Original Article

Associations of osteoclastogenesis and nerve growth in subchondral bone marrow lesions with clinical symptoms in knee osteoarthritis

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ABSTRACT

Background/objective: Subchondral bone marrow lesions (BMLs) are common magnetic resonance imaging (MRI) features in joints affected by osteoarthritis (OA), however, their clinical impacts and mechanisms remain controversial. Thus, we aimed to investigate subchondral BMLs in knee OA patients who underwent total knee arthroplasty (TKA), then evaluate the associations of osteoclastogenesis and nerve growth in subchondral BMLs with clinical symptoms.

Methods: Total 70 patients with primary symptomatic knee OA were involved, then separated into three groups based on MRI (without BMLs group, n = 14; BMLs without cyst group, n = 37; BMLs with cyst group, n = 19).

Volume of BMLs and cyst-like lesions was calculated via the OsiriX system. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire was used to assess clinical symptoms. Histology and immunohistochemistry were deployed to assess subchondral osteoclastogenesis and nerve distribution. Pearson’s correlation coefﬁcient was used to evaluate the associations between volume of BMLs and joint symptoms, and to assess the associations of osteoclastogenesis and nerve growth in subchondral BMLs with joint symptoms.

Results: In BMLs combined with cyst group, patients exhibited increased osteoclastogenesis and nerve distribution in subchondral bone, as shown by increased expression of tartrate resistant acid phosphatase (TRAP) and protein gene product 9.5 (PGP9.5). Volume of subchondral cyst-like component was associated with joint pain (p < 0.05).

Subchondral osteoclastogenesis and nerve distribution were positively associated with joint pain in BMLs with cyst group (p < 0.05).

Conclusion: The subchondral cyst-like lesion was an independent factor for inducing pain in OA patients; osteoclastogenesis and nerve growth in subchondral cyst-like lesions could account for this joint pain.

The translational potential of this article: Our results indicated that the increased osteoclastogenesis and nerve growth in subchondral cyst-like lesions could account for the pain of OA joints. These findings may provide valuable basis for the treatment of OA.

1. Introduction

As a highly prevalent disorder of the musculoskeletal system, osteoarthritis (OA) is mainly characterized by joint pain, which greatly affects the quality of life [1]. In addition to pain, OA patients are susceptible to other clinical symptoms including joint stiffness and
functional disability [2].

Bone marrow lesions (BMLs) in subchondral bone are common pathological features during the progression of OA [3,4]. Basically, BMLs show a hyperintense signal in magnetic resonance imaging (MRI), similar to the edema-like signal. A specific proportion of BMLs exhibit well-defined regions of rounded hyperintense signals in MRI, commonly described as cyst-like lesions [5]. Although the underlying mechanism of subchondral bone cyst remains unclear, there are several existing theories including synovial fluid influx, bone contusion, vascular ageing and endothelial dysfunction [6].

Evidence has shown that BMLs in OA could up-regulate the expression of genes related to nerve growth, bone turnover, and inflammation [7]. Hence, subchondral BMLs have been demonstrated to be potentially associated with the clinical symptoms in OA patients [8]. In fact, studies have demonstrated that knee pain was associated with subchondral BMLs [9,10]. Moreover, the volume of subchondral cyst-like lesions was associated with knee pain [11]. However, the mechanism of pain in OA joints aroused from subchondral BMLs (including cyst-like lesions) remains unclear.

Osteoclastogenesis and nerve growth in subchondral bone were demonstrated to play important roles in causing pain of OA joints [12,13]. Osteoclasts induce sensory nerve axonal growth via secreting netrin-1, while reduction of osteoclast formation in subchondral bone could inhibit the growth of sensory nerve and pain hypersensitivity in OA joints [12]. Moreover, osteoclasts are important sources of nerve growth factor (NGF), which could sensitize primary afferents in subchondral bone and lead to joint pain [13,14]. Therefore, we hypothesized that subchondral osteoclast densities might be increased in OA patients when combined with BMLs, and that the increased osteoclastogenesis could cause subchondral nerve growth, further arousing pain in OA joints.

In this study, we enrolled symptomatic knee OA patients who underwent total knee arthroplasty (TKA). We aimed to determine the association of osteoclastogenesis and nerve growth in subchondral BMLs (including the subtypes) with clinical symptoms in OA patients. By analyzing the relationships between histological parameters and clinical symptoms, we hoped to clarify how subchondral BMLs could cause joint pain in OA patients.

2. Materials and methods

2.1. Subjects

This cross-sectional study was approved by the Ethics Committee of Shanghai Ninth People's Hospital (NO. 2018-151-T137). All patients provided written informed consent. Participants involved were diagnosed with primary symptomatic knee OA based on clinical and radiographic criteria. Specifically, weightbearing anteroposterior radiographs of the knee joints were obtained, then the severity of OA was graded based on the Kellgren–Lawrence (K-L) classification system [15]. Patients involved in this study were in K-L grade III/IV. Those who had other forms of arthritis such as rheumatoid arthritis (RA), or who were taking medications that could affect bone remodeling (including estrogen and bisphosphonates) were excluded. Subjects underwent TKA in the Department of Orthopaedics Surgery, Shanghai Ninth People's Hospital. Total 70 patients were involved and divided into three groups based on MRI [11]. One group was without BMLs (n = 14, mean age 63.93 ± 9.65), one group had subchondral BMLs without cyst-like lesions (n = 37, mean age 65.14 ± 8.57), and the third group had subchondral BMLs with cyst-like lesions (n = 19, mean age 66.47 ± 9.81).

2.2. Assessment of OA clinical symptoms

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire was deployed to assess pain, stiffness, and function of the OA knee joints. Briefly, in the Likert format, the subscale scores are: pain (0–20, 5 items), stiffness (0–8, 2 item), and physical function (0–68, 17 item); each item is scored on a 0–4 scale. In the visual analogue scale (VAS) format, the ranges for three subscale scores are: pain, 0–500; stiffness, 0–200; and physical function, 0–1700. WOMAC total score was the sum of pain, stiffness, and function score [16]. In this study, participants were required to associate their scores with clinical symptoms within the last 48 h for getting WOMAC score. Then, the WOMAC score was recorded in VAS format and normalized to 100 units.

2.3. MRI acquisition

The index knee joints were pre-operatively scanned with a MAGNETOM Skyra 3.0 T MRI system (Siemens, Germany), utilizing a dedicated phased-array knee coil. A proton density-weighted (PD) fat-suppressed (FS) fast spin echo (FSE) sequence was applied [17]. The parameters were as follows: sagittal, repetition time (TR) 4500 ms, echo time (TE) 34 ms, slice thickness 4 mm, slice gap 0.2 mm, acquisition matrix 288 × 192, and axial, TR 3600 ms, TE 22 ms, slice thickness 4 mm, slice gap 0.2 mm, and acquisition matrix 256 × 192.

2.4. MRI based quantitative assessment

BMLs were characterized as ill-defined hyperintense (edema-like) regions on PD FS MRI sequence. To verify the subchondral BMLs, the edema-like hyperintense signal must have been adjacent to the articular cartilage or the cartilage loss area on at least a single MRI slice [11]. BMLs were subdivided into two subtypes based on with or without cyst-like component. More specifically, BMLs combined with cyst-like components were defined as those in which the cyst-like components were seen in more than two adjacent slices in MRI images [11].

The volumetric calculation of BMLs was performed by an experienced radiologist using the OsiriX system (edition 10.0, PixMeo SARL) in a blinded manner. Briefly, BMLs in each subregion were manually marked on MRI. Then, 3D post-processing techniques in the OsiriX system were applied to calculate the volume of BMLs [18]. 3D slice (edition 4.11.20200930) was used to reconstruct the structure of the knee joint, including subchondral BMLs. When calculating the total volume of BMLs, all subchondral BMLs with or without cyst-like components were included. The cyst-like component volume was calculated, excluding hyperintense signals within osteophytes and the cyst-like components without surrounding ill-defined hyperintense signal.

2.5. Sample processing

After TKA, mid-coronal regions of the middle third of the medial tibial plateau, which were important for weight-bearing, were segmented [13]. The samples were fixed with 4% paraformaldehyde for 48 h then rinsed overnight. The specimens were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) in 10 mM Tromethamine (Tris) buffer for 28 days and then embedded with paraffin.

2.6. Histological observation

Samples were sliced at 5 μm and then processed with hematoxylin and eosin (H&E) and safranin O/fast green (S&F) staining. The Osteoarthritis Research Society International (OARSI) score which can reflect cartilage damage was calculated as described previously [19]. Briefly, the OARSI scoring was based on six grades (0–6) reflecting lesion depth and four stages (0–4) reflecting lesion extent. OARSI score = grade × stage, this method produces OA score with a range from 0 to 24. The OARSI score in this study was performed by two experienced observers in a blind way; there was no significant difference in the results obtained by the two observers.

2.7. Immunohistochemistry

Five sequential slides in each specimen were stained and evaluated.
Tartrate resistant acid phosphatase (TRAP) was chosen as a marker for osteoclasts, and protein gene product 9.5 (PGP9.5) as a marker for nerve [20,21]. Sections were first blocked with 5% bovine serum albumin (BSA), then incubated with anti-TRAP antibody (Abcam, mouse, dilution 1:200) for 12 h. After being washed with phosphate buffered saline (PBS) three times, samples were incubated with anti-PGP9.5 antibody (Sigma–Aldrich, rabbit, dilution 1:100) for another 12 h. Green (Abcam, Alexa Fluor® 488, donkey anti-mouse, 1:500) and red (Abcam, Alexa Fluor® 594, goat anti-rabbit, dilution 1:500) fluorescent secondary antibodies were in turn deployed for 1 h. Anti-matrix metalloprotein 13 (MMP-13) antibody (Abcam, mouse, dilution 1:200) was administrated for 12 h then stained with green fluorescent secondary antibody (Abcam, Alexa Fluor® 488, donkey anti-mouse, 1:500). Isotype controls including mouse IgG (Abcam, dilution 1:200) and rabbit IgG (Abcam, dilution 1:200) were applied to exclude the non-specific staining. Finally, the sections were stained with 4,6-diamidino-2-phenylindole (DAPI) for 15 min then photographed using a confocal fluorescence microscope (Leica, Germany). The red/green fluorescence represents positively stained cells, then number of positive cells per unit area was calculated.

2.8. Statistical analysis

The data were expressed as mean ± standard deviation and presented in a box–whisker plot. The top line of the box–whisker plot represents the maximum value, while the bottom line represents the minimum value. The upper margin of the box is the upper quartile, the middle line is the median, lower margin of the box is the lower quartile. The data were tested for normality by the Shapiro–Wilk test. The two-sided Student’s t test was used to compare normally distributed clinical variables. Otherwise, the Kruskal–Wallis (K–W) test was utilized for analyzing non-normally distributed data. One-way analysis of variance (ANOVA) was applied to compare the variables in various groups. Variables were compared again after adjusting for each potential covariate by a multiple linear regression analysis. Covariates independent from each other were selected, including age, height, weight, body mass index (BMI) and K-L grade. Pearson’s correlation coefficient was deployed to analyze the relationships between BMLs (including the subtypes) and OA symptoms. For all the analyses, a two-tailed p < 0.05 was regarded as statistically significant. All data analyses were conducted using the SPSS 22.0 statistical software package (SPSS Inc, Chicago, USA).

3. Results

Patients were divided into three groups based on BMLs and the combination of cyst-like lesions (Fig. 1a–c). 3D reconstructed images showed the location of BMLs with cyst in subchondral bone (Fig. 1d–f). Age, height, weight, and BMI in the three groups showed no significant differences (p > 0.05). In looking at clinical symptoms, patients in BMLs with cyst group demonstrated significant increased WOMAC pain score compared with patients in the other two groups (p < 0.05). Although the WOMAC stiffness and function scores were increased in BMLs with cyst group, they showed no significant differences compared with the other two groups (p > 0.05) (Table 1).

Pearson’s correlation coefficient analysis showed that the volume of subchondral BMLs with or without cyst-like lesions demonstrated no significant association with WOMAC total, pain, stiffness, and function score (p > 0.05). Volume of the subchondral cyst-like component itself was significantly associated with WOMAC total, pain, and function score (p < 0.05), while it demonstrated no obvious association with WOMAC stiffness score (p > 0.05) (Table 2). These results suggested that the subchondral cyst itself was an important and independent factor for causing joint pain and functional disability.

Compared with patients without BMLs or with simple BMLs, patients with BMLs combined with cyst-like lesions exhibited necrotic bone fragments accompanied by surrounding fibrous connective tissue in H&E staining (Fig. 2a). Cartilage damage revealed by S&F staining

Figure 1. MRI images of various types of BMLs. (a) OA patient without BMLs. (b) Subchondral BMLs without cyst-like component (white arrows) in the tibia. (c) BMLs combined with cyst-like component (white arrows) in the tibia. (d) 3D image of subchondral BMLs combined with cyst (e, f) 3D models of the knee joint were reconstructed based on the MRI images.
Table 1
Patients and basic information.

| Characteristics                  | Without BMLs (n = 14) | BMLs without cyst (n = 37) | BMLs with cyst (n = 19) |
|----------------------------------|-----------------------|---------------------------|-------------------------|
| Age (years)                     | 63.93 ± 9.65          | 65.14 ± 8.57             | 66.47 ± 9.81           |
| Sex, n (male/female)            | 4/10                  | 14/23                     | 5/14                    |
| Height (cm)                     | 164.36 ± 7.01         | 162.43 ± 7.52            | 160.58 ± 6.31          |
| Weight (kg)                     | 65.36 ± 5.67          | 66.46 ± 7.33             | 64.37 ± 8.33           |
| Hypertension, n (%)             | 4 (28.57)             | 11 (29.73)                | 6 (31.58)               |
| Diabetes, n (%)                 | 2 (14.29)             | 7 (18.92)                 | 3 (15.79)               |
| BMI (kg/m²)                     | 24.25 ± 2.31          | 25.21 ± 2.55             | 24.97 ± 3.00           |
| Index knee, n (right/left)      | 8/6                   | 17/20                     | 9/10                    |
| Kellgren-Lawrence grade, n (II/IV) | 7/7               | 22/15                     | 13/6                    |
| WOMAC total score               | 35.33 ± 13.25         | 36.50 ± 8.35             | 40.37 ± 7.33           |
| WOMAC pain score                | 30.29 ± 4.50          | 31.73 ± 6.17             | 36.63 ± 8.18           |
| WOMAC stiffness score           | 43.57 ± 17.03         | 44.73 ± 13.69            | 46.05 ± 15.15          |
| WOMAC function score            | 35.42 ± 19.17         | 36.93 ± 11.20            | 40.80 ± 8.18           |
| Total BMLs volume (mm³)         | —                     | 1794.15 ±                | 1351.95 ±              |
| Subchondral BMLs without cyst (mm³) | —          | 1815.47 ±                | 808.50 ±               |
| Subchondral BMLs with cyst (mm³) | —                     | 1815.47 ±                | 808.50 ±               |
| Volume of the cyst (mm³)        | —                     | —                        | 152.48 ±               |

Symbol “*” indicates comparisons with the first group, “**” indicates p < 0.05, “##” indicates p < 0.01. Symbol “*#” indicates comparisons with the second group, “*##” indicates p < 0.01.

Table 2
Correlation coefficient between volume of BMLs and symptoms of OA.

| Group                  | WOMAC total score | WOMAC pain score | WOMAC stiffness score | WOMAC function score |
|------------------------|-------------------|------------------|-----------------------|----------------------|
| BMLs (n = 56)          | 0.213             | 0.101            | 0.145                 | 0.193                |
| BMLs without cyst (n = 37) | 0.290           | 0.156            | 0.201                 | 0.252                |
| BMLs with cyst (n = 19) | 0.109             | 0.224            | 0.012                 | 0.069                |
| Cyst (n = 19)          | 0.713**           | 0.685**          | 0.148                 | 0.668**              |

Symbol “**” indicates correlation between various types of BMLs and symptoms of OA, “##” indicates p < 0.05, “##” indicates p < 0.01.

Figure 2. Histological observation of subchondral bone and articular cartilage. (a) H&E staining showed the structure of subchondral bone. (b) S&F indicated the cartilage damage among the three groups of patients. (c) OARSI score of the cartilage in the tibia, compared by K-W test. “*” indicated p < 0.05, “##” indicated p < 0.01, “CC” indicated cystic cavity, “SB” indicated subchondral bone.

demonstrated more severe damage in patients with BMLs combined with cyst-like lesions, as shown by increased destruction and decreased thickness of the articular cartilage (Fig. 2b). Comparison of OARSI score showed that there was no significant difference between the group without BMLs and the group with simple BMLs, while OARSI score was obviously increased in the group with BMLs combined with cyst-like lesions (p < 0.01) (Fig. 2c).

To examine the mechanism of joint pain caused by subchondral cyst-like lesions, we deployed TRAP and PGP9.5 double immunofluorescence staining to detect subchondral osteoclastogenesis and nerve distribution. Enhanced red and green fluorescence staining revealed that osteoclast density and nerve distribution were obviously increased in patients with BMLs combined with cyst-like lesions. Moreover, most of the red fluorescence (nerve fiber) was co-localized with green fluorescence (osteoclast), indicating that the subchondral nerve growth may have been induced by the osteoclasts (Fig. 3a). Quantitative analysis showed that the number of TRAP/PGP9.5 positive cells had no obvious difference between the group without BMLs and the group with simple BMLs (p > 0.05), however, it was significantly increased in the group with BMLs combined with cyst-like lesions (p < 0.01) (Fig. 3b and c).

We further detected the expression of MMP-13 which is a key factor for promoting osteoclast differentiation and activation in subchondral bone [22,23]. Similarly, subchondral MMP-13 expression was significantly increased in the BMLs with cyst-like lesions group (p < 0.01) (Fig. 4a and b). These results demonstrated that osteoclasts were increased in patients with BMLs accompanied with subchondral cyst-like lesions, which could account for the increased nerve growth in the subchondral bone.

We detected the association of clinical symptoms of OA with subchondral osteoclast density and nerve growth via Pearson’s correlation coefficient. Results demonstrated that TRAP, PGP9.5, and MMP-13 expression were significantly associated with WOMAC total, pain, and function score in BMLs combined with cyst group (p < 0.05). As for the other two groups, subchondral TRAP, PGP9.5, and MMP-13 expression showed no statistically significant association with WOMAC total, pain, stiffness, and function score (p > 0.05) (Table 3). These results suggested that osteoclastogenesis and nerve growth in subchondral cysts might account for the pain and functional disability of OA joint.
4. Discussion

BMLs have been shown as an important feature in OA, however, the relationship between BMLs and OA joint pain has remained controversial [24]. Two observational studies demonstrated that knee pain was associated with increased subchondral BMLs [9,10]. However, in a secondary study of a randomized trial, results showed that there was no significant association between the volume of BMLs and OA joint pain [11]. Our data were consistent with results of the later research, in that there was no significant correlation between subchondral total BML volumes and pain. The association between subchondral cyst-like lesions and pain was also an uncertainty. Two cross-sectional studies which used a semi-quantitative approach had proposed that subchondral cysts were not associated with symptoms of OA [25,26]. Nevertheless, another study indicated that increased subchondral cyst volume was associated with more severe knee pain [11]. We showed that knee pain and function was associated with volume of the cyst-like component. Different results in these studies may have been induced by differences in the populations, assessment methods, and type of MRI sequence.

Subchondral cyst-like lesions are a common phenomenon in knee patients with OA. However, the cause of cyst-like lesion formation remains unclear. The synovial fluid intrusion theory proposed that increased intra-articular pressure causes joint fluid to infiltrate into subchondral bone through the fissured or ulcerated cartilage, which then develops into a cyst [27]. Based on this theory, subchondral cysts should develop in the regions with existing cartilage loss or fissuring. Another theory, the bone contusion theory, put forward that a subchondral cyst was a consequence of a traumatic bone contusion [28]. In fact, a previous study demonstrated that subchondral cysts were commonly observed in areas of pre-existing BMLs [29]; this could support the bone contusion theory. Pok Man Boris Chan et al. provided a new angle for understanding the pathophysiology of subchondral bone cysts. They proposed that subchondral cyst-like lesions especially in non-load-bearing region are results of vascular ageing and endothelial dysfunction. In particular, they proposed that ischemic episodes, endothelial senescence, insufficient H-type (CD31hi, EMCNhi) vessel, loss of pericyte and osteoprogenitors were associated with subchondral cysts [6].

Histologically, literature has revealed that most simple BMLs were subchondral bone with normal structure; only a small proportion demonstrated non-characteristic abnormalities such as bone marrow necrosis, bone marrow fibrosis and edema, and abnormal trabeculae [30]. However, the cyst-like lesions were usually composed of necrotic bone fragments and dead denuclearized cells. Moreover, the cystic cavities were demonstrated to be surrounded by fibrous connective tissue, which contained osteoblasts and adipocytes [31]. In this study, we found that the cyst-like lesions showed necrotic bone fragments which were surrounded by fibrous connective tissue. In addition, when BMLs were combined with a cyst, there was destruction of the subchondral structure. We also found that TRAP positively stained cells were increased mainly on the surface of cysts, indicating that osteoclasts were activated around the cysts. This might account for the formation of cyst-like lesions.

Subchondral bone has been demonstrated to be an important supporting structure for the upper cartilage, and disturbance of subchondral bone remodeling was closely related to the progression of OA [32,33]. A study has demonstrated that MMP-13, an important bone resorptive factor, was elevated in OA subchondral bone [17]. Other researches have supposed that subchondral MMP-13, mainly driven from osteoblasts, could work synergistically with cathepsin K and cause subchondral bone resorption [34]. Moreover, osteocytes were also demonstrated to be an important source of MMP-13 [35]. MMP-13 could cause type I collagen synthesis, leading to the formation of cyst-like lesions.
degradation and contribute to osteoclast differentiation and the resultant increased bone resorption process [22,23]. In the present study, MMP-13 was found to be significantly increased in the subchondral bone of OA patients with BMLs combined with cyst-like lesions. Moreover, the cartilage damage was more serious in the BMLs with cyst-like lesion group. The increased expression of subchondral MMP-13 caused increased osteoclast density and resultant enhanced bone resorption, which may have accounted for the more severe cartilage damage.

Several subsets of immune cells are involved in subchondral BMLs in OA joints. Macrophage infiltration and osteoclast formation in the subchondral bone were characteristic pathological changes in subchondral BMLs in OA [3,36]. Macrophages (both M1 and M2 type) could stimulate angiogenesis via releasing vascular endothelial growth factor (VEGF), causing lesions in subchondral bone [37]. On the other hand, it was demonstrated that M1 phenotype macrophages may be a transient state similar to pre-osteoclast, which could finally turn into osteoclasts [38]. Besides macrophages, lymphocytes including T cells and B cells were also demonstrated to infiltrate in subchondral bone of OA. T cells and B cells as the major effectors in the adaptive immunity are involved in osteoclastogenesis and bone erosion [39].

Osteoclasts can cause pain in OA joints via inducing nerve growth in subchondral bone. For one thing, osteoclasts could induce sensory nerve axonal growth via secreting netrin-1 and cause pain hypersensitivity in OA joints [12]. For another, osteoclasts were demonstrated to be an important source of NGF, which is capable of sensitizing subchondral primary afferents [13]. NGF may have accounted for the generation of joint pain, as it could directly activate sensory neurons by combining with tyrosine kinase A (TrkA) [40]. Moreover, the deployment of

![Figure 4](image_url)

**Figure 4.** Expression of MMP-13 in subchondral bone. (a) MMP-13 immunofluorescence staining in subchondral bone. (b) Quantitative analysis of MMP-13 among the three groups, compared by K-W test. “*” indicated p < 0.05, “**” indicated p < 0.01, “CC” indicated cystic cavity, “SB” indicated subchondral bone.

### Table 3
Correlation coefficient between subchondral osteoclast/nerve growth and symptoms in various groups.

| Group              | WOMAC score | TRAP   | PGP9.5 | MMP-13   |
|--------------------|-------------|--------|--------|----------|
| **Without BMLs**   |             |        |        |          |
| WOMAC total score | -0.335      | 0.068  | -0.087 |          |
| WOMAC pain score  | 0.445       | -0.158 | 0.155  |          |
| WOMAC stiffness score | -0.017   | -0.023 | 0.382  |          |
| WOMAC function score | -0.360   | 0.051  | -0.117 |          |
| **BMLs without cyst** |           |        |        |          |
| WOMAC total score | -0.025      | -0.126 | 0.234  |          |
| WOMAC pain score  | 0.240       | 0.183  | 0.305  |          |
| WOMAC stiffness score | 0.052     | 0.271  | 0.124  |          |
| WOMAC function score | -0.072    | -0.201 | 0.178  |          |
| **BMLs with cyst** |             |        |        |          |
| WOMAC total score | 0.850**     | 0.586**| 0.726**|          |
| WOMAC pain score  | 0.653**     | 0.520* | 0.472* |          |
| WOMAC stiffness score | 0.367     | 0.053  | 0.151  |          |
| WOMAC function score | 0.803**    | 0.577**| 0.747**|          |

Symbol "*" indicates correlation between subchondral osteoclast/nerve growth and clinical symptoms of OA, "**" indicates p < 0.05, "***" indicates p < 0.01.
Therefore, angiogenesis was supposed to contribute to pain in OA. Nerve growth by producing several regulators of nerve growth [14]. Moreover, angiogenesis and nerve growth are closely interacting processes. Vascular cells could induce nerve growth by producing several regulators of nerve growth [14]. Therefore, angiogenesis was supposed to contribute to pain in OA. However, pain is the most common symptom in OA patients is mediated by sensory nerve [44]. Thus, we paid more attention to nerve growth rather than angiogenesis in this study.

We must emphasize that osteoclasts may cause joint pain through multiple channels. Osteoclasts may directly change subchondral structure, hence affecting subchondral biochemical properties [45]. Osteoclasts may secrete angiogenic factors which lead to blood vessel formation in the subchondral bone, further contributing to the pain of OA [14,44]. Other factors besides osteoclasts could also account for joint pain. For example, osteocytes are potential players in OA pain, as they can produce NGF particularly in an inflammatory environment [46]. For another, synovitis has been proven to be a notable factor for causing OA joint pain [47]. In the present study, we focused on the pathology of BMLs and cyst-like lesions in subchondral bone. Therefore, we evaluated the changes of subchondral osteoclasts and the following nerve growth.

Some limitations of this study need to be considered. First, there are other types of BMLs such as ligament-based BMLs [11], while we focused on subchondral BMLs and cyst-like lesions as we wanted to explore how subchondral BMLs could cause joint pain. Moreover, subjects involved in this study were all symptomatic OA knee patients, we did not evaluate the subchondral osteoclastogenesis and nerve growth in asymptomatic patients with OA. Despite these limitations, our study is valuable in suggesting that subchondral cyst-like lesions could cause joint pain.

In conclusion, our study demonstrated the subchondral changes and increased cartilage damage in OA patients who had BMLs with cyst-like lesions. Moreover, the relationships between BMLs and clinical symptoms of OA indicated that the volume of cyst-like lesions was associated with pain in asymptomatic OA knee patients. Furthermore, the increased subchondral osteoclastogenesis and nerve growth induced by the cyst-like lesions may have accounted for the pain of OA joints. Thus, our study supported the potential explanation for the mechanism of subchondral cyst-like lesions in causing joint pain.

Declaration of competing interest
The authors have no conflicts of interest to disclose in relation to this article.

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