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ORIGINAL ARTICLE

Correlation between magnetic resonance imaging (MRI) findings and the new bone formation factor Dkk-1 in patients with spondyloarthritis

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Abstract

In recent years, MRI has been regarded as a major diagnostic tool for spondyloarthritis (SpA), and anti-TNF therapy has been widely confirmed as an effective treatment strategy. This study was designed to investigate the correlation between the secreted protein dickkopf-1 (Dkk-1) and abnormal findings on magnetic resonance imaging (MRI) through a prospective study of 30 cases of SpA. Thirty patients with active SpA were included, all treated with recombinant human tumor necrosis factor (TNF) receptor-antibody fusion protein (YiSaiPu) injection at 50 mg/week for 6 months. All patients were also examined for their clinical, serological, and imaging manifestations of the condition before and after treatment. In patients receiving TNF inhibitor treatment, the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and clinical activity indices BASDAI, BASFI, BASMI, ASDAS-CRP were significantly decreased ($p < 0.01$). Serum Dkk-1 concentration was also significantly decreased ($p < 0.05$), as were the scores of bone marrow edema of the sacroiliac joints and the spine ($p < 0.05$). The score of sacroiliac joint backfill was significantly increased ($p < 0.05$), and the baseline and changes in the serum Dkk-1 concentration were significantly correlated with the baseline and changes in spinal bone marrow edema levels. Inhibition of the level of serum Dkk-1 by TNF inhibitors may be the molecular basis for inhibiting the formation of new bone in SpA patients. In addition, spinal marrow edema may have significance for predicting new bone formation.

Keywords Dkk-1 · Magnetic resonance imaging (MRI) · Spondyloarthritis (SpA) · TNF inhibitors

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Abbreviation

SpA	Spondyloarthritis
axSpA	Axial spondyloarthritis
AS	Ankylosing spondylitis
MRI	Magnetic resonance imaging
STIR	Short time inversion recovery
TNF	Tumor necrosis factor
ASAS	Assessment of Spondyloarthritis International Society
SPARCC	Spondyloarthritis Research Consortium of Canada
CANDEN	Canada-Denmark
FASSS	Fat Spondyloarthritis Spine Score
mSASSS	Modified Stroke AS Spine Score
DVU	Discovertebral unit
BME	Bone marrow edema
Dkk-1	Dickkopf-1
CTX	Collagen type I carboxy-terminal peptide
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

Introduction

In recent years, the latest advances in the diagnosis and treatment of spondyloarthritis (SpA) have been using MRI as the major diagnostic tool and administering anti- tumor necrosis factor (TNF) therapy as the major treatment strategy. Recent prospective data suggest that the spinal inflammatory damage in patients with ankylosing spondylitis (AS) will be eventually converted into fat. In these complex inflammatory lesions, bone formation and inflammation are not synchronized. Researchers have also found fat infiltration to be a repair process and accelerated by some treatments; potentially causing no harm to patients. AS is a disease characterized by new bone formation, and Wnt/β-catenin signaling has been commonly considered the major pathway regulating new bone formation. Thus, the serum level of Dkk-1, the natural inhibitor of Wnt protein, may be a main factor in blocking new bone formation. Therefore, we prospectively analyzed the correlation between MRI findings and Dkk-1 levels of 30 patients with SpA. Current research suggests that spinal inflammation repair, such as fat infiltration, may further stimulate syndesmophyte formation or calcification [1, 2]. To further determine the therapeutic effects of TNF inhibitors on each vertebra, a total of 690 vertebrae of the 30 patients were also individually evaluated. This study was designed to investigate the molecular mechanism underlying the TNF inhibitors' blockade on new bone formation, and to provide information for evaluating prognosis and choosing appropriate therapy for patients with SpA.

Materials and methods

Collection of cases

This study enrolled 30 patients with axial spondyloarthritis (axSpA) who fulfilled the Assessment of Spondyloarthritis International Society (ASAS) axSpA criteria. All patients received an injection of recombinant human tumor necrosis factor receptor-antibody fusion protein (YiSaiPu) at a dose of 50 mg/week for 6 months. Physical examination, blood tests, and a spinal and sacroiliac MRI were performed on all patients.

Exclusion criteria were the following: a history of infectious disease 2 weeks before admission, a history of other rheumatic diseases, and spinal surgery or spinal tumor.

Our study was approved by the ethics committee of PLA General Hospital. The reference number was C2014–004-01. Consent for publication was obtained from all patients.

Instruments, software, and imaging assessment protocol

Sagittal spine MRI images were obtained using a 1.5 T MRI scanner (GE, USA), and a ClearCanvas Workstation 2.0 was used for image reading. The sacroiliac joints and spinal MRI imaging of the patients before and after the treatment were blindly reviewed and scored using the Spondyloarthritis Research Consortium of Canada (SPARCC), Canada-Denmark (CANDEN) MRI definitions, and Fat Spondyloarthritis Spine Score (FASSS) scoring system by two individuals who were familiar with the system. We also calculated the difference between before and after treatment scores (the scores at baseline—the scores at 24 weeks).

Scoring of sacroiliac joint inflammatory lesions [3]

The SPARCC scoring method for active inflammatory lesions in the sacroiliac joint relies on the use of a T2-weighted sequence that incorporates the suppression of normal marrow fat signals. Each sacroiliac joint is divided into four quadrants: upper iliac, lower iliac, upper sacral, and lower sacral. The presence of an increased signal on short-time inversion recovery (STIR) sequence in each of these four quadrants was scored on a dichotomous basis, where 1 represents an increased signal and 0 represents a normal signal. The maximal score for an abnormal signal in the two sacroiliac joints of one coronal slice was, therefore, 8. Joints that included a lesion exhibiting an intense signal were each given an additional score of 1 per slice to demonstrate this feature. Similarly, each joint that included a lesion demonstrating a continuous increased signal of a depth of 1 cm from the articular surface was also given an additional score of 1. These additional measures bring the maximal score for a single coronal slice to 12. The scoring was repeated in each of the six consecutive coronal slices leading to a maximal score of 72.

Scoring of sacroiliac joints structural lesions [4]

In this analysis, the SPARCC sacroiliac joint structural score was used to assess the structural lesions of erosion, backfill, fat metaplasia, and ankylosis on T1W MRI. Paired baseline and 24-week T1W MRI scans were scored independently by two different readers blinded to patients.

The presence or absence of lesions in sacroiliac joint quadrants (erosion, fat metaplasia) or sacroiliac joint halves

(backfill, ankylosis) was recorded with a yes or no using direct online data entry based on a schematic of the sacroiliac joint. Erosion and fat metaplasia were each scored from 0 to 8 per slice on a total of five slices (total score 0–40). Backfill and ankylosis were each scored from 0 to 4 per slice on a total of five slices (total score 0–20).

Scoring of spine inflammatory lesions [5, 6]

Inflammation scores were developed from the CANDEN definitions of lesions and scoring [5, 6] in a way similar to FASSS. The development and validation of FASSS based on the CANDEN MRI definitions has previously been reported [7, 8].

Vertebral bodies In central sagittal slices, anterior and posterior corner inflammatory lesions were scored as 0 (absent) or 1 (present). In DVUs in thoracic and lumbar spine (T1/T2 and below), a score of 1 was added for large lesions (≥ 25% of the anteroposterior (AP) diameter of the vertebral endplate and/or height of the vertebral body, perpendicular to the endplate). Non-corner inflammatory lesions (bone marrow edema, or BME) located between the corners of a vertebral body were scored as 0 (absent) or 2 (present). In DVUs in the thoracic and lumbar spine, a score of 2 was added for large non-corner lesions (≥ 25% of the height of the vertebral body, perpendicular to the endplate). We decided to assign a score of 2 for non-corner lesions, and 1 for corner lesions (both doubled if large, see definitions), because the area of this anatomical region is typically larger than the corner areas, and because each vertebral endplate contains two corner regions but only one non-corner region, thereby giving the same total weight to the two lesion types in the central sagittal slices. If a corner lesion in any central slice involved > 50% of the AP diameter of the vertebra, it was counted as a combined corner and non-corner lesion.

In lateral sagittal slices in the thoracic and lumbar spine, BME was scored if it was located at the anterior or posterior corner. These antero-lateral and postero-lateral inflammatory lesions were scored as 0 (absent) or 1 (present). All lesions were scored based on anatomical location. For example, if a lesion was visible at the anterior corner on a central sagittal slice and in the anterior half of the vertebral body on a lateral slice, it was scored as the combination of an anterior corner lesion (sagittal slice) and an antero-lateral corner lesion (lateral slice).

Posterior elements For each of the 23 levels from C2/C3 to L5/S1, facet joint inflammatory lesions on the left and right sides, spinous process inflammatory lesions and soft tissue inflammatory lesions were each scored as 0 (absent) or 1 (present). These lesions were identified by a hyper intense signal on STIR at ligaments and entheses at the posterior elements of the vertebrae. For each of the 17 vertebrae from T1 to L5, rib/transverse process inflammatory lesions were scored as 0 (absent) or 1 (present) on the left and right sides. Inflammation in the costovertebral joints was not scored separately but as an inflammation of the rib/transverse process and/or the postero-lateral corner inflammation when present at these locations.

The total scoring range for the CANDEN spine inflammation score was 0–582. The range for the vertebral body sub score was 0–456, and the range for the posterior elements sub score was 0–126.

Scoring of spine fat metaplasia [7, 8]

Fat metaplasia (FASSS) was assessed at each DVU, which constitutes the region between two horizontal lines drawn across the midpoint of adjacent vertebrae in the sagittal orientation. Vertebral corner fat metaplasia in either central or lateral slices was scored dichotomously (lesion present or absent = 1 or 0). In DVUs in the thoracic and lumbar (but not cervical) spine, a score of 1 was added if corner fat metaplasia could be classified as large (see above) in central slices. Corner and non-corner lesions in the cervical spine were not assessed for size, because the vertebral bodies here are much smaller when compared with the vertebral bodies of the thoracic and lumbar segments. By definition, there are no lateral slices in the cervical spine, because the pedicles here are located lateral to the vertebral body and, therefore, both structures cannot be seen on the same sagittal slices. Corner fat metaplasia in lateral slices was not assigned a weighting for size, because it was difficult to assess size in relation to anterior–posterior diameter on this curved part of the vertebral body.

Non-corner fat metaplasia was scored only in central slices and assessed dichotomously (lesion present or absent = 2 or 0). In DVUs in the thoracic and lumbar (but not cervical) spine, a score of 2 was added if the non-corner lesion could be classified as large (see above). If corner fat metaplasia in any central slice involved more than 50% of the anterior–posterior diameter of the vertebra, it was considered a combined corner and non-corner fat lesion. DVUs where the height of the disc was reduced unequivocally by ≥ 50% were not assessed since the fat metaplasia here may be caused by coincidental or secondary degenerative disc disease.

Each DVU from C2/C3 to L5/S1 was assessed systematically for the different categories of fat metaplasia. The scoring range for each thoracic and lumbar DVU was 0 to 24, where the central slices provided a maximum score of 16 (four large corner lesions each scoring 2 = 8, and two large non-corner lesions each scoring 4 = 8). The lateral slices provided a maximum score of 8 (four right + four left corner lesions each scoring 1). The maximum score for each cervical DVU was 8 (four corner lesions each scoring 1 = 4, and two non-corner

lesions each scoring 2 = 4). The scoring range for all 23 DVUs that comprised the total FASSS spine score was 0 to 456.

Measurement of clinical factors

All patients' erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Metrology Index (BASMI), and Ankylosing Spondylitis Disease Activity Score (ASDAS-CRP) were tested before and after treatment.

Measurement of the serum Dkk-1

A Dkk-1 ELISA kit (R&D) was used to detect the serum Dkk-1 levels in the 30 patients before and after treatment. All samples were processed in accordance with the manufacturer's instructions. Changes in serum Dkk-1 levels before and after treatment were calculated (the serum Dkk-1 levels at baseline—the serum Dkk-1 levels at 24 weeks).

Statistical analysis

SPSS 19.0 software was used for statistical analysis. Qualitative data are described as number (%) and quantitative data are described as mean (±SD) and median (IQR). A *t* test or rank sum test was used for statistical analysis. The correlation between serum levels and radiographic score variables was evaluated by Spearman's correlation coefficient.

Results

Subjects

Thirty active SpA patients were included, consisting of 28 male and 2 female patients, ranging in age from 22 to 41 (31.17 ± 5.48) years. The duration of disease ranged from 6 to 251 months (93.54 ± 75.75), and 29 patients were human leukocyte antigen B27 (HLA-B27)-positive (96.7%).

Changes before and after the treatment of clinical indicators

ESR, CRP, BASDIA, BASFI, BASMI, ASDAS-CRP rates, and serum Dkk-1 concentration were all decreased significantly after the treatment. Results are shown in Table 1.

Changes before and after the treatment of imaging scores

Changes on MRI before and after treatment of sacroiliac joints

All patients were scored for bone marrow edema, fat infiltration, bone erosion, backfill, and stiffness on MRI imaging of the sacroiliac joints. As shown in Table 2, after the treatment, the scores of bone marrow edema and backfill were significantly decreased and increased, respectively. Although the scores of fat infiltration and bone erosion showed a slight increasing trend, they did not change significantly.

Changes on spinal MRI before and after treatment

All patients were scored for bone marrow edema and fat infiltration on spinal MRI imaging. As shown in Table 3, after the treatment, the spinal MRI bone marrow edema scores were significantly decreased, while fat infiltration scores have shown a slight increasing trend.

As shown in Table 4, before treatment, 158 (22.9%) of the vertebrae showed bone marrow edema, of which 137 showed a reduction or resolution BME after treatment. While among those vertebrae which showed edema improvement, 28 had increased fat infiltration, though the degrees of fat infiltration before and after treatment showed no statistical significance

Table 1 Changes of clinical indexes and serum Dkk-1 concentration before and after treatment

	Before treatment mean (±SD)	Before treatment median (IQR)	After treatment mean (±SD)	After treatment median (IQR)
ESR (mm/h)	23.78 ± 22.27	15.00 (6.00, 39.75)	5.03 ± 4.63**	2.50 (2.00, 8.00)
CRP (mg/dl)	2.59 ± 2.90	1.26 (0.38, 2.96)	0.40 ± 0.52**	0.20(0.10, 0.33)
BASDAI	6.23 ± 1.29	6.32 (5.40, 6.67)	2.52 ± 1.84**	2.13(1.33, 3.94)
BASFI	5.78 ± 1.44	5.60 (4.95, 6.35)	2.69 ± 1.72**	2.28(1.40, 3.40)
BASMI	2.46 ± 1.91	1.50 (1.00, 2.75)	0.69 ± 1.21**	0.00(0.00, 1.00)
ASDAS-CRP	3.77 ± 0.83	3.83 (2.99, 4.48)	1.58 ± 0.74**	1.60(1.02, 1.97)
Dkk-1 (ng/ml)	98.23 ± 113.41	55.41 (27.95, 138.16)	51.88 ± 41.90*	32.32(22.40, 59.22)

*$p < 0.05$,**$p < 0.01$ after vs. before treatment

Table 2 Changes of sacroiliac joints imaging scores before and after treatment

	Before treatment mean (±SD)	Before treatment median (IQR)	After treatment mean (±SD)	After treatment median (IQR)
BME	7.50 ± 12.02	2.00 (0.00, 11.25)	2.13 ± 5.48*	0.00 (0.00,1.75)
FAT	15.37 ± 13.93	10.50 (4.00, 29.00)	18.47 ± 17.19	14.50 (3.25, 29.00)
EROSION	4.50 ± 4.57	4.00 (2.00, 7.25)	4.13 ± 4.49	3.00 (0.25, 5.00)
BACKFILL	3.10 ± 2.87	2.50 (0.00, 5.00)	4.23 ± 3.85*	3.50 (0.50, 7.00)
ANKYLOSIS	7.03 ± 10.00	0.00 (0.00, 6.50)	7.37 ± 10.78	0.00 (0.00, 13.25)

*$p < 0.05$ after vs. before treatment

(Table 4). The changes found by spine-MRI are shown in Figs. 1 and 2.

Moreover, 38 (5.5%) of these vertebrae had no bone marrow edema before treatment but showed fat infiltration after treatment.

The correlation between serum Dkk-1 and clinical factors

Correlation analysis was performed to determine the relation between serum Dkk-1 concentration and the clinical factors (ESR, CRP, BASDIA, BASFI, BASMI, and ASDAS-CRP) at baseline. The results indicate that, before treatment, there was no significant correlation between serum Dkk-1 concentration and the clinical factors (Table 5).

The correlation between serum Dkk-1 and imaging scores

The correlation between serum Dkk-1 concentration and sacroiliac joint MRI imaging

Correlation analysis was performed to determine the relation between serum Dkk-1 concentration and the scores of bone marrow edema, fat infiltration, bone erosion, and backfill seen on MRI imaging of the sacroiliac joints before treatment. Results are shown in Fig. 3.

The results indicate that, before treatment, there was no significant correlation between the serum Dkk-1 concentration and the sacroiliac MRI bone marrow edema, fat infiltration, bone erosion, and backfill ($r = -0.075$, $p = 0.703$; $r = -0.173$, $p = 0.399$; $r = 0.260$, $p = 0.181$; $r = -0.174$, $p = 0.376$). Correlation analysis was also performed to determine the relation between the changes in serum Dkk-1 levels before and after treatment and the difference before and after treatment in bone marrow edema, fat infiltration, bone erosion, and backfill scores of the sacroiliac joints. There was no significant correlation between the serum Dkk-1 concentration and the sacroiliac MRI scores ($r = 0.017$, $p = 0.931$; $r = 0.104$, $p = 0.597$; $r = -0.156$, $p = 0.427$; $r = 0.080$, $p = 0.684$). Results are shown in Fig. 4.

The correlation between serum Dkk-1 concentration and lesions on spinal MRI imaging

Correlation analysis was performed between the serum Dkk-1 concentration before treatment and the scores of bone marrow edema and fat infiltration on spinal MRI imaging. Results are shown on Fig. 5. The results show that, before treatment, the serum Dkk-1 concentration is significantly correlated with the spinal bone marrow edema scores ($r = 0.606$, $p = 0.001$) but not with fat infiltration scores ($r = 0.144$, $p = 0.465$).

Correlation analysis was also performed between the changes in serum Dkk-1 levels before and after treatment and the difference before and after treatment in spinal bone marrow edema or fat infiltration scores, and the results are shown on Fig. 6. The results show that the changes in serum Dkk-1 levels before and after treatment significantly correlate with the difference before and after treatment in spinal MRI BME scores ($r = 0.434$, $p = 0.021$, but not with the difference before and after treatment in fat infiltration scores ($r = -0.080$, $p = 0.684$).

Discussion

Data have shown that TNF-α inhibitors are effective for patients with Ankylosing Spondylitis (AS) [9]. Besides

Table 3 Changes of imaging scores before and after treatment

	Before treatment mean (±SD)	Before treatment median (IQR)	After treatment mean (±SD)	After treatment median (IQR)
BME	20.27 ± 23.53	17.50 (3.00, 28.25)	6.08 ± 8.09*	4.00 (0.00, 9.50)
FAT	10.08 ± 10.38	5.50 (3.00, 17.50)	13.81 ± 15.34	9.00 (3.00, 16.75)

*$p < 0.01$ after vs. before treatment

Table 4 Changes after treatment in 158 vertebrae showed bone marrow edema before treatment

	Bone marrow edema decreased	Bone marrow edema not decreased	Total
Fat infiltration reduced or did not change	109	1	110
Fat infiltration increased	28	20	48
Total	137	21	158

improving patient symptoms, they may also improve quality of life and slow the radiographic progression of the condition [10]. In our study, all patients received a TNF-α inhibitor developed by Sunshine Guojian Pharmaceutical (Shanghai) Co., Ltd. The generic name of this inhibitor is Recombinant Human Tumor Necrosis Factor α Receptor: IgG Fc Fusion Protein for Injection (brand name: YiSaiPu). After TNF inhibitor treatment, improvements were demonstrated by significant decreases in BASDAI, BASFI, BASMI, EST, CRP, and ASDAS-CRP, which were observed in all patients.

Various classification methods have been used by scholars to describe the sacroiliac joints and spinal MRI findings of patients with SpA. A commonly accepted method of scoring is the SPARCC method, which divides the SpA-associated lesions into acute inflammation and chronic structural lesions. The acute inflammatory lesions mainly refer to bone marrow edema, while the chronic structural lesions include bone erosion, fat infiltration, backfilling, bone spurs, and bone bridge formation [11, 12]. To better assess the lesions shown on MRIs of SpA patients, the SPARCC and CANDEN methods were used

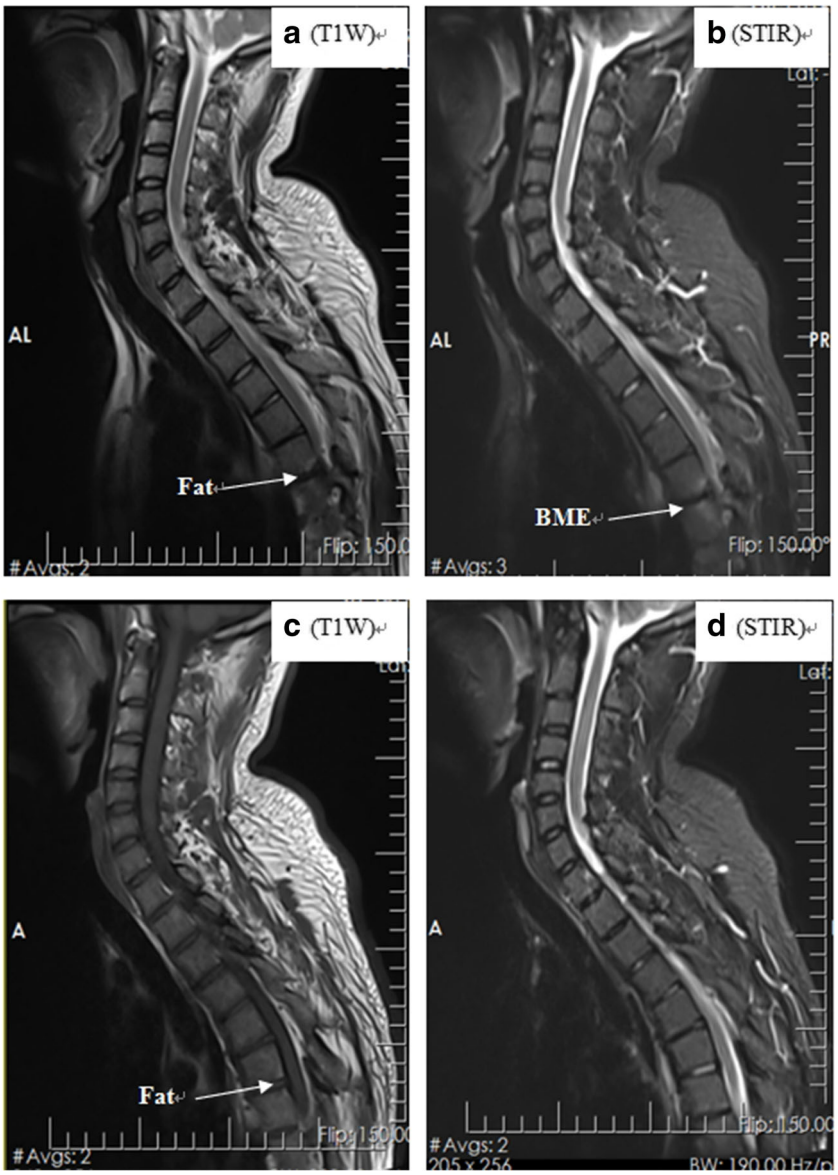

Fig. 1 There are fat infiltration and BME in T1W and STIR, respectively, at baseline (**a**, **b**, white arrow). After treatment, fat infiltration was still present (**c**, white arrow) and BME had disappeared (**d**)

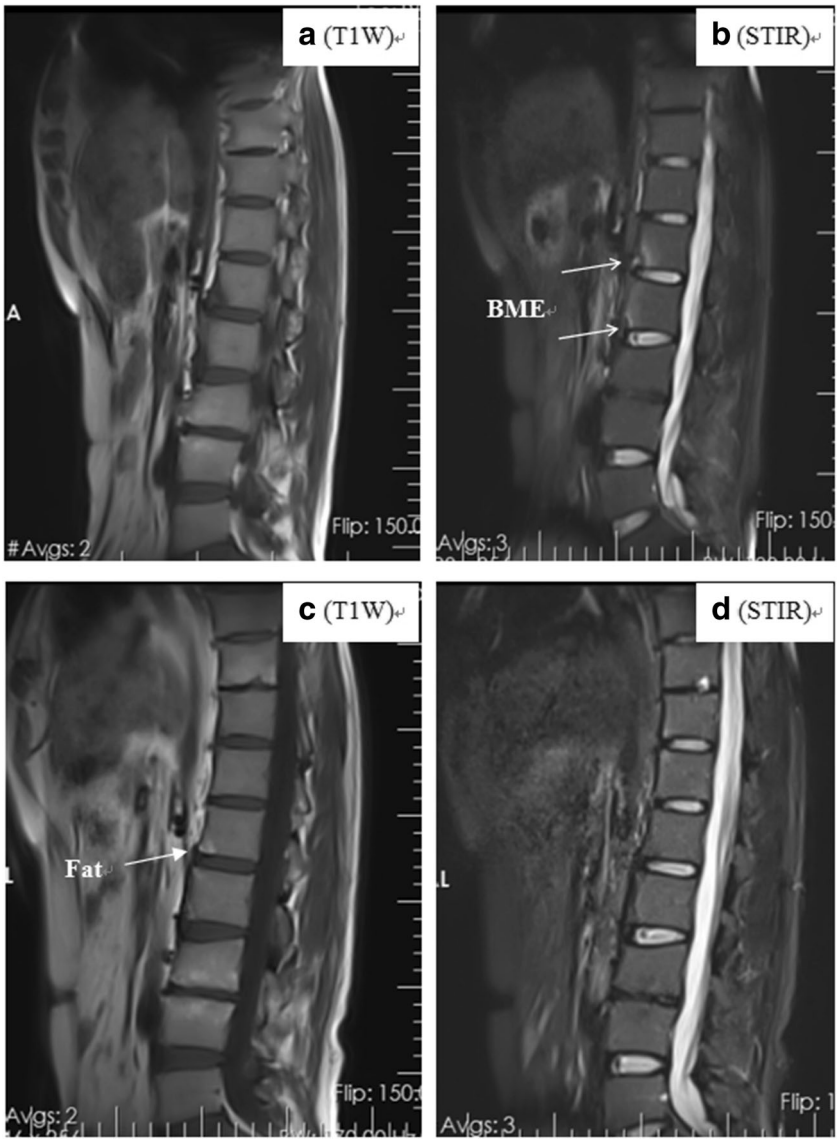

Fig. 2 There is BME in STIR at baseline (**b**, white arrow). After treatment, fat infiltration appeared at T1W (**c**, white arrow), while BME disappeared (**d**)

to score the acute and chronic inflammations of the sacroiliac joints and spinal MRIs in our patients. The results show that TNF inhibitors can significantly improve BME in both the sacroiliac joints and spine, and increase sacroiliac joints backfilling scores; but have no effect on fat infiltration and bone erosion in either the sacroiliac joints or the spine. A total of 690 vertebrae of the 30 patients were then evaluated individually. The results show that a total of 158 vertebrae had BME before treatment, which either decreased or resolved in 137 (86.7%) of those vertebrae after treatment. Among the vertebrae which showed improvement in BME, fat infiltration was not observed in 110 (69.6%). In addition, a total of 38 vertebrae from 18 patients having no BME before treatment developed fat infiltration after treatment. This result indicates that TNF inhibitors are not able to completely prevent chronic lesions like fat infiltration in some vertebrae. In a 5-year study following AS patients who received TNF inhibitor treatment [13], it has been found that, though the incidences of fat infiltration in the second year was high in vertebrae that showed inflammations at baseline, no new bone formation was noted until the fifth year. In addition, research has also found that the occurrence rates of fat infiltration slowed from the second year to the fifth

Table 5 Correlation between Dkk-1 serum level and characteristics of SpA patients

	Spearman r_s	p value
ESR	0.028	0.887
CRP	− 0.014	0.943
BASDAI	− 0.001	0.997
BASFI	− 0.166	0.398
BASMI	− 0.295	0.127
ASDAS-CRP	0.060	0.760

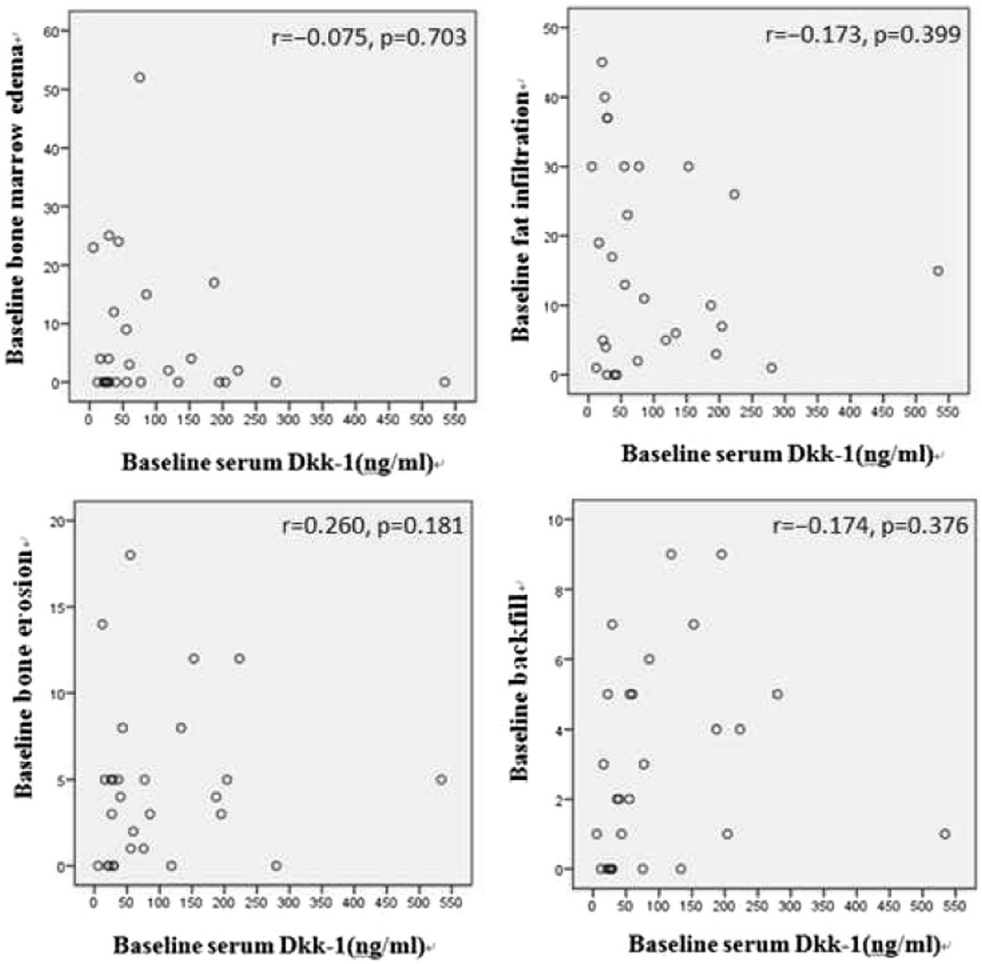

Fig. 3 Serum Dkk-1 level before treatment is not correlated with bone marrow edema, fat infiltration, bone erosion, or backfilling

year during the process of TNF inhibitor treatment, which may indicate that it does no harm to the patient by potentially accelerating the repair process of fat infiltration through some treatments.

To further understand the relationship between the MRI changes and new bone formation in SpA patients, we analyzed their serum Dkk-1 levels and found that those levels in active SpA patients before treatment were significantly higher than those in remission after the treatment. Korkosz's study also found that serum Dkk-1 decreased significantly after TNF inhibitor treatment in AS patients with high disease activity [14]. Dkk-1 is an antagonist of the classical Wnt signaling pathway by binding to the LRP5/6 receptor of osteoblasts and inhibiting bone formation by acting at several of differentiation stages of the osteoblasts [15, 16]. Papapoulos and his team from Leiden University Medical Center of Netherlands [17] found that the increase of Dkk-1 level was a compensatory response to the lack of sclerostin and the increase in bone formation. Their research indicated that the Dkk-1 levels in sclerosteosis patients were significantly increased when compared to healthy controls. Further, serum Dkk-1 level and (CTX) levels are positively correlated in sclerosteosis patients. This finding also explains why Dkk-1 levels were increased in our active SpA patients.

Biological agents are some of the most effective treatments for SpA; however, the key factor affecting the prognosis of patients with SpA is new bone formation. It has been proved that TNF antagonists do not inhibit bone formation directly. In a mice arthritis model, TNF antagonists promoted new bone formation by inhibiting the effect of TNF-α on osteoblasts. In the same study, even though etanercept inhibited inflammation effectively, it failed to prevent new bone formation [18]. The ultimate goal of SpA treatment is to improve the prognosis of the patients, and this result complicates the choice of SpA treatment strategies. Two years ago, the German researcher Baraliakos [19] investigated two cohorts, which were DIKAS that continuously used full doses of infliximab, and Herne that did not use TNF inhibitors. The differences of their modified stroke AS spine scores (mSASSS) were compared in the 8th year of the study. The result revealed that the differences of mSASSS

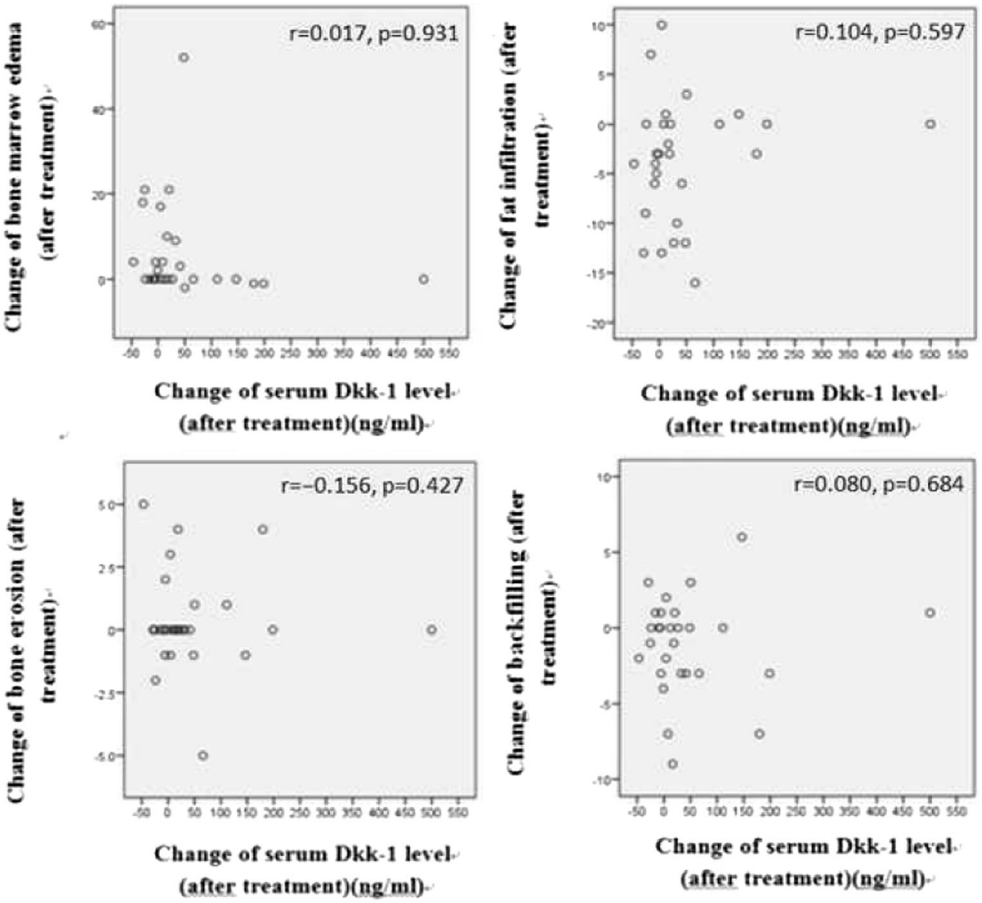

Fig. 4 Difference in serum Dkk-1 levels before and after treatment is not correlated with the differences in bone marrow edema, fat infiltration, bone erosion, or backfilling

between the two cohorts were not significantly increased during the first 4 years, but gradually increased from the fourth year to the eighth year, and became statistically significant at the eighth year. This study indicates that a prolonged observation period may prove that TNF inhibitors can inhibit new bone formation. However, long-term usage of biological reagents will undoubtedly bring a huge economic burden to society and individuals. For this reason, it is of particular importance for rheumatologists to decide the proper timing to start and stop the application of biological agents. Our research found that, after TNF antagonist treatment, the patients' clinical symptoms were relieved, and the serum Dkk-1 levels were significantly reduced, which may be the molecular mechanism of TNF antagonists' inhibitory effects on new bone formation. Nocturne [20] and his team found that Dkk-1 serum level was correlated with CRP level and the presence of sacroiliitis on radiography, but none of the other

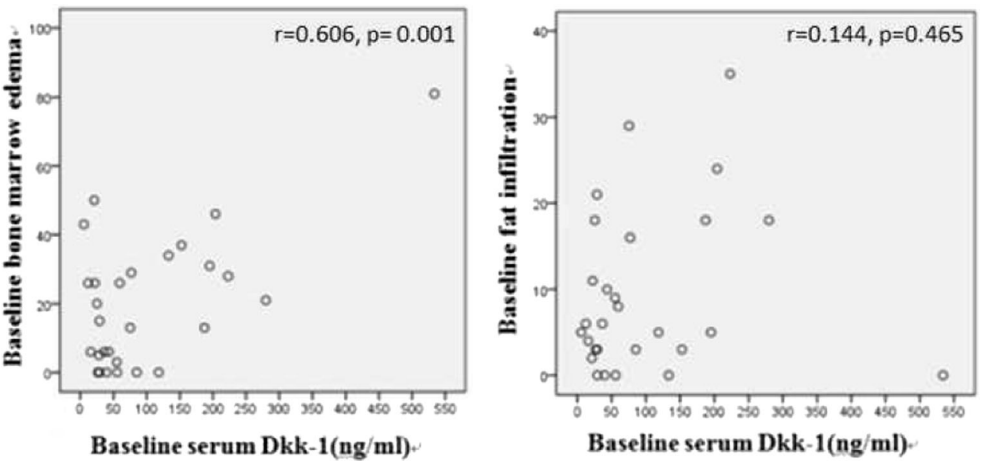

Fig. 5 Serum Dkk-1 level before treatment is correlated with bone marrow edema, but not correlated with fat infiltration

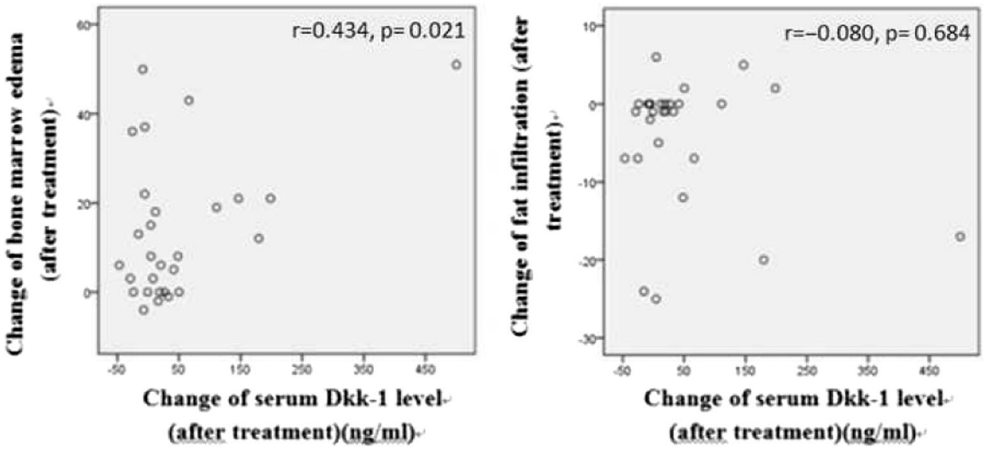

Fig. 6 Difference in Serum Dkk-1 levels before and after treatment is correlated with the differences in bone marrow edema, but not correlated with the differences in fat infiltration

studied variables (age, gender, weight, and BASDAI) were significantly correlated with Dkk-1 serum level. However, our research found that Dkk-1 serum level was not correlated with systemic inflammation or disease activity assessed by ESR, CRP level, ASDAS-CRP, BASDAI, BASFI, or BASMI, but positively correlated with the spinal MRI acute inflammation. The change levels of serum Dkk-1 were also positively correlated with the change levels of the spinal MRI acute inflammation, but not with the fat infiltration scores. These same correlations were not found in MRI images of the sacroiliac joints, which indicates that spinal bone marrow edema may predicate new bone formation and can be used as better evidence for prognostic evaluation. Thus, for patients with spinal bone marrow edema, a more progressive treatment may be administered for better prognosis. However, further research is needed on patients who have received long-term TNF antagonist treatment to find the time points when serum Dkk-1 level could reach a stabilized plateau. Increased knowledge in this area will be helpful when assessing a predictive marker for the timing of treatment withdrawal.

In conclusion, this is the first work to find a significant correlation between the spinal bone marrow edema shown on MRI and the serum Dkk-1 levels. Although patient sample size is relatively small, our result still reveal the molecular basis responsible for new bone formation in SpA patients, and provides a theoretical basis for determining the timing of biological agents treatment as well as evaluating patient prognosis.

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Compliance with ethical standards

Disclosures None.

References

1. Maksymowych W, Chiowchanwisawakit P, Lambert R (2010) TNF blocking agents promote resolution of structural lesions in patients with spondyloarthritis. Ann Rheum Dis 69:262
2. Chiowchanwisawakit P, Lambert RG, Conner-Spady B, Maksymowych W (2011) Focal fat lesions at vertebral corners on magnetic resonance imaging predict the development of new syndesmophytes in ankylosing spondylitis. Arthritis Rheum 63: 2215–2225
3. Maksymowych WP, Inman RD, Salonen D, Dhillon SS, Williams M, Stone M, Conner-spady B, Palsat J, Lambert RGW (2005) Spondyloarthritis research consortium of Canada magnetic resonance imaging index for assessment of sacroiliac joint inflammation in ankylosing spondylitis. Arthritis Rheum 53:703–709
4. Maksymowych WP, Wichuk S, Chiowchanwisawakit P, Lambert RG, Pedersen SJ (2015) Development and preliminary validation of the spondyloarthritis research consortium of Canada magnetic resonance imaging sacroiliac joint structural score. J Rheumatol 42: 79–86
5. Pedersen SJ, Østergaard M, Chiowchanwisawakit P et al (2009) Validation of definitions for active inflammatory lesions detected by magnetic resonance imaging in the spine of patients with spondyloarthritis. J Rheumatol 36(suppl 84):35–38
6. Krabbe S, Sørensen IJ, Jensen B, Møller JM, Balding L, Madsen OR, Lambert RGW, Maksymowych WP, Pedersen SJ, Østergaard M (2018) Inflammatory and structural changes in vertebral bodies

and posterior elements of the spine in axial spondyloarthritis: construct validity, responsiveness and discriminatory ability of the anatomy-based CANDEN scoring system in a randomised placebo-controlled trial. RMD Open 4(1):e000624

7. Chiowchanwisawakit P, Østergaard M, Pedersen SJ et al (2009) Validation of definitions for structural lesions detected by magnetic resonance imaging in the spine of patients with spondyloarthritis. J Rheumatol 36(suppl 84):39–47
8. Pedersen SJ, Zhao Z, Lambert RGW, Wichuk S, Østergaard M, Weber U, Maksymowych WP (2013) The FAt Spondyloarthritis Spine Score (FASSS): development and validation of a new scoring method for the evaluation of fat lesions in the spine of patients with axial spondyloarthritis. Arthritis Res Ther 15(6):216
9. Calin A, Dijkmans BAC, Emery P, Hakala M, Kalden J, Leirisalo-Repo M, Mola EM, Salvarani C, Sanmartí R, Sany J, Sibilia J, Sieper J, van der Linden S, Veys E, Appel AM, Fatenejad S (2004) Outcomes of a multicentre randomized clinical trial of etanercept to treat ankylosing spondylitis. Ann Rheum Dis 63: 1594–1600
10. Inman RD, Clegg DO, Davis JC, Whitmore JB, Solinger A (2006) Etanercept in adult patiens with early onset ankylosing spondylitis1. J Rheumatol 33(8):1634–1636
11. Robert GW, Lambert SJ, Chiowchanwisawakit P, Østergaard M (2009) Active inflammatory lesions detected by magnetic resonance imaging in the spine of patients with spondyloarthritis — definition, assessment system and reference image set. J Rheumatol 36(Suppl 84):3–17
12. Østergaard M, Maksymowych WP, Susanne J, Chiowchanwisawakit P, Lambert RG (2009) Structural lesions detected by magnetic resonance imaging in the spine of patients with spondyloarthritis —definition, assessment system and reference image set. J Rheumatol 36(Suppl 84):18–34
13. Baraliakos X, Heldmann F, Callhoff J, Listing J, Pappas D. No evidence for a role of the hypothesized sequence inflammation-fatty degeneration-new bone formation in patients with ankylosing spondylitis treated with infliximab or other anti-TNF agents over 5 years. EULAR 2012 abstract [THU0285]
14. Korkosz M, Gąsowski J, Leszczyński P, Pawlak-Buś K, Jeka S, Siedlar M, Grodzicki T (2014) Effect of tumour necrosis factor-α inhibitor on serum level of dickkopf-1 protein and bone morphogenetic protein-7 in ankylosing spondylitis patients with high disease activity. Scand J Rheumatol 43(1):43–48
15. Wang FS, Ko JY, Lin CL, Wu HL, Ke HJ, Tai PJ (2007) Knocking down diekkopg-1 alleviates estrogen deficiency induction of bone loss. A histomorphological study in ovarietomized rats. Bone 40(2): 458–492
16. McCarthy HS, Marshall MJ (2010) Dickkopf-1 as a potential therapeutic target in Paget's disease of bone. Expert Opin Ther Targets 14(2):221–230
17. van Lierop AH, Moester MJ, Hamdy NA, Papapoulos SE (2014) Serum Dickkopf 1 levels in sclerostin deficiency. J Clin Endocrinol Metab 99(2):E252–E256
18. Lories RJ, Derese I, de Bari C, Luyten FP (2007) Evidence for uncoupling of inflammation and joint remodeling in a mouse model of spondylarthritis. Arthritis Rheum 56:489–497
19. Baraliakos X, Haibel H, Listing J, Sieper J, Braun J (2014) Continuous long-term anti-TNF therapy does not lead to an increase in the rate of new bone formation over 8 years in patients with ankylosing spondylitis. Ann Rheum Dis 73(4):710–715
20. Nocturne G, Pavy S, Boudaoud S, Seror R, Goupille P, Chanson P, van der Heijde D, van Gaalen F, Berenbaum F, Mariette X, Briot K, Feydy A, Claudepierre P, Dieudé P, Nithitham J, Taylor KE, Criswell LA, Dougados M, Roux C, Miceli-Richard C (2015) Increase in Dickkopf-1 serum level in recent spondyloarthritis. Data from the DESIR cohort. PLoS One 10(8):e0134974