays a major role in the pathogenesis of axial spondyloarthritis (Ramos et al., 2002), being used to synthesize molecules biological with utility in the therapeutic management of patients. Clinical trials with agents targeting the IL-23/IL-17 axis provide important data, which help us improve the evolution, prognosis and quality of life of these patients (Ruiz de Morales et al., 2020).

IL-23, a cytokine with subunits IL-12B (IL-12p40) and IL-23A (IL-23p19) (Aydin et al., 2005), is also secreted by the intestinal epithelium, dendritic cells and macrophages and has a major role in the regulation, activation and proliferation of IL-17-expressing Th17 cells, as well as IL-22 secretion (Cui et al., 2002). The IL-23-IL-17 connection has been proven in murine models, by studying the differentiation of T cells into T helper cells (Tan et al., 2009; Benham et al., 2014). IL-17 production is potentiated by IL-23, but exclusively after their activation (Bettelli et al., 2006).

Experimental studies have proven that the differentiation of non-pathogenic Th-17 cells takes place under the action of IL-6 and TGF-β, in the absence of IL-23 (Lee et al., 2012). Moreover, for mast cells, NK or γ-δ T cell subpopulations, IL-23 is the main regulator of IL-17 or IL-22 expression (Lee et al., 2012; Jansen et al., 2015). Regarding the therapeutic approach, it has been suggested that IL-23 plays an important role in the initiation of pathological processes, and not in the perpetuation of destructive lesions. Administration of IL-23 inhibitors provided less significant results compared to IL-17. Studies using ustekinumab or risankizumab, which included TNF-naive subjects, did not reveal significant data; moreover, ustekinumab was administered to patients with prior anti-TNF therapy without achieving notable results (Baeten et al., 2018).

In addition, with regard to radiological progression, there are insufficient data available. The enthesal inflammation, characteristic of the spondyloarthritis group, is potentiated by IL-23, by means of a CD3+ CD4-CD8- lymphocyte population. The lack of therapeutic response, compared to anti-IL17 agents, emphasizes the importance of similarities and differences between IL-17 and IL-23, especially in terms of cellular differentiation, inflammation, bone destruction as well as bone neoformation (Sieper & Poddubnyy, 2020). Regarding cell differentiation, the first event is the activation of IL-17 cells, via IL-1 and IL-6, subsequent to inflammation, followed by the increase in the number of Th17 cells and γ-δ T cells; the presence of IL-23 can be a promoter in the maturation of pathogenic Th17 cells (Burton et al., 2007).

IL-23 production occurs in intestinal epithelial and immunological cells, including dendritic cells and macrophages. IL-23 has a central role in the regulation, activation and proliferation of Th17 cells, which explains its role in the expression of IL-17 and IL-22 during inflammation (Chu et al., 2013). Furthermore, IL-23 regulates the inflammatory involvement of IL-17 and IL-22 by suppressing their expression in innate lymphoid cells, the gamma-delta T cell (Tγ/δ) subpopulation, NK cells, and mast cells. Suppression of IL-23 in individuals with features of rheumatoid disease shows a drastic expression of clinical symptoms. Specifically, such an intervention reduces joint effusion, bone erosion and improves bone regeneration through osteoclast activity. In addition, actions that neutralize IL-23p19 subunits reduce the clinical manifestation of AS and down-regulate mediators and genes involved in joint effusion and bone erosion, including RANKL, IL-6, IL-1β and matrix metalloproteinases (Chu et al., 2013).

Following the therapeutic advances in the field of spondyloarthritis, which can have a major impact on the evolution and can stop the progression of joint destruction, when they are applied as early as possible, the need to establish some biomarkers, which can be integrated in a clinical context, can facilitate a correct early diagnosis and can provide insight into the evolution and prognosis of patients.

Numerous serum markers have been evaluated to assess disease activity, response to treatment, or as predictors of radiological progression. In order to determine disease activity, in addition to the evaluation of C-reactive protein, the most studied were interleukins (IL-17 and IL-23), matrixmetalloproteinases (MMP), especially MMP3. Assessment of radiological progression can be done using markers of bone metabolism, cartilage degradation products or adipose tissue.

The difficulty lies in the fact that no biomarker can

be used individually for the purpose of assessing disease activity or radiological progression, but must be integrated in a clinical context, in order to establish an algorithm that can help us to optimize individualized therapeutic behavior and improve quality the lives of these patients.

Conclusions

1. Due to the almost 100% specificities obtained for IL-17 and IL-23, we can consider that they can be a diagnostic alternative to the already known cytokines, TNF-α and IL-6 and other scores such as ASDAS, BASDAI and BASFI, which evaluate AS activity.

2. We can also use these cytokines with determined threshold values to differentiate patients with high activity from those with moderate activity.

Acknowledgment
The article capitalizes on partial results from the doctoral thesis of Laura-Ioana Crînguș, currently underway at the University of Medicine and Pharmacy of Craiova.

Conflict of interest
None to declare.

References

Aydin T, Karacan I, Demir SE, Sahin Z. Bone loss in males with ankylosing spondylitis: its relation to sex hormone levels. Clin Endocrinol (Oxf). 2005;63(4):467-469. doi:10.1111/j.1365-2265.2005.02369.x.

Baeten D, Østergaard M, Wei JC, et al. Risankizumab, an IL-23 inhibitor, for ankylosing spondylitis: results of a randomised, double-blind, placebo-controlled, proof-of-concept, dose-finding phase 2 study. Ann Rheum Dis. 2018;77(9):1295-1302. doi:10.1136/annrheumdis-2018-213328.

Benham H, Rehaume LM, Hasnain SZ, et al. Interleukin-23 mediates the intestinal response to microbial β-1,3-glucan and the development of spondyloarthritis pathology in SKG mice. Arthritis Rheumatol. 2014;66(7):1755-1767. doi:10.1002/art.38638.

Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441(7090):235-238. doi:10.1038/nature04753.

Burton PR, Wellcome Trust Case Control Consortium; Australo-Anglo-American Spondylitis Consortium (TASC), et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet. 2007;39(11):1329-1337. doi:10.1038/ng.2007.17.

Calin A, Garrett S, Whitelock H, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol. 1994;21(12):2281-2285.

Cauli A, Piga M, Floris A, Mathieu A. Current perspective on the role of the interleukin-23/interleukin-17 axis in inflammation and disease (chronic arthritis and psoriasis). Immunotargets Ther. 2015;4:185-190. doi:10.2147/ITT.S62870.

Chu WM. Tumor necrosis factor. Cancer Lett. 2013;328(2):222-225. doi:10.1016/j.canlet.2012.10.014.

Cui X, Hawari F, Alsaaty S, et al. Identification of ARTS-1 as a novel TNFR1-binding protein that promotes TNFR1 ectodomain shedding. J Clin Invest. 2002;110(4):515-526. doi:10.1172/JCI13847.

Evans DM, Spencer CC, Pointon JJ, et al. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility [published correction appears in Nat Genet. 2011;43(9):919. Opperman, Udo [corrected to Oppermann, Udo]; Moutsianis, Loukas [corrected to Moutsianas, Loukas]]. Nat Genet. 2011;43(8):761-767. doi:10.1038/ng.873.

Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol. 1994;21(12):2286-2291.

Gravallese EM, Schett G. Effects of the IL 23 IL 17 pathway on bone in spondyloarthritis. Nat Rev Rheumatol. 2018;14(11):631-640. doi: 10.1038/s41584-018-0091-8.

Heijde DV, Ramiro S, Landewe R, Xenofon B, Bosch FV, Sepriano A, Regel A, Ciurea A, Dougados M, Gaalen F, Her P, Horst-Bruinsma I, Inman RD, Jongkees M, Kiltz U,Kvien TK, Machado PM, Marzo-Ortega H, Molto A, Navarro-Compan V, OzgocmenS, Pimentel-Santos FM, Reveille J, Rudwaleit M, Sieper J, Sampaio-Barros P, WorkD, Bruan J. 2016 Update of the ASAS-EULAR ManagementRecommendation for Axial Spondyloarthritis. Ann Rheum Dis. 2016;76(6):978-991. doi:10.1136/annrheumdis-2016-210770.

Jansen DT, Hameetman M, van Bergen J, et al. IL-17-producing CD4+ T cells are increased in early, active axial spondyloarthritis including patients without imaging abnormalities. Rheumatology (Oxford). 2015;54(4):728-735. doi:10.1093/rheumatology/keu382.

Jethwa H, Bowness P. The interleukin (IL)-23/IL-17 axis in ankylosing spondylitis: new advances and potentials for treatment. Clin Exp Immunol. 2016;183(1):30-36. doi:10.1111/cei.12670.

Keller C, Webb A, Davis J. Cytokines in the seronegative spondyloarthropathies and their modification by TNF blockade: a brief report and literature review. Ann Rheum Dis. 2003;62(12):1128-1132. doi:10.1136/ard.2003.011023.

Lau MC, Keith P, Costello ME, et al. Genetic association of ankylosing spondylitis with TBX21 influences T-bet and pro-inflammatory cytokine expression in humans and SKG mice as a model of spondyloarthritis. Ann Rheum Dis. 2017;76(1):261-269. doi:10.1136/annrheumdis-2015-208677.

Lee Y, Awasthi A, Yosef N, et al. Induction and molecular signature of pathogenic TH17 cells. Nat Immunol. 2012;13(10):991-999. doi:10.1038/ni.2416.

Lukas C, Landewé R, Sieper J, et al. Development of an ASAS-endorsed disease activity score (ASDAS) in patients with ankylosing spondylitis. Ann Rheum Dis. 2009;68(1):18-24. doi:10.1136/ard.2008.094870.

Machado PM, Landewé RB, van der Heijde DM. Endorsement of definitions of disease activity states and improvement scores for the Ankylosing Spondylitis Disease Activity Score: results from OMERACT 10. J Rheumatol. 2011;38(7):1502-1506. doi:10.3899/jrheum.110279.

Mei Y, Pan F, Gao J, et al. Increased serum IL-17 and IL-23 in the patient with ankylosing spondylitis. Clin Rheumatol. 2011;30(2):269-273. doi:10.1007/s10067-010-1647-4.

Pedersen SJ, Maksymowych WP. The pathogenesis of ankylosing spondylitis: An update. Curr Rheumatol Rep. 2019;21(10):58. doi: 10.1007/s11926-019-0856-3.

Ramos M, Alvarez I, Sesma L, Logean A, Rognan D, López de Castro JA. Molecular mimicry of an HLA-B27-derived ligand of arthritis-linked subtypes with chlamydial proteins. J Biol Chem. 2002;277(40):37573-37581. doi:10.1074/jbc.M205470200.

Ranganathan V, Gracey E, Brown MA, Inman RD, Haroon N. Pathogenesis of ankylosing spondylitis - recent advances and

future directions. Nat Rev Rheumatol. 2017;13(6):359-367. doi: 10.1038/nrrheum.

Reveille JD, Zhou X, Lee M, et al. HLA class I and II alleles in susceptibility to ankylosing spondylitis. Ann Rheum Dis. 2019;78(1):66-73. doi:10.1136/annrheumdis-2018-213779.

Ruiz de Morales JMG, Puig L, Daudén E, et al. Critical role of interleukin (IL)-17 in inflammatory and immune disorders: An updated review of the evidence focusing in controversies. Autoimmun Rev. 2020;19(1):102429. doi:10.1016/j.autrev.2019.102429.

Sieper J, Poddubnyy D. Pathogenesis of spon dylarthritis: Relevance for treatment. Z Rheumatol. 2020;79(1):5-12, (In German). doi: 10.1007/s00393-019-00708-y.

Tan ZY, Bealgey KW, Fang Y, Gong YM, Bao S. Interleukin-23: immunological roles and clinical implications. Int J Biochem Cell Biol. 2009;41(4):733-735. doi:10.1016/j.biocel.2008.04.027.

van Gaalen FA, Verduijn W, Roelen DL, et al. Epistasis between two HLA antigens defines a subset of individuals at a very high risk for ankylosing spondylitis. Ann Rheum Dis. 2013;72(6):974-978. doi:10.1136/annrheumdis-2012-201774

Wendling D, Cedoz JP, Racadot E, Dumoulin G. Serum IL-17, BMP-7, and bone turnover markers in patients with ankylosing spondylitis. Joint Bone Spine. 2007;74(3):304-305. doi:10.1016/j.jbspin.2006.11.005.

Yin J, Sternes PR, Wang M, Song J, Morrison M, Li T, Zhou L, Wu X, He F, Zhu J et al. Shotgun metagenomics reveals an enrichment of potentially cross-reactive bacterial epitopes in ankylosing spondylitis patients, as well as the effects of TNFi therapy upon microbiome composition. Ann Rheum Dis. 2020;79(1):132-140. doi: 10.1136/annrheumdis-2019-215763.

Zhu W, He X, Cheng K et al. Ankylosing spondylitis: etiology, pathogenesis, and treatments. Bone Res. 2019;7:22. doi:10.1038/s41413-019-0057-8.

Websites

(1) Ankylosing Spondylitis Disease Activity Score (ASDAS). Available at: https://www.asas-group.org/instruments/asdas-calculator/. Accessed online: 22 January 2019.