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BIOMARKERS

Serum CCL11 level is associated with radiographic spinal damage in patients with ankylosing spondylitis

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Abstract

The clinical significance of C–C motif chemokine11 (CCL11) in bone metabolism in ankylosing spondylitis (AS) is not clearly elucidated. Thus, this cross-sectional study aimed to compare serum levels of CCL11 between patients with AS and healthy controls and to investigate the relationship between serum levels of CCL11 and radiographic spinal damage in patients with AS. We consecutively recruited 55 male patients with AS and 26 age- and sex-matched healthy controls. Serum levels of CCL11, tumor necrosis factor-α (TNF-α), interleukin-17, and Dickkopf-1 (DKK-1) were measured with commercially available enzyme-linked immunosorbent assay kits. Radiographs were scored according to the modified Stoke ankylosing spondylitis spine score (mSASSS), and syndesmophytes were defined as mSASSS ≥ 2. The serum levels of CCL11 in AS patients with syndesmophytes were significantly higher than those in AS patients without syndesmophytes ($p = 0.007$) and healthy controls ($p = 0.006$). In AS patients, the serum levels of CCL11 were significantly and positively correlated with mSASSS ($p = 0.006$), number of syndesmophytes ($p = 0.029$). After adjusting for confounding factors, elevated serum levels of CCL11 were associated with increased mSASSS ($\beta = 0.007$, $p = 0.03$) and higher risk for the presence of syndesmophytes (OR 2.34 per 50 pg/ml increase, $p = 0.012$) in AS patients. We found that the serum level of CCL11 was associated with structural damage in patients with AS, suggesting that CCL11 may serve as a promising biomarker for new bone formation in AS.

Keywords Ankylosing spondylitis · CC chemokines · Osteogenesis · Biomarkers · Tumor necrosis factor

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease that predominantly affects the axial skeleton such as the sacroiliac joint and spine. New bone formation in the form of syndesmophytes and bony bridges occurring at the cortical bone compartment of the axial skeleton is a distinguishing characteristic of AS, potentially leading to decreased spinal mobility and loss of function [1, 2]. Otherwise, inflammation-associated trabecular bone loss in the spine is another important clinical and pathophysiological features of AS [3], which is strongly associated with increased risk of fragility fracture [4]. Accordingly, impaired bone metabolism is evident during the disease course and

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can contribute to a significant clinical burden for patients with AS. New bone formation leading to structural damage is assumed to occur as a result of repair process secondary to inflammation-associated bone loss [1, 5], but the mechanism underlying bone metabolism in AS remains to be clarified. In addition, many studies have attempted to identify promising molecules for bone-related outcomes in AS including Dickkopf-1 (DKK-1), sclerostin, and adipokines [6–10]. However, these markers have not been validated and are not yet available in clinical practice [11–13]. Thus, further research is necessary to explore novel biomarkers that reflect structural bone damage in patients with AS.

C–C motif chemokine11 (CCL11), also known as eotaxin-1, exerts its action by binding CCR3 and is a potent chemoattractant for eosinophils. Thus, it plays an important role in the pathogenesis of allergic conditions such as bronchial asthma [14] and allergic rhinitis [15]. CCL11 can also recruit mast cells, Th2 cells, basophils, and macrophages and is produced by various cell types including eosinophils, endothelial cells, epithelial cells, fibroblasts, and chondrocytes [16]. Beyond its association with allergic diseases, elevated levels of CCL11 have also been identified in patients with other inflammatory diseases such as inflammatory bowel disease [17] and rheumatoid arthritis (RA) [18]. Although less is known about the effect of CCL11 on bone metabolism, a recent study has reported that osteoblasts express CCL11 during inflammatory conditions and osteoclastic bone resorption is activated by CCL11, suggesting a potential role of CCL11 in inflammatory bone diseases [16]. The association between elevated serum levels of CCL11 and less radiographic progression in early RA was previously reported [19], but the clinical significance of CCL11 in bone metabolism in AS remains poorly defined. Hence, in the present study, we aimed to compare the serum levels of CCL11 between patients with AS and healthy controls and to investigate the relationship between serum levels of CCL11 and radiographic spinal damage in patients with AS.

Methods

Study design and subjects

This observational and cross-sectional study involved 55 consecutively recruited male patients with AS and 26 age- and sex-matched healthy controls. The participants were recruited from a university-affiliated rheumatology center in Korea from March 2016 to December 2017. All patients with AS fulfilled the modified New York criteria for AS [20] and were aged ≥ 20 years. Exclusion criteria were as follows: (1) patients with other autoimmune rheumatic diseases except for AS; (2) AS patients having known allergic diseases including asthma, allergic rhinitis, and atopic dermatitis; (3) AS patients with chronic diseases of kidney, liver, thyroid, or parathyroid; (4) AS patients with active infection; (5) AS patients taking bisphosphonates or other anti-osteoporosis medications which could affect bone metabolism; and (6) AS patients who refuse to participate in the present study. Healthy controls had no history of autoimmune rheumatic diseases or any chronic diseases and were not receiving any medications which could affect bone metabolism. The present study was approved by the Research and Ethical Review Board of Pusan National University Hospital (IRB No. 1603-005-039). All study participants provided written informed consent in accordance with the principles of the Declaration of Helsinki.

Clinical, laboratory and radiographic evaluations

Demographic data, such as age, body mass index (BMI), and current smoking status, and laboratory markers, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), CCL11, tumor necrosis factor-α (TNF-α), IL-17, and DKK-1, in patients with AS and healthy controls were obtained at baseline visit. BMI was determined as weight divided by squared height (kg/m^2). All laboratory assays were performed with blood samples obtained after overnight fasting. CRP levels were measured using particle-enhanced immunoturbidimetric assay (Tina-Quant C-reactive protein assay; Roche Diagnostics, Zurich, Switzerland) with a P800 Module (Roche Diagnostics). The blood samples were centrifuged at 3000 rpm for 10 min at 4 °C, and the serum was separated and stored at − 80 °C until quantitative assessment of chemokine and cytokines. The serum levels of CCL11, TNF-α, IL-17, and DKK-1 were measured with commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA).

The following additional clinical parameters were assessed in patients with AS: disease duration, HLA-B27 status, Bath ankylosing spondylitis disease activity index (BASDAI), Bath ankylosing spondylitis functional index, Bath ankylosing spondylitis metrology index (BASMI), ankylosing spondylitis disease activity score (ASDAS)-CRP [21], ASDAS-ESR [21] and current medications.

Radiographs of lateral cervical and lumbar spine of all patients with AS were obtained at baseline and scored according to the modified Stoke ankylosing spondylitis spine score (mSASSS) [22]. Briefly, the anterior corners of C2 lower to T1 upper and T12 lower to S1 upper were scored for squaring, erosion, and/or sclerosis (1 point), nonbridging syndesmophyte (2 points), and bridging syndesmophyte (3 points) [22]. Syndesmophytes were defined as nonbridging or bridging syndesmophytes (mSASSS of 2 or 3, respectively) in at least 1 vertebral corner, and the number of syndesmophytes per patient with AS was recorded. All

radiographs were scored and analyzed by a highly experienced rheumatologist (Lee) who was blinded to clinical data.

Assessment of bone mineral density

Bone mineral density at the lumbar spine (L1–L4) and left hip (femoral neck and total hip) was measured in a subgroup of 27 patients with AS and 11 healthy subjects using dual energy X-ray absorptiometry (DEXA) equipment (GE-Lunar Prodigy or Lunar Prodigy advance, Madison, MA, USA). BMD was presented as g/cm^2 and the standard deviation (SD) from the age-matched healthy population (*Z*-score). Unlike women, there is no consensus regarding appropriate reference data in the assessment of BMD in male patients. Use of women reference population when calculating *Z*-scores in men is supported by the WHO [23], Scientific Advisory Council of Osteoporosis Canada [24], National Osteoporosis Guideline Group [25] and International Society for Clinical Densitometry [26]. Thus, calculation of the *Z*-score in this study was performed based on South Korean female reference data provided by the manufacturers. BMD examinations were carried out according to the standardized procedures provided by the manufacturer. The coefficient of variance was 0.33% at the spine and 0.4% at the hip in our center.

Statistical analyses

Quantitative variables are presented as mean ± SD or median (interquartile range; IQR) as appropriate and qualitative variables as numbers (percentages). To evaluate the normality of data distribution, we performed Kolmogorov–Smirnov test. For group comparisons, the Student's *t* test, Mann–Whitney *U* test, or Kruskal–Wallis test for quantitative variables and the Chi-squared test or Fisher's exact test for qualitative variables were performed as appropriate. Spearman's correlation analyses were conducted to assess the correlations among clinical and laboratory variables. To investigate independent relationship between the serum levels of CCL11 and mSASSS in patients with AS, we used stepwise multivariable linear regression models including variables with $p < 0.1$ in univariable analysis and a priori confounding factors such as TNF-α blockers. Because mSASSS was not normally distributed, log-transformed mSASSS determined as ln(mSASSS + 1) was included as an outcome variable (dependent variable) in our multivariable linear regression models. The association between the serum levels of CCL11 and the presence of syndesmophytes was assessed using backward multivariable logistic regression models. All statistical analyses were carried out with PASW version 18.0 (SPSS Inc., Chicago, IL, USA) and STATA version 11.0 (StataCorp LP, College Station, TX, USA). A *p* value less than 0.05 was considered statistically significant.

Results

Comparisons of clinical and laboratory characteristics between patients with AS and healthy controls are summarized in Table 1. For patients with AS, the mean ± SD disease duration and BADAI were 77.8 ± 51.7 months and 4.2 ± 2.2, respectively. All but one of the patients with AS showed positive results for HLA-B27, and the median (IQR) mSASSS was 5 (2–16). Twenty-six (47.3%) patients had one or more syndesmophytes. At baseline visit, 28 (50.9%) patients with AS were receiving TNF-α blockers and 16, 8, 2, 1, 1 and 1 patients were treated with adalimumab, etanercept, infliximab biosimilar, infliximab and golimumab, respectively. Serum levels of ESR, CRP, and DKK-1 in patients with AS were significantly higher than those in healthy subjects, whereas serum levels of TNF-α and IL-17 did not differ between these two groups. No statistically significant difference was observed in the serum levels of CCL11 between patients with AS and controls. However, the median serum levels of CCL11 in AS patients with syndesmophytes were significantly higher than those in AS patients without syndesmophytes [158.5 (131.3–185.8) vs. 106.1 (92.8–139.6) pg/mL, $p = 0.007$] and healthy controls [158.5 (131.3–185.8) vs. 105.6 (94.1–148.6) pg/mL, $p = 0.006$], as depicted in Fig. 1. In addition, as compared with healthy controls, patients with AS showed significantly lower total hip BMD and *Z*-score but exhibited comparable lumbar spine BMD and *Z*-score.

Table 2 shows differences in clinical and laboratory characteristics in patients with AS according to the presence of syndesmophytes. AS patients with syndesmophytes had significantly longer disease duration and a higher BASMI than those without syndesmophytes. In contrast to CCL11, no significant differences were noted in the serum levels of TNF-α, IL-17, and DKK-1 in patients with AS according to the presence of syndesmophytes. The *Z*-scores of the lumbar spine in AS patients with syndesmophytes were significantly higher than those in AS patients without syndesmophytes. However, no statistically significant differences were found in BMD and *Z*-scores at the femoral neck and total hip. We also compared laboratory markers between AS patients with BADAI ≥ 4 and those with < 4, but the differences in the serum levels of CCL11, TNF-α, IL-17, and DKK-1 were not statistically significant according to disease activity (data not shown).

Correlations of serum levels of CCL11 with other clinical and laboratory variables in patients with AS and healthy subjects are described in Table 3. In patients with AS, the serum levels of CCL11 were significantly and positively correlated with mSASSS ($\rho = 0.366$, $p = 0.006$), number of syndesmophytes ($\rho = 0.295$, $p = 0.029$), and BASMI ($\rho = 0.348$, $p = 0.01$). However, the serum levels

Table 1 Comparisons of clinical and laboratory characteristics between patients with ankylosing spondylitis and healthy controls

	AS patients ($n=55$)	Controls ($n=26$)	p value
Age, years, mean ± SD	37.8 ± 10.8	35.6 ± 6.8	0.492
BMI, kg/m^2, mean ± SD	25.1 ± 4.1	23.8 ± 3.2	0.173
Current smoker, *n* (%)	13 (23.6)	6 (23.1)	1
ESR, mm/h, median (IQR)	15 (6–30)	4 (2–9.5)	< 0.001
CRP, mg/dL, median (IQR)	0.26 (0.08–0.7)	0.04 (0.02–0.07)	< 0.001
CCL11, pg/mL, median (IQR)	132.8 (98.7–167.7)	105.6 (94.1–148.6)	0.127
TNF-α, pg/mL, median (IQR)	3.7 (2.6–14.1)	3.1 (2.6–5.9)	0.164
IL-17, pg/mL, median (IQR)	1.6 (1.2–2)	1.7 (1.2–2.7)	0.554
DKK-1, pg/mL, median (IQR)	2052.1 (1232.7–2790.5)	1358.7 (994.5–1960.3)	0.039
L1-4 BMD[a], g/cm^2, median (IQR)	1.25 (1.09–1.34)	1.2 (1.14–1.33)	0.808
L1-4 *Z*-score[a], median (IQR)	− 0.3 (− 1.7 to 1.2)	− 0.2 (− 0.5 to 1.01)	0.509
Femoral neck BMD[a], median (IQR)	0.96 (0.83–1.02)	1 (0.96–1.08)	0.211
Femoral neck *Z*-score[a], median (IQR)	− 0.2 (− 1.3 to 0.8)	0.4 (0.1–1.1)	0.074
Total hip BMD[a], g/cm^2, median (IQR)	0.96 (0.85–1.1)	1.1 (1.01–1.13)	0.035
Total hip *Z*-score[a], median (IQR)	0.1 (− 0.9 to 1.1)	1.1 (0.7–1.5)	0.007
Disease duration, months, mean ± SD	77.8 ± 51.7		
HLA-B27, *n* (%)	54 (98.2)		
BASDAI, mean ± SD	4.2 ± 2.2		
BASFI, mean ± SD	2.1 ± 2		
BASMI, mean ± SD	3.4 ± 1.8		
ASDAS-ESR, mean ± SD	2.63 ± 1.08		
ASDAS-CRP, mean ± SD	2.51 ± 1.03		
mSASSS, median (IQR)	5 (2–16)		
Number of syndesmophyte, median (IQR)	0 (0–6)		
Current medications			
NSAIDs, *n* (%)	38 (69.1)		
SSZ, *n* (%)	26 (47.3)		
MTX, *n* (%)	7 (12.7)		
TNF-α blockers, *n* (%)	28 (50.9)		

BMI body mass index, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *TNF-α* tumor necrosis factor-α, *DKK-1* Dickkopft-1, *BMD* bone mineral density, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BASMI* Bath Ankylosing Spondylitis Metrology Index, *ASDAS* Ankylosing Spondylitis Disease Activity Score, *mSASSS* modified Stoke Ankylosing Spondylitis Spine Score, *NSAIDs* non-steroidal anti-inflammatory drugs, *SSZ* sulfasalazine, *MTX* methotrexate

[a]Twenty-seven patients with ankylosing spondylitis and 11 health controls underwent dual energy X-ray absorptiometry

did not show correlations with disease activity indices, such as BASDAI, ASDAS-ESR, and ASDAS-CRP, or acute-phase reactants, such as ESR and CRP. In addition, the serum levels of CCL11 showed significant positive correlations with TNF-α ($\rho=0.331$, $p=0.013$) and DKK-1 ($\rho=0.32$, $p=0.017$) and a trend of positive correlation with IL-17 ($\rho=0.257$, $p=0.059$) in patients with AS. The serum levels of CCL11 were significantly positively correlated with lumbar spine BMD ($\rho=0.408$, $p=0.035$) and *Z*-scores ($\rho=0.488$, $p=0.01$) in patients with AS, but showed a trend of negative correlation with lumbar spine *Z*-scores ($\rho=-0.41$, $p=0.091$) in healthy controls.

Results of linear regression analyses for the log-transformed mSASSS in patients with AS are described in Table 4. In univariable linear regression models, increased serum levels of CCL11 were associated with higher log-transformed mSASSS in patients with AS. This association remained significant in multivariable linear regression analyses after adjusting for confounding factors including disease duration and use of medications, such as nonsteroidal anti-inflammatory drugs, and TNF-α blockers ($\beta=0.007$, SE = 0.003, $p=0.03$). Table 5 shows logistic regression models for the presence of syndesmophytes in patients with AS. After adjusting for confounding factors, the odds ratio

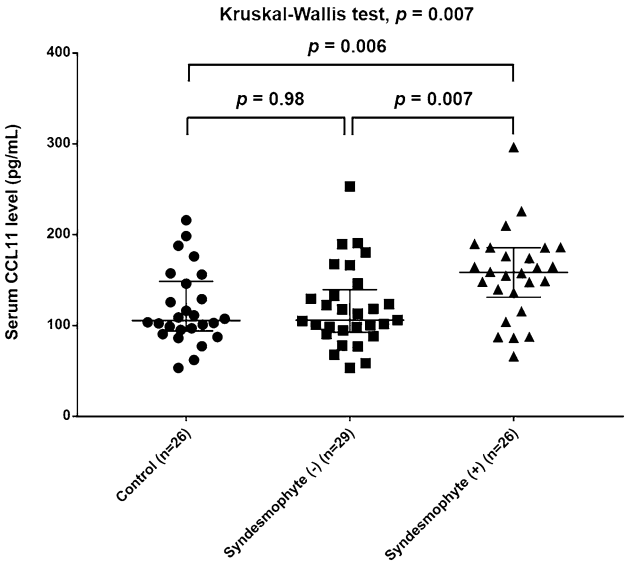

Fig. 1 Comparisons of serum CCL11 levels among healthy controls and two groups of patients (with and without syndesmophytes)

(95% confidence interval) for the presence of syndesmophytes was 2.34 (1.2–4.53) per 50 pg/mL increase in the serum levels of CCL11 in patients with AS.

Discussion

In the present cross-sectional study, AS patients with syndesmophytes had significantly higher serum levels of CCL11 than those without syndesmophytes and healthy subjects. In patients with AS, elevated serum levels of CCL11 were independently associated with higher risk for increased mSASSS as well as the presence of syndesmophytes after adjusting for confounding factors. This result indicates a potential role of this chemokine in the pathogenesis of new bone formation in AS. In addition, the serum levels of CCL11 in patients with AS were positively correlated with the serum levels of TNF-α, DKK-1, and IL-17, all of which are known to be associated with bone metabolism in AS. Otherwise, no correlations of serum levels of CCL11 with

Table 2 Differences in clinical and laboratory features in patients with ankylosing spondylitis according to the presence of syndesmophytes

	AS patients without syndesmophyte ($n=29$)	AS patients with syndesmophyte ($n=26$)	p value
Disease duration, months, mean ± SD	63 ± 34.1	94.3 ± 62.6	0.029
BMI, kg/m^2, mean ± SD	24.5 ± 3.9	25.7 ± 4.4	0.295
ESR, mm/h, median (IQR)	11 (3.5–25)	18.5 (10–39.5)	0.185
CRP, mg/dL, median (IQR)	0.26 (0.12–0.63)	0.23 (0.06–0.8)	0.993
BASDAI, mean ± SD	4.5 ± 2.3	4 ± 2.3	0.393
BASFI, mean ± SD	1.7 ± 1.7	2.5 ± 2.3	0.157
BASMI, mean ± SD	2.2 ± 1.2	4.6 ± 1.6	< 0.001
ASDAS-ESR, mean ± SD	2.6 ± 1.1	2.7 ± 1.1	0.826
ASDAS-CRP, mean ± SD	2.6 ± 1	2.5 ± 1.1	0.729
NSAIDs use, *n* (%)	23 (79.3)	15 (57.5)	0.134
TNF-α blockers use, *n* (%)	15 (51.7)	13 (50)	0.898
Current smoker, *n* (%)	5 (17.2)	8 (30.8)	0.343
CCL11, pg/mL, median (IQR)	106.1 (92.8–139.6)	158.5 (131.3–185.8)	0.007
TNF-α, pg/mL, median (IQR)	3.3 (2.5–18.9)	5.3 (3.1–12.3)	0.177
IL-17, pg/mL, median (IQR)	1.6 (1.2–3.6)	1.6 (1.2–1.8)	0.516
DKK-1, pg/mL, median (IQR)	1811 (922.4–2903.5)	2119.8 (1631.5–2579.4)	0.324
L1-4 BMD[a], g/cm^2, median (IQR)	1.29 (1.08–1.45)	1.18 (1.07–1.27)	0.116
L1-4 *Z*-score[a], median (IQR)	1.2 (−1.6 to 1.9)	−0.9 (−1.9 to 0.1)	0.033
Femoral neck BMD[a], median (IQR)	0.96 (0.82–1.06)	0.9 (0.83–1.05)	0.756
Femoral neck *Z*-score[a], median (IQR)	0.6 (−1 to 1)	−0.7 (−1.3 to 0.5)	0.155
Total hip BMD[a], g/cm^2, median (IQR)	0.95 (0.76–1.03)	0.96 (0.89–1.14)	0.402
Total hip *Z*-score[a], median (IQR)	0.1 (−1 to 1)	0.1 (−0.8 to 1.2)	0.83

BMI body mass index, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BASMI* Bath Ankylosing Spondylitis Metrology Index, *ASDAS* Ankylosing Spondylitis Disease Activity Score, *NSAIDs* non-steroidal anti-inflammatory drugs, *TNF-α* tumor necrosis factor-α, *DKK-1* Dickkopft-1, *BMD* bone mineral density

[a]Fourteen AS patients without syndesmophyte and 13 AS patients with syndesmophyte underwent dual energy X-ray absorptiometry

Table 3 Correlations of serum CCL11 levels with other clinical and laboratory variables in patients with ankylosing spondylitis and healthy subjects

Variables	Patients with AS ($n=55$)		Healthy subjects ($n=26$)	
	Correlation coefficient	p value	Correlation coefficient	p value
mSASSS	0.366	0.006	–	–
Number of syndesmophytes	0.295	0.029	–	–
Disease duration, months	0.109	0.43		
BASDAI	−0.073	0.598	–	–
BASFI	0.185	0.173	–	–
BASMI	0.348	0.01	–	–
ASDAS-ESR	0.106	0.44	–	–
ASDAS-CRP	−0.021	0.881	–	–
ESR, mm/h	0.217	0.112	0.431	0.028
CRP, mg/dL	0.027	0.845	0.063	0.758
TNF-α, pg/mL	0.331	0.013	0.11	0.591
IL-17, pg/mL	0.257	0.059	−0.016	0.942
DKK-1, pg/mL	0.32	0.017	−0.075	0.716
BMI, kg/m^2	−0.078	0.574	0.233	0.252
L1-4 BMD[a], g/cm^2	0.408	0.035	−0.286	0.25
L1-4 *Z*-score[a]	0.488	0.01	−0.41	0.091
Femoral neck BMD[a], g/cm^2	0.147	0.446	−0.23	0.358
Femoral neck *Z*-score[a]	0.276	0.164	−0.307	0.215
Total hip BMD[a], g/cm^2	0.023	0.911	−0.216	0.39
Total hip *Z*-score[a]	0.097	0.629	−0.407	0.093

mSASSS modified Stoke Ankylosing Spondylitis Spine Score, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *BASFI* Bath Ankylosing Spondylitis Functional Index, *BASMI* Bath Ankylosing Spondylitis Metrology Index, *ASDAS-ESR* Ankylosing Spondylitis Disease Activity Score-erythrocyte sedimentation rate, *ASDAS-CRP* Ankylosing Spondylitis Disease Activity Score-C-reactive protein, *TNF-α* tumor necrosis factor-α, *DKK-1* Dickkopft-1, *BMI* body mass index, *BMD* bone mineral density

[a]Twenty-seven patients with ankylosing spondylitis and 11 health controls underwent dual energy X-ray absorptiometry

disease activity markers, such as BASDAI and ASDAS, or acute-phase reactants, such as ESR and CRP, were observed. This finding suggests that the effect of CCL11 on inflammatory process in AS may be minimal. We also performed a subgroup analysis to investigate the association between serum levels of CCL11 and BMD. The result showed that the serum levels of CCL11 were positively correlated with lumbar spine *Z*-scores in patients with AS but showed a trend of negative correlation with lumbar spine *Z*-scores in healthy controls, suggesting a contradictory effect of CCL11 in patients with AS and healthy subjects on BMD.

To our knowledge, this study is the first to evaluate the serum levels of CCL11 in patients with AS. The major finding of our study was that increased serum levels of CCL11 were associated with structural damage, suggesting that CCL11 may contribute to osteoproliferation in AS. Although a recent experimental study has shown that CCL11 increases pre-osteoclast migration and concomitant bone resorption [16], a significant association was also reported between higher baseline serum levels of CCL11 and less radiographic progression in patients with early RA [19], which supports our hypothesis that CCL11 contributes to osteogenesis in AS. The reasons for this discrepancy remain unclear, but it may be due to a complex process of bone metabolism in AS. The pathologic process of new bone formation in AS is not well established. However, it has been recently proposed that inflammatory and biomechanical stress initially leads to bone loss and reduces bone strength via activation of osteoclast, which subsequently triggers a stabilizing anabolic effort resulting in new bone formation during the disease course [1]. Based on this concept, our results may be interpreted that CCL11 primarily activates osteoclast at early stage and sequentially induces osteoproliferation as a reactive process at later stage in AS. Taken together, the effect of CCL11 on bone metabolism in inflammatory arthritis, such as AS and RA, seems to be extremely complex and warrants clarification in further studies.

In this study, the serum levels of CCL11 were significantly and positively correlated with those of TNF-α and showed a trend of positive correlation with the serum levels of IL-17 in patients with AS. Although CCL11 may have a direct and specific role in osteogenesis, this finding suggests that the biologic action of CCL11 in bone metabolism may be connected with or mediated by TNF-α and/or IL-17, which are key inflammatory cytokines in AS. TNF-α and IL-17 are considered to have a mostly negative impact on bone formation in AS [1] because they have potent osteoclastogenic activity by upregulating the receptor activator of NF-κB ligand, which promotes differentiation and proliferation of osteoclast precursors [27] and inhibiting osteogenesis by suppressing the WNT signaling pathway, which is responsible for osteoblastogenesis [28–30]. In contrast to their classical action in osteoclastic bone loss, recent data have suggested that TNF-α and IL-17 could possibly enhance osteogenesis [31–33]. Lower levels of TNF-α moderately increase the expression of osteogenic transcription factors although higher levels of TNF-α treatment inhibit osteogenic differentiation [31]. IL-17 enhances bone matrix formation on human bone marrow-derived mesenchymal stem cells [32] and this effect is synergistic with bone morphogenetic protein 2 [33]. Thus, the effect of TNF-α and IL-17 on bone metabolism in AS may be heterogeneous. Further studies are needed to elucidate the exact role of CCL11 along with TNF-α and IL-17 in the pathogenesis of bone formation in AS.

Table 4 Linear regression models for the log transformed the modified Stoke Ankylosing Spondylitis Spinal Score in patients with ankylosing spondylitis

Variables	Univariable model		Multivariable model	
	β (SE)	p value	β^{a} (SE)	p value
Serum CCL11, pg/mL	0.007 (0.003)	0.025	0.007 (0.003)	0.03
Disease duration, months	0.009 (0.003)	0.006	0.008 (0.003)	0.007
NSAIDs use	−0.823 (0.344)	0.02	–	–
TNF-α blockers use	0.088 (0.335)	0.793	–	–
Current smoker	0.225 (0.393)	0.568		
BASDAI	−0.01 (0.075)	0.898		
ASDAS-ESR	0.146 (0.155)	0.351		
ASDAS-CRP	0.044 (0.164)	0.791		
ESR, mm/h	0.012 (0.009)	0.162		
CRP, mg/dL	0.16 (0.196)	0.418		
TNF-α, pg/mL	0.002 (0.002)	0.387		
IL-17, pg/mL	−0.006 (0.047)	0.901		
DKK-1, pg/mL	0 (0)	0.402		
BMI, kg/m^2	0.051 (0.04)	0.215		

NSAIDs non-steroidal anti-inflammatory drugs, *TNF-α* tumor necrosis factor-α, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *ASDAS-ESR* Ankylosing Spondylitis Disease Activity Score-erythrocyte sedimentation rate, *ASDAS-CRP* Ankylosing Spondylitis Disease Activity Score-C-reactive protein, *mSASSS* modified Stoke Ankylosing Spondylitis Spine Score, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *DKK-1* Dickkopft-1, *BMI* body mass index

[a]Estimated using stepwise linear regression models including serum CCL11 levels, disease duration, NSAIDs use and TNF-α blocker use

Table 5 Logistic regression models for the presence of syndesmophyte in patients with ankylosing spondylitis

Variables	Univariable model		Multivariable model	
	Crude OR (95% CI)	p value	Adjusted OR[a] (95% CI)	p value
Serum CCL11, per 50 pg/mL	2.41 (1.23–4.71)	0.01	2.34 (1.2–4.53)	0.012
Disease duration, months	1.01 (1–1.03)	0.03	1.01 (1–1.03)	0.042
NSAID use	0.36 (0.11–1.17)	0.088	–	–
TNF-α blockers use	0.93 (0.32–2.67)	0.898	–	–
Current smoker	2.13 (0.6–7.62)	0.244		
BASDAI	1 (0.7–1.14)	0.386		
ASDAS-ESR	1.06 (0.65–1.74)	0.822		
ASDAS-CRP	0.91 (0.54–1.53)	0.723		
ESR, mm/h	1.01 (0.98–1.04)	0.407		
CRP, mg/dL	1.04 (0.56–1.93)	0.91		
TNF-α, pg/mL	1 (0.99–1.01)	0.402		
IL-17, pg/mL	1 (0.84–1.15)	0.843		
DKK-1, pg/mL	1 (1–1)	0.282		
BMI, kg/m^2	1.08 (0.94–1.23)	0.296		

NSAIDs non-steroidal anti-inflammatory drugs, *TNF-α* tumor necrosis factor-α, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *ASDAS-ESR* Ankylosing Spondylitis Disease Activity Score-erythrocyte sedimentation rate, *ASDAS-CRP* Ankylosing Spondylitis Disease Activity Score-C-reactive protein, *mSASSS* modified Stoke Ankylosing Spondylitis Spine Score, *BASDAI* Bath Ankylosing Spondylitis Disease Activity Index, *DKK-1* Dickkopft-1, *BMI* body mass index

[a]Estimated using backward logistic regression models including serum CCL11 levels, disease duration NSAIDs use and TNF-α blocker use

In addition to new bone formation at the cortical bone compartment, AS is characterized by trabecular bone loss [34]. In subgroup analyses of our study, the serum levels of CCL11 were positively correlated with lumbar spine BMD and *Z*-scores in patients with AS, but this correlation was not observed in the femoral neck and total hip. Because lumbar

BMD measured by DEXA can be overestimated by syndesmophytes and/or bony bridges, which were associated with serum levels of CCL11 in our data, whether CCL11 directly acts as a protective factor for systemic bone loss at the trabecular bone or simply reflects the osteoproliferation at the cortical bone in AS is unclear. In contrast to patients with AS, a trend of negative correlation between serum levels of CCL11 and lumbar *Z*-scores was found in healthy controls. Thus, CCL11 seems to have different effect on BMD between patients with AS and healthy population. However, due to the small sample size that underwent BMD examination in our data, this interpretation should be taken with care, and larger studies should be performed to clarify the relationship between CCL11 and BMD in AS.

CCL11 has long been recognized as a proinflammatory chemokine involved in allergic and other inflammatory diseases including bronchial asthma [14], allergic rhinitis [15], atomic dermatitis [35] and inflammatory bowel disease [17]. With regard to chronic inflammatory arthritis, recent experimental studies have shown the increased level of CCL11 in synovial fluid and elevated expression of CCR3 in fibroblast-like synoviocytes in patients with RA [36] and osteoarthritis (OA) [37]. This finding implicates that CCL11–CCR3 may play an important role in the pathogenesis of synovitis in these conditions. In contrast to its association with bone metabolism, our data showed that the serum level of CCL11 was not significantly related to markers for disease activity, such as BASDAI, ASDAS, ESR, and CRP, in patients with AS. Thus, in contrast to RA and OA, the contribution of CCL11 to inflammation in AS may be minimal.

The present study has several limitations. First, due to its cross-sectional nature, our data could not provide evidence whether CCL11 can lead to radiographic progression in patients with AS and thus further longitudinal studies are needed to confirm our findings. Second, this study only evaluated male patients with AS; the role of CCL11 in bone metabolism in female counterparts also needs to be determined in further studies. Third, besides allergic diseases, production of CCL11 and its circulating levels can be affected by psychiatric disorders, such as schizophrenia, major depression, and bipolar disorder [38–40]. However, this study did not fully adjust the effect of psychiatric diseases on the serum levels of CCL11 in study subjects. Finally, due to the small size of the examined sample in the present study, further largers studies are needed to validate our results.

In conclusion, we found that the serum levels of CCL11 in AS patients with syndesmophytes were significantly higher than those in AS patients without syndesmophytes or healthy subjects. In addition, the elevated serum levels of CCL11 were independently associated with increased mSASSS and higher risk for the presence of syndesmophytes, which reflect spinal damage in patients with AS. This notion suggests that CCL11 may be exploited as a promising biomarker for new bone formation of AS. Although in vitro and animal studies as well as longitudinal studies are required to validate our results more conclusively, we believe that this study provides a novel insight into a potential role of chemokines in the pathogenesis involved in bone metabolism of AS.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

Ethical approval The present study was approved by the Research and Ethical Review Board of Pusan National University Hospital (IRB No. 1603-005-039). All study participants provided written informed consent in accordance with the principles of the Declaration of Helsinki.

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