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The natural initiation and progression of osteoarthritis in the anterior cruciate ligament deficient feline knee

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SUMMARY

Objective: The aim of this study was to document the natural history of development and long-term progression of osteoarthritis (OA) in the feline knee after minimally invasive anterior cruciate ligament (ACL) transection.
Design: ACL transections of the left knee joint of 14 skeletally mature cats were performed. Radiographic scores, tibiofemoral and patellofemoral joint space and anterior tibial translation were assessed before, immediately and every 3 months after ACL transection (longest follow-up: 93 months).
Results: After 26 months, all ACL transected knees had developed definite OA. The earliest changes were observed on the tibia plateau starting as early as 2 months after ACL transection, and at 12 months signs of OA were present in more than 80% of cats in the medial and in almost 80% of cats in the lateral compartment. In the first 24 months, medial tibiofemoral joint space decreased by 0.88 mm (95% confidence interval [−0.55;−1.21] mm) and lateral tibiofemoral joint space by 0.55 mm ([−0.26;−0.85] mm). In the same interval, the joint space in the patellofemoral joint increased by 0.98 mm ([0.59; 1.37] mm). Throughout the entire observation period, the anterior tibial translation was on average 5.3 mm greater than in the contralateral knee ([4.5; 6.0]mm).
Conclusions: Immediate changes in anterior tibial translation during an anterior drawer test clearly showed joint instability that persisted throughout the lifetime of the animals. Degenerative changes were observed on radiographs within 4 months of the injury only in the transected but not the contralateral limb suggesting the role of mechanical instability for the development and progression of knee OA.

Introduction

Although knee osteoarthritis (OA) affects more than 20% of the older human population[1], to date the pathomechanics underlying knee OA are still poorly understood. In humans, anterior cruciate ligament (ACL) rupture is one of the strongest risk factor for the development of premature degeneration of the articular cartilage at the knee termed posttraumatic OA[2]. Independent of the type of treatment (surgical or conservative), 40% of patients will develop tibiofemoral OA within the first 10 years after ACL rupture[3]. While ACL rupture leads to mechanical instability of the knee[4,5], other injury related factors such as the blunt impact of articulating surfaces, contusion and other associated injuries of the menisci and or collateral ligaments may initiate the detrimental cascade and influence to the prognosis of posttraumatic OA.[6]

To study the complex nature of this degenerative disease, different animal models have been proposed and implemented. In particular, ACL transected cat and dog models have been useful in studying the effects of pain associated with ACL deficiency on limb movement[7,8]. Especially cats showed a slow initiation of OA similar to that observed in humans[9]. Moreover, significant changes in joint

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kinetics and kinematics from the normal locomotion pattern have been observed as early as 1 week following ACL transection that recovered to near pre-surgical patterns within the first year after ACL transection in cat[8,10]. The advantage of these models is that joint instability is caused by isolated transection of the ACL while all other structures remain intact allowing to study the effect of instability without other injury on joint degeneration. However, while Suter *et al.*[8] reported radiographic changes within the first year after ACL transection, data on the natural progression of development of OA in the feline cat knee beyond the first year after ACL transection are lacking. The aim of this study was to document the natural history of development and long-term progression of OA in the feline knee after minimally invasive ACL transection. We tested the hypothesis that signs of OA develop in the ACL transected knee and not in the contralateral knee.

Methods

Fourteen skeletally mature, outbred female cats (nonpregnant, nonlactating; age at beginning of study, 1.7 ± 1.2 years, i.e., young adults; body mass, 4.8 ± 1.5 kg) were included in this study. Animal care was supervised by a veterinarian, and this study was approved by the Committee of Animal Ethics of the University of Calgary. Animals were housed in a 4 m by 10 m room allowing for unrestricted movement including on wooden-tree-like structures. Minimally invasive ACL transections of the left knee joint were performed after acquisition of the first measurement set as previously described[11,12]. Briefly, an anterolateral capsulotomy was performed with a scalpel between the tendon of the extensor digitorum longus and the patellar tendon directly exposing the ACL. The ACL was hooked with a rounded, blunt probe and transected with a curved scalpel. All ACL transections were confirmed by probing of the intercondylar notch and by a positive anterior drawer test, and if present any remnant fibers were cut. The skin was closed using single sutures. The intact right knee joint served as a contralateral control. Animals were sacrificed by over-dose of barbiturate at 2 years ($n = 4$), 4 years ($n = 2$), 5 years ($n = 4$), 6 years ($n = 2$) or 7 years ($n = 2$) post-ACL transection. The knees of the two oldest cats (sacrified after 7 years) were transected and documented. Specimens were treated with Indian ink staining for morphological analysis and with O/fast green staining for histological analysis.[13]

Radiographic assessments

Radiographs were taken in regular time intervals (every 3.2 months) to document the joint status, any joint degeneration and persistent ACL insufficiency for a maximum follow-up time of 93 months. At each assessment, four standardized radiographs were taken of each knee (ACL transected knee and contralateral knee): anterior-posterior view in extended position and lateral view at 110° knee flexion (180° being a fully extended knee joint) under axial load mimicking standing position; lateral view at 140° and 110° knee flexion under anterior drawer force of 30 N (Kobayashi *et al.*, 1993). The reader was blinded to the knee (ACL transected or contralateral) and the time after ACL transection.

Radiographic scores

From the radiographic images, scores were assessed by two readers (AL and MH) who were blinded to the other reader's score, the knee (ACL transected or contralateral) and the time after ACL transection. For all animals, both the affected and the contralateral knee were assessed. Degradation in the medial and lateral compartment of the tibiofemoral joint and for the patellofemoral joint was classified according to the recommendations of the Osteoarthritis Research Society International (OARSI)[14]. Joint space narrowing and osteophytes were graded on a scale from 0 to 3 (with 0 indicating no evidence of joint space narrowing or bony changes) using a radiographic atlas[15]. The Kellgren–Lawrence grade was determined based on radiographic data (0—no osteophytes; 1—possible osteophyte lipping; 2—definite osteophytes and possible joint space narrowing; 3—moderate multiple osteophytes and definite joint space narrowing, as well as some sclerosis and possible bone contour deformity; 4—large osteophytes, marked joint space narrowing, severe sclerosis, and definite bone contour deformity)[16]. Definite OA was defined as Kellgren–Lawrence grade 2. Additional parameters assessed were presence of cyst formation, sclerosis and loose bodies, joint space width in the medial and lateral tibiofemoral compartments (anterior-posterior view radiographs) and patellofemoral joint (lateral view radiograph)[14,15]. Joint space width was measured at the center of each compartment as the distance between the lines of the subchondral bone plate indicating the margin of cartilage to bone.

Anterior translation of the tibia relative to the femur under anterior drawer force of 30 N at 110 and 140° (Kobayashi *et al.*, 1993) was measured using a custom made device similar to a human knee ligament arthrometer (Fig. 1). Briefly, the femur was firmly strapped to femur and thigh blocks that were rigidly mounted onto a ½ inch clear acrylic plate. The tibial block had a strap that fixes the tibia to the block so that during force application via the sprocket/load cell the tibia/tibia block were translated forward with respect to the femur. The load applied was controlled by a read out from the load cell amplifier facilitating repeatable measurements. The anterior displacement was measured as the perpendicular distance between a line approximating the posterior border of the tibia, and a line parallel to the posterior border of the tibia through the estimated mid-point of the most posterior aspects of the femoral condyles.[8]

Statistical analysis

All statistical analyses were performed in IBM SPSS Statistics Version 21.0 (IBM Corporation, Amonk, NY). Between-tester reliability for OARSI and Kellgren–Lawrence scores were assessed

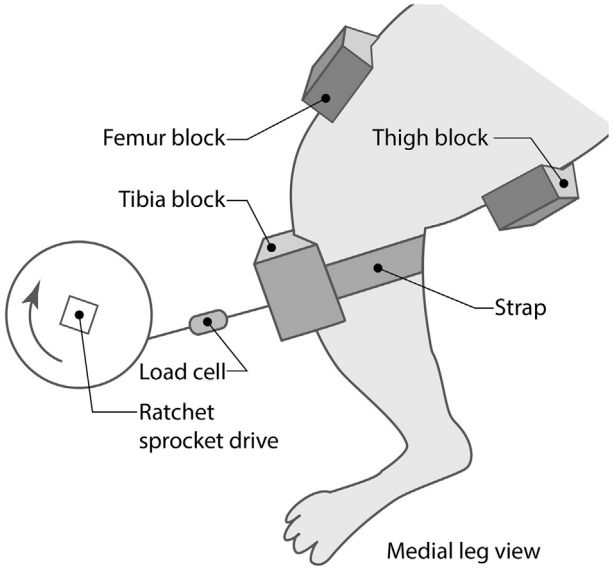

Fig. 1. Illustration of the experimental setup to measure anterior translation using radiographs and a loading frame at 110 and 140° knee flexion.

using intraclass correlation coefficients (ICCs). Kaplan–Meier analysis was performed to illustrate the onset of definite OA and signs of OA after ACL transection. Generalized Wilcoxon tests were performed to compare survival curves between sides, compartments, and between parameters describing signs of osteoarthritis, respectively. All parameters were tested for normal distribution and then compared between the affected and contralateral side using linear mixed models with animal ID, side and time as within animal effects. Paired Student's *t*-tests and 95% confidence interval (CI) of the side to side differences were used as posthoc test at all time points after ACL transection ($P < .01$).

Results

Radiographic scores were assessed with a good to excellent between-reader reliability. ICCs for OARSI scores were 0.914, 0.890 and 0.853 for the medial and lateral tibiofemoral compartments and patellofemoral joint, respectively. The ICC for Kellgren–Lawrence scores was 0.904.

Kaplan–Meier analysis illustrated that after 26 months, all ACL transected knees had developed definite OA (Fig. 2). None of the contralateral knees developed definite OA during the lifetime of the animals. The change in Kellgren–Lawrence scores over time differed significantly between the ACL transected and the contralateral side (Wilcoxon test: $Z = 4.554$, $P < 0.001$). Radiographs of one animal are shown in Fig. 3.

The earliest changes according to the OARSI were observed on the tibia plateau starting as early as 2 months after ACL transection, and at 12 months signs of OA were present in more than 80% of cats in the medial compartment and almost 80% of cats in the lateral compartment (Fig. 4). The development of OA presence in the tibia plateau, joints space narrowing and attrition differed significantly between the medial and lateral compartments (Wilcoxon test: $Z = 5.282$, $P < 0.001$; $Z = 5.282$, $P = 0.007$; and $Z = 4.105$, $P < 0.001$). The prevalence of joint space narrowing and attrition in the medial compartment were similar (Wilcoxon test: $Z = 1.500$, $P = 0.134$), and the prevalence of sclerosis and joint space narrowing in the lateral compartment were similar (Wilcoxon test: $Z = 1.040$, $P = 0.298$). In contrast to the tibiofemoral compartments, only nine animals showed any changes in the patellofemoral joint. Joint space narrowing, sclerosis and attrition in the patellofemoral joint were only present in two animals throughout their lifetime. Radiographic findings were confirmed with photographic and histologic evidence of severe joint destruction in the ACL transected but not in the contralateral knee (Figs. 5 and 6 show extracted examples).

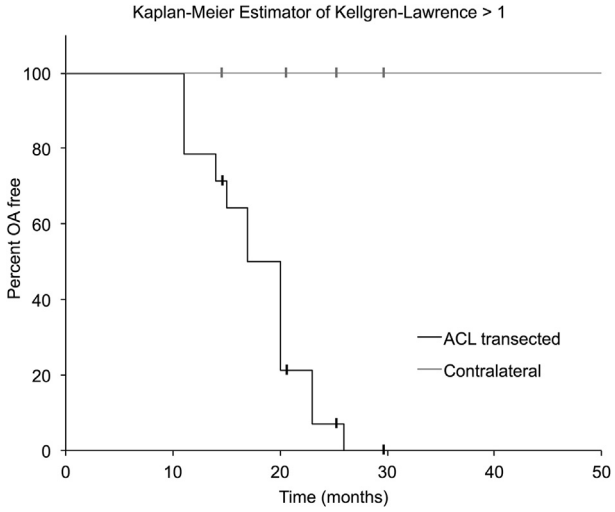

Fig. 2. Kaplan–Meier survival analysis of definite OA assessed as Kellgren–Lawrence grade >1. Kaplan–Meier curves for the ACL transected knees and the contralateral knees are shown. Curves are shown up to 50 months because by then all ACL transected knees had developed OA. During this period, four animals were right censored because they were sacrificed represented by small vertical tick-marks.

A significant side by time interaction in joint space width was observed for the medial ($P < 0.001$) and lateral ($P < 0.001$) tibiofemoral compartment and for the patellofemoral joint ($P = 0.001$). Joint space width decreased in the first 24 months after ACL transection in the medial and lateral tibiofemoral compartment of the ACL transected knee and remained constant thereafter (Fig. 7). In the first 24 months, medial tibiofemoral joint space decreased by 0.88 mm (95% CI [−0.55; −1.21] mm; $P < .001$) and lateral tibiofemoral joint space by 0.55 mm (95% CI [−0.26; −0.85] mm; $P < .001$). In the same interval, the joint space in the patellofemoral joint increased by 0.98 mm (95% CI [0.59; 1.37] mm; $P < .001$). In the contralateral knee, the joint space in medial tibiofemoral joint decreased by 0.27 mm (95% CI [−0.03; −0.50] mm; $P = .029$) and remained constant beyond 24 months after ACL transection. Joint space in the lateral tibiofemoral compartment and patellofemoral joint of the contralateral knee did not change after ACL transection.

Anterior tibial translation relative to the femur in the ACL transected knee during the anterior drawer test increased significantly after transection (Fig. 8). Throughout the entire observation period, anterior tibial translation was on average 5.3 mm and 6.0 mm greater than in the contralateral knee at 110° and 140° flexion (95% CI, [4.5; 6.0] mm and [5.1; 6.8] mm), respectively, reaching an average of 10 mm difference 5 years after ACL transection.

Discussion

The aim of this study was to document the natural history of development of OA in the feline knee after minimally invasive ACL transection. Our results clearly showed early osteoarthritic changes within a few months of the ACL transection. Greater anterior drawer was established with ACL transection and maintained throughout the lifetime of the animals. Joint space narrowing was observed in the tibiofemoral compartments of the knee, and an increase in joint space was observed in the patellofemoral joint. These results provide evidence to support the ACL transected cat as useful model for knee OA.

Interestingly, degenerative changes were consistent among animals as well as between the medial and lateral tibiofemoral compartments. A previous study on this cohort[8] showed that 1 week following ACL transection significant deviation from the normal locomotion pattern occur. Presumably, these changes are associated with a sudden reduction in anterior-posterior stability indicated by greater tibial translation in the anterior drawer test. This instability persisted during the entire study period of up to 93 months after ACL transection indicating that the ACL did not heal. Despite of this continuing instability, ground reaction forces and hind limb kinematics recovered to near pre-surgical patterns within the first year after ACL transection[8], yet long-term kinematic changes in these animals are currently unknown.

Different factors appear to contribute to the initiation of OA. For instance, a change in knee kinematics in the ACL deficient knee may play an important role[17–19], and in the sheep model the loading direction of articular cartilage changes after ACL transection[18]. Because articular cartilage is designed to best absorb loading in a predefined direction, subtle changes in the loading direction may initiate degeneration[20]. With every loading cycle, the extracellular network of collagen, glucosaminoglycans and proteoglycans is loaded in an unphysiological manner, the cartilage loses its excellent properties, and fissures develop that progress to severe OA[21,22].

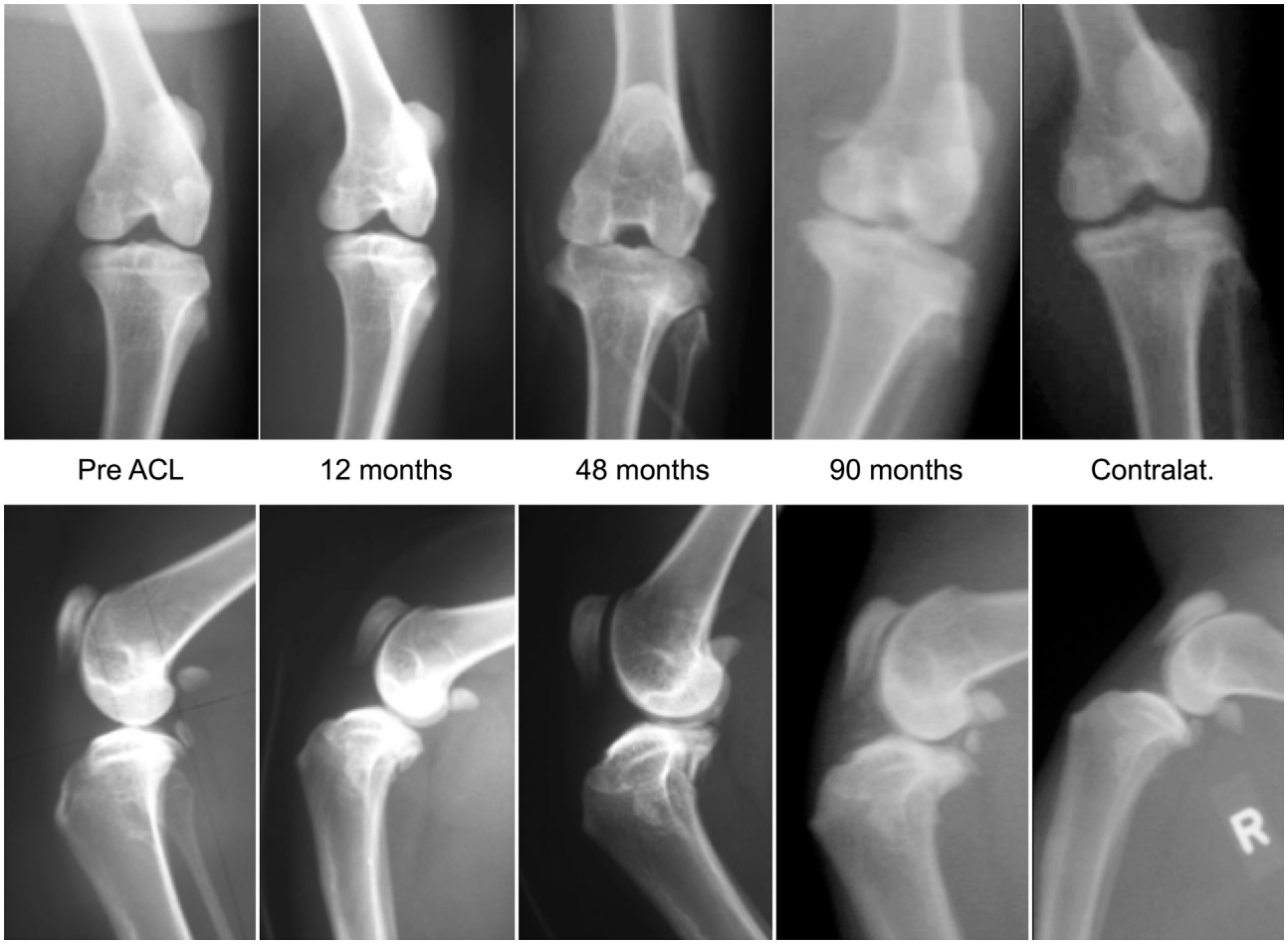

Fig. 3. Radiographs of one animal taken before and after ACL transection of the ACL transected and the contralateral knee. The radiograph was taken while the tibia was pulled forward and the load maintained by locking the sprocket device.

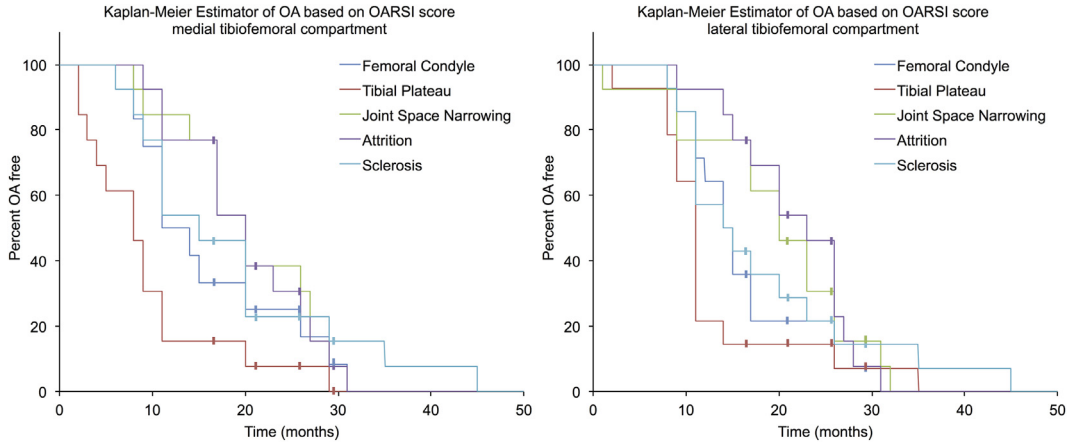

Fig. 4. Kaplan–Meier survival analysis of signs of OA classified according to the recommendations of the Osteoarthritis Research Society International (OARSI) (femoral condyle, tibial plateau, joint space narrowing, attrition and sclerosis)[15]. Left—medial tibiofemoral compartment; right—lateral tibiofemoral compartment. Curves are shown up to 50 months because by then all ACL transected knees had developed OA. During this period, four animals were right censored because they were sacrificed represented by small vertical tick-marks.

Interestingly, in our study OA progressed despite of apparent normalization of kinematic and kinetic parameters.[8]

It is possible that subtle changes in the micro joint kinematics cannot be measured with the kinematic analyses methods employed by Suter *et al.*[8] As previously suggested[17] the location of contact regions within the joint may change after ACL transection and regions are loaded that were previously unloaded and hence not predisposed to sustaining ambulatory loads thereby accelerating degeneration processes. Moreover, radiological changes observed in our study suggest that degeneration occurs already

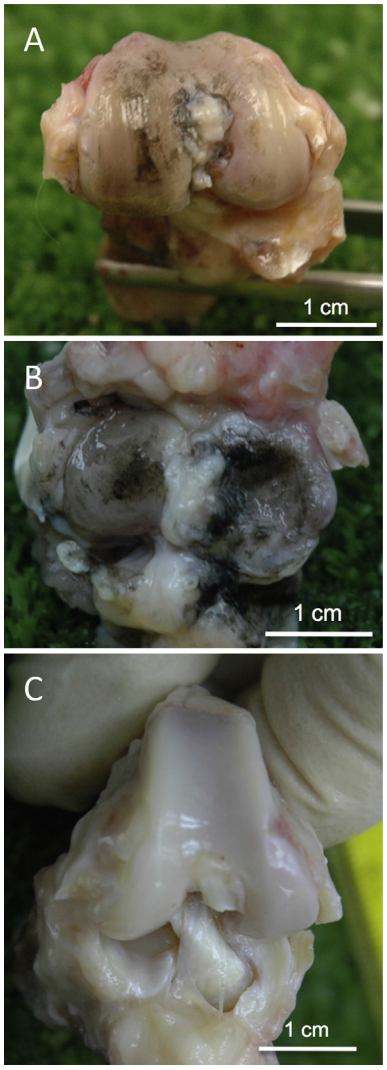

Fig. 5. Photographs of the ACL transected and contralateral knee after scarification in one animal. Surface staining using Indian ink for better visibility. (A) femur condyle with almost complete loss of articular cartilage and osteophytes; (B) tibia plateau with distinct medial and lateral defect; (C) contralateral femur.

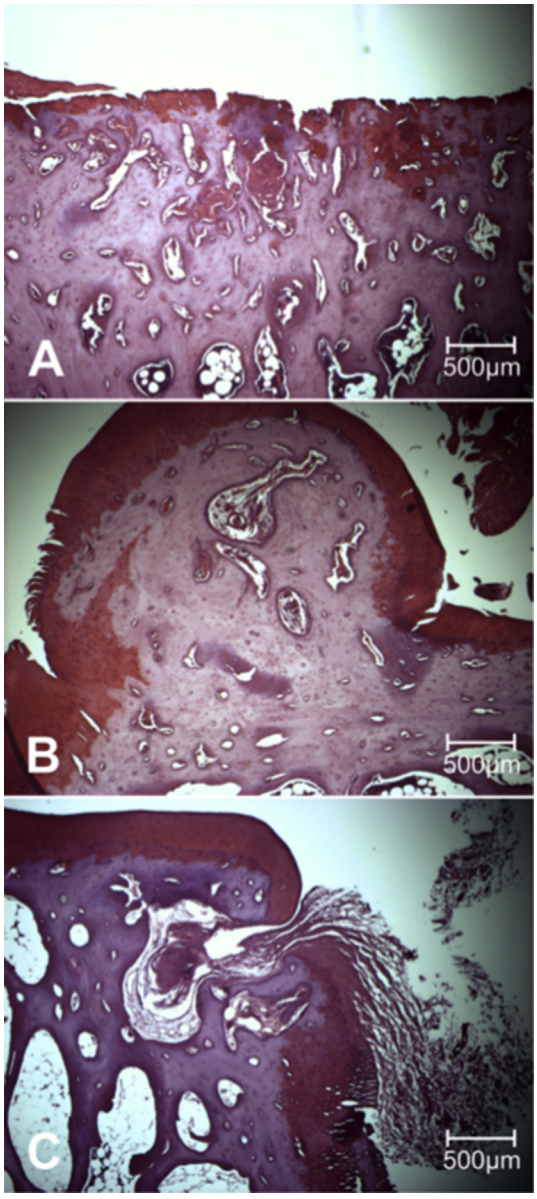

Fig. 6. Histology with safranin O staining. (A) completely depredated medial tibia plateau without any articular cartilage and subchondral sclerosis; (B) large osteophyte in the notch; (C) cyst formation at the periphery of the tibia plateau.

within the first 4 months after ACL transection. These degenerative changes may lead to an altered biochemical milieu (e.g., elevated IL-1, matrix metalloproteinases) with an overproduction of catabolic factors that irreversibly triggers the degenerative cascade.[23]

The contusion that is often observed in an ACL rupture and contributes to the initiation of OA[24] was not present in our experiment because the ACL rupture was iatrogenically established. It is well known that the initial injury initiates OA and that the development of OA occurs faster in more complex injuries. For instance, multi-ligament knee injuries and associated meniscus injuries[25] have been especially deleterious. Hence, the early osteoarthritic changes in our ACL transected model are startling. It is known that a surgical intervention and the associated hemarthrosis and cytokine cascade can already influence osteoarthritic degeneration[26], and hence a surgical intervention may be a sufficient manipulation of the joint to generate an environment that is vulnerable to degenerative processes.

Several animal studies have shown that in the early degenerative phase articular cartilage undergoes a hypertrophic effect, and a subsequent degeneration leads to a loss of the articular cartilage[8,27].

However, we can not confirm these results as the joints space decreased in the tibiofemoral compartments and increased in the patellofemoral cartilage throughout our study. While it is possible that the anterior translation of the tibia changes the anatomy of the patellofemoral joint, we did not observe obvious signs of subluxation in the radiographs. Moreover, we observed an immediate increase in anterior drawer after the ACL transection, yet joint space width in the patellofemoral joint increased only 10 months after the ACL transection indicating a physiological change rather than a mechanical change in the patellofemoral joint. These results suggest that degenerative changes are joint specific and possibly related to the specific loading environment of the respective joint. Moreover, changes to the articular cartilage may not only be specific to the site but also to the location within the tissue. For instance, Clark *et al.*[27] observed extensive adaptation processes in the patellofemoral joint 4 months after ACL resection. Chondrocytes increased in size and were organized in clusters. The number of chondrocytes increased in the middle and deep cartilage

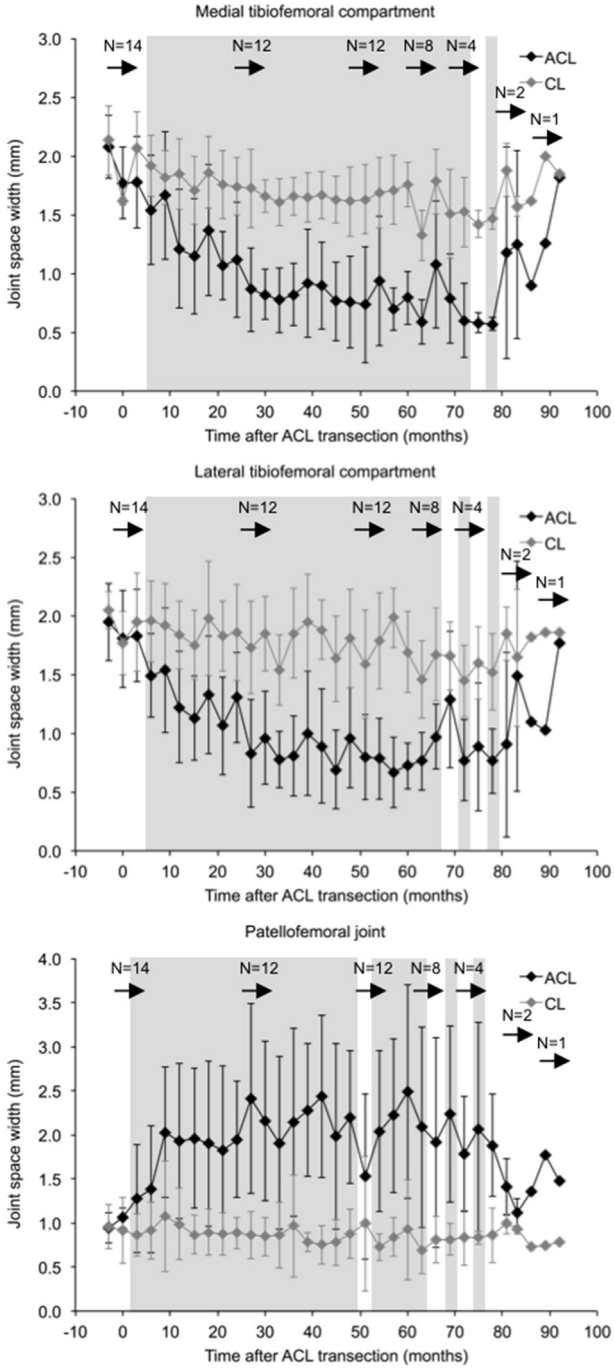

Fig. 7. Joint space width (mean ± 1 standard deviation) over time for the medial (top) and lateral (middle) tibiofemoral compartment and the patellofemoral joint (bottom) of the ACL transected (left) and contralateral knee (right). Time points with significant differences ($P < 0.01$) are highlighted by grey boxes.

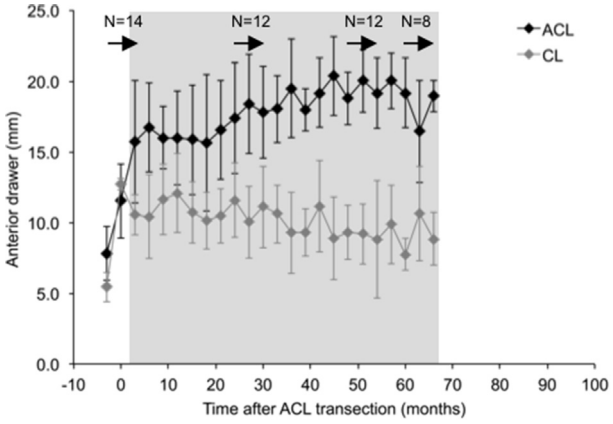

Fig. 8. Anterior translation (mean ± 1 standard deviation) during the drawer test at 110° knee flexion for the ACL transected (black) and the contralateral (dark grey) knee. Time points with significant differences ($P < 0.01$) are highlighted by grey boxes.

layers and decreased in the superficial layer, and the articular cartilage became thicker.

Interestingly, we did not observe radiographic signs of OA in any of the contralateral limbs suggesting that joint degeneration in the transected knees was directly attributed to the instability established in these joints. Moreover, any potential biochemical changes appear to remain local and did not affect the contralateral limb through systemic pathways.

The strength of our study is that the animals lived in a very controlled environment explaining the consistent results among animals. However, because of ethical issues only few animals were studied, and these were sacrificed at different ages. Although sample size calculations were not performed a priori for this exploratory study, the study balanced the need to investigate long term effects of ACL transection (which had not previously been studied) with the desire to minimize animal expenditure and resources. However, the large effects observed in our study despite the small sample size warrants further investigation. Moreover, the contralateral knee served as control joint. We cannot eliminate the possibility that changes occur at the contralateral knee due to systemic factors. However, we did not observe any radiographic signs of OA in the contralateral knees. To completely exclude this possible factor, sham operated control animals would be required, which was not feasible for animal protection reasons. However, joint spaces and anterior tibial translation during the anterior drawer test remained relatively constant throughout the study suggesting that our experimental approach was appropriate. Moreover, morpholocial and histological data were only available for two specimen but these data confirmed our radiographic findings. Finally, we used the ACL transected cat model as model for ACL injury in the human. However, because joint mechanics, joint geometry and tissue properties differ between species the results of our study may not be directly transferrable to humans.

In conclusion, our results describe the natural progression of osteoarthritic change in articular cartilage of the feline knee after ACL transection for a follow-up of up to 93 months. Immediate changes in anterior tibial translation during an anterior drawer test clearly showed joint instability that persisted throughout the lifetime of the animals. Degenerative changes were observed on radiographs within 4 months of the injury only in the transected but not the contralateral limb suggesting the role of mechanical instability for the development and progression of knee OA.

Author contributions

AL evaluated all radiographs, analyzed and interpreted the data and wrote the manuscript; TL was involved in the study design, animal care, data collection and revised the manuscript; CN performed the statistical analysis, interpreted the data and critically reviewed the manuscript; MH was involved in data analysis and interpretation and revised the manuscript; AM performed the statistical analysis, interpreted the data and wrote the manuscript; WH conceived the study, was involved in data collection and interpretation and revised the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Funding sources

The grant agency was not involved in the study design or in any aspect of the manuscript.

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