Association between musculoskeletal ultrasonography and bone remodelling markers and its role in disease monitoring of gout and hyperuricaemia

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Abstract

Objective

To evaluate associations between bone destruction markers and musculoskeletal ultrasonography (MU) findings in patients with gout and hyperuricaemia and clarify the role of MU in treatment responsiveness.

Methods

One-hundred and fifty patients with gout and 100 patients with hyperuricaemia were divided into five groups according to MU manifestations. Circulating Dickkopf-1 (DKK-1) and receptor activator of nuclear factor-κB ligand (RANKL) levels were measured. Thirty patients from the gout group and 10 from the hyperuricaemia group, were treated for 1 year with urate-lowering therapy (ULT).

Results

Patients with gout and tophus and/or bone erosion had the highest DKK-1 and RANKL levels. Patients with gout and MU-evidenced aggregates and/or double-contour signs had higher DKK-1 and RANKL levels than the normal MU group ($p<0.001$). Patients with hyperuricaemia and abnormal MU findings had significantly higher DKK-1 and RANKL levels than those with normal MU findings. DKK-1 and RANKL levels positively correlated with disease duration in patients with gout ($r=0.430$, $p<0.001$; $r=0.359$, $p<0.001$, respectively) and hyperuricaemia ($r=0.446$, $p<0.001$; $r=0.379$, $p<0.001$, respectively). After ULT, MU abnormalities disappeared in 12 and 8 patients with gout and hyperuricaemia, respectively. The largest tophus diameter decreased in patients with gout ($t=6.092$, $p<0.001$). DKK-1 and RANKL concentrations significantly decreased in all patients. Lower serum urate levels corresponded with higher ratios of normal MU features in all patients.

Conclusion

In patients with gout and hyperuricaemia, MU manifestations were associated with DKK-1 and RANKL levels and were ameliorated after ULT. Thus, MU could be a useful tool in assessing bone remodelling and monitoring disease responsiveness.

Key words

gout, hyperuricaemia, ultrasonography

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Received on June 29, 2019; accepted in revised form on October 1, 2019.

Funding: this work was supported by grants from the National Natural Science Foundation of China (no. 81771678).

Competing interests: none declared.

Introduction

Gout is a metabolic disease in which a disturbance in purine metabolism causes excessive production and/or reduced excretion of uric acid (1). This leads to a subsequent continuous rise in blood uric acid concentration, resulting in deposition of urate crystals in soft tissues. Clinical manifestations include recurrent acute and chronic arthritis, formation of tophi caused by hyperuricaemia, and urate crystal deposition in connective tissues (especially in the cartilage and synovium), which lead to joint erosion and deformities.

Musculoskeletal ultrasonography (MU) is a feasible, non-invasive, reproducible imaging technique with clear visualisation of soft tissues. It was included in the new 2015 gout classification criteria developed by American College of Rheumatology and the European League Against Rheumatism (2). It has gained increasing importance for the diagnosis and monitoring of gout. The study by Ogdie *et al*. showed that the sensitivity and specificity of double-contour sign (DCS) on US were 0.83 (95% CI: 0.72–0.91) and 0.76 (95% CI: 0.68–0.83), respectively, and the sensitivity and specificity of tophus on US were 0.65 (95% CI: 0.34–0.87) and 0.80 (95% CI: 0.38–0.96), respectively (3).

Gout and hyperuricaemia cause deposition of monosodium urate monohydrate (MSU) in synovial fluid and other tissues, which would cause disordered bone remodelling (4). Dickkopf-1 (DKK-1) and receptor activator of nuclear factor-κB ligand (RANKL) are two common mediators of bone remodelling (5, 6). DKK-1 is a secreted glycoprotein abnormally expressed in diseases such as osteoporosis (7), rheumatoid arthritis (8), and multiple myeloma (9). It can block the transduction of Wnt signals into cells, which reduces bone formation (10). Studies have shown that DKK-1 may be involved in bone destruction in gout (11). RANKL is mainly expressed in the bone tissue and in the immune system (6, 12). It can promote osteoclast differentiation, enhance the viability of mature osteoclasts, and prevent osteoclast apoptosis (13). Ashika Chhana found that the RANKL is independently associated with bone erosion in gout (14).

However, there are few studies on the relationship between musculoskeletal ultrasonography and bone destruction-related cytokines/proteins in patients with gout and hyperuricaemia. Therefore, this study examined the correlations between indicators of bone destruction and various ultrasonic manifestations of joints in gout and hyperuricaemia, by monitoring the levels of serum DKK-1 and RANKL. We aimed to further explore the usefulness of MU in the diagnosis and monitoring of articular pathological changes occurring in gout and hyperuricaemia.

Materials and methods

Patients

A total of 150 patients with gout and 100 with hyperuricaemia were recruited at the Department of Rheumatology and Immunology, Peking University People's Hospital from July 2016 to June 2017. Patients with rheumatoid arthritis, osteoarthritis, osteoporosis, and other rheumatisms were excluded. All patients were men. The diagnosis was made on the basis of the new gout classification standard issued by EULAR and ACR in 2015. Musculoskeletal ultrasonography of bilateral first metatarsophalangeal, bilateral ankle, and bilateral knee joints was performed in the enrolled patients. Circulating DKK-1 and RANKL were measured in all patients. Among them, 30 patients from the gout group and 10 from the hyperuricaemia group were followed up for 1 year with urate-lowering therapy (ULT). Our study was complied with the Declaration of Helsinki. The Medical Ethics Committee of the Institute of Peking approved the research protocol, and informed consent was obtained from all subjects.

Ultrasound examination and serological tests

Patients with gout were divided into three groups according to US manifestations: normal group (Group A), aggregates and/or double-contour signs group (Group B), and tophus and/or bone erosion group (Group C). Similarly, patients with hyperuricaemia

Table I. General characteristics of patients with gout and hyperuricaemia.

Characteristics	Patients with gout				Patients with hyperuricaemia		
	Group A	Group B	Group C	*p*-value	Group D	Group E	*p*-value
Age (y)	46.1 ± 17.0	41.2 ±14.9	46.4 ±14.3	$p \geq 0.05$	40.8 ±16.0	44.1 ± 14.7	$p \geq 0.05$
Disease duration (y)	3.1 (1.7, 5.9)	4.2 (2.9, 6.7)	7.5 (3.4, 10.7)	$p < 0.05$	3.2 (1.5, 5.2)	4.0 (2.9, 6.8)	$p < 0.05$
SUA (μmol/L)	526.1 ±116.4	545.8 ±140.2	498.4 ± 117.6	0.153	524.7 ± 128.4	538.8 ± 131.2	0.187
FPG (mmol/L)	5.3 ± 0.6	5.4 ± 0.7	5.3 ± 0.8	0.583	5.1 ± 0.8	5.2 ± 0.7	0.621
TG (mmol/L)	2.41 ± 1.30	2.30 ± 1.22	2.38 ± 1.25	0.495	2.27 ± 1.78	2.34 ± 1.24	0.427
TC (mmol/L)	4.95 ± 1.02	4.78 ± 1.05	4.81 ± 0.99	0.232	4.68 ± 1.01	4.81 ± 1.02	0.530
ALT (U/L)	26.00 (18.00, 50.25)	28.00 (17.50, 44.25)	29.00 (19.00, 51.25)	0.156	27.00 (16.00, 46.25)	28.00 (17.00, 44.25)	0.368
AST (U/L)	21.50 (16.75, 29.25)	20.50 (15.75, 27.25)	20.00 (14.00,28.25)	0.325	20.50 (15.75, 29.50)	21.00 (17.75,28.25)	0.303
BUN (mmol/L)	5.71 ± 1.08	5.83 ±1.28	5.97 ± 1.10	0.418	5.95 ± 2.62	5.84 ±1.22	0.406
Crea (μmol/L)	82.13 ± 11.77	87.55 ±11.53	90.75 ± 12.67	0.102	84.4 ± 24.9	88.7 ± 22.8	0.233

SUA: serum uric acid; FPG: fasting plasma glucose; TG: triglyceride; TC: cholesterol; ALT: alanine transaminase; AST: aspartate transaminase; BUN: blood urea nitrogen; Crea: creatinine. $p<0.05$: statistically significant.

were classified into two groups: normal US group (Group D) and abnormal US group with at least one of the following manifestations: aggregate, double-contour signs, tophus, or bone erosion (Group E). Each subject was examined by the same physician qualified in joint ultrasonography. Serum DKK-1 and RANKL concentrations were measured via ELISA. The DKK-1 and RANKL kits were purchased from Cloud-Clone Corp (US). The specific experimental procedures were carried out according to the instructions of the manufacturer. Clinical data including demographic data and laboratory tests were collected. After 1 year of urate-lowering therapy, the patients whose fasting serum uric acid levels were <360 μmol/L met the criteria for enrolment. Therefore, a total of 18 patients with gout in group B, 12 patients with gout in group C, and 10 patients with hyperuricaemia in group E were selected.

Statistical analysis

The statistical analysis was performed using Statistical Package of Social Science (SPSS) software v. 21.0. The normal distribution of variables was verified using the Shapiro-Wilk test. Continuous variables were expressed as mean ± standard deviation (SD) and discrete variables were expressed as median (interquartile range). For statistical comparisons, the independent t-test, the paired t-test, one-way ANOVA, and rank sum tests were used. Spearman's tests were used for correlation analysis. $p<0.05$ was considered statistically significant.

Results

Patient characteristics

The patients with gout were all men, with a mean age of 44±16 years and a median disease course of 4.0 (2.1,6.2) years. The patients with hyperuricaemia were also all men, with a mean age of 42.6±15.6 years and a median disease course of 3.7 (2.0,5.6) years. Age, serum uric acid, liver and kidney functions, blood glucose, and lipid were well matched among groups. However, the duration of disease was significantly different in gout groups and hyperuricaemia groups (Table I).

Comparison of levels of DKK-1 and RANKL among patients with gout and hyperuricaemia

The serum DKK-1 levels in group A, group B, and group C were (807.9±373.8) ng/L, (1309.3±496.4) ng/L, and (1722.2±482.7) ng/L, respectively. As shown in Figure 1, the serum concentration of DKK-1 in group C was significantly higher than that in group B ($p<0.001$), and the serum concentration in group B was significantly higher than that in group A ($p<0.001$). The serum RANKL concentrations in group A, group B, and group C were (0.10+0.09) ng/mL, (0.35+0.29) ng/mL, and (0.78+0.47) ng/mL, respectively. As indicated in Figure 1, Group C had the highest concentration of serum RANKL, followed by group B and group A ($p<0.001$).

The serum DKK-1 levels in group D and group E were (889.7±235.2) ng/mL and (1478.8±367.9) ng/mL, respectively. The serum RANKL levels in group D and group E were (0.19±0.11) and (0.48±0.24), respectively. Levels of both DKK-1 and RANKL in group E were significantly higher than those in group D (Fig. 1).

Correlations between DKK-1 and RANKL levels and disease duration

In patients with gout, the longer the disease course in patients with gout, the higher the level of serum DKK-1 (Pearson r=0.430, $p<0.001$, Fig. 2). There was no correlation between serum DKK-1 and age, and serum uric acid level (Pearson r=0.095, $p=0.234$; Pearson r=-0.103, $p=0.197$). There was a positive correlation between the duration of gout and the level of serum RANKL (Pearson r=0.359, $p<0.001$, Fig. 2); however, there was no correlation between serum RANKL and age and serum uric acid (Pearson r=0.018, $p=0.871$; Pearson r=0.022, $p=0.783$).

As seen in Figure 2, the serum levels of DKK-1 and RANKL, respectively, were positively correlated with the course of hyperuricaemia (Pearson r=0.446, $p<0.001$; Pearson r=0.379, $p<0.001$), whereas they were not correlated with age and serum uric acid levels.

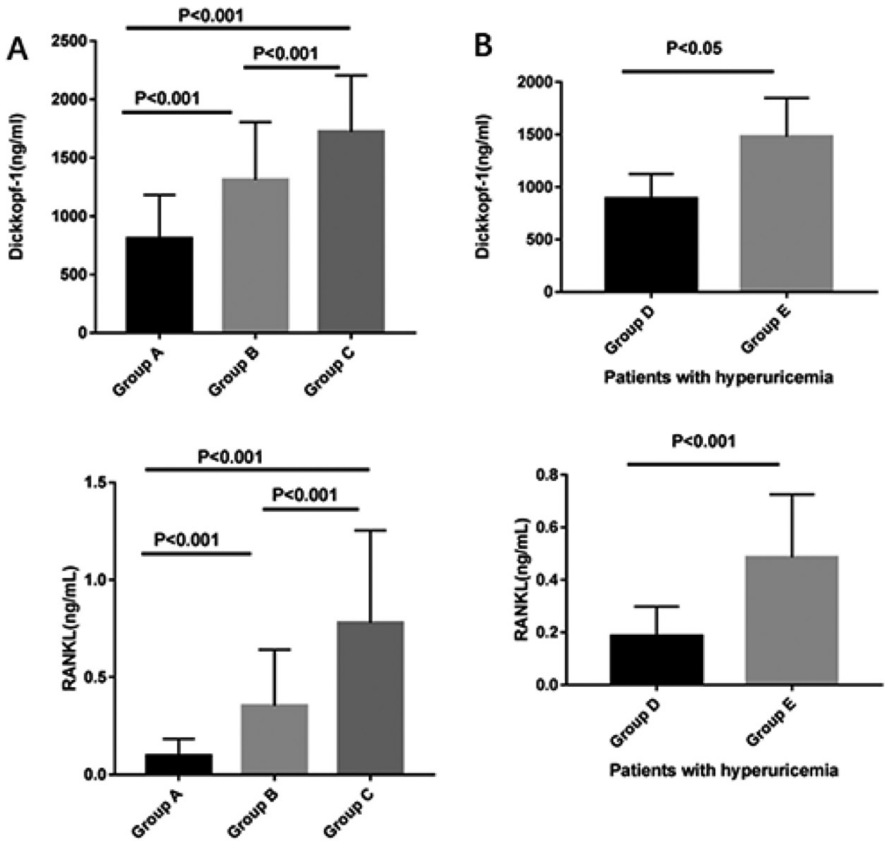

Fig. 1. Comparison of Dickkopf-1 and RANKL levels among patients with gout and hyperuricaemia RANKL: receptor activator of nuclear factor-κB ligand.
A: Comparison of Dickkopf-1 and RANKL levels among patients with gout.
B: Comparison of Dickkopf-1 and RANKL levels among patients with hyperuricaemia.

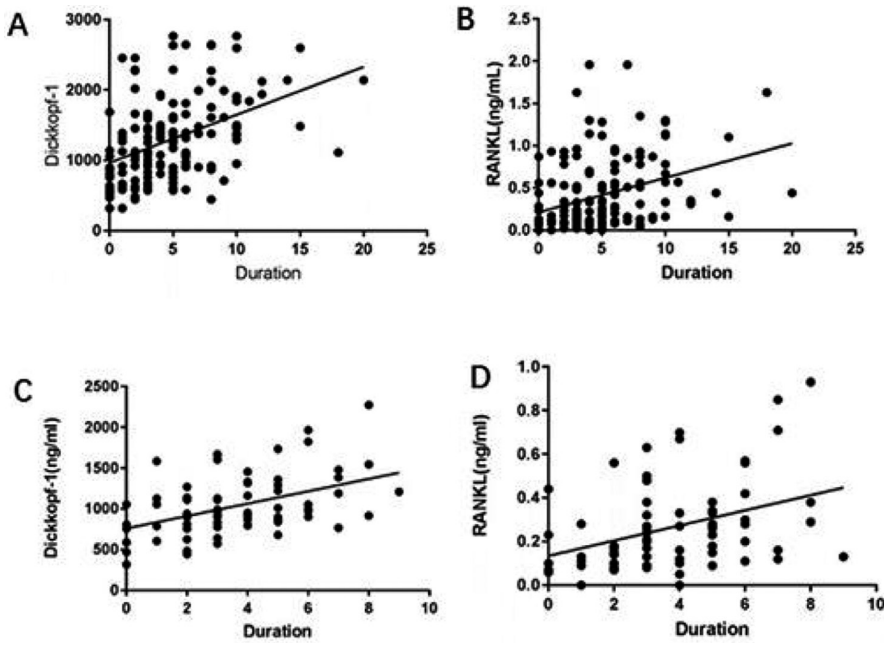

Fig. 2. Correlations between Dickkopf-1 and RANKL levels and disease duration in patients with gout and hyperuricaemia. RANKL: receptor activator of nuclear factor-κB ligand.
A: Correlations between the levels of Dickkopf-1 and duration in patients with gout.
B: Correlations between the levels of RANKL and duration in patients with gout.
C: Correlations between the levels of Dickkopf-1 and duration in patients with hyperuricaemia.
D: Correlations between the levels of RANKL and duration in patients with hyperuricaemia.

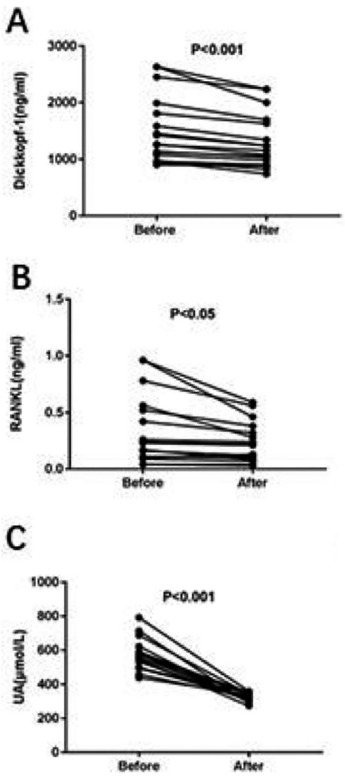

Fig. 3. Analysis of Dickkopf-1, RANKL, and uric acid levels in group B after urate-lowering therapy for 1 year. RANKL: receptor activator of nuclear factor-κB ligand.
A: Analysis of Dickkopf-1 in group B after urate-lowering therapy for 1 year.
B: Analysis of RANKL in group B after urate-lowering therapy for 1 year.
C: Analysis of uric acid levels in group B after urate-lowering therapy for 1 year.

Analysis of DKK-1, RANKL, and uric acid with 1-year urate-lowering therapy

After 1 year of ULT, US abnormalities disappeared in 12 patients in group B and in 8 patients in group E. Besides, the diameter of the largest tophus was shortened from (14.83±5.41) mm to (11.67±3.94) mm after treatment in group C (t=6.092, $p<0.001$). Moreover, the concentrations of serum DKK-1, RANKL, and uric acid significantly decreased after treatment in group B (Fig. 3), group C (Fig. 4), and group E (Fig. 5).

Discussion

Through this study, we evaluated the association between bone remodelling markers and MU manifestations in patients with gout and hyperuricaemia to clarify the role of MU in treatment responsiveness. Our study demonstrates that in patients with gout, serum

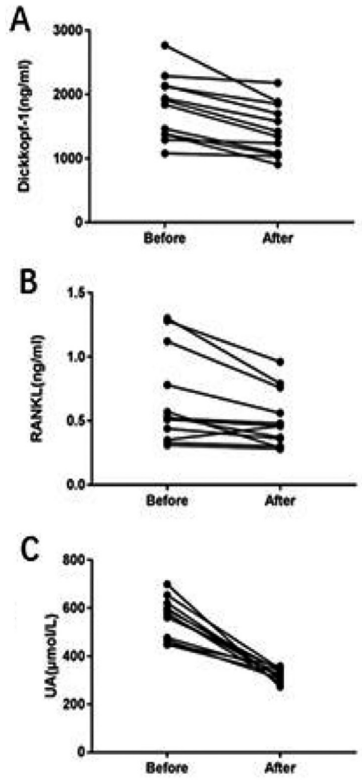

Fig. 4. Analysis of Dickkopf-1, RANKL, and uric acid levels in group C after urate-lowering therapy for 1 year. RANKL: receptor activator of nuclear factor-κB ligand.
A: Analysis of Dickkopf-1 in group C after urate-lowering therapy for 1 year.
B: Analysis of RANKL in group C after urate-lowering therapy for 1 year.
C: Analysis of uric acid levels in group C after urate-lowering therapy for 1 year.

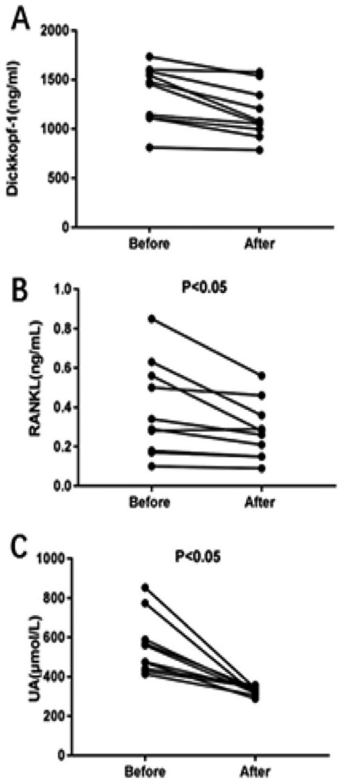

Fig. 5. Analysis of Dickkopf-1, RANKL, and uric acid levels in group E after urate-lowering therapy for 1 year. RANKL: receptor activator of nuclear factor-κB ligand.
A: Analysis of Dickkopf-1 in group E after urate-lowering therapy for 1 year.
B: Analysis of RANKL in group E after urate-lowering therapy for 1 year.
C: Analysis of uric acid in group E after urate-lowering therapy for 1 year.

DKK-1 and RANKL levels were higher in those with tophi and bone erosion than in those with only aggregates and double-contour sign and higher in those with only aggregates and double-contour sign than in those without aggregates. Similarly, the levels of DKK-1 and RANKL in patients with hyperuricaemia and abnormal MU were significantly higher than those of patients with hyperuricaemia and normal MU.

In recent years, the prevalence rate of gout has been progressively increasing with age, with 7% of men aged more than 65 years and 3% of women aged more than 85 years (15). The MSU crystal status in synovial fluid or nodule aspirate was the gold standard (16). However, its sensitivity is relatively low. Moreover, synovial fluid aspiration (SFA) is usually unfeasible and invasive. Sometimes, tophi are next to the femoral condyles or in the soft tissue, which could increase the false negative result of SFA. Hence, imaging is essential to confirm the diagnosis, particularly for those whose diagnosis was not supported by microscopy. MU has been widely used in both clinical practice and research activity as it is less expensive, safe, and free of radiation. Besides, it can be used repeatedly within a short period of time for disease monitoring. In 2015, the OMERACT (Outcome Measures in Rheumatology Clinical Trials) gout ultrasonography task group published an international consensus on a standardised definition of ultrasound (US) findings in gout (17): double-contour sign, tophus, aggregate, and bone erosion. Additionally, the new classification standards for gout published by EULAR and ACR in 2015 have already included the "double-contour sign" in ultrasound findings (2). Therefore, we can determine the bone erosive effect of urate crystals on the joints by measuring the indicators of bone metabolism in patients with gout and hyperuricaemia with different ultrasonic findings.

A recent study has also found a connection among bone loss, tophus, and formation of new bone in gout (18). In patients with gout, serum DKK-1 levels are positively correlated with TRAP5b, which reflects osteoclast activity and function, suggesting that DKK-1 is one of the important factors involved in bone destruction in gout (11). In addition, DKK-1 can increase factors that promote osteoclast differentiation, which will, consequently, lead to bone destruction.

RANKL is a driver of the RANKL-RANK-OPG signalling system and a member of the TNF ligand superfamily (19). High levels of RANKL are present in lymphoid tissues (lymph nodes, thymus, spleen, and foetal liver) and bone tissues (skeleton and bone marrow), but minimal levels are expressed in non-lymphoid tissues such as the heart, placenta, skeletal muscles, stomach, and thyroid (2, 13, 17, 20). Although currently there is limited evidence on the correlation between gout and RANKL, studies have shown that RANKL knockout mice develop osteosclerosis due to the complete absence of mature osteoclasts, indicating the important role of RANKL in osteoclast formation (21). Therefore, bone destruction in gout can also be detected by monitoring the level of RANKL. Studies have found that cytokines that are associated with bone resorption, such as IL-1 and transforming growth factor α (TNF-α) and transforming growth factor β (TGFβ), are expressed in tophus (22-24). These inflammatory factors in tophus can promote the expression of RANKL, thus enhancing osteoclast differentiation.

It has been reported in the literature that urate crystal deposition in bone tissue can lead to a decreased osteoblastic activity and an increased osteoclastic activity (4, 25). Blood uric acid level is not only one of the important factors for diagnosis and treatment of gout but also a crucial indicator for evaluating drug efficacy (26). Serum uric acid and urate were deposited in bone tissues, which

will lead to bone destruction. Therefore, in this study, we used MU to observe the deposition of urate in joints.

We grouped patients according to the different MU findings and measured levels of serum DKK-1 and RANKL in different groups, to determine whether urate deposition will lead to bone destruction. After examination, we found no significant difference in serum uric acid levels in patients with different MU manifestations, and serum DKK-1 and RANKL concentrations did not directly correlate with blood uric acid levels. Thus, we can conclude that blood uric acid level does not represent the extent of bone destruction. In addition, in this study, we found that the longer the course of disease, the higher the level of serum DKK-1 and RANKL, indicating that the longer the disease course, more serious may have been the degree of bone destruction.

Moreover, in patients whose serum uric acid level is less than 360 μmol /L after ULT for 1 year, their MU findings showed the disappearance of "double-contour sign" and intra-articular strong echoes and the decrease of gout stone diameter. Besides, the serum DKK-1 and RANKL has significantly decreased after ULT. These results suggest that ULT can reduce the deposition of intra-articular uric acid crystals and thus reduce the bone destruction. Therefore, MU can be regarded as an effective modality of disease monitoring and therapeutic effect evaluation. A recent study showed that MU can be used as the first choice of imaging technique to assess MSU crystal deposition, with comparable specificity and higher sensitivity than dual-energy computed tomography (DECT) (27-30).

This study has some limitations. First, the number of patients followed-up in this study is limited, and the duration of follow-up is short. Secondly, our study is a single-centre study; thirdly, these groups can be subtyped by MUS if the asymptomatic hyperuricaemia study size is larger, therefore, additional multicentre studies involving a larger population must be conducted to confirm our results.

In conclusion, musculoskeletal ultrasonography manifestations are remarkably associated with levels of DKK-1 and RANKL and will be ameliorated after ULT for patients with gout and hyperuricaemia. Articular pathological changes occurring in patients with gout and hyperuricaemia have been accurately assessed (31). Thus, musculoskeletal ultrasonography could be a useful tool for reflecting bone remodelling and monitoring disease responsiveness to treatment.

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