

Original Article

Assessment of ankylosing spondylitis by serum cytokine profile

Yi Zhong^{1,2*}, Bingjun Zhang^{3*}, Zetao Liao¹, Jieruo Gu¹

Departments of ¹Rheumatology, ³Neurology, Third Affiliated Hospital, Sun Yat-sen University, Guangzhou, China;
²Department of Dermatology, Guangzhou Women And Children's Medical Center, Guangzhou, China. *Equal contributors.

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Abstract: Ankylosing spondylitis (AS) is characterized by new bone formation at sites of inflammation in ankylosis. The aim of this study was to differentiate the cytokines that results in poor outcomes and the major bone turnover cytokines that lead to new bone formation and, paradoxically, to osteoporosis. Thirty-seven selected cytokines were detected using a highly sensitive multiplex system in 46 AS patients and 20 healthy controls (HC) to identify specific profiles that could discriminate inflammatory activity, syndesmophyte formation and changes in bone mineral density. A clinical questionnaire, dual energy x-ray absorptiometry (DEXA) and spinal x-ray radiography were assessed simultaneously. Multiple logistic regressions were performed to confirm the characteristic risk cytokines. Based on Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores, 24 patients (52.17%) had active disease stage. Overall, 24 patients (52.17%) showed the presence of syndesmophytes, and 13 patients (28.26%) met the Osteoporosis (OP) criteria. Multivariate analysis of the serum cytokine profile showed that AS patients had higher concentrations of Osteopontin (odd ratio [OR]=0.99; P=0.024) and thymic stromal lymphopoietin (TSLP) (OR=0.842; P=0.032) than controls. Patients with active disease score presented a higher concentration of Chitinase-3 (OR=1.007; P=0.011). The concentration of Chitinase-3 also strongly correlated with total hipbone bone mineral density (BMD), expressed as g/cm². The presence of syndesmophytes was related to a lower level of Osteocalcin (OCN) (P=0.026). In conclusions, a specific cytokine profile of AS could be identified in patients with AS for the assessment of disease. Chitinase-3 combined with OCN may be an integrality predictive biomarker of AS activity, new bone formation and BMD loss.

Keywords: Ankylosing spondylitis, cytokine, Bio-Plex, inflammation, bone turnover

Introduction

Ankylosing spondylitis (AS) is a chronic, potentially disabling inflammatory rheumatic disease that affects the axial skeleton and peripheral joints and can lead to structural changes and functional impairments [1]. The major pathogenic events of AS include 1. inflammation of the enthesis, 2. pathological new bone formation and 3. Osteoporosis [2]. Although genetic factors are important in disease development, there is no clear manner of predicting a severe disease prognosis.

A number of studies have demonstrated the fundamental role of inflammatory cytokines in the disease process and in increased bone formation at sites of inflammation in ankylosis.

One of the best-studied groups of cytokines is the tumor necrosis factor superfamily (TNFSF) [3]. In AS, Tumor Necrosis Factor- α (TNF- α) is widely accepted as the primary cytokine involved in the early stages of disease. Most reports have validated that TNF blockade significantly reduces joint inflammation and destruction. Furthermore, the TNFSF member TNF-like protein 1A (TL1A, TNFSF15) has been identified as a potential modulator of AS in genome-wide association studies (GWAS) analysis [4]. In an analysis of the gene profile in AS macrophages, 55% of the differentially expressed genes are interferon (IFN)-regulated [5]. Moreover, a recent study suggested that a defect in IFN- γ signaling in antigen-presenting cells in spondyloarthritis (SpA) patients may result in T helper 17 (Th17) expansion and T regulatory

cells (Tregs) alteration, which may contribute to activation of the interleukin 23/17 (IL-23/IL-17) axis [6]. Although the preventive role of Treg cells in autoimmunity has been widely studied and it is known that an imbalance of Treg cells and inflammatory Th17 cells plays a role in AS pathogenesis, the underlying mechanism remains poorly understood [7].

Various studies have attempted to explain the mechanisms underlying the imbalance in bone turnover, which leads to syndesmophytes formation and, paradoxically, to osteoporosis [2, 8]. Several biomarkers, such as matrix Metalloproteinases (MMPs), Osteocalcin (OCN), and Osteopontin (OPN), are widely accepted as being related to bone turnover in AS [9-11]. The role of serum cytokines in the pathogenesis of new bone formation and bone destruction has not been fully elucidated.

With the technology for measuring the expression of many different biomarkers simultaneously from small serum samples, we have conducted an investigation of the relation between a large panel of inflammatory cytokines/bone turnover cytokines and disease characteristic in AS patients. Poor patient outcomes may be associated with discrete cytokine profiles, and combinations of cytokines and associated markers may be more informative than individual markers.

Methods

Patients and study design

Forty-six consecutive Chinese patients with AS who met the modified New York criteria were recruited for this study among patients who underwent dual energy x-ray absorptiometry (DEXA) for bone mineral density between March 2015 and August 2015 [12]. Plain radiographs of the spine (C1-L5), femoral neck and total hip were also performed on these patients every 12 months. Patient exclusion criteria were age younger than 18 years, clinical infection symptoms in the preceding 2 weeks, malignant disease, pregnancy or other autoimmune disorders that may affect cytokine concentrations. Twenty self-reported healthy volunteers with matched age and gender were chosen from the physical examination center in our hospital as controls, and all participants were selected by a face-to-face health questionnaire indicating

that respondents were without recent systemic disease or discomfort symptoms.

Clinical data and laboratory measurements

A clinical assessment of the disease characteristics was performed that included age, gender, disease duration, smoking habits, human leukocyte antigen B27 gene (HLA-B27) subtype, and disease-related scores (Bath Ankylosing Spondylitis Disease Activity Index [BASDAI], C-reactive Protein Ankylosing Spondylitis Disease Activity Score [ASDAS-CRP], Erythrocyte Sedimentation Rate Ankylosing Spondylitis Disease Activity Score [ASDAS-ESR]). Disease activity was assessed using the BASDAI as the standard instrument. The BASDAI is a self-administered instrument with six questions regarding individual domains of fatigue, spinal pain, joint pain and swelling, areas of local tenderness, and morning stiffness. BASDAI scores ≥ 4 indicate active disease stage. Two radiologists identified the presence and/or number of spinal syndesmophytes on plain radiographs. Bone mineral density (BMD) was evaluated based on the parameters of BMD (g/cm^2): T score and Z score. According to the International Society for Clinical Densitometry, patients with a Z-score < 2 standard deviation (SD) were considered to have osteopenia [13]. At enrollment, blood samples were collected to analyze inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and to perform the cytokine assay. All blood samples were immediately centrifuged at 3,000 rpm for 10 min at 4°C , and the sera were frozen at -20°C until analysis.

Measurement of inflammatory chemokine

Researchers measured the concentrations of 37 key biomarkers of inflammation from the TNF superfamily and IFN family proteins, Tregs, and MMPs: proliferation-inducing ligand (APRIL), B cell activating factor (BAFF), sCD30, sCD163, Chitinase-3-like 1 (CHI3L1), sIL-6R β , IFN- $\alpha 2$, IFN- β , IFN- γ , IL-2, sIL-6R α , IL-8, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-19, IL-20, IL-22, IL-26, IL-27 (p28), IFN- $\lambda 2$, IFN- $\lambda 1$, IL-32, IL-34, IL-35, LIGHT/TNFSF14, MMP-1, MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, thymic stromal lymphopoietin (TSLP), and tumor necrosis factor-like weak inducer of apoptosis (TWEAK). Researchers used Bio-Plex Pro Human Inflammation Assays

Cytokine profile in assessment of AS

Table 1. Clinical characteristics and cytokine concentrations in the AS group and healthy control group

	Univariate analysis		Multivariate analysis		
	AS group (n=46)	Control group (n=46)	P	OR (95% CI)	P
Age (years)	31.74±10.55	35.10±11.51	0.255		
Gender (male: female)	43:3	43:3	1		
Cytokines (pg/ml)	Median (IQR)				
Pentraxin-3	182.01 (121.00-308.97)	89.70 (68.76-153.29)	<0.001		
APRIL	2590.92 (2170.06-7202.76)	2170.06 (1063.13-3085.13)	0.001		
IFN- γ	4.56 (3.86-6.73)	3.86 (3.16-4.56)	0.002		
IL-20	5.12 (3.59-7.48)	2.94 (1.90-4.24)	<0.001		
IL-35	25.44 (19.08-35.53)	12.57 (5.81-19.17)	<0.001		
Osteocalcin	552.55 (375.00-780.14)	347.57 (266.87-488.34)	0.002		
Osteopontin	7836.12 (5584.87-10348.21)	5181.34 (3743.44-7061.28)	0.001	0.99 (0.99-1)	0.024
MMP1	1477.70 (890.10-2021.91)	616.24 (392.38-913.58)	<0.001		
MMP2	7822.11 (5636.71-12231.57)	11666.96 (8632.04-13197.27)	0.027		
LIGHT	2.12 (2.11-3.33)	2.12 (1.72-2.71)	0.048		
TSLP	18.29 (12.94-28.44)	13.15 (10.63-14.81)	0.001	0.842 (0.72-0.98)	0.032

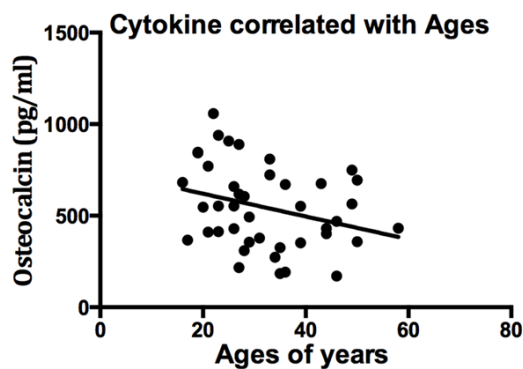


Figure 1. Association between Osteocalcin (pg/ml) and the age of AS patients ($r=-0.328$, $P=0.026$).

(Bio-Rad Laboratories, Inc., USA) and Bio-Plex Manager software version 6.0 in the Bio-Plex 200 System (Bio-Rad Laboratories, Inc., USA). Cytokines were determined according to the manufacturer's instructions. In brief, serum samples were thawed, centrifuged at 2,265 g for 5 min, and incubated with micro beads labeled with antibodies specific to the above-mentioned cytokines for 30 min. After a wash step, the beads were incubated with the detection antibody cocktail, with each antibody specific to a single cytokine. After another wash step, the beads were incubated with streptavidin-phycoerythrin for 10 min and washed again, and the concentration of each cytokine was then determined using the Bio-Plex Suspension Array Reader.

Statistical analysis

The Kolmogorov-Smirnov test was used to confirm that the data were within the ranges of a normal distribution. Descriptive statistics were shown as mean \pm standard deviation (SD), and the cytokine concentrations were presented as medians and interquartile ranges (IQRs). The correlations between the concentration of each cytokine and the clinical data were tested using Spearman's correlation test. Comparisons of the cytokine profiles in the clinical feature groups were analyzed using the Mann-Whitney test. To control for confounding factors, multiple logistic regressions were performed to confirm the relationships between serum cytokine profiles and disease diagnosis, severity, and the presence of syndesmophytes. For all tests, the differences were considered statistically significant at $P<0.05$. Statistical Product and Service Solutions (SPSS) software version 17 was used for all data management and statistical analysis.

Ethical considerations

This study was conducted in compliance with the Declaration of Helsinki to protect human subjects and was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University. Written informed consent was obtained from all study participants or a qualified family member.

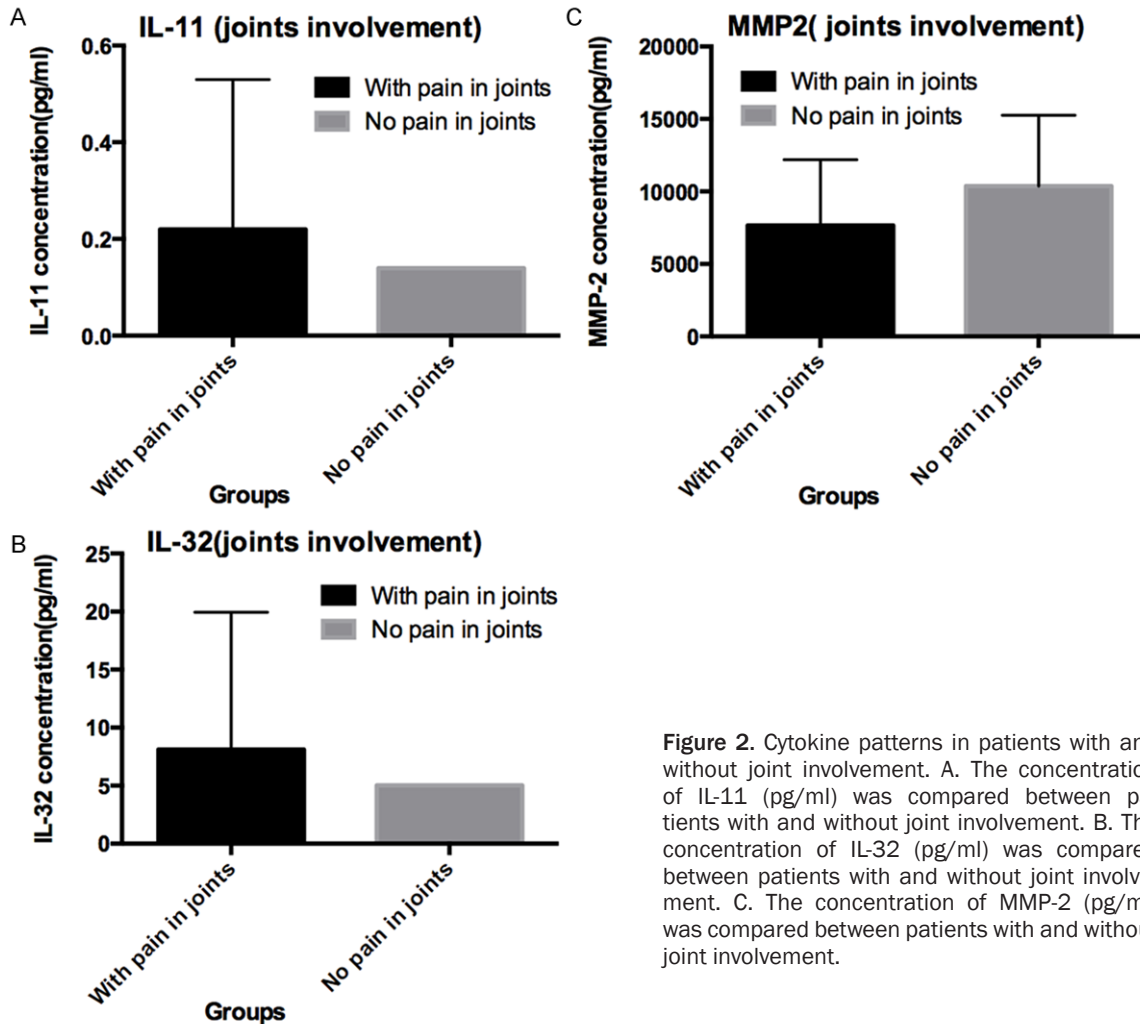


Figure 2. Cytokine patterns in patients with and without joint involvement. A. The concentration of IL-11 (pg/ml) was compared between patients with and without joint involvement. B. The concentration of IL-32 (pg/ml) was compared between patients with and without joint involvement. C. The concentration of MMP-2 (pg/ml) was compared between patients with and without joint involvement.

Result

Cytokine profiles of AS patients and healthy individuals

A total of 46 Chinese patients with AS who met the modified New York criteria and 20 healthy controls were enrolled in the study between March 2015 and August 2015. The mean age of 46 patients was 31.76 ± 10.55 years, and the majority of the patients were male (43; 91.67%) (**Table 1**). Comparisons of cytokine concentrations by univariate analysis demonstrated higher levels of Pentraxin-3, APRIL, IFN- γ , IL-20, IL-35, Osteocalcin, Osteopontin, MMP1, MMP2, LIGHT and TSLP in the AS patients than in the healthy controls (HC) ($P < 0.05$). Multiple logistic regression results indicated that higher concentrations of Osteopontin (odd ratio [OR] 0.99) and TSLP (OR 0.842) were significant markers

for AS. To control for the effect of gender, the same analysis was repeated for the male patients and the male control group. In this analysis, the serum concentrations of the cytokines referred to above remained significantly higher.

The average disease duration in the patients was 9.19 ± 7.60 years. Osteocalcin was observed to have a negative correlation with age ($r = -0.328$, $P = 0.026$) but not with disease duration (**Figure 1**). HLA-B27 was detected in 42 patients (91.30%); the cytokine profiles of patients with positive/negative HLA-B27 were similar ($P > 0.05$). In a subgroup analysis of 19 patients (41.3%) who were smokers, the serum concentrations of sCD30 ($P = 0.017$), Chitinase-3 ($P = 0.035$), MMP3 ($P = 0.026$) and sTnfr1 ($P = 0.009$) were elevated (**Figure 3**). In 24 patients with peripheral arthritis, the concentrations of IL-11

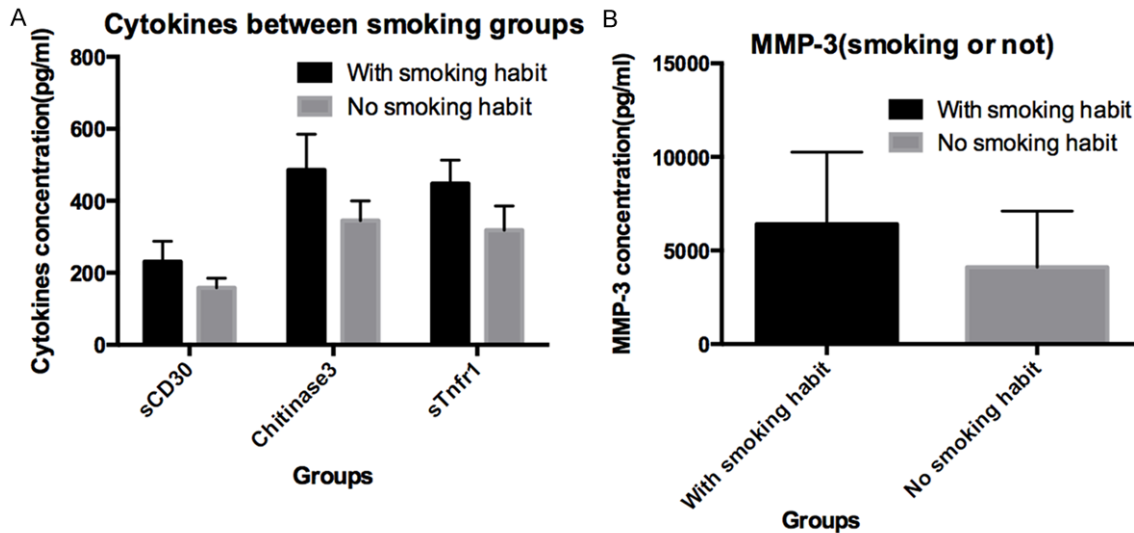


Figure 3. Cytokine patterns in smoking and non-smoking patients. A. The concentrations of sCD30, Chitinase-3, sTnfr1 were compared between smoking and non-smoking patients. B. The concentration of MMP-3 (pg/ml) was compared between smoking and non-smoking patients.

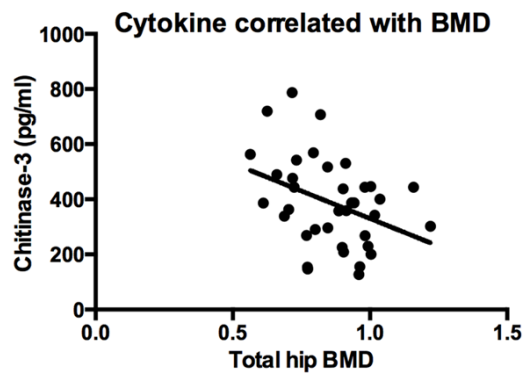


Figure 4. Association between Chitinase-3 (pg/ml) and total hip bone mineral density T score in AS patients ($r=-0.351$, $P=0.031$).

($P=0.048$) and IL-32 ($P=0.048$) were increased, and the level of MMP2 ($P=0.031$) was significantly decreased (**Figure 2**). Thus, age, smoking habits and joint pain were included in the multiple logistic regression models to be controlled as possible variables. Finally, the criteria for osteoporosis (OP) were met in 13 patients (28.26%) based on their lumbar spine, femoral neck or/and total hip results although no correlation was observed between these results and cytokine concentrations ($P>0.05$). The total hipbone BMD value was the only parameter that correlated with the level of Chitinase-3 ($r=-0.351$, $P=0.031$) (**Figure 4**).

AS disease activity and serum cytokine profile

Based on BASDAI scores, 22 patients were classified as having mild-moderate disease activity ($\text{BASDAI}<4$), whereas 24 patients had active disease stage ($\text{BASDAI}\geq 4$) (**Table 2**). Patients in the active disease group were older than those in the mild-moderate group; however, the difference was not statistically significant ($P=0.077$). Patients in the active disease group had the disease for a longer time ($P=0.001$) and showed a greater presence of syndesmophytes ($P=0.017$); however, the ratios of smokers to non-smokers ($P=0.245$) and the proportions of peripheral arthritis involvement ($P=0.77$), HLA-B27 positivity ($P=1$) and bone mineral density were similar. The serum concentrations of BAFF, IL-32, TSLP, Chitinase-3 and MMP3 were significantly higher in the active disease group than in the HC group ($P<0.05$). Multivariate analysis demonstrated a specific profile related to clinical severity, characterized by increased Chitinase-3 levels. The majority of the cytokine concentrations were similar in the two groups. Further analysis showed that the level of Chitinase-3 correlated with the disease activity biomarkers CRP ($r=0.485$, $P=0.001$), ESR ($r=0.319$, $P=0.031$), and the ASDAS-CRP ($r=0.409$, $P=0.005$) and the ASDAS-ESR ($r=0.349$, $P=0.017$) assessment scores.

Table 2. Clinical characteristics and cytokine concentrations according to disease activity in AS patients

	Univariate Analysis			Multivariate Analysis	
	Mild-Moderate (22)	Active (24)	P	OR (IC)	P
Age (yrs)	30.59±11.16	32.83±9.03	0.077		
Gender (male: female)	20:2	23:1	0.467		
Disease duration (yrs)	5.78±5.48	12.31±8.03	0.001	1.198 (1.051-1.366)	0.007
Smoking habit	7	12	0.245		
CRP	15.13±12.29	31.04±29.15	0.007		
ESR	15.57±12.01	31.05±25.97	0.010		
ASDAS-CRP	2.45±0.99	4.04±0.72	<0.001		
ASDAS-ESR	2.30±0.81	3.55±0.86	<0.001		
BASDAI	3.16±1.68	5.47±1.88	<0.001		
Presence of syndesmophytes	7	17	0.017		
Pain in joints	12	12	0.77		
HLA-B27 positive	20	21	1		
BMD (g/cm ²)					
Lumbar spine	0.89±0.14	0.91±0.12	0.73		
Femoral neck	0.78±0.12	0.72±0.16	0.20		
Total hip	0.897±0.12	0.82±0.16	0.13		
T score					
Lumbar spine	-1.75±1.34	-1.69±1.15	0.88		
Femoral neck	-1.03±0.87	-1.51±1.16	0.17		
Total hip	-0.83±0.81	-1.37±1.0	0.097		
Z score					
Lumbar spine	-1.61±1.37	-1.58±1.1	0.93		
Femoral neck	-0.87±0.86	-1.27±1.09	0.22		
Total hip	-0.75±0.85	-1.28±1.07	0.10		
Cytokines (pg/ml)					
BAFF	1976.08 (1747.27-2756.05)	2575.00 (2038.95-3647.24)	0.039		
IL-11	0.14 (0.14-0.15)	0.14 (0.14-0.16)	0.048		
IL-32	5.03 (5.02-5.04)	5.45 (5.03-6.188)	0.048		
Chitinase 3	304.52 (228.57-400.78)	443.74 (344.92-561.00)	0.004	1.007 (1.002-1.013)	0.011
MMP3	2923.04 (2115.54-4874.19)	5762.28 (3450.51-7509.37)	0.014		
TSLP	13.88 (12.42-27.06)	20.10 (16.50-29.95)	0.026		

Serum cytokine profile and presence of syndesmophytes

With regard to bone destruction, the presence of syndesmophytes and spinal fusion on spine radiographs was observed in 24 (52.17%) and 11 patients (23.91%), respectively. Patients who had syndesmophytes in the lumbar spine were compared with patients without that condition. Patients with syndesmophytes were older ($P<0.001$) and had a longer disease duration ($P<0.001$), higher HLA-B27 positivity ratio ($P=0.019$) and higher smoker to non-smoker ratio ($P=0.08$); for the latter, the difference approached statistical significance by univariate analysis. However, BMD and T scores in the femoral neck ($P=0.015$) and total hip ($P=0.03$) reached statistical significance, as shown in

Table 3. The only cytokine that correlated with the presence of syndesmophytes was Osteocalcin ($P=0.026$). On multiple logistic regressions, patient age had an OR=1.281 (95% CI=1.112-1.476), and total hip T score had an OR=0.485 (95% CI=0.23-1.019).

Discussion

In the current study, the serum concentrations of 37 cytokines were measured using a highly sensitive multiplex system in 46 AS patients and 20 healthy controls to identify specific cytokine profiles that could reflect the presence of inflammatory activity, syndesmophyte formation and bone loss. The data show that higher concentrations of OPN and TSLP can discriminate AS patients from healthy controls. The

Cytokine profile in assessment of AS

Table 3. Clinical characteristics and cytokine concentrations according to the presence of syndesmophytes in AS patients

	Univariate Analysis			Multivariate Analysis	
	Presence of syndesmophytes (24)	No of syndesmophytes (22)	P	OR (95% CI)	P
Age (years)	38.54±9.33	24.36±5.76	<0.001	1.307 (1.090-1.576)	0.004
Gender (male: female)	23:1	20:2	0.60		
Disease duration (yrs)	12.77±7.69	5.27±5.31	<0.001		
Smoking habit	13	6	0.08		
HLA-B27 positive	24	17	0.019		
Pain in joints	12	12	0.77		
BMD (g/cm ²)					
Lumbar spine	0.913±0.14	0.888±0.13	0.506		
Femoral neck	0.705±0.147	0.793±0.129	0.017		
Total hip	0.811±0.16	0.902±0.118	0.040		
T score					
Lumbar spine	-1.64±1.29	-1.79±1.18	0.563		
Femoral neck	-1.63±1.10	-0.96±0.92	0.015		
Total hip	-1.45±1.1	-0.81±0.77	0.03		
Z score					
Lumbar spine	-1.52±1.26	-1.66±1.19	0.506		
Femoral neck	-1.24±1.06	-0.94±0.94	0.201		
Total hip	-1.28±1.11	-0.81±0.84	0.146		
Cytokines (pg/ml)					
Osteocalcin	450.89 (353.73-667.92)	702.47 (412.84-894.52)	0.026		

Chitinase-3 is a dual biomarker that is strongly related to inflammatory activity and the bone mineral density (g/cm²). Indeed, the presence of syndesmophytes accompanied by low BMD associated with Osteocalcin.

The study demonstrated that elevated concentrations of TSLP and OPN are strongly correlated with AS. This is the first report to identify TSLP in the pathogenesis of AS. TSLP is involved in T cell development in the activation of human classical dendritic cells (DCs) and mediates the interaction between tissue cells and immune cells belonging to the innate and adaptive immune system [14]. In rheumatoid arthritis (RA), TSLP-stimulated sCDs in joints induce Th2 cell proliferation and secretion of Th1. During the production of Th1/Th2, the Th17-associated cytokines IFN- γ and IL-17 combine to attract CD4⁺ T cells to the site of inflammation [15, 16]. It is important to note that Th17 cytokines (IL-17 and IL-22) can contribute to bone erosion, osteitis and new bone formation, which are hallmark skeletal features associated with the pathophysiology of AS [17]. In our study, the

higher concentration of TSLP suggests that potent T cell activation and expansion, which supplement up-stream mechanisms, promote TH1/TH17-associated inflammation in AS, as well as in RA, systemic sclerosis and lupus nephritis [18].

With regard to OPN, our findings are also consistent with some previous studies, noting that AS patients overexpressed OPN [19]. However, S.T. Choi demonstrated that the level of OPN was independent of disease activity in AS [19]. Additionally, in our study, the level of OPN was observed to be similar between disease activity groups. The mechanism by which OPN contributes to the AS process remains poorly understood. Clinical studies have suggested that the OPN level is correlated with serum levels of alkaline phosphatase (ALP), OCN and C-terminal telopeptide fragments of type I collagen (CTX-I) in AS patients. Moreover, a recent study in AS patients demonstrated the presence of OPN in fibroblast-mesenchymal cells, which suggests a change of enthesal cells to an osteoblast phenotype [20]. Thus, OPN may be recognized

as a biomarker for bone remodeling rather than as an inflammatory cytokine in AS.

In the analysis of disease activity, patients with a higher score had a prolonged disease duration and a higher level of Chitinase-3 (CHI3L1). Further analysis indicates that our study is consistent with the finding that CHI3L1 shows a significant correlation with CRP, ESR, ASDAS-CRP and ASDAS-ESR, suggesting that CHI3L1 correlates positively with inflammatory activity and disease activity [21, 22]. Although originally described as a biomarker of inflammation, it was recently reported that up-regulation of CHI3L1 expression can counteract TNF- α -mediated inflammation by inhibiting Nuclear factor-KB (NF-kB) activation in human skeletal muscle cells [23]. Immune response studies have linked CHI3L1 to the down-regulation of the inflammatory mediators MMP1, MMP3 and IL-8, suggesting a protective role in inflammatory environments. The increase in CHI3L1 in response to inflammatory cytokines triggers a negative feedback loop that can control the inflammatory response.

Analysis of the relation between the cytokine profile and bone mineral density showed that only CHI3L1 was negatively correlated with total hipbone mineral density T scores and total hipbone mineral density, expressed in g/cm². As the inflammatory biomarker CHI3L1 increased, the BMD of total hipbone decreased and osteoporosis worsened (**Figure 4**). It was first reported that CHI3L1 correlated with BMD in AS. It had been previously suggested that the production of CHI3L1 is restricted to an injury response of the tissue. In rheumatoid arthritis, osteoarthritis and scleroderma, elevated CHI3L1 has been associated with a disorder characterized by increased connective tissue turnover [24]. In myeloma patients, it has also been observed that elevated CHI3L1 is accompanied by increased bone resorption, hastening bone destruction. The following findings may account for this phenomenon. It has been reported that silencing CHI3L1 resulted in a significant decrease in bone resorption activity, indicating that CHI3L1 is involved in promoting bone resorption. Moreover, during osteoclast differentiation, transfection with CHI3L1 decreases the levels of the pro-differentiating MMP9, which plays a critical role in osteoclast formation and bone resorption [25]. In addition, inflammatory

bone loss is a crucial pathological destruction that frequently occurs in AS. It is known that the activation of the IL-17 axis to the receptor activator of nuclear factor kappa-B ligand (RANKL) is a critical initiating event leading to an imbalance of bone modeling and remodeling, which is mediated by osteoclasts and osteoblasts [26]. Therefore, because osteoclasts are known to play a major role in pathological bone resorption in AS, osteoclast differentiation may account for the increased expression of CHI3L1.

The ages of patients with spinal syndesmophytes were higher whereas the ages of patients with BMD and osteocalcin (OCN) were lower than the ages of patients without syndesmophytes. The CRP, ESR, BASDAI and ASDAS between the two groups were not significantly different. A great number of studies observed the OCN associated with syndesmophytes; however, the tendency is controversial. Our result was consistent with a radiographic study that reported that the serum levels of OCN showed a downward trend in male AS patients with syndesmophytes compared with patients without syndesmophytes [27]. Controversially, a follow-up study in SpA patients treated with TNF- α inhibitor also reported that the patients developed new syndesmophytes and had greater increases in serum OCN from baseline to week 22 than patients with no new syndesmophytes [28]. OCN levels increased in patients with major improvement in ASDAS and decreased in patients with no clinically important improvement [10]. However, another cross-sectional study reported that the OCN was similar between the syndesmophytes group and the no-syndesmophytes group [29]. Even in a diagnosis predictor selection study, OCN was not significantly higher in AS patients than the control group. These studies considered either the BMD or disease activity; however, none of the studies combined both indexes. In early research, OCN was elevated in 34% of patients but was not associated with disease activity or BMD [30]. Previously, treatment with infliximab showed that baseline OCN levels at week 2 were significantly associated with increased BMD scores in the spine (week 102) and hip (weeks 24 and 102) of patients with AS [31]. In this study, patients with syndesmophytes had a lower BMD. The decrease in OCN suggested that the lower osteoblast activity and decreased

bone turnover rate play predominant roles in the process of OP that accompanies syndesmophyte formation in AS.

Our study also suggested risk factors other than inflammatory activity that affect the serum cytokine profile in AS. Not only did patients who are smokers have higher serum concentrations of sCD30, Chitinase-3, MMP3 and sTnfr1 but patients in the active disease group also had a higher ratio of smokers to non-smokers. These results are consistent with previous findings indicating that smoking hastens both inflammation and bone damage.

With regard to peripheral arthritis involvement, IL-11 and IL-32 were increased; however, MMP2 was significantly decreased in AS. In our study, MMP-2 declined in the AS group compared with the HC group based on multivariate regression analysis. These results were the first showing AS patients with peripheral joint disease having lower MMP-2 than patients without peripheral joint disease. MMP-2 expression is up regulated in autoimmune diseases with varying degrees of disruption of the immune response [32]. In an AS study by Derek L Matthey, MMP-2 was observed to be negatively associated with the CRP level [33], suggesting an anti-inflammatory role for this MMP, which is consistent with other studies in inflammatory arthritis [34, 35]. It was observed that MMP-2 affects the clearance of recruited immune cells, which is necessary for resolving inflammatory reactions [36].

There were several limitations to our study. First, because this was a single center case-control study, the number of AS patients included was relatively small. Further studies with more subjects are necessary to confirm our findings. Second, because this study was only conducted in southern Chinese patients, the serum levels of inflammatory and bone turnover cytokines were likely influenced by genetics, ethnicity, air pollution, dietary habits and gene-environment interactions; thus, the findings may not be generalizable to all patients. Third, the control group of health volunteers was not a 1:1 match; a study that included a 1:1 match could strengthen the study design and findings. The function (Bath ankylosing spondylitis functional index) and global health (Bath ankylosing spondylitis global health) assessment of the AS patients was not investi-

gated. Finally, because the included patients must undergo dual energy x-ray absorptiometry (DEXA) for bone mineral density in the last half-year, bias is inevitable.

In conclusion, our result implied that higher concentrations of Osteopontin and TSLP in a serum cytokine profile can discriminate AS patients from healthy controls and may be additional biomarkers for the diagnosis of AS. The Chitinase-3 serum concentration is a dual biomarker that is strongly related to inflammatory activity and bone mineral density (g/cm²). Indeed, poor disease prognosis is based on the presence of syndesmophytes accompanied by low BMD associated with Osteocalcin. Chitinase-3, combined with OCN, could integrally reflect activity, new bone formation and BMD loss. Finally, some disease features, such as age, gender, smoking habits and peripheral arthritis involvement, may modify the expression of cytokines and should be considered in future studies.

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Disclosure of conflict of interest

None.

Address correspondence to: Jieruo Gu, Department of Rheumatology, Third Affiliated Hospital of Sun Yat-sen University, No 600 Tianhe Road, Guangzhou 510630, Guangdong, China. Tel: +86-208525-2205; E-mail: gujieruo@163.com

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