Knee joint ultrasonography of the ACLT rabbit experimental model of osteoarthritis: relevance and effectiveness in detecting meniscal lesions

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Summary

Objectives: To develop a protocol for rabbit knee joint ultrasonography (US); to grade ultrasonographically the meniscal injuries of the anterior cruciate ligament transection (ACLT) rabbit model of osteoarthritis (OA); to assess with US the effectiveness of the ACLT; to compare final US with macroscopy for the evaluation of medial and lateral meniscal injuries depending on the age and weight when ACLT is performed.

Methods: Twenty-two skeletally mature and adolescent New Zealand white rabbits were housed during the same period at the Institut Claude-Bourgelat, Lyon, France. Surgical ACLT was performed in the left knee of nine adolescent and five adult rabbits. Final US and macroscopic semi-quantitative grading of the meniscal injuries were compared 5 months after ACLT.

Results: A standardised protocol was developed to evaluate the rabbit knee joint. US was performed in both control and ACLT knees. Normal and abnormal meniscal US appearances were described. A semi-quantitative scale to grade US meniscal injuries was created. Macroscopic and US total meniscal scores were significantly positively correlated (P < 0.001, r = 0.70). US detection of meniscal injuries was 92% sensitive and 87.5% specific compared to macroscopy. Positive and negative predictive values of US were, respectively, 92% and 87.5%. US detection of the ACLT effectiveness was 100% specific and 78.5% sensitive.

Conclusion: A significant relationship was found between ultrasonographic and macroscopic grading of meniscal injuries. US was both specific and sensitive in detecting meniscal lesions. We propose US as a non-invasive, non-expensive, in vivo imaging technique for preclinical studies in the ACLT rabbit OA model.

Key words: Osteoarthritis, Ultrasonography, Experimental model, Meniscus.

Introduction

Osteoarthritis (OA) is the most common disease of the musculoskeletal system in man and is characterised by a progressive degradation of the whole joint organ, including cartilage, menisci, synovial capsule, subchondral bone and ligaments. Clinically, in humans, meniscal injuries are correlated with higher OA severity and cartilage loss in the knee joint. In numerous cases only one of these tissues is damaged and it remains unclear whether meniscal injuries are a cause or a consequence of more severe OA. A chronological evaluation of events is needed to understand the pathogenesis of the disease. Studies on the initiation and on the early progression of OA are inherently difficult in humans. Consequently, a number of animal models have been developed to establish the pathogenesis of OA and to evaluate the potential of new disease structure modifying drugs. The transection of the anterior cruciate ligament (ACL) in rabbits is a widely used experimental model with a known time of initiation of OA. The resulting changes closely resemble those observed naturally in humans. Studies in the canine and rabbit models of OA have shown meniscus damage in the unstable knee after ACL transection (ACLT) and the rabbit ACLT model, Hélie LeGraverand et al. recently studied cellular, matrix and molecular menisci alterations. However, they have not been characterised to what extent meniscal injuries contributed to the underlying cartilage degradation, a distinctive feature of OA. The detection of in vivo meniscal injuries following the experimental ACLT would be useful for further morphological and biochemical analyses of the OA pathogenesis.
In addition, in the ACLT rabbit model of OA there are no available data on the correlation of meniscal injuries or cartilage degradation with age and weight of the OA induction, two of the major risk factors for the development of OA.13,14–16

In humans, musculoskeletal US plays an important role in detecting the minimal soft tissue changes.1,2,17–19 US has the ability to differentiate intra-articular and extra-articular soft tissue structures. Recently, US has been recommended as the first-line diagnostic method for non-invasive exploration of meniscal injuries.20 In the rabbit OA model, US has only been used to evaluate the articular cartilage ex vivo.21,22 To date, no protocol for in vivo rabbit knee joint US exists. Yet, this is a quick, readily available, inexpensive and non-invasive imaging technique which is particularly adapted in longitudinal in vivo experimental model studies.

The purpose of this study was to develop a protocol for in vivo joint US of the ACL transection rabbit model of OA; to detect with US the effectiveness of ACLT; and to compare final US evaluation of medial meniscal injury (MMI) and lateral meniscal injury (LMI) with macroscopy depending on the age and weight when ACLT is performed.

Material and method

All work was conducted in full compliance with Ecole Nationale Vétérinaire de Lyon (ENVL) Ethical Committee Guideline (protocol agreement number 0634) in accordance with current European and French Legislation for Animal Protection.

EXPERIMENTAL ANIMAL MODEL

Twenty-two New Zealand white rabbits were used for this study and housed during the same period at the Institut Claude-Bourgelat, Lyon, France. They were kept in individual cages. Baseline radiographs revealed that the rabbits could be divided into 13 adolescent rabbits (opened proximal tibial and distal femoral physes) and nine adult rabbits (closed physes). After 10 days of acclimation, experimental OA was surgically induced by ACLT in the left knee of 14 rabbits. The operated group was composed of five adult rabbits (5.2 ± 0.1 kg) and nine adolescent rabbits (1.9 ± 0.1 kg). Four adolescent (2.1 ± 0.2 kg) and four adult rabbits (5.5 ± 0.2 kg) were not operated on and formed the control group.

SURGICAL PROCEDURE

Anaesthesia of the operated rabbits was induced by intramuscular sedation using a combination of xylazine (Rompun 2%©, Bayer, 2 mg/kg) and ketamine (Imalgene 1000©, Merial, 35 mg/kg) and maintained with isoflurane in oxygen through a face mask. Lateral arthrotomy was performed under sterile conditions, the patella was retracted medially and the knee fully flexed to visualise the ACL. The ACL was sectioned close to its tibial insertion without affecting the surrounding structures. The anterior drawer test was consistently positive after the operation. The contralateral knee was left intact. Analgesia was provided before surgery with morphine (3 mg/kg) and after surgery with morphine (2 mg/kg) and a fentanyl patch on the interscapular space (25 μg/h). The operated leg was not immobilised. Rabbits were allowed free activity in their cages. Recovery was closely monitored by veterinarians.

Control and operated rabbits were euthanised 5 months post-surgery after clinical examination. Radiography of the knee joints revealed that adolescent rabbits had reached skeletal maturity.

KNEE JOINT RANGE OF MOTION AND MENISCAL “CLICKING”

Rabbits were sedated by intramuscular injection of ketamine (Imalgine 1000©, Merial, 30 mg/kg) and xylazine (Rompun 2%©, Bayer, 3 mg/kg) and without intubation or ventilation. Goniometric measurements were performed monthly by the same observer. They gave the angle between the left femoral and tibial bone long axis. The range of motion of left knee resulted from the difference between full extension and full flexion angles. The decrease in the range of motion was defined as the difference between the final and the first measurements. Meniscal “clicking” (“snapping”) was recorded.23,24

US protocol

Data are presented in Table I. For this study, US were done in vivo under sedation with the same drug combination as above and on fresh cadavers. During a complementary study, eight control rabbits with closed physes (four young rabbits and four adult) were scanned twice by the veterinarian ultrasonomgrapher of the Institut Claude-Bourgelat ICI-B, in vivo under sedation as for our study, and then 5–6 h after euthanasia. No noticeable difference in echogenicity and texture in intra- and extra-articular structures of the knee was observed.

A protocol for the rabbit knee joint US was created based on our routine canine and equine stifle US protocol used at the ENVL.25–27 The examination was always performed in the same order by the referring veterinarian for small animal musculoskeletal US of the ENVL. The left knee was clipped. Isopropyl alcohol and ultrasound coupling gel were applied to ensure good contact between the skin and the transducer. Rabbits were positioned in lateral recumbency with the imaged knee up, or on the back with the imaged

<table>
<thead>
<tr>
<th>Probe location</th>
<th>Standard scan</th>
<th>Knee joint positioning</th>
<th>Anatomic structures observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sagittal suprapatellar [Fig. 2(A)]</td>
<td>Longitudinal Transverse</td>
<td>Neutral and flexed</td>
<td>Suprapatellar pouch</td>
</tr>
<tr>
<td>Sagittal infrapatellar [Fig. 2(B)]</td>
<td>Longitudinal Transverse</td>
<td>Neutral</td>
<td>Femoral trochlear ridges</td>
</tr>
<tr>
<td>Antero-lateral [Fig. 2(C)]</td>
<td>Longitudinal</td>
<td>Neutral and Extended</td>
<td>Anterior horn of the LM</td>
</tr>
<tr>
<td>Posterolateral [Fig. 2(E)]</td>
<td>Longitudinal</td>
<td>Lateral collateral ligament</td>
<td></td>
</tr>
<tr>
<td>Medial [Fig. 2(G)]</td>
<td>Longitudinal</td>
<td>Medial collateral ligament</td>
<td></td>
</tr>
<tr>
<td>Postero-median [Fig. 2(F)]</td>
<td>Longitudinal</td>
<td>Anterior horn of the LM</td>
<td></td>
</tr>
</tbody>
</table>

Dynamic examination — from proximal to distal with the knee in flexion/extension; varus/valgus
Table II
US and macroscopic semi-quantitative grading of the meniscal lesions

<table>
<thead>
<tr>
<th>Grades</th>
<th>Macroscopic lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal gross external appearance</td>
</tr>
<tr>
<td>1</td>
<td>Longitudinal surface striation or tear</td>
</tr>
<tr>
<td>2</td>
<td>Bucket handle tear</td>
</tr>
<tr>
<td>3</td>
<td>US</td>
</tr>
<tr>
<td>0</td>
<td>Normal meniscus</td>
</tr>
<tr>
<td>1</td>
<td>Heterogeneous, or mottled echogenicity</td>
</tr>
<tr>
<td>2</td>
<td>Protruded or not visualised</td>
</tr>
</tbody>
</table>

US semi-quantitative grading of the meniscal injuries

US menisci grading chart is presented in Table II. Normal US aspect of menisci corresponded to grade 0. Any change in the shape, echogenicity or position of the menisci was considered as indicative of meniscal injuries (grade 1). When the meniscus could not be visualised, grade 2 was reported as we observed they corresponded macroscopically to severe meniscal injuries (protruded or even absence of menisci).

GROSS MACROSCOPY

Evaluation of the cartilage damages was based on the Société Française d’Arthroscopie recommendations and was performed by two observers. Qualitative macroscopic evaluation of meniscal injuries was performed by one investigator. Meniscal injuries were reported in a semi-quantitative grading table modified from the human literature with a four-grade scale (0 = normal; 1 = longitudinal surface striations or tears; 2 = bucket handle or transverse tears and 3 = fibrillation of the entire meniscus, protrusion or even absence of meniscus) (Table II).

STATISTICAL ANALYSES

All analyses were planned to be performed at 5% significance level. Comparisons between ordinal variables were made with Spearman’s rank correlation coefficient. Statistical analyses were performed using a statistical software package (R software version 2.2.1, Ihaka, 1996).

Results

KNEE JOINT RANGE OF MOTION AND MENISCAL “CLICKING”

No meniscal clicking was heard.

Final cross-sectional comparison of the knee joint range of motion

At 5 months, operated rabbits had a significantly lower knee range of motion than control rabbits ($P < 0.0001$). No statistically significant difference was observed between the adolescent and adult operated knee range of motion ($P > 0.05$).

Longitudinal comparison of the knee joint range of motion

In control and operated rabbits the range of motion of the left knee joint decreased. The decrease in the range of motion was significantly higher in operated knees than in control knees, respectively, $–47° (± 14°)$ and $–12° (± 8°)$ ($P = 0.0001$) (Fig. 1).

In the control group, the decrease in knee range of motion was not statistically significant ($P > 0.05$). No statistically significant difference was observed between the adolescent and adult operated knee range of motion ($P > 0.05$).

US at 5 months after ACLT

US observations

Each knee joint could be examined within 20 min. A stand-off pad was unnecessary but a thorough clipping and a significant amount of coupling gel were needed. The left knee was viewed sequentially in the longitudinal and transverse planes, with the transducer placed on the anterior, antero-lateral, posterolateral, posteromedial, antero-medial and posterior aspects of the knee joint in extension and then flexion. The posterior view was not efficient to see the menisci. If possible, a dynamic examination of the suprapatellar compartment – particularly of the trochlear groove and of the patella – followed the stationary examination. The lateral and medial ridges of the femoral trochlea, the patella, the lateral and medial tibial and femoral condyles and the tibio-patellar tendon were useful anatomical and US landmarks to position the probe. Probe knee relative positioning is shown in Fig. 2.

The tibio-patellar and the quadriiceps tendons [Fig. 3(A)], the tibial and femoral condyles, the femoral trochlear ridges, the patella and the infrapatellar fat pads were consistently and easily observed in all animals on US images. The tibio-patellar tendon was readily identified in transverse and longitudinal images as a homogeneously hypoechoic structure [Fig. 3(B)]. The medial and lateral (fibular) collateral ligaments were seen as thin echogenic lines [Fig. 3(C)] and were used to locate the anterior and posterior horns of the menisci. The anterior horn of the meniscus was defined as meniscal tissue anterior to the collateral ligament and the posterior horn of the meniscus as meniscal tissue posterior to the collateral ligament. The medial collateral ligament was seen immediately superficial to the MM. The proximal tendon of the long digital extensor ran superficially to the anterior horn and to the posterior horn of the lateral meniscus (LM), respectively. The lateral collateral ligament was separated proximally from the LM by the tendon of the popliteal muscle.

Fig. 1. Box-plot comparison of the decrease in the knee range of motion between operated and control rabbits. In ordinate: difference in the knee range of motion between initial and final measurements (in degree angle).
Fig. 3. Ultrasonographic images. For all US examination and images labelling, longitudinal images were defined as a scan in the same direction of the fibre of the imaged muscle or tendon. The proximal aspect of the joint is right on the screen and the distal aspect is left. (A) Longitudinal US image of the suprapatellar area. Control rabbit knee joint. Sagittal view of the quadriceps tendon (arrows) and its insertion on the patella. (a) Acoustic shadow of the patella; (b) acoustic shadow of the distal femoral bone; and (c) Quadriceps muscle. (B) Longitudinal US image of the infrapatellar area. Operated rabbit knee joint. Sagittal view at the level of the patellar tendon (dotted black arrows). (a) Acoustic shadow of the proximal tibial bone; (b) acoustic shadow of the distal femoral bone. Transected ACL (long white arrow). Synovial effusion (double black arrow). Interrupted trochlear bone surface (small white arrows). Large infrapatellar fat pad (*) with a mottled, heterogeneous echogenicity, between the patellar tendon (dotted arrows show upper outline) and the femoro-tibial joint space. (C) Longitudinal US image of the antero-lateral area. Operated rabbit knee joint. (a) Acoustic shadow of the lateral tibial condyle; (b) acoustic shadow of the lateral femoral condyle. Collateral lateral ligament (double white arrow). Anterior horn of the LM (within arrows) which is protruded. Note the hypoechogenic heterogeneous central area in the protruded meniscus (US grade 1). (D) Longitudinal US image of the postero-lateral area. Control rabbit knee joint. (a) Acoustic shadow of the lateral tibial condyle; (b) acoustic shadow of the lateral femoral condyle. Normal posterior horn of the LM (within arrows) (US grade 0). (E) Longitudinal US image of the postero-medial area in an operated rabbit knee joint. (a) Acoustic shadow of the medial tibial condyle; (b) Acoustic shadow of the medial femoral condyle. Heterogeneous posterior horn of the MM (within black arrows) (US grade 1). Note the interrupted (dotted white arrows) hyperechogenic line representing the tibial condyle subchondral bone surface (bone erosion).
The posterior cruciate, the meniscal ligaments and the joint capsule could not be identified with absolute certainty.

US of the control group knees

For this study, the left knees of eight control rabbits (four adolescent and four adult rabbits) were imaged.

US aspect of the normal ACL. In control rabbits, the ACL was identified in the infrapatellar anteromedial area with the knee in flexion after 15–20° lateral rotation of the probe from the sagittal view. ACL distal portion was recognised at the anterior margin of the tibial plate as a thin band echogenic surrounded by thin hyperechogenic lines. ACL proximal attachment to the femoral condyle was not seen due to the narrow space between femoral condyles and limited transducer access.

US aspect of the normal menisci. Menisci were best imaged in the coronal plane (lateral to medial for the lateral meniscus (LM) and medial to lateral for the medial meniscus (MM)) with a longitudinal view. Normal menisci filled the joint space with homogeneous echogenicity and echo texture between the hyperechoic subchondral bone surfaces of the femoral and tibial condyles [Fig. 3(D)]. The anterior and posterior horns of both medial and lateral menisci had a triangular radial section with homogeneous echogenicity similar to the echogenicity of the body of the meniscus. No difference in echogenicity was observed between the medial and lateral menisci. The evaluations were slightly enhanced during dynamic examination: valgus stress and/or external rotation of the tibia, for the MM; and varus stress and/or internal rotation of the tibia, for the LM. However, in the majority of the cases, menisci were well visualised with the knee in moderate extension without dynamic examination.

US of the ACLT knees 5 months after surgery

For this study, the operated knees of 14 rabbits were imaged.

All operated knees showed multiple US evidence of severe OA. Disruption of the hyperechoic linear line representing the bone surfaces (i.e., bone erosion) was seen at the tibial and femoral medial and lateral condyles and at the trochlear ridges [Fig. 3(B and F)].

US of the transected ACL. In all except 3 of the 14 operated knees (78.5% sensitivity—100% specificity), the distal portion of the transected ACL was seen and appeared hyperechoic on the contrary to normal ACL.

In the remaining three knees, the ACL was obscured by a hyperechoic area.

US of the menisci of the ACLT knees. Data are presented in Tables III and IV. Abnormal menisci showed a heterogeneous, mottled echogenicity with hypo- and hyperechoic foci. Meniscal protrusion or subluxation could be identified as shown in Fig. 3(C).

The total US meniscal score (1.5 ± 0.6 out of 2) was significantly higher in the operated group than in the control group (0.1 ± 0.5) (P < 10⁻⁶). In the operated group, the medi-AL US meniscal injuries were slightly lower (1.4 ± 0.6 out of 2) than lateral US meniscal injuries (1.5 ± 0.5 out of 2). In the adolescent operated group, medial US meniscal injuries were slightly lower than the lateral US meniscal injuries, respectively, 1.2 ± 0.6 and 1.6 ± 0.5 out of 2. In the adult operated group, medial US meniscal injuries were slightly higher than lateral injuries, respectively, 1.8 ± 0.4 and 1.4 ± 0.5 out of 2. None of these differences were statistically significant (P > 0.05).

MACROSCOPIC FINDINGS AT 5 MONTHS AFTER ACLT

Data are detailed in Table IV and illustrated in Fig. 4. Macroscopic data of one out of the 14 operated rabbits are missing (menisci damaged during the dissection of the knee joint) thus a total of 26 menisci were graded macroscopically.

Experimental OA changes

The ACL was intact in all of the control rabbit knees and contralateral knees of the operated rabbits. The ACLT was completely ruptured in all of the operated rabbits. The operated knees exhibited severe changes consistent with the development of chronic OA. Evidence of severe degradation of the menisci and of the femoral and tibial cartilage surfaces were present in 100% of the operated knees. The medial tibial plateau underwent extensive remodelling, with prominent osteophytes, at the posterior lip of the medial tibial condyle.

Macroscopic semi-quantitative evaluation of the menisci

Macroscopy of the menisci of the control knees. A total of 16 menisci were evaluated. Menisci of the control rabbits were intact in all except in one adult rabbit. The MM of this adult rabbit presented a bucket handle tear (grade 2 out of 3).

Macroscopy of the menisci of the ACLT knees. Menisci of the ACLT rabbits were all macroscopically severely injured except in one adolescent rabbit presenting an intact LM. The lesions ranged from complex longitudinal to bucket handle tears, extrusion to complete destruction of the meniscus. In the operated knees, MMs and LMs (respectively, 2.8 ± 0.4 and 2.4 ± 1 out of 3) were significantly higher than meniscal injuries in the control knees (respectively, 0.25 ± 0.7 and 0.0 ± 0.0 out of 3) (P < 0.0001).
US AND MACROSCOPIC MENISCAL INJURY SCORES COMPARISON

Data are presented in Tables IV and V. The US and macroscopic scores were available from the comparison of 42 menisci (21 lateral and 21 medial menisci).

The total US meniscal score was significantly positively correlated with the total macroscopic meniscal score ($P < 0.001, r = 0.70$). False negatives were observed in two medial menisci macroscopically prolapsed. False positives were observed in two lateral menisci. These menisci were not visualised with US but were macroscopically intact. US detection of meniscal injuries was 92% sensitive and 87.5% specific compared to macroscopy. US meniscal injuries positive predictive value (PPV) was 92% and the negative predictive value (NPV) was 87.5%.

Table IV

<table>
<thead>
<tr>
<th>US grading (N=22 knees)</th>
<th>US MMI (three-grade scale)</th>
<th>US LMI (three-grade scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Operated knee</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult (N=5)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Adolescent (N=9)</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Control knee</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult (N=4)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Adolescent (N=4)</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Macroscopic grading (N=21)

<table>
<thead>
<tr>
<th>Macroscopic MMI (four-grade scale)</th>
<th>Macroscopic LMI (four-grade scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Operated knee</td>
<td></td>
</tr>
<tr>
<td>Adult (N=5)</td>
<td>0</td>
</tr>
<tr>
<td>Adolescent (N=8)</td>
<td>0</td>
</tr>
<tr>
<td>Control knee</td>
<td></td>
</tr>
<tr>
<td>Adult (N=4)</td>
<td>3</td>
</tr>
<tr>
<td>Adolescent (N=4)</td>
<td>4</td>
</tr>
</tbody>
</table>

NB: Macroscopic meniscal injuries are missing for one adolescent operated rabbit (menisci injured during the dissection).

MMI: medial meniscal injury; LMI: lateral meniscal injury.

US and macroscopic LMI and MMI are in bold.

Fig. 4. Macroscopic aspect of operated rabbit left knees at 5 months. (A) Multiple longitudinal tears of the LM. (B) LM with multiple longitudinal tears. (C) Protrusion and fibrillation of the entire LM.
Discussion

Menisci are intra-articular fibrocartilaginous structure with essential roles in normal function of the knee joint. They distribute loads within the joint which decrease the stresses on the tibia which is regarded as essential for cartilage protection. The added effects from ACLT and meniscal injuries in knee kinematics lead to more severe and rapid OA changes. Concomitance of meniscal and articular cartilage lesions in the femoro-tibial joint is well known in preclinical and clinical OA studies. In vivo animal experiments require maximum animal comfort with a minimum protocol set up time to limit or avoid anesthesia which are conditions US can satisfy. In 1997, Kramer et al. stated that direct US examination of ligaments in small animals was not possible. However, recent technical improvements allow for high axial and lateral resolutions of the image even in the field close to the probe. The results of this study demonstrate the value of ultrasound in the evaluation of meniscal injuries in the rabbit experimental model of OA. A reproducible US protocol for the ACLT rabbit knee joint was standardised. US images of the extra- and intra-structures corroborated anatomic situation. Normal menisci were echogenic with a moderately fine echotexture only if the ultrasound beam was placed perpendicular to their abaxial surface. In control rabbits, the cartilage appeared as a smooth hypoechoic band between two hyperechoic lines corresponding to the interfaces between soft tissue and cartilage and between cartilage and subchondral bone. In operated rabbits, the delineation of the cartilage layer was blurred and the sharpness of its margins was lost. However, it was not possible to precisely identify and quantify fibro-femoral cartilage damages. Assessment of the effectiveness of the ACLT was 78.5% sensitive and 100% specific and was based on the direct visualisation of the hyperechogenic distal portion of the transected ACL. A small hyperechoic irregular area close to the antero-proximal aspect of the tibial plateau was present in 100% of the operated knees. Macroscopy revealed that this could well be US evidence of fibrotic tissue around the distal portion of the transected ACL. In the dog, Grunli and Berton found that this was a significant US sign of joint instability following clinical ACL rupture. This tissue might be the result of chronic and hyperplastic synovitis. In the ACLT rabbit model, this could also be considered as an indirect indicator of the chronicity and/or of the intensity of the OA inflammatory process. Other US evidence of severe OA were present including synovial capsule thickening, synovial effusion (Fig. 3) and bone erosion. Five months after ACLT surgery in the rabbit experimental model of OA, the sensitivity and specificity for ultrasonographic diagnosis of meniscal injuries were 92% and 87.5%, respectively, compared to macroscopy. PPV and NPV were 92% and 87.5%, respectively. In humans, Najafi et al. found that detection of meniscal tear detection, compared to arthroscopy, for the MM, their PPV was 95%, and NPV was 100% and for the LM 93% and 100%, respectively. In the dog, Mahn et al. found that the detection of meniscal damage of both menisci with US had 90.0% sensitivity, 92.9% specificity, 90.0% PPV and 92.9% NPV compared to arthroscopy. They found that MM was easier to evaluate than the lateral. However, in the rabbit knee joint, we found technically easier to locate the LM than the medial and for both medial and lateral meniscus, the anterior horn was easier to locate than the posterior horn (BC, personal observation).

The use of US does have some limitations in the evaluation of the rabbit knee joint particularly when severe OA lesions are present added to the inherent small size of the rabbit knee joint. In the operated knees, US was sometimes difficult due to the significantly reduced range of motion and to the joint space narrowing induced by the OA changes (Fig. 1). In addition, soft tissue fibrosis might alter the echogenicity of intra-articular structures which might have impaired the full evaluation of the meniscus and modified its echogenicity. US examinations were performed at the end of the study to evaluate the feasibility and usefulness of our US protocol and validate it. All operated rabbits exhibited severe US and macroscopic MMIs and LMIs, and concomitant macroscopic medial and lateral tibial condyle cartilage lesions. Whether meniscal structural damage or cartilage degradation appears first remains debatable both in human and animal models. In humans, meniscal injuries are strongly associated with cartilage defects. In the dog OA model 2 years after ACLT, Anderst et al. stated that MM damages were correlated to medial articular cartilage damage, while Smith et al. did not find any correlation between the severity of the articular cartilage damages and the meniscal injuries 12, 24 or 32 weeks after ACLT. Smith et al. observed that MM damages did not precede gross articular cartilage. In the rabbit OA model, 3 weeks after ACLT, Helio LeGraverand et al. observed bucket handle or longitudinal tears of the MM in eight out of 12 ACLT knees. By 8 weeks after ACLT, all operated rabbit knees had transverse or bucket handle tear of the MM while evident fibrillation of the articular cartilage was still not observed. None of the lateral menisci presented severe lesion. They deduced structural and biochemical OA changes of the menisci occurred prior to cartilage damages and that the MM was one of the first tissues involved in the development of OA. In the ACLT models of experimental OA in rabbits in contrast to dogs, structural meniscal damage might develop more rapidly than structural articular cartilage damage after ACLT. Smith et al. stated that Helio LeGraverand et al. assessed very early changes in the meniscus as the used expression of message for specific molecules. Our study confirmed Helio LeGraverand findings at a later stage of OA and for structural changes. However, we cannot conclude definitely on the temporal sequence of the OA changes. We do not know whether US could identify less severe types of meniscal lesions and detect meniscal structural changes before macroscopy. Comparison of US with macroscopic and/or histological sections of the meniscus and cartilage might also be useful in the evaluation of the OA changes in the ACLT rabbit experimental OA model. We believe that earlier and

Table V

Comparison US/macroscopy (N = 42 menisci)

<table>
<thead>
<tr>
<th>Menisci (N = 42)</th>
<th>Macroscopic meniscal injury (N = 26)</th>
<th>Normal macroscopy (N = 16)</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>US detected meniscal injury (N = 26)</td>
<td>24</td>
<td>2</td>
<td>PPV 92%</td>
</tr>
<tr>
<td>Normal US (N = 16)</td>
<td>2</td>
<td>14</td>
<td>NPV 87.5%</td>
</tr>
</tbody>
</table>

Sensitivity 92% Specificity 87.5%
longitudinal US examination of the rabbit ACLT model would add to our knowledge on the OA pathogenesis additionally to more classical evaluation procedures.

Conclusion
In the present study, we have shown that US examination of the menisci was possible in the ACLT rabbit experimental OA model. We demonstrated that the value of ultrasound in the evaluation of meniscal injuries in the rabbit experimental model of OA and in the assessment of the ACLT effectiveness 5 months after surgery. A standardised protocol and a chart for the rabbit knee joint examination were created. A US semi-quantitative grading scale of meniscal injuries was developed and proved as being both specific and sensitive compared to gross macroscopy. US were performed on sedated rabbits, but based on our clinical experience we believe that this should even be possible without sedation with appropriate animal handling.

Further studies are now required to qualify and locate precisely US meniscal injuries and to create a semi-quantitative grading scale similar to arthroscopic and/or macroscopic scales. Earlier US examination of the rabbit experimental model would enhance our knowledge of the temporal sequence of the OA pathogenesis.

We propose US as a non-invasive, non-expensive, in vivo imaging technique for preclinical studies in the ACLT rabbit OA model.

Acknowledgements
The authors wish to thank the Institut Claude-Bourgelat (ICI-B), Lyon, France, for the animal care. We are grateful to Tobias Schwarz, University of Wisconsin and T. Avison (ENVL) for linguistic help.

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