Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2017, Vol. 45(6) 2023–2035

DOI: 10.1177/0300060517730452
journals.sagepub.com/home/imr
SAGE

Diagnostic value of combined serum marker changes and quantitative MRI evaluation of cartilage volume of tibial plateau in a surgically-induced osteoarthritis dog model

Jiangdong Ren[1], Jinsai Ma[3], Xiaogang Zhang[1], Abdusami aimaiti[1], Maimaitiming Saiyiti[2], Yuming Chen[4] and Li Cao[1]

Abstract

Objective: To evaluate the combined diagnostic value of two serum osteoarthritis (OA) markers and quantitative magnetic resonance imaging (MRI) evaluation of the cartilage volume of the tibial plateau in a canine model of experimental OA.

Methods: A total of 18 male Beagle dogs were used in this longitudinal study. OA was surgically induced via anterior cruciate ligament transection (ACLT) of the right knee in 10 dogs. The remaining eight dogs formed the sham operation control group and underwent the same procedure without ACLT. At various times after surgery, enzyme-linked immunosorbent assay was used to measure serum C-telopeptide of type II collagen (CTX-II) and type X collagen (ColX) levels. Quantitative evaluation of the tibial plateau volume was undertaken using MRI and ImageJ software.

Results: The serum CTX-II levels were significantly higher in the OA group at weeks 8, 12 and 16 after surgery, but not at week 4, compared with the control group. The serum ColX levels in the OA group were significantly higher than in the control group at weeks 8 and 12. The tibial plateau cartilage volumes in the OA group were significantly lower than in the control group at weeks 8 and 16.

[1]Department of Joint Surgery, First Affiliated Hospital of Xinjiang Medical University, Urumqi, China
[2]Department of Orthopaedics, First Affiliated Hospital of Xinjiang Medical University, Urumqi, China
[3]Department of Orthopaedics, Changji Branch of the First Affiliated Hospital of Xinjiang Medical University, Changji, China
[4]Department of Pain Management, Changji Branch of the First Affiliated Hospital of Xinjiang Medical University, Changji, China

Corresponding author:
Li Cao, Department of Joint Surgery, First Affiliated Hospital of Xinjiang Medical University, 137 Liyushan Road, Urumqi, Xinjiang 830054, China.
Email: xjbone@sina.com

Conclusion: Serum CTX-II and ColX levels combined with quantitative MRI evaluation of the tibial plateau cartilage volume in a canine model of OA demonstrated the potential to detect and monitor OA progression.

Keywords
Osteoarthritis, monitoring, magnetic resonance imaging, anterior cruciate ligament transection (ACLT) model

Date received: 5 January 2017; accepted: 14 August 2017

Introduction

Osteoarthritis (OA) is a common degenerative joint disease involving the hip, knee, spine, and other weight-bearing joints. It seriously affects the activities of daily living and often leads to disability.[1] At present, joint replacement is the most effective treatment for OA; no other method is effective to improve subchondral bone and cartilage structure.[2] The majority of treatments are symptomatic, being designed to relieve pain, but they do not stop or delay disease progression, so ultimately, many patients require artificial joint replacement surgery. Therefore, early diagnosis of OA and accurate prediction of disease development is extremely important.

With the rapid development of molecular biology, considerable research has been undertaken in the evaluation of biochemical markers for the early diagnosis of knee OA and disease prediction. To date, markers of cartilage metabolism, C-telopeptide of type II collagen (CTX-II)[3–5] and type X collagen (ColX),[6] have been identified. These markers are important for medical and pharmaceutical research. CTX-II is a specific degradation product of cartilage collagen type II catabolism that can be released into the blood.[7]Research has found that patients with hip OA exhibit significantly increased urine CTX-II levels than do healthy individuals; and patients with rapid OA progression presented with urine CTX-II levels that were significantly higher than those of patients with slow progression.[8] In addition, CTX-II was associated with radiographic OA progression in diseased knee and hip joints.[9,10] The specific expression of ColX in OA has attracted considerable attention from researchers in this field as another potential serum biomarker for cartilage degradation.[6] ColX, a non-fibrillar collagen consisting of three identical alpha 1 chains transcribed from the *COL10A1* gene, is a well-established marker for hypertrophic chondrocyte differentiation.[11] Research has demonstrated that ColX mRNA and protein levels are significantly increased in patients with OA.[12] ColX is also a specific marker of hypertrophic chondrocytes in OA.[13–15] This evidence supports the theory that ColX might have potential as a specific diagnostic biochemical marker for OA.[11–16]

Magnetic resonance imaging (MRI) is being increasingly used for the accurate evaluation of articular cartilage injury. Longitudinal and cross-sectional studies of knee OA have confirmed notable sensitivity and accuracy when using quantitative MRI analysis of structural joint changes.[17,18] Long-term structural changes and associated morphological changes in knee OA can be accurately assessed via an OA model through quantitative analysis using MRI software.

This current longitudinal study aimed to evaluate the combined diagnostic value of

the serum levels of two biochemical markers (CTX-II and ColX) and the quantitative evaluation of tibial plateau cartilage volume using MRI software in an anterior cruciate ligament transection (ACLT) animal model of OA.

Materials and methods

Animal model induction

This longitudinal animal study was conducted in the Department of Joint Surgery, First Affiliated Hospital of Xinjiang Medical University, Urumqi, China and it was approved by the Ethics Committee of Xinjiang Medical University (no. 20150507-05). It was undertaken in strict accordance with the regulations outlined by the Animal Protection Committee. All procedures complied with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care, and the protocol was approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Xinjiang Medical University (protocol no. IACUC20160616-08).

A total of 18 male Beagle dogs, aged 2.0–2.5 years and weighing 15–18 kg, were used in the study. The dogs were weighed and organised into increasing weight order from 1 to 18. They were then randomly assigned to either the experimental ($n = 10$) or control group ($n = 8$) using a random number table. The animals were housed in a 12-h light/12-h dark cycle with free access to water and food. In the experimental group, the 10 randomly assigned dogs underwent ACLT of the right knee joint under general anaesthesia following induction of anaesthesia using an intramuscular injection of Zoletil® 50 (0.5 ml/kg) mixed with Su-Mian-Xin (0.5 ml/kg) as described in brief here. As dogs have spontaneous breathing, there was no need for mechanical ventilation during surgery. Intravenous antibiotics were administered to prevent infection. Along the medial patella skin incision, the subcutaneous tissue was opened, and the capsule and fat pad were fully exposed in order to cut the anterior cruciate ligament (ACL) (Figure 1). Close attention was paid to ensure no damage was caused to the surrounding tissue when the ACL was cut. A medial lateral stress test and drawer test were performed and the success of the ACLT was confirmed by a positive result. The articular capsule, subcutaneous tissue and the skin were sutured layer-by-layer, paying attention to haemostasis. A further

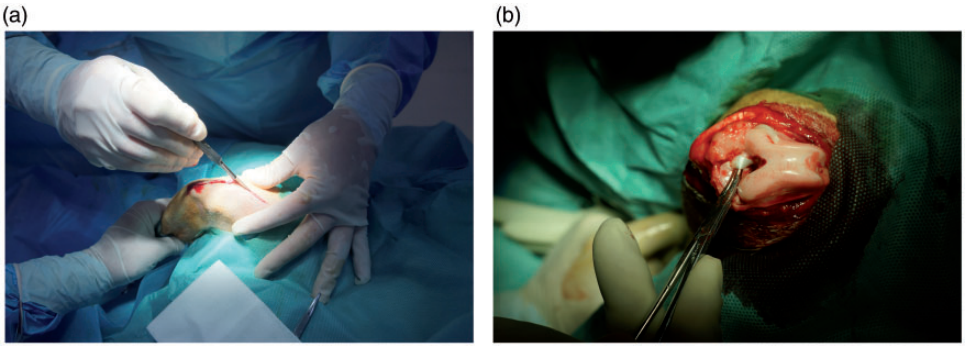

Figure 1. Beagle dogs underwent unilateral anterior cruciate ligament transection in order to induce osteoarthritis in the right knee joint. (a) The medial patellar surgical approach of the knee. (b) The intraoperative incision of the knee capsule after the full exposure of the anterior cruciate ligament.

eight male Beagle dogs formed the control group and received sham surgery. Sham surgery involved opening the knee joint capsule, fully exposing the ACL without any treatment, and closing the joint capsule before suturing the subcutaneous tissue and the skin. Oxygen saturation, blood pressure, and heart rate were continuously monitored during surgery. Postoperative follow-up was performed to determine whether infection or gastrointestinal complications occurred. Following surgery, the two groups of dogs underwent the same forced twice daily walk (1 h each time) for 16 weeks.

Biochemical marker measurements

At different time-points after surgery, venous blood samples (approximately 5 ml) were collected from the necks of all dogs. The blood samples were labelled, set aside for 1 h, and then centrifuged at room temperature (1000 ***g***, 10 min) using a Sigma 1-15K centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) prior to the storage of serum at –80℃.

Serum levels of CTX-II and ColX were determined using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (Canine CTX-II ELISA Kit and Canine COL-X ELISA Kit; R&D Systems, Minneapolis, MN, USA). In brief, the microplates provided by these kits were precoated with anti-canine CTX-II- and ColX-specific antibodies. Using a purified polyclonal antibody to the coat the microtiter plate wells, a solid-phase antibody was used, then the biochemical marker was added to the wells and combined with a horseradish peroxidase (HRP)-labelled polyclonal antibody. This formed an antibody-antigen-enzyme-antibody complex that, after washing completely, was supplemented with 3,3′,5,5′-tetramethylbenzidine substrate solution that became blue as the HRP enzyme catalysed the reaction. The reaction was terminated by the addition of a sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450 nm using a full wavelength Benchmark Plus microplate reader (Bio-Rad, Hercules, CA, USA). The concentration of the two biochemical markers in the serum samples was determined by comparing the optical density of the samples to the standard curve. The minimum detectable concentrations were 6ng/ml for CTX-II and 0.1 pg/ml for ColX. Intra- and interassay coefficients of variation for all ELISAs were<9% and <11%, respectively.

MRI protocol and image processing

High resolution MRI using a wrist coil and a Siemens 1.5 T scanner (MAGNETOM® Avanto 1.5T; Siemens, Erlangen, Germany) was undertaken preoperatively and at postoperative weeks 4, 8 and 16 to examine the right knee joints. After checking the standard equipment used, the dog was placed in the supine position, the right leg was stretched and fixed, and the knee joint was placed in the wrist circle for scanning. In addition, a cushion was used to assist the dog in maintaining the supine position and the right hind leg in a slightly internal rotation hold. MRI was undertaken under general anaesthesia using Zoletil® 50 (0.5 ml/kg) mixed with Su-Mian-Xin (0.5 ml/kg). As dogs have spontaneous breathing, there was no need for mechanical ventilation during the MRI. The dogs were monitored under the supervision of a veterinarian.

The MRI examination of the right knee joint included two image acquisition sequences. The left knee was not checked. A sagittal 3-dimensional volume interpolation T1-weighted (t1-vibe-we-sag) MRI with fat saturation (TR, 14.8ms; TE, 6.5 ms; flip angle, 10 u; slice thickness, 1mm)

was used for cartilage analysis (cartilage volume and defects). A coronal fast spin echo T2-weighted (t2-tse-cor) with fat saturation (TR, 2000 ms; TE,36 ms; slice thickness, 2 mm; flip angle, 180 u) was utilized for the subchondral bone lesions. The total acquisition time was approximately 60 min.

Evaluation was performed in a blinded fashion by two physicians experienced with musculoskeletal system MRI. The final diagnosis was made by a consensus following evaluation of the changes of the articular cartilage and subchondral bone structure of the knee joint between the operation and sham-operation groups. The presence and location of bone marrow oedema and cartilage of tibial plateau alterations were used as the indicators for MRI evaluation. The cartilage volume of the lateral and medial tibia was assessed by quantitative measurement, which was performed using the 3-dimensional FLASH images and ImageJ software (National Institutes of Health) as previously described.[19,20] The volume of the tibial plateau cartilage was measured using ImageJ at different time-points after surgery. The absolute volumes at each time-point were compared with those obtained prior to surgery (baseline) and expressed as a percentage difference (%).

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 22.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Quantitative data are expressed as mean ± SD. Serum CTX-II and ColX levels and MRI tibial plateau cartilage volumes in the OA group were compared with those of the control group using an independent sample *t*-test (2-detailed, 95% confidence interval). Using Shapiro–Wilk for normality testing, $P > 0.1$ was considered to indicate that the data were normally distributed. A $P \leq 0.05$was considered statistically significant.

Results

Changes in the levels of the two serum biomarkers at multiple time-points after surgery are shown in Figures 2 and 3. ColX levels peaked at week 8 in the group with OA, after which the levels decreased. The difference between the two groups was significant at weeks 8 and 12 ($P = 0.005$ and $P = 0.018$, respectively). The highest serum CTX-II level was observed at week 12 in the group with OA, after which the levels decreased, but remained significantly higher than the control group ($P \leq 0.05$). The difference between the two groups was significant at weeks 8, 12 and 16 ($P = 0.012$, $P = 0.001$, and $P = 0.002$ respectively).

Over the course of 16 weeks post-surgery, there was a progressive loss of cartilage volume on the tibial plateaus in the group with OA (Figure 4; Table 1). These changes were first detected at week 8 post-

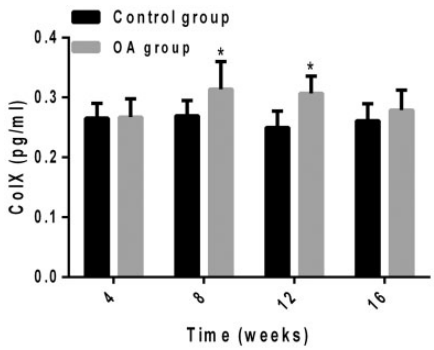

Figure 2. Serum type X collagen (ColX) levels at different time-points after surgery in dogs who either underwent unilateral anterior cruciate ligament transection in order to induce osteoarthritis (OA) in the right knee joint (OA group) or a sham operation (Control group). Data presented as mean ± SD. *$P \leq 0.05$ versus the control group, as determined by an independent-samples *t*-test.

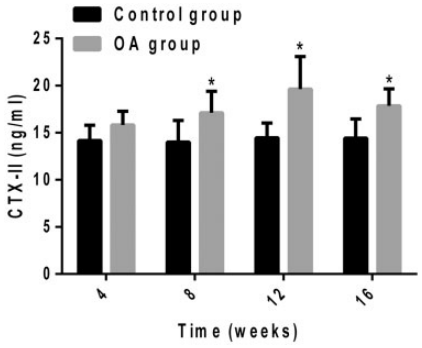

Figure 3. Serum C-telopeptide of type II collagen (CTX-II) levels at different time-points after surgery in dogs who either underwent unilateral anterior cruciate ligament transection in order to induce osteoarthritis (OA) in the right knee joint (OA group) or a sham operation (Control group). Data presented as mean ± SD. *$P \leq 0.05$ versus the control group, as determined by an independent-samples *t*-test.

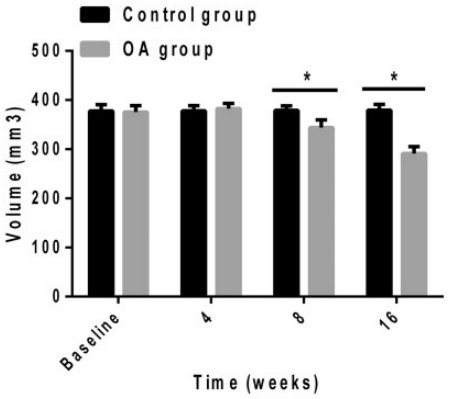

Figure 4. Tibial plateau volume at different time-points before (baseline) and after surgery in dogs who either underwent unilateral anterior cruciate ligament transection in order to induce osteoarthritis (OA) in the right knee joint (OA group) or a sham operation (Control group) as determined by quantitative magnetic resonance imaging evaluation with ImageJ software. Data presented as mean ± SD. *$P \leq 0.05$ versus the control group, independent-samples *t*-test.

surgery and became more apparent over time. The difference between the two groups was significant at weeks 8 and 16 ($P < 0.001$ for both comparisons). According to the gross morphology (Figure 5), the cartilage lesion that increased over time predominantly occurred on the central and posterior portion of the tibial plateaus, which included the weight-bearing areas.

The changes in subchondral bone marrow oedema over time are illustrated in Figure 6. At week 4, MRI revealed subchondral bone marrow oedema in the posteromedial tibia in three dogs (OA group), causing a hyperintense signal on the t2-tsecor sequences. No bone marrow oedema was detected in the sham-operated knees.

Discussion

Articular cartilage degeneration is the primary concern in patients with OA, as the homeostasis and integrity of the articular cartilage rely on its biochemical and biomechanical interplay with the subchondral bone and other joint tissues.[21] Subchondral bone provides the mechanical support for the overlying articular cartilage during the movement of load-bearing joints, and undergoes constant adaptation in response to changes in the mechanical environment through modelling or remodelling.[22] As a result of the instability of mechanical loading on such joints, the subchondral bone and calcified cartilage zone undergo changes.[23]During the progression of OA in its early stage, the abnormal proliferation of subchondral bone and the overlying articular cartilage are subjected to the combined effects of abnormal mechanical pressure, biochemical and other complex factors leading to the occurrence of hypertrophic degeneration of chondrocytes.[21] Hypertrophic degeneration of chondrocytes results in the secretion of large amounts of ColX when attempting to repair damaged chondrocytes.[24]

Table 1. Tibial plateau cartilage volume (mm3) and change from baseline at three postoperative time-points in dogs who either underwent unilateral anterior cruciate ligament transection in order to induce osteoarthritis (OA) in the right knee joint (OA group) or a sham operation (Control group).

		Postoperative time-point		
Group	Baseline	4 weeks	8 weeks	16 weeks
Control group	379.9 (13.0)	377.9 (10.6)	378.8 (9.4)	379.4 (11.6)
Percentage change from baseline		–0.5 (1.0)	–0.3 (1.8)	–0.1 (1.6)
Statistical significancea		NS	NS	NS
OA group	377.3 (12.9)	382.6 (10.6)	343.9 (16.0)	290.9 (14.3)
Percentage change from baseline		+1.4 (1.2)	–8.8 (3.9)	–22.8 (4.1)
Statistical significancea		NS	$P \leq 0.05$	$P \leq 0.05$

Data presented as mean (SEM).
[a]Versus the baseline value; independent sample *t*-test. NS, no significant difference ($P > 0.05$).

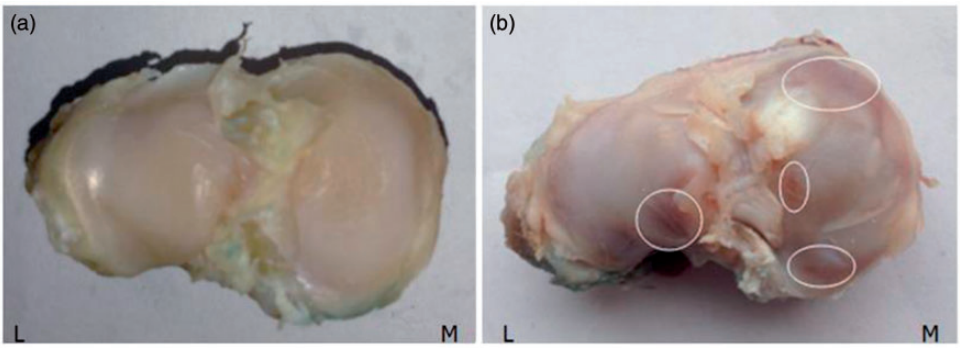

Figure 5. Gross macroscopic appearance of tibial plateau cartilage 16 weeks post-surgery. (a) A representative normal healthy knee tibial plateau from a dog in the control group, which is smooth with no obvious defects. (b) A representative tibial plateau from a dog with osteoarthritis, which shows that the surface is no longer smooth and has surface thinning and subchondral bone exposure (rings).

The critical role of hypertrophic chondrocytes has been summarized in several notable reviews.[25,26] Due to patients with OA continuously suffering from abnormal proliferation of subchondral bone and the comprehensive effect of complex abnormal biochemical factors, this may finally lead to the death of the chondrocyte and result in further degradation of articular cartilage.[23] Type II collagen is localized almost exclusively in cartilage, where it is a major structural component of the tissue.[7]With the deterioration of OA, type II collagen degradation increased in the cartilage, and CTX-II is the specific degradation fragment of type II collagen that is released into the blood.[27] Therefore, the combined detection of hypertrophic chondrocyte specific markers, ColX and type II collagen degradation marker CTX-II, as joint cartilage metabolism markers has a solid theoretical basis.[6]

Type X collagen is a well-established marker for hypertrophic chondrocyte differentiation, which is a non-fibrillar collagen consisting of three identical alpha 1

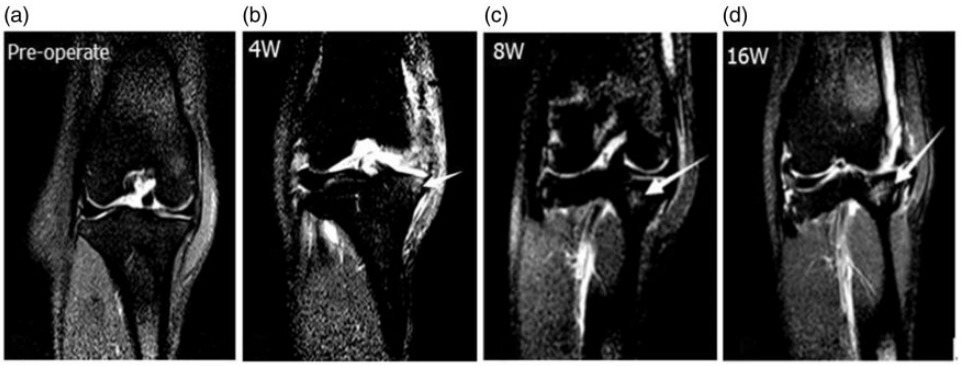

Figure 6. Representative magnetic resonance images of the right knee joint of dogs who underwent unilateral anterior cruciate ligament transection in order to induce osteoarthritis (OA). (a) No subchondral bone marrow oedema was detected on the coronal fast spin echo T2-weighted (t2-tse-cor) sequences prior to surgery. (b) Subchondral bone marrow oedema in the posteromedial tibia causing a hyperintense signal on the t2-tse-cor sequences after 4 weeks (arrow). (c) Subchondral bone marrow oedema in the posteromedial tibia causing a hyperintense signal on the t2-tse-cor sequences after 8 weeks (arrow). (d) Subchondral bone marrow oedema in the posteromedial tibia causing a hyperintense signal on the t2-tse-cor sequences after 16 weeks (arrow).

chains.[28] Each chain has three domains: a short triple helix domain flanked by a bigger globular domain (NC1 domain) at the carboxyl end and a short non-collagenous domain (NC2 domain) at the amino end. ColX is thought to be specifically synthesized by hypertrophic chondrocytes of the growth plate during the development of long bones.[23] Normal chondrocytes do not secrete ColX, but articular chondrocytes undergoing abnormal hypertrophic degeneration secrete large amounts of ColX.[24] A previous study found that anti-ColX monoclonal antibody was produced by using the specific peptide (SFSGFLVAPM), the truncated peptide (SFSGFLVA) without the last two amino acids and the nonsense peptide (DMDYLPRVPNQ), and through the detection of serum ColX levels in 261 cases of knee OA patients and 10 normal subjects, it was found that the serum levels of ColX in the knee OA group were significantly increased.[6] This present study found that the levels of serum ColX in the dogs with the ACLT model of OA were significantly higher than those of the sham operation control group at weeks 8 and 12. At week 16, the levels of serum ColX in the OA group were increased slightly compared with the control group, but the difference was not significant. These current findings suggest that in the OA group at week 4, the early stages of hypertrophic degeneration of the chondrocytes resulted in the secretion of ColX in an attempt to repair damaged chondrocytes, leading to a gradual increase in the secretion of ColX into the serum. By weeks 8 and 12, a large number of chondrocytes exhibited hypertrophic degeneration and ColX secretion markedly increased. At week 8, detection of ColX in the circulatory system was significantly increased and reached its peak. At week 16, as a result of the persistent effect of the mechanical instability of the articular cartilage, the number of chondrocytes that died due to the hypertrophy and degeneration gradually increased. Similarly, the volume of articular cartilage gradually reduced, leading to

subchondral bone exposure and joint space narrowing. Cartilage loss is a serious health risk, thus the secretion of ColX began to decline, and the level of ColX in the peripheral blood also decreased. Therefore, these current findings support the use of ColX as a potential marker of OA disease progression.

C-telopeptide of type II collagen is a micro-molecule polypeptide produced by the cleavage of type II collagen by activated protease, which finally accumulates in the urine via the circulatory system.[29,30] With the aggravation of articular cartilage injury, serum CTX-II levels may be increased.[31–34] Therefore, CTX-II is well-suited as a quantitative biomarker of OA cartilage degeneration and is a sensitive method for the detection of type II collagen degradation in patients with OA.[35] A previous study investigated the changes of serum CTX-II levels in OA induced by ACLT for up to 5 months.[3] The study found that the CTX-II concentration first peaked at 3 weeks post-surgery; but from the sixth week, a second peak in CTX-II concentration was noted during week 12 after surgery, which was then followed by a decline until the end of the study.[3]As a result, the authors concluded that OA began to deteriorate from the sixth week post-surgery.[3] Moreover, the peak value of CTX-II concentration may be significantly correlated with the aggravation of articular cartilage lesions.[3] Another study found that the CTX-II concentration in the synovial fluid of patients with knee joint injury is increased.[30] Other researchers have also reported a transient increase in CTX-II levels in the rabbit model of OA.[36,37] In a canine OA model, serum CTX-II levels were significantly higher than those of the control group.[38] The current investigation demonstrated that serum CTX-II levels were higher in the OA group compared with the control group at week 4 but the difference was not significant. At weeks 8, 12 and 16, serum CTX-II levels in the OA group were significantly higher than those of the control group. These findings suggest that, in the early stages of cartilage injury, type II collagen undergoes no obvious degradation. In the early stages of articular cartilage damage, compensatory hypertrophy of the chondrocytes occurs in an attempt to repair the damage, but with the increasing severity of OA, hyperplastic chondrocytes from the early stage undergo cell death and necrosis. As the chrondrocytes continue to die, there is also the continuous degradation of type II collagen, so that increasing amounts of CTX-II are released into the blood leading to an increase in CTX-II levels between weeks 8 and 16. Therefore, CTX-II has potential as a biochemical marker of cartilage type II collagen degradation for monitoring the progression of OA.

In recent years, with the improvements in the spatial resolution of MRI technology, the software that can quantitatively evaluate MRI cartilage volume has increased in accuracy. ImageJ software is a Java-based public image processing and analysis program developed by Professor Wayne Rasband at the National Institutes of Health. It supports image stacks, a series of images that share a single window in image processing, and is multithreaded. This present study used lmageJ software to measure the cartilage volume of the tibial plateau of the knee joint in a canine MRI T1-vibe sequence. The results showed that in the control sham operation group, the cartilage volume of the tibial plateau was similar before and after the sham surgery. In the OA group at week 4, the cartilage volume of the tibial plateau increased by 1.4%compared with the preoperative baseline values, although there was no significant difference between the two groups. At weeks 8 and 16, the cartilage volume of the tibial plateau in the OA group decreased by 8.8% and 22.8%, respectively,

compared with the preoperative baseline values; and the difference between the two groups was significant. We hypothesize that, at week 4, in the early stages of cartilage injury, ACLT leads to mechanical instability of the knee joint and injury to the articular cartilage surface. Simultaneously, the articular chondrocytes undergo compensatory hypertrophy when trying to repair the damaged chondrocytes. Therefore, the volume of the tibial plateau cartilage was slightly increased compared with the preoperative values. However, as time goes on, with the death of articular chondrocytes from compensatory hypertrophy due to decompensation gradually increasing, the thickness of the articular cartilage gradually reduced. Therefore, at weeks 8 and 16, the volume of the tibial plateau was significantly decreased compared with the preoperative baseline values. However, at week 4, the differences were not significant when compared with preoperative baseline values. The restricted sample size may be a limitation of the present study, so these conclusions require further investigation.

Although the ACLT model has been frequently used in the past, there are fewer studies on the pathological mechanism of cartilage damage in OA from the viewpoint of serology and imaging. This present study, from the serological point of view, explores the compensatory hypertrophic degeneration of chondrocytes after injury, which attempts to repair damaged chondrocytes. However, the continuous mechanical instability acting on the articular cartilage alters the mechanism from compensatory hypertrophy to decompensated death and subsequently leads to the degeneration of articular cartilage.[39] The current quantitative MRI analysis of the tibial plateau cartilage volume also found that the cartilage volume increased lightly during the early stage of injury because of the compensatory hypertrophy of chondrocytes. However, under the continuous pressure of mechanical instability, the death of cartilage cells gradually increased, eventually resulting in a markedly decreased articular cartilage volume compared with the preoperative values (baseline). The changes in articular cartilage volume were also consistent with the serological test results. It should be noted that a potential limitation of this study is the fact that subchondral bone marrow oedema is not a common occurrence detected using MRI in Beagles. Among the dogs that exhibited bone marrow oedema in the current study, the oedema was not increased with the degeneration of the cartilage. Some dogs exhibited obvious oedema after week 4 in the medial tibial plateau. Although the oedema was reduced by postoperative week 8, the oedema markedly increased again after week 16. One dog presented with oedema at postoperative week 4 and the oedema was resolved at the week 8 and 16 scans, with no obvious presentation during follow-up. A previous study found that the degeneration of articular cartilage is closely related to bone marrow injury.[40] However, the present study found that there was no universal prevalence of subchondral bone marrow oedema in this canine model of cartilage degeneration OA. Furthermore, in the small number of dogs that exhibited oedema, the oedema did not gradually increase with the deterioration of OA disease. Therefore, the present study did not demonstrate an obvious synergistic relationship between bone marrow oedema and OA cartilage degeneration. Additional research is required to investigate the latter stages of OA in terms of bone marrow oedema.

In conclusion, these current results indicate that combined serum marker changes and quantitative MRI evaluation of the cartilage volume of the tibial plateau in a canine model of experimental OA may potentially be used to detect and monitor

the progression of OA. Moreover, the results of this study suggest a novel direction for follow-up analysis of the biochemical pathogenesis of OA cartilage degeneration.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

Funding

This work was supported by the National Natural Science Foundation of China (no. u1503221).

References

1. Thomas E, Peat G and Croft P. Defining and mapping the person with osteoarthritis for population studies and public health. *Rheumatology (Oxford)* 2014; 53: 338–345.
2. Bachmeier CJ, March LM, Cross MJ, et al. A comparison of outcomes in osteoarthritis patients undergoing total hip and knee replacement surgery. *Osteoarthritis Cartilage* 2001; 9: 137–146.
3. Duclos ME, Roualdes O, Cararo R, et al. Significance of the serum CTX-II level in an osteoarthritis animal model: a 5-month longitudinal study. *Osteoarthritis Cartilage* 2010; 18: 1467–1476.
4. Henrotin Y, Addison S, Kraus V, et al. Type II collagen markers in osteoarthritis: what do they indicate? *Curr Opin Rheumatol* 2007; 19: 444–450.
5. Charni-Ben Tabassi N and Garnero P. Monitoring cartilage turnover. *Curr Rheumatol Rep* 2007; 9: 16–24.
6. He Y, Siebuhr AS, Brandt-Hansen NU, et al. Type X collagen levels are elevated in serum from human osteoarthritis patients and associated with biomarkers of cartilage degradation and inflammation. *BMC Musculoskelet Disord* 2014; 15: 309.
7. Christgau S, Garnero P, Fledelius C, et al. Collagen type II C-telopeptide fragments as an index of cartilage degradation. *Bone* 2001;29: 209–215.
8. Garnero P, Conrozier T, Christgau S, et al. Urinary type II collagen C-telopeptide levels are increased in patients with rapidly destructive hip osteoarthritis. *Ann Rheum Dis* 2003; 62: 939–943.
9. Reijman M, Hazes JM, Bierma-Zeinstra SM, et al. A new marker for osteoarthritis cross-sectional and longitudinal approach. *Arthritis Rheum* 2004; 50: 2471–2478.
10. Jordan KM, Syddall HE, Garnero P, et al. Urinary CTX-II and glucosyl-galactosyl-pyridinoline are associated with the presence and severity of radiographic knee osteoarthritis in men. *Ann Rheum Dis* 2006; 65: 871–877.
11. Gu J, Lu Y, Zheng Q, et al. Identification and characterization of the novel Col10a1 regulatory mechanism during chondrocyte hypertrophic differentiation. *Cell Death Dis* 2014; 5: e1469.
12. Shen G. The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. *Orthod Craniofac Res* 2005; 8: 11–17.
13. Mackie EJ, Ahmed YA, Tatarczuch L, et al. Endochondral ossification: how cartilage is converted into bone in the developing skeleton. *Int J Biochem Cell Biol* 2008; 40: 46–62.
14. Gelse K, Ekici AB, Cipa F, et al. Molecular differentiation between osteophytic and articular cartilage – clues for a transient and permanent chondrocyte phenotype. *Osteoarthritis Cartilage* 2012; 20: 162–171.
15. Saito M, Sasho T, Yamaguchi S, et al. Angiogenic activity of subchondral bone during the progression of osteoarthritis in a rabbit anterior cruciate ligament transaction model. *Osteoarthritis Cartilage* 2012; 20: 1574–1582.
16. Welting TJ, Caron MM, Emans PJ, et al. Inhibition of cyclooxygenase-2 impacts chondrocyte hypertrophic differentiation during endochondral ossification. *Eur Cell Mater* 2011; 22: 420–436.
17. Boileau C, Martel-Pelletier J, Abram F, et al. Magnetic resonance imaging can accurately assess the long-term progression of knee structural changes in experimental dog osteoarthritis. *Ann Rheum Dis* 2008; 67: 926–932.
18. Libicher M, Ivancic M, Hoffmann V, et al. Early changes in experimental osteoarthritis

using the Pond-Nuki dog model: technical procedure and initial results of in vivo MR imaging. *Eur Radiol* 2005; 15: 390–394.
19. Schneider CA, Rasband WS and Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012; 9: 671–675.
20. Abramoff MD, Magalhães PJ and Ram SJ. Image Processing with ImageJ. *Biophotonics International* 2004; 11: 36–42.
21. Zhen G and Cao X. Targeting TGFβ signaling in subchondral bone and articular cartilage homeostasis. *Trends Pharmacol Sci* 2014; 35: 227–236.
22. Crane JL and Cao X. Bone marrow mesenchymal stem cells and TGF-β signaling in bone remodeling. *J Clin Invest* 2014; 124: 466–472.
23. Goldring SR. Alterations in periarticular bone and cross talk between subchondral bone and articular cartilage in osteoarthritis. *Ther Adv Musculoskelet Dis* 2012; 4: 249–258.
24. Matsumoto T, Cooper GM, Gharaibeh B, et al. Cartilage repair in a rat model of osteoarthritis through intraarticular transplantation of muscle-derived stem cells expressing bone morphogenetic protein 4 and soluble Flt-1. *Arthritis Rheum* 2009; 60: 1390–1405.
25. Ding M, Lu Y, Abbassi S, et al. Targeting Runx2 expression in hypertrophic chondrocytes impairs endochondral ossification during early skeletal development. *J Cell Physiol* 2012; 227: 3446–3456.
26. Amano K, Densmore M, Nishimura R, et al. Indian hedgehog signaling regulates transcription and expression of collagen type X via Runx2/Smads interactions. *J Biol Chem* 2014; 289: 24898–24910.
27. Garnero P, Ayral X, Rousseau JC, et al. Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. *Arthritis Rheum* 2002; 46: 2613–2624.
28. Bruckner P and van der Rest M. Structure and function of cartilage collagens. *Microsc Res Tech* 1994; 28: 378–384.
29. Eyre DR and Wu JJ. Collagen structure and cartilage matrix integrity. *J Rheumatol Suppl* 1995; 43: 82–85.
30. Lohmander LS, Atley LM, Pietka TA, et al. The release of crosslinked peptides from type II collagen into human synovial fluid is increased soon after joint injury and in osteoarthritis. *Arthritis Rheum* 2003; 48: 3130–3139.
31. Vos LM, Kuijer R, Huddleston Slater JJ, et al. Alteration of cartilage degeneration and inflammation markers in temporomandibular joint osteoarthritis occurs proportionally. *J Oral Maxillofac Surg* 2013; 71: 1659–1664.
32. Chmielewski TL, Trumble TN, Joseph AM, et al. Urinary CTX-II concentrations are elevated and associated with knee pain and function in subjects with ACL reconstruction. *Osteoarthritis Cartilage* 2012; 20: 1294–1301.
33. Kalai E, Bahlous A, Charni N, et al. Increased urinary type II collagen C-telopeptide levels in Tunisian patients with knee osteoarthritis. *Clin Lab* 2012; 58: 209–215.
34. Mazzuca SA, Brandt KD, Eyre DR, et al. Urinary levels of type II collagen C-telopeptide crosslink are unrelated to joint space narrowing in patients with knee osteoarthritis. *Ann Rheum Dis* 2006; 65: 1055–1059.
35. Park YM, Kim SJ, Lee KJ, et al. Detection of CTX-II in serum and urine to diagnose osteoarthritis by using a fluoro-microbeads guiding chip. *Biosens Bioelectron* 2015; 67: 192–199.
36. Lindhorst E, Wachsmuth L, Kimmig N, et al. Increase in degraded collagen type II in synovial fluid early in the rabbit meniscectomy model of osteoarthritis. *Osteoarthritis Cartilage* 2005; 13: 139–145.
37. Rogart JN, Barrach HJ and Chichester CO. Articular collagen degradation in the Hulth-Telhag model of osteoarthritis. *Osteoarthritis Cartilage* 1999; 7: 539–547.
38. Matyas JR, Atley L, Ionescu M, et al. Analysis of cartilage biomarkers in the

early phases of canine experimental osteoarthritis. *Arthritis Rheum* 2004; 50: 543–552.
39. Goldring MB and Goldring SR. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Ann N Y Acad Sci* 2010; 1192: 230–237.
40. Hunter DJ, Zhang Y, Niu J, et al. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. *Arthritis Rheum* 2006; 54: 1529–1535.