# CORRELATING BIOCHEMICAL, HISTOLOGIC, AND MAGNETIC RESONANCE IMAGING MEASURES FOR EARLY DIAGNOSIS OF OSTEOARTHRITIS IN THE POND-NUKI MODEL IN DOGS

+*Cook JL, *Stoker AM, **Lerner A,***Totterman S, *Cook CR, *Fox DB
+*Comparative Orthopaedic Laboratory, University of Missouri, Columbia, MO,**University of Rochester, Rochester, NY,***Virtual Scopics LLC, Rochester, NY
cookjl@missouri.edu

**Introduction:** Articular cartilage degradation is the hallmark of osteoarthritis (OA). Articular cartilage degradation in OA can be accurately assessed; however, this cannot be done currently in a noninvasive, clinically-relevant manner. Magnetic resonance imaging (MRI) is readily available, noninvasive, and shows promise for assessing early articular cartilage degradation. If MRI can be used to consistently and accurately predict the extent and severity of cartilage degradation in the early, potentially reversible stages of disease, preventative and threrapeutic strategies for OA would be greatly enhanced. Therefore, the purpose of this work was to assess the ability of MRI to accurately assess early articular cartilage degradation as assessed by "gold standard" histologic and biochemical measures. We hypothesized that 1) articular cartilage degradation in OA can be assessed prior to gross evidence of pathology by MRI; and 2) MRI will directly correlate to biochemical and histologic changes associated with early OA.

**Methods:** The anterior cruciate ligament (ACL) of 4 adult mongrel dogs (mean weight 26.2 kg) was surgically transected (ACL-X). After surgery, the dogs were allowed unrestricted use of the limb. Lameness scoring was performed 4 weeks after surgery. The contralateral knee (stifle) was used as the nonoperated control. After 2 or 4 weeks, the dogs were euthanatized and evaluated. MRI was performed on the knees. Scans were acquired with a 1.5T unit and a custom-designed 3-coil phased-array surface coil. Four image sequences were acquired: T2W 3D gradient recalled echo (GRE), 3D spoiled gradient with fat suppression (SPGR), T1W spin echo sequences, and Dual Echo fast spin echo with fat suppression, which gives proton density and T2*W images. The knees were then disarticulated and evaluated for gross evidence of OA. The limbs were potted and positioned in a holder aligned with the imaging plane. A band saw was used to cut slabs parallel to the sagittal imaging plane to precisely match to MRI slices (Fig. 1). Slabs were then fixed, decalcified and embedded in paraffin for histologic processing. Histologic sections were stained with H&E and toluidine blue and subjectively evaluated for cell and matrix morphology and proteoglycan content and distribution, and scored using the modified Mankin system. Articular cartilage was collected from 8 areas of interest in both stifles for molecular and biochemical assessment. Total sulfated glycosaminoglycan (GAG) content was determined using the dimethylmethylene blue assay. Total collagen content was determined by measuring hydroxyprolene (HP) content using a spectrophotometric assay. Statistically significant differences in biochemical data were determined by performing a t-test with significance at $p<0.05$.

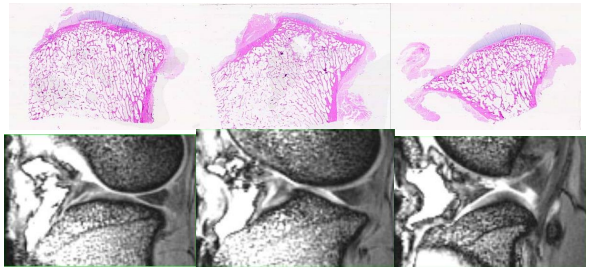

**Figure 1**. Matched MRI and histologic slices used for comparison

**Results:** At 2 weeks after surgery, significant differences were not observed in GAG or collagen content, and no evidence of gross or histologic pathology was seen in articular cartilage from either control or ACL-X tissues. In ACL-X joints at 4 weeks, MRI changes in articular cartilage and subchondral bone were noted, including articular cartilage thinning and surface irregularities, as well as signal intensity changes in subchondral bone. MRI changes were most prevalent in the caudomedial tibial plateau (CmTP). This corresponded well to histoloic changes both in location and character (Fig. 2) Fibrillations, erosions, and loss of cell and extraceullular matrix content and morphology were noted in the artciular cartilage from ACL-X knees and was subjectively most apparent in the caudomedial tibial plateau. Histologic sections from ACL-X knees had significantly ($p < 0.05$) higher Mankin scores (more severe pathologic changes) in the medial femoral condyle and all regions of the tibial plateau when compared to tissues from control knees At week 4, GAG content in nonoperated control cartilage was significantly ($p<0.01$) higher than in ACL-X cartilage (Fig. 3). Differences in collagen content of articular cartilage were not significant at week 4. Subjectively, biochemical data corresponded well to histologic assessment of proteoglycan content and distribution and MRI changes in matched sections. No hindlimb lameness was noted in any dog. No gross evidence of pathology was seen in any knee evaluated.

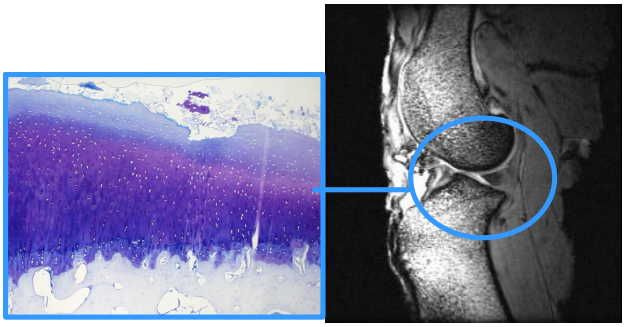

**Figure 2**. Corresponding histologic and MRI changes seen in the caudomedial tibial plateau of ACL-X dogs at week 4 after surgery

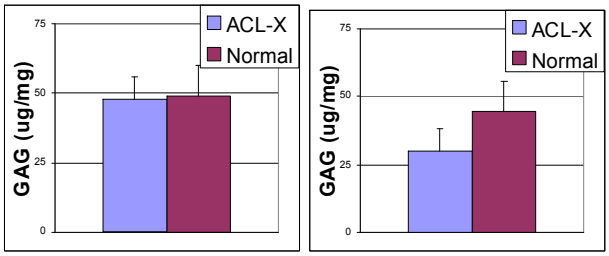


**Figure 3**. GAG content in ACL-X and contralateral control (normal) caudomedial tibial plateau cartilage of dogs at week 2 (left) and 4 (right)

**Discussion**: These data indicate that changes in "gold standard" measures of articular cartilage degradation in OA, histologic evaluation and measurement of GAG content, can be seen in specific regions of the knee joint 4 weeks after surgery using the ACL-X model in dogs. Importantly, these changes were detected prior to gross and clinical evidence for OA, and can be "matched" to definitive MRI changes seen 4 weeks after ACL transection. Taken together, these data indicate that the canine ACL-X model is useful for investigating changes in articular cartilage that may serve as markers for early changes in OA. The results of this study indicate that MRI has the potential to provide a noninvasive modality for assessing early articular cartilage degradation to accurately predict the extent and severity of cartilage degradation in the potentially reversible stages of disease. Current work in our laboratory is aimed at further assessing the usefulness of MRI for predicting and diagnosing OA through correlation to clinical, arthroscopic, histologic, biochemical, and molecular measures of disease.