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Qualitative macroscopic and microscopic evaluation of the knee joint in a dog osteoarthritis experimental model 90 days after cranial cruciate ligament transection

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SUMMARY

Cranial cruciate ligament transection (CCLT) is a well accepted experimental method used for initiating osteoarthritis (OA) in the canine femoro-tibial joint. The aim of the present study was to evaluate qualitatively gross and microscopic OA lesions 90 days after experimental CCLT in twenty-one young mature female beagle dogs. The femoral trochlear ridges showed the highest osteophyte score while the patella had the smallest. The medial femoral condyle exhibited higher osteophyte score than the lateral femoral condyle. Meniscal damages were observed only on the medial meniscus of five operated knees. Gross cartilage lesions (fibrillation) were seen in decreasing order of frequency on medial and lateral tibial condyles, lateral femoral condyle, femoral trochlea, medial femoral condyle and finally patella. Histologically, the superficial layer of the cartilage was fibrillated and discontinuous. Most of the cells were rounded and organised tangentially to the surface. In the transitional and deep layers, some chondrocytes were moderately hypertrophic and some clusters of chondrocytes were observed in the deep layer only. Concerning the synovial membrane, thickening of the mesothelium and a high density of collagen were observed and the ratio of thickness "mesothelium/ fibers" was between 1/0.15 and 1/0.10 in operated knees and 1/0.05 in control knees. The images provided here might be used as references for further work on disease modifying OA therapies.

Keywords: dog, osteoarthritis, experimental model, knee joint, cartilage erosion, osteophyte, collagen

RESUME

Evaluation qualitative macroscopique et microscopique du grasset chez un modèle expérimental d'arthrose canine 90 jours après section du ligament croisé crânial

La transection du ligament croisé crânial (CCLT) est une méthode couramment admise d'induction expérimentale d'arthrose (OA) au niveau du grasset chez le chien. Le but principal de cette étude était d'évaluer qualitativement les lésions d'arthrose induites par la CCLT par macroscopie et histologie 90 jours après chez 21 jeunes femelles de race beagle. Les lèvres de la trochlée fémorale présentaient le plus haut score ostéophytique tandis que la patelle présentait le plus bas et celui du condyle fémoral médial était supérieur à celui du condyle latéral. Les lésions méniscales ont été observées uniquement sur le ménisque médial de 5 genoux opérés. Les lésions macroscopiques du cartilage (stade de fibrillation) ont été notées dans un ordre de fréquence décroissant sur les condyles tibiaux médial et latéral, le condyle fémoral latéral, la trochlée fémorale, le condyle fémoral médial et enfin la patelle. L'examen histologique a révélé que la couche superficielle du cartilage était fibrillée et discontinue. La plupart des cellules étaient rondes et disposées tangentiellement à la surface. Dans les zones transitionnelle et profonde, quelques chondrocytes étaient modérément hypertrophiques et des amas de chondrocytes ont été uniquement observés dans la couche profonde. Concernant la membrane synoviale, un épaississement du mésothélium et une importante densité de collagène ont été notés et le rapport des épaisseurs "mésothélium/fibres" était entre 1/0.15 et 1/0.10 sur les genoux opérés et 1/0.05 sur les genoux témoins. Les images fournies ici pourront servir de références pour des travaux ultérieurs portant notamment sur les thérapies contre l'arthrose.

Mots-clés : chien, arthrose, modèle expérimental, grasset, érosion du cartilage, ostéophyte, collagène.

Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by progressive fibrillation and erosion of the cartilage tissue, sclerosis of the subchondral bone, osteophyte production at the joint margins, moderate synovitis and fibrosis of the joint capsule [15, 18, 31]. OA affects more than 20% of dogs older than one year and a post-mortem study on 2000 dogs estimated cartilage damage was present in 78% of the animals [15, 24].

Meniscal lesions are frequently associated with OA of the knee joint. In man, 50% of those with a diagnosed anterior

cruciate ligament lesion or meniscus tear have osteoarthritis with pain and functional impairment 10 to 20 years after the diagnostic [17].

In dogs, the prevalence of spontaneous rupture of cranial (anterior) cruciate ligament (CCL) is estimated at 1.8% [33]. This is the first cause of hindlimb lameness and the most frequent joint disease in the canine population [11]. Joint laxity following surgical section or spontaneous rupture of the CCL causes OA lesions [32]. Since 1973, transection of the CCL is a well-accepted experimental method used for initiating osteoarthritis (OA) in the canine femoro-tibial joint [2, 5, 7, 15, 20, 25]. The clinical, radiographical,

morphological and biochemical resulting changes closely mimic the naturally occurring OA in dogs and in humans [5, 7, 20].

Assessment of the OA joint lesions is crucial for monitoring OA progression [16]. Reference images would help the veterinary surgeon to diagnose and evaluate knee OA lesions during arthroscopy and/or arthrotomy [26]. The aim of the present study was thus to evaluate qualitatively gross and microscopic OA lesions 90 days after experimental CCL transection and to compare them to published data to enhance knowledge on OA pathogenesis process in the canine CCL transection experimental model of OA.

Materials and methods

All work was conducted in full compliance with the veterinary campus of VetAgro Sup (Lyon, France) ethical committee guidelines for animal protection in accordance with the European and French legislations. This study was part of a larger investigation on OA.

ANIMALS AND SURGICAL PROCEDURE

Twenty-one young female beagle dogs, around one year old, were used for the study. Each animal was identified with a number and pictures. They were skeletally mature with a mean body weight of 10.5 ± 1.2 kg at the beginning of the study and 11.8 ± 1.5 kg ninety days later. The dogs were housed by group of seven and were examined clinically once or twice daily. They were taken out twice daily. They were considered healthy based on both clinical and orthopaedic examinations. Follow up observations (orthopaedic exam and gait analysis) and treatments were reported individually and daily in a study book.

Analgesia was provided by intramuscular morphine injections (0.1 mg/kg) before, immediately after surgery and 4 hours later. A fentanyl patch was placed on the neck area for 2 days. Acepromazine (0.05 mg/kg) and glycopyrrolate (0.01 mg/kg) were administered by intramuscular injection. Anaesthesia was induced using intravenous injection of ketamine (10 mg/kg) and diazepam (0.5 mg/kg) and maintained with isoflurane (2%) and oxygen after intratracheal intubation. The right knee was aseptically prepared for surgery in a routine way. CCL of the right knee joint was transected using a medial arthrotomy approach based on SCHWARTZ and PALMOSKI methods [23, 30]. A particular care was taken to not traumatize the surrounding structures of the knee joint. Direct and indirect anterior drawer motion tests confirmed complete CCL transection. Joint capsule, subcutaneous tissue and skin were closed layer by layer with a strict attention to haemostasis. Antibiotic prophylaxis was made with amoxicillin (12.5 mg/kg IM) just after surgery. A sterile bandage was applied over the wound and a soft-padded bandage was placed on the right knee joint for 3 days. Skin sutures were removed at 12 days. Left knee joints were not operated and used as controls.

GROSS EXAMINATION

Three months after surgery the dogs were anaesthetized with the same protocol as for surgery and then euthanized by intravenous injection of 3 mL of pentobarbital sodium (Dolethal, Vetoquinol). Operated (right) and control (left) knee joints were resected with a saw from the top of the femoral trochlea to the proximal tibia (between tibial plateau and tibial crest). Knee joints were opened and carefully dissected. Medial and lateral menisci were carefully removed from knee joints. Tibial and femoral condyles, femoral trochlea, patella, menisci and synovial membranes were displayed in rows for immediate grading and were pictured methodically. Three experienced observers examined the specimens immediately; the scores resulted from their consensual agreement.

Osteophytes were classified as absent (grade 0); minimal or small (debatable) (grade 1); evident or moderate (grade 2); and large (grade 3). Cartilage evaluation was based on the BEGUIN and LOCKER classification and lesions were recorded according to their location, depth and extent [3]. Lesion depth was classified as follow: intact cartilage surface, discoloration or swelling (chondromalacia), fibrillation, fissuration and full thickness erosion (ulceration / denudation). The global score resulted from the product of the percentage of lesion area and a factor based on the grade of the lesion depth. Scores ranged from 0 indicating that the cartilage is intact to 100 meaning full-thickness cartilage erosion over the whole condyle. Synovial membrane thickness was measured with a cutimeter and its macroscopic aspect was described (presence or absence of synovial pannus, blood vessels diameter).

HISTOLOGY AND HISTOMORPHOMETRY

Specimens were fixed in a buffered formalin solution (alcohol, formalin and acetic acid) immediately after gross examination. They were decalcified in a media for rapid bone decalcification (RDO, Eurobio) for 21 to 28 days depending on thickness and density of the specimen. After decalcification, 3 to 4 sections of 5 mm were done perpendicularly to articular surfaces: coronal sections of tibial plateaus and coronal and transverse sections of femoral condyles and trochlea (perpendicular to the widest osteophyte). Samples were embedded in paraffin and cut with a microtome in slices of 5 μ m. Deparaffinized slices were stained with haematoxylin, eosin and safranin-O (HES). The synovial membrane samples were simply fixed in formalin without decalcification before sections were obtained. Slices coloured with HES were dehydrated and fixed with synthetic resins combined in a particularly rapid embedding agent (Entellan, Merck).

Regions of interest from cartilage and synovial membrane were pictured with a camera mounted on a microscope. A modified HHGS (Histological Histochemical Grading

System) score (Colombo score) was used to grade the histological samples (Table I). Colombo score is similar to HHGS and was modified by HOTTA *et al.* [29]. Synovial membranes were assessed on HES slices when just one mesothelial cells layer was present. Density and organisation of chondrocytes, synoviocytes and collagen fibers were noted. Histomorphometry of the medial tibial cartilage only was performed on the medial condyle edge and on the intercondylar area. Histomorphometry of the synovium assessed the thickness of the mesothelial layer in relation to the thickness of collagen fibers.

Results

All the dogs recovered uneventfully from the surgery and completed the study. Many were very active.

GROSS EVALUATION 90 DAYS FOLLOWING CCL TRANSECTION

None of the control knees presented osteophytes (figure 1A). Osteophytes were present in all operated knees: evident or large on the femoral trochlear ridges (figure 1B), small on medial femoral and medial tibial condyles (figure 1C), debatable or absent on lateral femoral and lateral tibial condyles and patella (figure 1D) (Table II).

In control knees, menisci were all intact (figure 2A). Meniscal damage was observed on the medial meniscus of 5 operated knees (23.8%) like “bucket-handle” tears in 4 cases (figure 2B) and like “parrot beak” in one case (figure 2C). No lateral menisci of operated knees were injured.

	Histological grading				
	0	1	2	3	4
Cartilage erosion					
Depth of the lateral border (maximal value, in µm)	> 750	700 - 750	650 - 700	600 - 650	< 600
Depth of the medial border (minimal value, in µm)	> 550	500 - 550	450 - 500	400 - 450	< 450
Total score	0	1 - 2	3 - 4	5 - 6	7 - 8
Superficial layer alteration	Tangential	Tangential	Rounded	NA	NA
Cell morphology	Continuous	Discontinuous	NA	NA	No cell
Layer continuity	0	1	2	3	4 - 6
Total score					
Chondrocyte hypertrophy	0	< 25	25 - 50	50 - 75	> 75
In the intermediate layer	0	< 25	25 - 50	50 - 75	> 75
Length (%)*					
Width (%)*	0	< 25	25 - 50	50 - 75	> 75
In the deep layer	0	< 25	25 - 50	50 - 75	> 75
Depth (%)*	0	1-4	5 - 8	9 - 12	13 - 16
Width (%)*					
Total score					

NA: Non applicable; *: a characteristic picture was chosen and observed X 100.

TABLE I: Methodology for histological grading of the osteoarthritis joint lesions experimentally induced by CCL (cranial cruciate ligament) transection in dogs.

Location	Osteophyte score	Cartilage lesion score
Femoral trochlear ridges	2.3 ± 0.6	5.5 ± 3.8
Medial femoral condyle	1.4 ± 0.5	4.7 ± 3.1
Medial tibial condyle	1.2 ± 0.6	15.0 ± 6.6
Lateral femoral condyle	0.9 ± 0.6	8.0 ± 5.0
Lateral tibial condyle	0.6 ± 0.5	9.2 ± 6.0
Patella	0.4 ± 0.5	1.6 ± 2.1
Total	6.9 ± 1.9	44.0 ± 12.8

TABLE II: Quantitative evaluation of the macroscopic lesions observed on operated knees, 90 days following CCL (cranial cruciate ligament) transection in dogs. Results are expressed as means ± standard deviations.

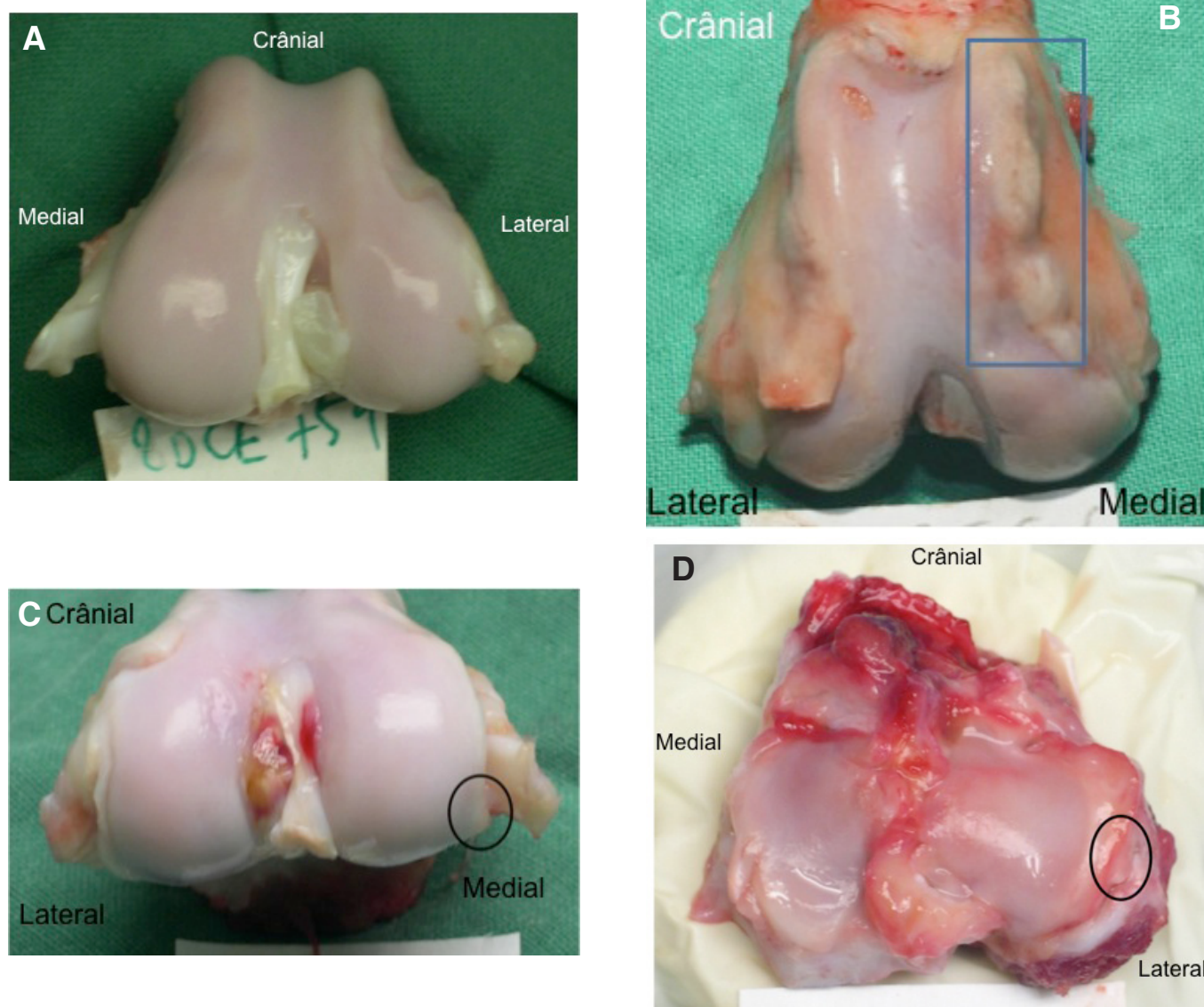


FIGURE 1: Gross observations of control and operated knees 90 days following CCL (cranial cruciate ligament) transection in dogs; Note the absence of osteophyte and of cartilage macroscopic lesion on the femoral trochlea and condyles in control (not operated) knee (1A) whereas it was observed for operated knees large osteophyte on the trochlear ridge (1B), small osteophyte on the medial femoral condyle (1C) and debatable osteophyte on the lateral tibial condyle (1D).

Histological lesions	Quantitative score
<i>Femoral condyle cartilage</i>	
Alteration of the superficial layer	2.1 ± 1.3
Osseous growth within the cartilage	0.8 ± 0.4
<i>Medial tibial condyle</i>	
Alteration of the superficial layer	1.2 ± 1.1
Cartilage erosion	3.2 ± 1.3
Chondrocyte hypertrophy	1.6 ± 1.0

TABLE III: Quantitative evaluation of the main histological lesions observed on operated knees, 90 days following CCL (cranial cruciate ligament) transection in dogs. Results are expressed as means ± standard deviations.

Fibrillation of the cartilage surface was observed in all operated knees but neither cartilage oedema (chondromalacia) nor more severe lesions were observed. Cartilage lesions were reported in decreasing order of frequency on the tibial condyles (medial > lateral) (figure 3A), then on femoral condyles (lateral > medial) (figure 3B) and trochlear ridge (figure 3C), and finally on patella (Table II) while the cartilage surface was intact in all control knees.

HISTOLOGY 90 DAYS FOLLOWING CCL TRANSECTION

In all operated knees, the superficial layer of the cartilage was fibrillated and discontinuous. This change did not extent into the transitional layer or deeper (figure 4A). Most of the cells were rounded and organized tangentially to the surface. In the transitional and deep layers, some chondrocytes

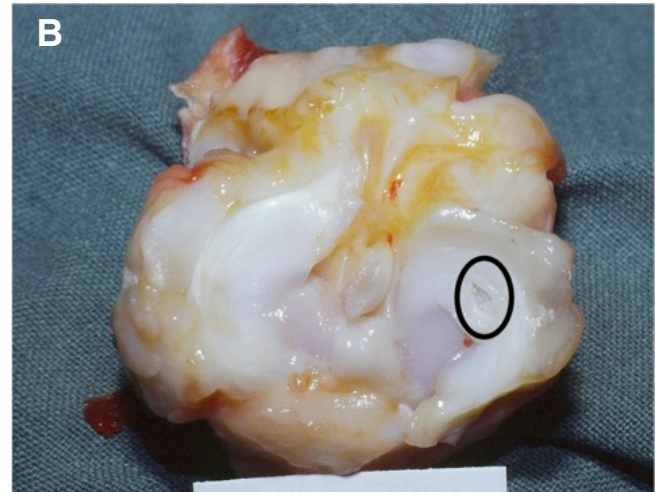


FIGURE 2: Gross observations of control and operated knees 90 days following CCL (cranial cruciate ligament) transection in dogs; Note the intact meniscus in control (not operated) knee (2A) whereas medial meniscus has appeared as a bucket-handle tear in 4 operated knees (2B), or as a "parrot beak" tear in one case (2C).

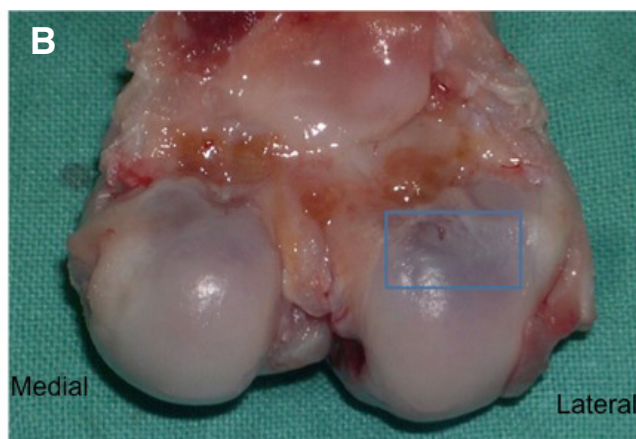
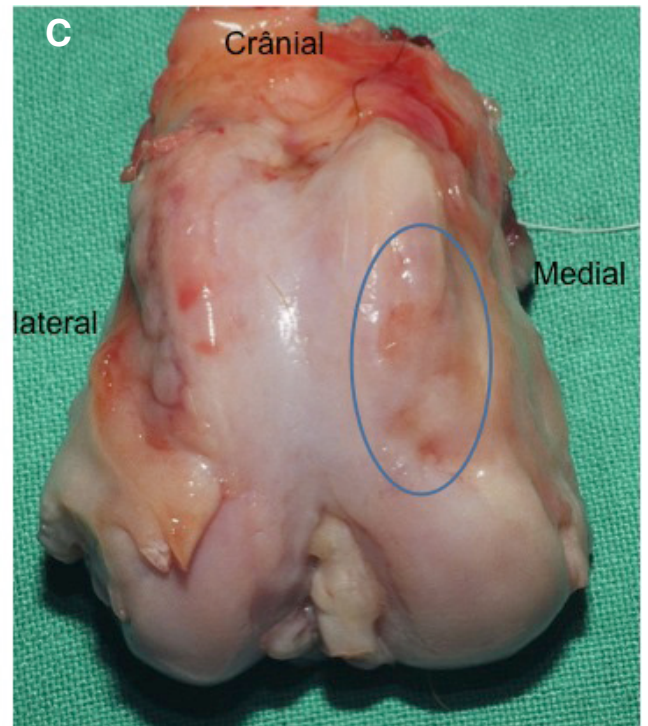
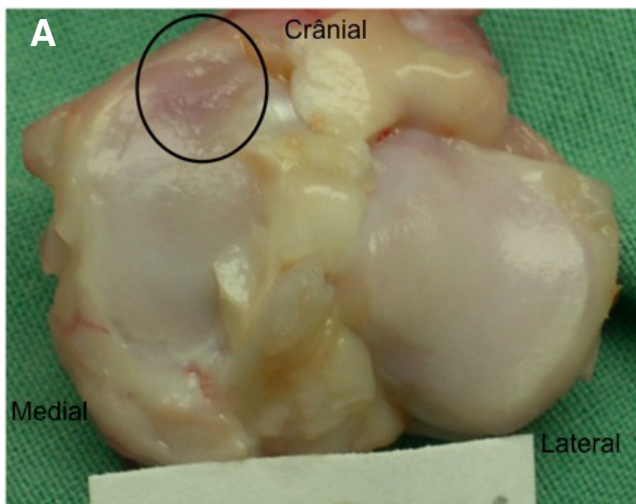


FIGURE 3: Gross observations of cartilage lesions on the medial tibial condyle (3A), the lateral femoral condyle (3B) and on the femoral trochlear ridge (3C) in operated knees 90 days following CCL (cranial cruciate ligament) transection in dogs.

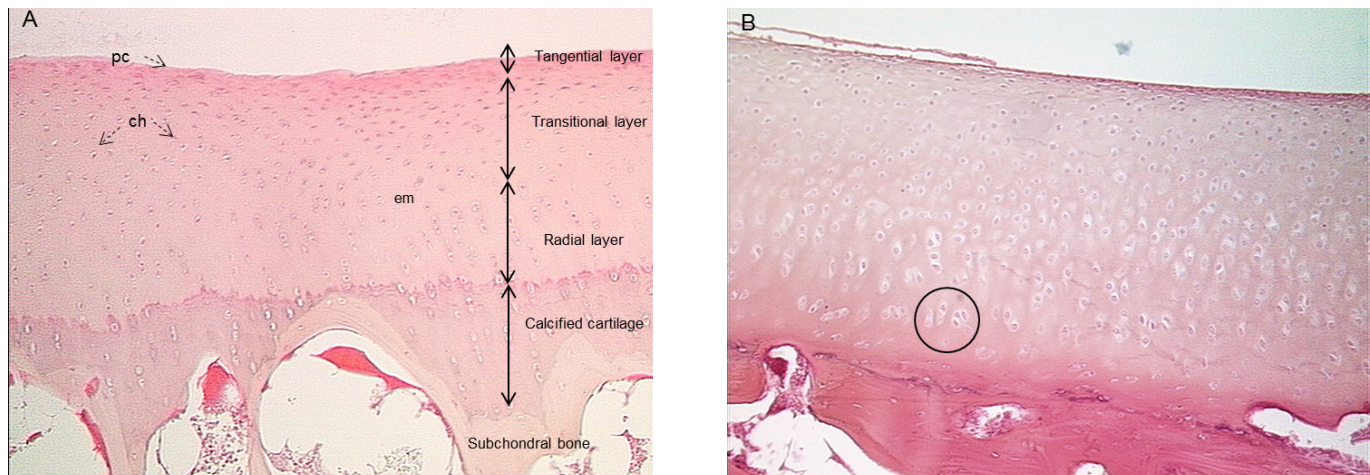


FIGURE 4: Histology of the cartilage (X 100) of the medial tibial condyle in operated knee 90 days following CCL (cranial cruciate ligament) transection in dogs. The fibres are not visible in the slide. Note the fibrillated superficial layer, with tangential and rounded cells and loss of superficial chondrocytes (4A) as well as the presence of a chondrocyte cluster (circle) in the deep layer (4B) in a medial tibial condyle. em: extracellular matrix, ch: chondrocyte, pc: perichondrium.

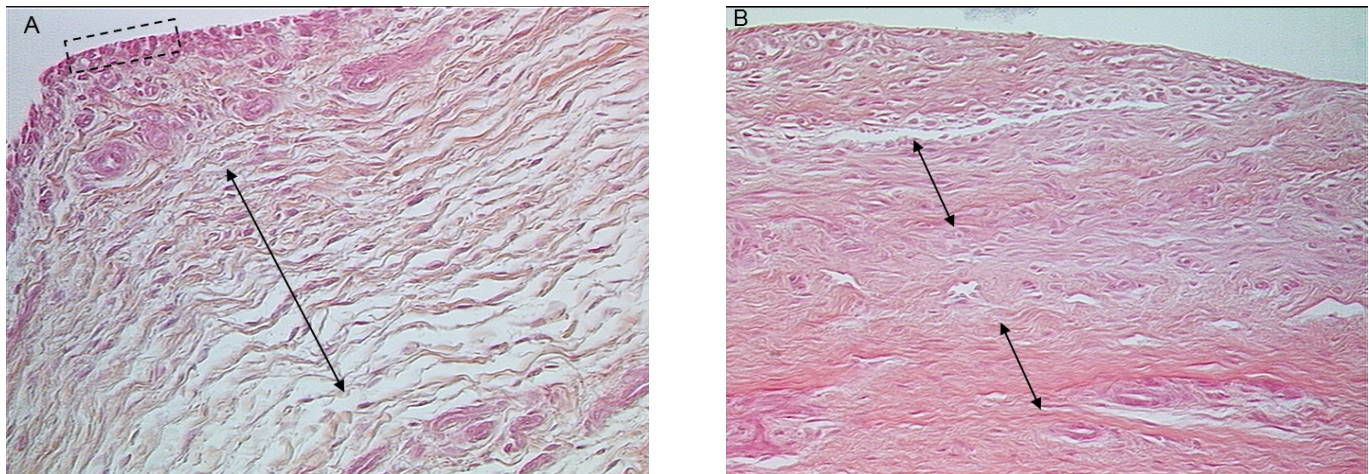


FIGURE 5: Histology of the synovium (X 100) in control and operated knees 90 days following CCL (cranial cruciate ligament) transection in dogs; Note the thin mesothelium (dashed rectangle) and the low density of highly undulated collagen fibers (double head arrow) in a control (not operated) knee (5A) whereas slightly wavy collagen fibers (double head arrow), localized deeply were evidenced in synovium from operated knee (5B).

were moderately hypertrophic. Clusters of chondrocytes were observed in the deep layer only (figure 4B). Cartilage histological lesions were essentially evidenced in the medial tibial condyles (Table III) whereas they were remained weak in the lateral condyles (data not shown). Concerning the articular cartilage of the femoral trochlea, at the level of the osteophytes, the osseous growth within cartilage was minimal, just slightly superior to the control knees (Table III).

In the control knees, mesothelium of the synovial membrane was thin and the density of the collagen fibers was lower, with fibers highly undulated (figure 5A). In operated knees, histology revealed a thickening of the mesothelium and a high density of collagen in the synovial membrane. These collagen fibers were slightly wavy and localized deeply in the synovial membrane (figure 5B). Histomorphometry showed the ratio of thickness "mesothelium/ fibers" was between 1/0.15 and 1/0.10 in operated knees and 1/0.05 in control knees. Inflammatory cells were observed neither in operated nor in control knees.

Discussion

In human patients, late-stage OA is characterized by joint space narrowing, full-thickness cartilage erosion, development of osteophytes, and increased subchondral sclerosis [24]. Early changes after joint trauma are less well known due to practical limitations because the time of onset is not usually known [22, 28]. There are similarities between the progression of post-traumatic OA in humans and in the canine experimental model [6, 9], but there are also discrepancies that have to be pointed out. This study provides an atlas of macroscopic and microscopic lesions of the knee joint in small dogs' models of OA, 90 days after cranial (anterior) cruciate ligament transection (CCLT). As observed by others, CCLT resulted mainly in osteophytes formation on the femoral trochlear ridges and histological changes of the tibial cartilage characteristic of early OA [4-8].

Gross examination showed obvious (large) osteophytes on the femoral trochlear ridges, debatable (small) on the medial femoral condyle and absent or small in the other sites. This is consistent with Mc DEVIT *et al.* [19] study in which osteophyte formation was noted at the proximal limit of the femoral trochlear ridges as early as two weeks after cruciate section.

Cartilage oedema (chondromalacia) and cartilage erosion were never observed which is consistent with other studies [1, 4]. Cartilage gross lesions were limited to superficial erosion of a greater or a lesser extension from the medial tibial condyle, lateral tibial condyle, lateral femoral condyle, femoral trochlear ridge, medial femoral condyle to the patella. After 12 weeks, BOYD *et al.* [4] found that the cartilage surface erosion was not yet at full thickness. Full erosion of the cartilage might take up to 4 to 5 years to appear after CCL injury at which the other symptoms of the developed disease are obvious in dogs [6, 8]. This might result from an active synthetic response by the chondrocytes resulting in hypertrophic cartilage repair for up to 64 weeks as suggested by ADAMS and BRANDT [1]. Gross cartilage lesions were predominantly on the medial femoral condyles in the study at 12 weeks as described by BOYD *et al.* [4]. Interestingly, in our study, gross cartilage lesions were higher in the lateral than medial femoral condyles.

In our study, five out of the 21 operated knees presented meniscal damage (23.8%), all lesions affected medial menisci, which is similar to reported cases [12, 21]. In the literature, 10 to 77% in dogs with CCL injuries present meniscal lesion [12, 13, 27]. The difference might be due to the young age and small weight of the dogs used in the present study thus avoiding degenerative lesions. The difference of activity of each dog might also have impacted the results.

Histology of the tibial condyles demonstrated intermediate modifications of the superficial layer of cartilage. Erosion of cartilage of tibial condyles had superior score to the mean of the evaluation scale. On the opposite, the score of the intermediate and deep layers chondrocyte hypertrophy of the tibial condyles was inferior (< 25%) to the mean of the interval between control (no hypertrophy) and terminal grade (> 75%). This phenomenon was therefore not much advanced.

Histology of the superficial layer of the femoral trochlear cartilage with osteophytes revealed a fibrous tissue of slightly below average cellularity thus between control and final stage lesions. The osseous growth within the articular cartilage did not cover the whole osteophyte basis revealing an early stage of sclerosis of the subchondral bone. DEDRICK *et al.* [10] studied bone changes at 3, 18, and 54 after CCL transection and found subchondral plate thickness increased only at 54 months, which correspond to increased subchondral sclerosis in human patients with late-stage OA [10]. The study by BOYD *et al.* [4] corroborated this finding and in addition, they observed periarticular bone adaptation as early as 3

weeks post-surgery and adaptation was more pronounced in the femur than in the tibia. The choice of the site of section (level with the biggest osteophytes for the femur) might have influenced the results but a reliable anatomical reference could not be found such as for the section of the tibial cartilage (level with the intercondylar eminences).

It is worth noting that on the opposite of the gross examination score, histology of medial femoral condyles showed more advanced superficial erosion than their lateral counterpart. The reason of this difference remains unclear. It is possible that the gross examination was less objective than the histology. This could explain the large standard deviation we observed between results of the gross examination. In addition, some dogs were much more active than others, this might account also for the large standard deviation.

The absence of histology and biochemistry of menisci is one of the limitations of the study as pathological changes can occur in grossly normal menisci [14]. One other limitation is the insufficiency of preparation of the synovial membranes before their fixation. This sometimes lead to a folding of the membrane, thus impairing its analysis.

As a conclusion, a large quite standard deviation in the results was observed which might be due to the difference of activity of each dog that could not be controlled. However, in this small dog model of induced OA, 90 days after the transection of the CCL, the femoral trochlear ridges showed the highest osteophyte score while the patella had the smallest. The medial tibial condyle had the highest macroscopic cartilage lesions. It is interesting to point out that the medial femoral condyle had higher osteophyte score than the lateral femoral condyle while it was the opposite concerning the cartilage gross lesion. In addition, histology of medial femoral condyles showed more advanced superficial erosion than their lateral counterpart.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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