Original article

How precisely does ultrasonographic evaluation reflect the histological status of the articular cartilage of the knee joint?

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ABSTRACT

The thickness and the grade of the articular cartilages of the knee of 34 patients who underwent total knee arthroplasty were evaluated by ultrasound (US) and by histology. The US grade correlated with the histological grade and the thickness of the articular cartilage measured by US. The thickness measured by US was significantly correlated with that measured by histology for the medial condyle. The US thickness was significantly less than the histological thickness for thicker articular cartilages. US grading and the thickness of the articular cartilages evaluated by US is sufficiently reliable to indicate their histological status.

1. Introduction

In degenerative joint disorders such as osteoarthritis (OA) and rheumatoid arthritis (RA), articular cartilages wear down and become thinner over time, and patients with severe degeneration eventually require total joint replacement. It is widely accepted that the state of the articular cartilage is well correlated with the symptoms and severity of the joint degeneration.¹ X-ray and magnetic resonance imaging (MRI) are commonly used to reliably evaluate the status and the severity of joint degeneration. However, ultrasonography (US) of the joint has been recently developed and has gradually gained wide acceptance as an advanced technology capable of observing subtle changes. Of these three modalities, however, X-ray cannot directly assess cartilages, and MRI is time-consuming and costly. Therefore, the advantages of US, including its low cost, lower demands for processing, and the lack of radiological exposure, have been widely appreciated.

US evaluation of the joints has gained credibility, especially for assessment of joint inflammation and destruction. Previous studies have shown a correlation between US features and pain or arthroscopic features.^{2,3} In particular, it has been shown that knee US performs excellently compared with arthroscopic grading for detection of degenerative changes in the articular cartilage.⁴ However, its concordance with direct histological evaluation has not been confirmed. It is crucial to know how the femoral articular cartilages have degenerated because this reportedly represents accurately the status of all the articular cartilages.^{2,3,5} Lee et al. reported that the histological grading was weakly

correlated with the US grading.⁶ However, that study evaluated the articular cartilages in a transverse plane, which in practice is not easily scanned by US, and moreover, did not compare the thickness of cartilages evaluated by US and histology. We have previously shown in an *in vitro* study using a specific US system to detect early changes in the articular cartilage that US grades correlate well with histological grades.⁷ However, we did not evaluate *in vivo* changes or analyze degenerative changes or the thickness of the articular cartilages.

Thus, the purpose of this study was to assess the reliability of US for evaluation of articular cartilage thickness and grades *in vivo* in patients with degenerative knee changes who underwent total knee arthroplasty.

2. Patients and methods

2.1. Patients

The protocol for this study was approved by the Institutional Review Board before the start of this study (Approval no. E1490). Informed consent for the study was obtained from all participating patients in accordance with the World Medical Association Declaration of Helsinki. Sequential patients undergoing total knee arthroplasty because of RA or OA were recruited for this study. The diagnoses of OA and RA were established based on the diagnostic criteria of the American College of Rheumatology⁸ and the European League Against Rheumatoid Arthritis/ACR,⁹ respectively. A total of 35 knees in 34 patients were

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Table 1
Demographic data.

parameters	
number of specimens (patients)	35 (34)
age $\pm$ SD (min-max)	72.3 $\pm$ 7.8 (61–87)
females (%)	27 (77.1)
OA / RA	31 / 4
BMI $\pm$ SD (min-max)	25.0 $\pm$ 4.0 (18.2–34.1)

OA; osteoarthritis, RA; rheumatoid arthritis, BMI; body mass index.

enrolled. The average age of the patients was 74.5 years (61–87 years) and 27 patients (77%) were women (Table 1). The demographic information for all patients was collected from their medical records.

2.2. Clinical evaluation

Knee joint symptoms were evaluated using the Knee Society Score (KSS), which was developed and revised for patients who undergo total knee arthroplasty.^{10–12} An X-ray of the knee joint was taken using a standardized protocol,¹³ and the level of joint destruction was graded using the Kellgren–Lawrence grading system¹⁴ by two experienced orthopedic surgeons (MF, HI), both of whom were board members of the Japanese Orthopaedic Association and were blinded to the US and histological findings for the articular cartilages.

2.3. US evaluation

Patients underwent US in the operating room just before total knee arthroplasty or one day before the operation at their bedside. Transverse scans of the intercondylar notch and sagittal longitudinal scans of the distal femoral condyles medially and laterally to the patella were performed with the patient lying supine with a fully flexed knee.^{4, 15, 16} The thickness of the cartilages was measured at three points on the images, and the cartilage thickness was defined as the average of the three measurements. As a reference, the thickness of the femoral cartilage of the patellofemoral joint was also recorded.¹⁷ The degree of degenerative change in the cartilages was classified into five grades using the grading system reported previously.⁴ The US machine used for this study was a NOBLUS (Hitachi Aloka, Tokyo, Japan) with a 5 MHz linear transducer.

2.4. Histological evaluation

The articular cartilage fragments attached to the subchondral bone of the distal femoral condyles were resected during the operation. During the placement of the US probe, lines were drawn with a marker pen to indicate the femoral articular cartilage medial and lateral to the patella with the knee fully flexed; the outlined portion was collected after the femoral resection for arthroplasty and used for the histological evaluation (Fig. 1). These specimens were first cut into approximately 3-mm-wide sections in the sagittal plane, corresponding to the plane used for US imaging. The specimens were fixed in 4% paraformaldehyde and decalcified in Morse's solution for 2–3 days. After decalcification, the tissue was embedded in paraffin wax, and 7- μ m sections were made. These sections were stained with 0.1% Safranin O and 0.06% fast green. Cartilage thickness was measured at three points of each section, similar to the measurement of cartilage thickness by US, and the cartilage thickness was defined as the average of the three measurements. In addition, the histological features were graded using the Osteoarthritis Research Society International grading system.¹⁸ The measurements and grades were independently evaluated by two researchers (KM, MF) and were strongly correlated (data not shown).

2.5. Statistical analyses

Statistical analyses were performed by an independent researcher (YM) who was blinded to the intraoperative findings and who did not assess any of the US, radiological, or histological grades or measure the thickness of the cartilage. Continuous data are expressed as means, standard deviation (SD), and ranges, whereas categorical data are presented as absolute numbers and percentages. Spearman's rank correlation coefficient was used to determine correlations between two grades. We also calculated Spearman's rank correlation coefficient to compare the values of the thickness measured by the two methods. The intraclass correlation coefficient (ICC) was also calculated for the values measured by the two methods. A p value < 0.05 was considered significant.

3. Results

The average cartilage thicknesses of the patellofemoral joint, and medial and lateral condyles measured by US were 1.18 ± 0.72 (0–2.76) mm, 0.49 ± 0.35 (0–1.47) mm, and 0.75 ± 0.45 (0.10–1.60) mm, respectively (Table 2). The knee function evaluated by KSS was weakly but significantly correlated with the histological grade for the medial femoral condyle but not with that for the lateral femoral condyle (data not shown), which is consistent with other reports.² Importantly, the US grades were strongly correlated with the histological grades for the femoral medial condylar cartilages (Fig. 2a), while there was no significant correlation for the femoral lateral condyles (data not shown). However, the US grades were significantly correlated with the thickness of the articular cartilage measured by US for both the medial and the lateral condyles (Fig. 2b).

Next, the cartilage thicknesses assessed by US and histology were compared. Fig. 3 shows that there was a significant correlation between the two evaluation methods for the medial condyle ($\rho = 0.42$, $p = 0.02$, ICC = 0.751), but not for the lateral condyle ($\rho = 0.15$, $p = 0.42$, ICC = 0.204). In addition, Fig. 4 shows that many specimens came from the area under the center line, especially those with thicker cartilages. The subtracted difference between the value measured by histology and that by US showed significant positive correlation with the thickness of the articular cartilage measured by histology (Fig. 4). Therefore, the thickness of the cartilages in this thick group appeared significantly thinner by US evaluation than by histological evaluation (Fig. 4).

4. Discussion

This study identified several important results concerning the measurement and grade of the articular cartilage of the knee joint evaluated by US. First, the US grades were strongly correlated with the histological grades, which also supports the validity of the standardized US grading system currently used. Second, the measurements of cartilage thickness showed that knee symptoms were also correlated with the thickness of the medial but not of the lateral femoral condyle as measured by US. Third, for the medial but not the lateral condylar cartilage, there was a significant correlation between the thickness of the articular cartilage as measured by US and the histological evaluation. Lastly, as a practically important point, the thickness of the thickest cartilages appeared thinner on US than on histological evaluation.

The grading system for articular cartilages aims to indicate the overall degree of degeneration with sufficient reliability. Both the radiological Kellgren–Lawrence grading and the US grading have been frequently reported to have excellent practicality and reliability.^{2, 19} However, the correlation between the US and histological grading may not have been adequately demonstrated. This study shows that the US grading system correlates well with histological grading. Although each system has its own weaknesses, the three systems seem to complement each other. Although we did not have the opportunity to obtain MRI for

Fig. 1. Osteochondral fragment taken at the total knee arthroplasty for histological evaluation. Articular cartilage fragments with subchondral bone of the distal femoral condyles were cut during the operation. As the US probe was placed, a line was drawn using a marker pen medially and laterally to the patella with the knee fully flexed, and the outlined portion was collected after the femoral resection.

Table 2
Thickness of the articular cartilages of the femoral condyles and intercondylar area in the knee joint.

	US thickness (mm)	Histological thickness (mm)
Medial femoral condyle	0.49 ± 0.35	0.60 ± 0.76
Lateral femoral condyle	0.75 ± 0.45	1.68 ± 0.76
Inter condylar area	1.18 ± 0.72	

the patients in this study, evaluation by MRI also reportedly correlates with the status of the articular cartilages.^{20–22} US has major advantages over the other modalities because of its low cost, lower demands for processing, and lack of radiological exposure, and provides valuable practical information about the current status of the knee joint.

There are a number of concerns about the usefulness and precision of the measurement of articular cartilages. One study reported that the composition, thickness, and mechanical properties of the cartilage varied significantly across the tibial plateau²³; another study showed that US was not valid for thickness assessment.²⁴ However, the former study analyzed only the tibial cartilages, and the latter was a nonhuman study. Although it was reported that 3-D reconstructed images obtained by US were reproducible and well correlated with those obtained using MRI for measurement of femoral cartilage thickness,²⁵ the present

study has confirmed that 2-D images from a standard US device can reliably show the thickness of the femoral articular cartilage *in vivo*. The US grading of the articular cartilage may be sufficiently reliable to assess degeneration because it was shown that the thickness of the cartilages was strongly correlated with the US grade for both the medial and lateral condyles of the femur. However, we also confirmed that the absolute values of the thickness measured by US were well correlated with those measured by histology.

The results of this study also demonstrate that caution should be used in measuring the thickness of the articular cartilage by US, as the thickness of thicker cartilage may be underestimated by US. This indicates that the degenerative or damaged status of the femoral articular cartilage in the knee joint should not be overestimated. To our knowledge, this effect has not been described previously. This finding raises an interesting question: why do thicker cartilages appear thinner on US than in reality? One plausible answer might be that the thickness of an artefact on the cartilage surface was excluded by the examiner and thereby the total thickness was underestimated. However, in this case, the examiner was well trained by an experienced ultrasonographer before the examination, and even the thicker cartilages were relatively degenerate with little artefact. This possibility is therefore unlikely. Another plausible reason might be that the examiner included a layer of synovial fluid over the degenerate articular cartilage and overestimated the thickness of the thinner cartilages. However, this possibility is also

Fig. 2. Correlation between the ultrasonographic (US) and histological grades for the articular cartilages of the knee joints. (a) The correlation between the US and the histological grades for the articular cartilage in the medial condyle of the femur was calculated. (b) The correlation between the US grades and the thickness of the cartilage in the medial condyle of the femur was calculated.

Fig. 3. Correlations between the articular cartilage thickness calculated from the ultrasonographic (US) and histological measurements. (a) Medial condyle. (b) Lateral condyle.

Fig. 4. Correlations between the thickness of the articular cartilages measured by histology and the subtracted difference between the values measured by ultrasonographic (US) and histology. (a) Medial condyle. (b) Lateral condyle.

unlikely because the operators underwent sufficient training before the examination and there is no reason why only the thickness of thinner cartilages would be overestimated. Nevertheless, examiners and researchers should bear our results in mind when dealing with the thickness of the articular cartilage.

This study has several limitations. First, the sample size was relatively small. Second, both OA and RA patients were included. The grading and measurement might differ because of differences in pathology. Third, all the studied specimens were moderately to severely damaged cartilages collected from patients who underwent total knee arthroplasty. Less damaged cartilages may show a different pattern of measurements. Fourth, this study only analyzed the articular cartilage of the knee joint. The cartilages of other joints might be measured or graded differently. These issues should be investigated in future studies.

In conclusion, the US grading of the femoral medial condylar cartilage is well correlated with the histological grading. The thickness measured by US was significantly and reliably correlated with the thickness measured by histology only for the medial condyle of the knee joint, while the thickness of thick articular cartilage in the distal femoral condyle evaluated by US tends to appear thinner than that measured by histology.

Conflicts of interest

HI has received research grants and/or speaker fees from Mitsubishi-Tanabe, Bristol-Myers, Asahi-Kasei, and Astellas. KM, YM,

MF, TF, MA, AO, KN, SK, SN, and SM have declared no conflicts of interest for this study. The sponsors were not involved in the study design; in the collection, analysis, interpretation of data; in the writing of this manuscript; or in the decision to submit the article for publication. The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

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References

- Pollard TC, Gwilym SE, Carr AJ. The assessment of early osteoarthritis. *J Bone Joint Surg Br.* 2008;90:411–421.
- Podlipská J, Koski JM, Kaukinen P, et al. Structure-symptom relationship with wide-area ultrasound scanning of knee osteoarthritis. *Sci Rep.* 2017;7:44470.
- Serban O, Porojan M, Deac M, et al. Pain in bilateral knee osteoarthritis - correlations between clinical examination, radiological, and ultrasonographical findings. *Med Ultrason.* 2016;18:318–325.

4. Saarakkala S, Waris P, Waris V, et al. Diagnostic performance of knee ultrasonography for detecting degenerative changes of articular cartilage. *Osteoarthritis Cartilage*. 2012;20:376–381.
5. Hada S, Kaneko H, Sadatsuki R, et al. The degeneration and destruction of femoral articular cartilage shows a greater degree of deterioration than that of the tibial and patellar articular cartilage in early stage knee osteoarthritis: a cross-sectional study. *Osteoarthritis Cartilage*. 2014;22:1583–1589.
6. Lee CL, Huang MH, Chai CY, et al. The validity of in vivo ultrasonographic grading of osteoarthritic femoral condylar cartilage: a comparison with in vitro ultrasonographic and histologic gradings. *Osteoarthritis Cartilage*. 2008;16:352–358.
7. Nishitani K, Kobayashi M, Kuroki H, et al. Ultrasound can detect macroscopically undetectable changes in osteoarthritis reflecting the superficial histological and biochemical degeneration: ex vivo study of rabbit and human cartilage. *PLoS One*. 2014;9:e89484.
8. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum*. 1986;29:1039–1049.
9. Aletaha D, Neogi T, Silman AJ, et al. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum*. 2010;62(2010):2569–2581.
10. Scuderi GR, Bourne RB, Noble PC, et al. The new knee society knee scoring system. *Clin Orthop Relat Res*. 2012;470:3–19.
11. Noble PC, Scuderi GR, Brekke AC, et al. Development of a new knee society scoring system. *Clin Orthop Relat Res*. 2012;470:20–32.
12. Hamamoto Y, Ito H, Furu M, et al. Cross-cultural adaptation and validation of the Japanese version of the new knee society scoring system for osteoarthritic knee with total knee arthroplasty. *J Orthop Sci*. 2015;20:849–853.
13. Ravaud P, Giraudeau B, Auleley GR, et al. Radiographic assessment of knee osteoarthritis: reproducibility and sensitivity to change. *J Rheumatol*. 1996;23:1756–1764.
14. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthritis. *Ann Rheum Dis*. 1957;16:494–502.
15. Wakefield RJ, Balint PV, Szkudlarek M, et al. Musculoskeletal ultrasound including definitions for ultrasonographic pathology. *J Rheumatol*. 2005;32:2485–2487.
16. Backhaus M, Burmester GR, Gerber T, et al. Guidelines for musculoskeletal ultrasound in rheumatology. *Ann Rheum Dis*. 2001;60:641–649.
17. Schmidt WA, Schmidt H, Schicke B, et al. Standard reference values for musculoskeletal ultrasonography. *Ann Rheum Dis*. 2004;63:988–994.
18. Pritzker KP, Gay S, Jimenez SA, et al. Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis Cartilage*. 2006;14:13–29.
19. Okano T, Filippucci E, Di Carlo M, et al. Ultrasonographic evaluation of joint damage in knee osteoarthritis: feature-specific comparisons with conventional radiography. *Rheumatology (Oxford)*. 2016;55:2040–2049.
20. Yoon CH, Kim HS, Ju JH, et al. Validity of the sonographic longitudinal sagittal image for assessment of the cartilage thickness in the knee osteoarthritis. *Clin Rheumatol*. 2008;27:1507–1516.
21. Wang Y, Wluka AE, Jones G, et al. Use magnetic resonance imaging to assess articular cartilage. *Ther Adv Musculoskelet Dis*. 2012;4:77–97.
22. McGibbon CA, Trahan CA. Measurement accuracy of focal cartilage defects from MRI and correlation of MRI graded lesions with histology: a preliminary study. *Osteoarthritis Cartilage*. 2003;11:483–493.
23. Aula AS, Töyräs J, Tiitu V, et al. Simultaneous ultrasound measurement of articular cartilage and subchondral bone. *Osteoarthritis Cartilage*. 2010;18:1570–1576.
24. Jurvelin JS, Räsänen T, Kolmonen P, et al. Comparison of optical, needle probe and ultrasonic techniques for the measurement of articular cartilage thickness. *J Biomech*. 1995;28:231–235.
25. Ohashi S, Ohnishi I, Matsumoto T, et al. Measurement of articular cartilage thickness using a three-dimensional image reconstructed from B-mode ultrasonography mechanical scans feasibility study by comparison with MRI-derived data. *Ultrasound Med Biol*. 2012;38:402–411.