Association of serum interleukin-17 and interleukin-23 levels with disease activity in Chinese patients with ankylosing spondylitis

Wei-Sheng Chen, Yu-Sheng Chang, Kuan-Chia Lin, Chien-Chih Lai, Shu-Hung Wang, Kai-Hung Hsiao, Hui-Ting Lee, Ming-Han Chen, Chang-Youh Tsai, Chung-Tei Chou

Abstract

Background: Ankylosing spondylitis (AS) is a chronic arthritis with a pathogenesis which is not fully understood. A third subset of IL-17-producing T helper cells, called Th17 cells, has been discovered and characterized. We investigated whether IL-17 and IL-23, two Th17-related cytokines, play any roles in the pathogenesis of, and have any correlations with, disease activity and clinical manifestations in AS.

Methods: This cross-sectional study included 49 AS patients and 25 healthy control subjects. The serum IL-17 and IL-23 levels were measured using enzyme-linked immunosorbent assay kits. At the same time, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Patient Global Score (BAS-G) levels were measured, and physical examinations were performed on study participants to determine their extent of physical mobility.

Results: The serum IL-17 and IL-23 levels of the AS patients were significantly higher than those of the healthy controls. In the AS patients, the BASDAI scores had a better correlation with the serum IL-17 or IL-23 levels (IL-17, r = 0.351, p = 0.014; IL-23, r = 0.398, p = 0.005) than with ESR (r = 0.078, p = 0.600) and CRP (r = 0.012, p = 0.993). IL-17 or IL-23 correlate to the BASFI, BAS-G and parameters related to physical mobility. In the receiver operating characteristic (ROC) analysis, the serum IL-17 and IL-23 levels act better in discriminating patients with BASDAI > 4 (AUC value 0.88, p = 0.001) than ESR and CRP (AUC value 0.727, p = 0.008).

Conclusion: Serum IL-17 and IL-23 levels were significantly higher in AS patients than in healthy controls and the levels correlate to disease activity measured by BASDAI scores, but not parameters of functional ability and spinal mobility. These results suggest the existence of a role of IL-17 and IL-23 in the pathogenesis of inflammation in AS.

Keywords: ankylosing spondylitis; C-reactive protein; erythrocyte sedimentation rate; interleukin-23; interleukin-17

1. Introduction

Spondyloarthritis (SpA) is a family of chronic arthritis diseases characterized by inflammatory back pain, peripheral arthritis, and enthesitis. It comprises five major subtypes, including ankylosing spondylitis (AS), reactive arthritis, psoriatic arthritis (PsA), arthritis associated with inflammatory bowel disease, and undifferentiated spondyloarthritis. AS is the prototype of spondyloarthritides, and it predominantly involves the axial joints and bilateral sacroiliac joints. AS occurs predominantly in young adult males and has a strong association with human leukocyte antigen (HLA)-B27.
exact pathogenesis is still not fully understood, while the role of tumor necrosis factor-α (TNF-α) was proven by its increased level in serum and sacroiliac joint and the dramatic response after anti-TNF-α therapy.2,3

Recently, the discovery of CD4+ Th17 T cells and the interleukin-23 (IL-23)/IL-17 axis has challenged the existing paradigms and the role of Th1 T cells in many autoimmune diseases.4 Th17 is a third CD4+ T effector subset characterized by the production of IL-21, IL-22, TNF-α and IL-17, the hallmark proinflammatory cytokine. Other than host defense, IL-17 has been proven to be critical in the pathogenesis of various autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus, systemic sclerosis, psoriasis and inflammatory bowel disease.5–9

IL-23 is a member of the IL-12 family, which is composed of p40 and p19 subunits, in contrast to the p35/p40 heterodimer which forms the bioactive IL-12. IL-23 not only synergizes with IL-6 and IL-1 to promote Th17 development, but also stimulates Th17 expansion and prolongs IL-17 production.10 A growing body of evidence has revealed the possible role of the IL-23/IL-17 axis in the pathogenesis of AS.11–14 In RA, the serum IL-17 levels correlated strongly with disease activity and CRP concentration.15 The correlation of IL-17 and IL-23 with AS disease activity, function and spinal mobility is not fully known. In this study, other than comparing the serum levels of IL-23 and IL-17 between AS patients and healthy controls, we investigated the relationship of the two serum cytokine levels with parameters related to disease activity, function and spinal mobility.

2. Methods

2.1. Patients

A total of 49 ethnic Chinese AS patients, who fulfilled the 1984 modified New York criteria, were consecutively enrolled from the outpatient department of the Division of Allergy, Immunology, and Rheumatology, Taipei Veterans General Hospital.16 As a control group, blood samples were obtained from 25 age- and sex-matched healthy ethnic Chinese controls. This research was approved by the ethics committee of Taipei Veterans General Hospital. Before the study, informed consent was obtained from all participants.

2.2. Study design

Clinical and laboratory assessments of the patients were performed on the same day. We evaluated the disease activity, functional ability and the AS patients’ global assessment using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Bath Ankylosing Spondylitis Patient Global Score (BAS-G), with visual analog scale. Meanwhile, physical examinations were performed on the subjects to determine physical mobility, including tragus-to-wall distance (TWD), lumbar flexion (modified Schober index, MSI), intermalleolar distance (IMD), cervical rotation (CROT), fingertip-to-floor distance (FFD), chest expansion (CE), and occiput-to-wall distance.17–19 The BASDAI, BASFI, or BAS-G scores had a range of 0 to 10. Acute-phase reactants, including ESR and CRP levels, were also measured in the AS patients.

Samples of peripheral blood were allowed to clot and were then centrifuged at 2400 × g for 10 minutes. The sera were frozen at −80 °C immediately after sample collection. Estimation of serum levels of IL-17A and IL-23 was performed with commercial enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, San Diego, CA, USA).

2.3. Statistical analysis

Statistical analyses were carried out using the SPSS (17th edition; IBM Corp., New York, NY, USA) statistical package. The independent-samples t test was used to analyze group differences. The Mann-Whitney U test was applied in comparison for nonparametric analysis. Correlations between variables were determined by Spearman’s rank correlation test. We used receiver operating characteristic (ROC) plot analysis to evaluate and compare the performance of each biomarker. ROC analysis is a non-parametric method used to quantify the accuracy of the prediction. Two-graph ROC (TG-ROC) analysis was used to compare the whole spectrum of sensitivity and specificity of different markers and to choose an optimal cut-off value for each marker. A p value of 0.05 was provisionally regarded as significant.

3. Results

3.1. Serum levels of IL-17 and IL-23 in AS patients and healthy controls

This study enrolled 49 AS patients and 25 healthy controls (20 men and 5 women, mean age = 35.1 years) who were matched for age and sex (Table 1). We compared the serum IL-17 and IL-23 levels between the AS patients and the healthy controls, and both were significantly higher in the 49 AS patients than in the 25 healthy controls [IL-23: mean (SD) = 208.27 pg/mL (59.1) vs. 121.12 pg/mL (41.5), p < 0.001; IL-17: mean (SD) = 66.03 pg/mL (17.8) vs. 31.37 pg/mL (8.4), p < 0.001]. Of the AS group, 28 patients were in the active status with a BASDAI score > 4, while 21 patients were in the inactive status. A comparison between the active AS group, the inactive AS group and healthy controls revealed that active AS patients had the highest levels of both serum IL-17 and IL-23, while healthy controls had the lowest levels, and that there were significant differences between each group (Fig. 1).

3.2. Correlations between biomarkers and clinical parameters

We further calculated the correlation between the serum IL-17 and IL-23 levels and clinical parameters in the 49 AS patients (Table 2). The serum IL-17 levels did not correlate
with ESR, BASFI, BAS-G, MSI, FFD, CE, OWD, TWD, IMD and disease duration. However, the serum IL-17 levels correlated significantly with BASDAI ($r = 0.351$, $p = 0.014$) and showed a trend toward correlating with CRP ($r = 0.270$, $p = 0.061$). Serum IL-23 levels did not correlate with BASFI, BAS-G, MSI, FFD, CE, OWD, TWD, IMD and disease duration, but did correlate significantly with CRP ($r = 0.284$, $p = 0.048$), ESR ($r = 0.358$, $p = 0.012$), and BASDAI score ($r = 0.398$, $p = 0.005$).

### 3.3. Use of IL-17 and IL-23 to determine disease activity

Finally, we performed ROC analysis and calculated the area under the curve (AUC) at BASDAI cut-off values of 4, 5, and 6 to evaluate the ability of IL-17, IL-23, ESR, and CRP to discriminate disease activity (Fig. 2). At each BASDAI cut-off, the two cytokines had superior discriminative ability to the traditional biomarkers. ESR and CRP were the most effective in the discrimination of patients with BASDAI scores < 6 from those with BASDAI scores of 6. However, the AUC values were only 0.611 ($p = 0.326$) and 0.612 ($p = 0.319$), respectively. The best AUC was obtained from use of IL-17 to discriminate between patients with BASDAI scores < 6 and those with BASDAI scores of 6 ($AUC = 0.880$, $p = 0.001$). The best AUC value for IL-23 was 0.727 at the BASDAI cut-off of 5 ($p = 0.008$). IL-23 had slightly greater AUC values than IL-17 at BASDAI cut-offs of 4. The sensitivity, specificity, negative predictive value, and positive predictive value in discriminating at the BASDAI cut-off values of 4 revealed that serum cytokine levels of IL-17 and IL-23 were better at discriminating disease activity than the traditional biomarkers (Table 3).

### 4. Discussion

The discovery of CD4+ Th17 cells and related cytokines has challenged existing paradigms and the role of Th1 T cells in many autoimmune diseases. However, in the earlier study by Wendling et al, the serum and synovial fluid levels of p40 IL12/23 in spondyloarthropathy patients were not significantly different from those in normal controls.14 But other studies all showed the possible roles of IL-17 and IL-23 in the pathogenesis in AS. Wang et al found significantly higher serum levels of IL-17 and IL-23 in psoriatic arthritis.20 Our data showed that serum IL-17 and IL-23 levels were significantly higher in AS patients compared with age- and gender-matched healthy controls, and this was not limited in patients with BASDAI scores > 4.

We also found that the serum IL-17 and IL-23 levels both correlated with BASDAI, but did not correlate with BASFI,

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AS patients ($n = 49$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39.0 (12.3)</td>
</tr>
<tr>
<td>Male/female</td>
<td>43/6</td>
</tr>
<tr>
<td>HLA-B27 (+)</td>
<td>45</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>15.08 (13.2)</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (mm/h)</td>
<td>25.35 (24.0)</td>
</tr>
<tr>
<td>BASDAI</td>
<td>4.47 (1.9)</td>
</tr>
<tr>
<td>BASFI</td>
<td>2.46 (2.2)</td>
</tr>
<tr>
<td>BAS-G</td>
<td>4.76 (2.9)</td>
</tr>
<tr>
<td>Targus-to-wall (cm)</td>
<td>13.45 (4.5)</td>
</tr>
<tr>
<td>Modified Schober index (cm)</td>
<td>3.03 (1.6)</td>
</tr>
<tr>
<td>Fingertip-to-floor distance (cm)</td>
<td>25.00 (26.1)</td>
</tr>
<tr>
<td>Chest expansion (cm)</td>
<td>4.32 (2.8)</td>
</tr>
<tr>
<td>Occiput-to-wall distance (cm)</td>
<td>3.97 (6.4)</td>
</tr>
<tr>
<td>Intermalleolar distance (cm)</td>
<td>112.54 (22.9)</td>
</tr>
<tr>
<td>Disease duration (y)</td>
<td>9.10 (7.6)</td>
</tr>
</tbody>
</table>

Values are shown as mean (SD).

BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; BASFI = Bath Ankylosing Spondylitis Functional Index; BAS-G = Bath Ankylosing Spondylitis patient Global score.

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We also found that the serum IL-17 and IL-23 levels both correlated with BASDAI, but did not correlate with BASFI,
BAS-G, MSI, FFD, CE, OWD, TWD, and IMD in our data. The latter parameters are related to functional ability and spinal mobility, which are the results of the long-term progression of new bone formation instead of the current disease activity of AS. It is of interest that, other than its correlation to BASDAI, IL-23 levels also have a significant correlation to the ESR and CRP in our data, while IL-17 only showed a non-significant trend with these two methods.

Furthermore, compared with ESR and CRP, the most currently used biomarkers for disease activity, the serum IL-17 and IL-23 levels, have a better correlation with BASDAI and worked better to discriminate between patients with higher disease activity at BASDAI cut-off values from 4 to 6. CRP and ESR are the most common laboratory methods for evaluating AS disease activity in clinical practice.\(^2\) An elevated ESR or CRP level is present in only 30–40% of patients with ankylosing spondylitis.

### Table 2: Spearman’s correlation between clinical parameters and serum IL-17 and IL-23 levels in the 49 ankylosing spondylitis patients.

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>IL-17 (pg/mL)</th>
<th>IL-23 (pg/mL)</th>
<th>CRP (mg/L)</th>
<th>ESR (mm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-23</td>
<td>0.015 (0.919)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CRP</td>
<td>0.270 (0.061)</td>
<td>0.284 (0.048)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ESR</td>
<td>0.032 (0.829)</td>
<td>0.358 (0.012)</td>
<td>0.669 (&lt;0.001)</td>
<td>—</td>
</tr>
<tr>
<td>BASDAI</td>
<td>0.351 (0.014)</td>
<td>0.398 (0.005)</td>
<td>0.012 (0.993)</td>
<td>0.078 (0.600)</td>
</tr>
<tr>
<td>BASFI</td>
<td>0.140 (0.342)</td>
<td>−0.066 (0.655)</td>
<td>0.083 (0.573)</td>
<td>0.089 (0.549)</td>
</tr>
<tr>
<td>BAS-G</td>
<td>0.248 (0.089)</td>
<td>0.136 (0.358)</td>
<td>0.190 (0.195)</td>
<td>0.106 (0.475)</td>
</tr>
<tr>
<td>Disease duration</td>
<td>−0.102 (0.537)</td>
<td>−0.050 (0.763)</td>
<td>−0.111 (0.501)</td>
<td>−0.160 (0.330)</td>
</tr>
</tbody>
</table>

Values are shown as r (p value). r is determined by Spearman’s rank correlation test.

BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; BASFI = Bath Ankylosing Spondylitis Functional Index; BAS-G = Bath Ankylosing Spondylitis patient Global score; CRP = C-reactive protein; ESR = erythrocyte sediment rate; IL = interleukin.

\(^a\) p < 0.05.

**Fig. 2.** Receiver operating characteristic (ROC) analysis of four biomarkers in the diagnosis of high disease activity in patients with ankylosing spondylitis (AS) based on three cut-off points for the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). (A) BASDAI score of \(\geq 4\) (AUC\(_{\text{ESR}}\) = 0.581, \(p = 0.341\); AUC\(_{\text{CRP}}\) = 0.530, \(p = 0.722\); AUC\(_{\text{IL-17}}\) = 0.677, \(p = 0.038\); AUC\(_{\text{IL-23}}\) = 0.686, \(p = 0.082\)); (B) BASDAI score of \(\geq 5\) (AUC\(_{\text{ESR}}\) = 0.552, \(p = 0.540\); AUC\(_{\text{CRP}}\) = 0.501, \(p = 0.992\); AUC\(_{\text{IL-17}}\) = 0.675, \(p = 0.040\); AUC\(_{\text{IL-23}}\) = 0.727, \(p = 0.008\)); (C) BASDAI score of \(\geq 6\) (AUC\(_{\text{ESR}}\) = 0.611, \(p = 0.326\); AUC\(_{\text{CRP}}\) = 0.612, \(p = 0.319\); AUC\(_{\text{IL-17}}\) = 0.880, \(p = 0.001\); AUC\(_{\text{IL-23}}\) = 0.719, \(p = 0.053\)). AUC = area under the curve; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; IL-17 = interleukin-17; IL-23 = interleukin-23.
AS, and normal values do not rule out the presence of inflammation. In this study, the correlation of ESR or CRP to BASDAI is quite poor. Consistent with previous studies, ESR and CRP are not sufficiently capable of reflecting AS disease activity. The poor discriminative ability of ESR and CRP may be due to the complexity of AS pathogenesis. Serum level of IL-6, a major cytokine related to ESR or CRP, was reported significantly higher in AS patients and the level did not correlate with serum levels of IL-17 or IL-23. Moreover, compared with other interfering factors of inflammatory biomarkers, the inflammation of AS, especially axial arthritis, might cause only mild elevation of the two acute phase reactants in some patients. This can be supported by the finding that there was much overlap of ESR or CRP levels between AS patients and normal controls in our data. Therefore, it is not surprising that ESR and CRP have an inadequate capacity to differentiate disease activity.

In our AS cohort, we found no correlation between serum levels of IL-17 and IL-23, contrary to expectation. However, there was a significant correlation of IL-17 and IL-23 if healthy controls were included in the analysis (r = 0.59, p < 0.001). Other than a small sample size, the lack of correlation in AS patients might be because serum IL-17 is produced by other cells in addition to Th17 cells, such as CD4+ T cells, γδ T cells, and mast cells.

There are some limitations to our study. Firstly, the patient number in our study is relatively small. The small sample size also led to a greater male to female ratio than the expectation (3:1). However, there was no difference between males and females in our cohort after analysis. Furthermore, our cohort has homogeneity in view of treatment and ethnicity, and various parameters of AS were analyzed and determined the potential role of IL-17 and IL-23 in the disease activity. Secondly, this is only a cross-sectional study and we did not follow the response to treatment and the serum IL-17 and IL-23 levels after treatment. Furthermore, we did not examine Th17 cells and other related cytokines in our study. Consequently, further study is needed to define the role of Th17 in AS.

In conclusion, our study demonstrated that the serum IL-17 and IL-23 levels were significantly higher in AS patients than in healthy controls and the levels correlate to disease activity measured by BASDAI scores, but not parameters of functional ability and spinal mobility. These results imply the role of IL-17 and IL-23 in the pathogenesis of inflammation in AS.

IL-17 and IL-23 may be potential treatment targets besides TNF-α. However, further studies are warranted.

Acknowledgments

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References

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<table>
<thead>
<tr>
<th>Cut off point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tbody>
<tr>
<td>ESR 17.50 mm/h</td>
<td>0.643</td>
<td>0.5</td>
<td>0.643</td>
<td>0.5</td>
</tr>
<tr>
<td>CRP 0.9105 mg/dL</td>
<td>0.643</td>
<td>0.4</td>
<td>0.6</td>
<td>0.444</td>
</tr>
<tr>
<td>IL-17 59.4 pg/mL</td>
<td>0.679</td>
<td>0.6</td>
<td>0.704</td>
<td>0.571</td>
</tr>
<tr>
<td>IL-23 179.42 pg/mL</td>
<td>0.786</td>
<td>0.6</td>
<td>0.733</td>
<td>0.667</td>
</tr>
</tbody>
</table>

BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; CRP = C-reactive protein; ESR = erythrocyte sediment rate; IL = interleukin; NPV = negative predictive value; PPV = positive predictive value.