Validation of diffusion tensor imaging of articular cartialge in an animal model of posttraumatic osteoarthritis

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Purpose and background: To validate diffusion tensor imaging (DTI) of articular cartilage to detect cartilage degradation during the onset and early progression of posttraumatic osteoarthritis (PTOA) in an animal model. Low energy injuries, such as anterior cruciate ligament (ACL) rupture, are responsible for the development of PTOA in young subjects (<60 years).[1] At 10 to 20 years after ACL rupture the prevalence of PTOA is 50–70%, regardless of whether surgical intervention was performed.[1] This suggests that acute changes in the joint following injury trigger the cascade of events leading to PTOA. There is strong need for better understanding of early pathological changes in after injury. In this work we validate DTI of articular cartilage as a biomarker for cartilage degeneration since it has potential to discriminate degradation of the most important components of the cartilage matrix: proteoglycan (PG) and collagen.[2,3]

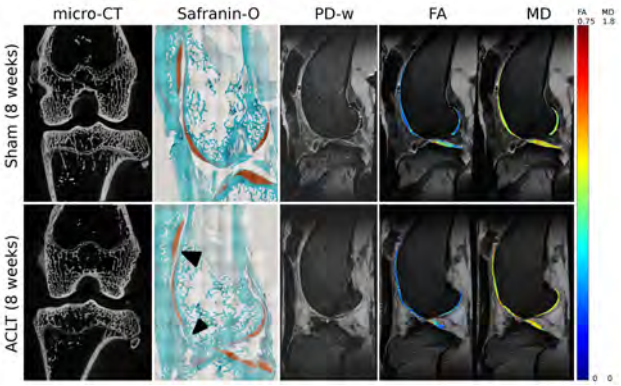

Figure 1: micro-CT, histology, and MRI (PDw, FA and MD maps) of both sham and ACLT hindlimbs of a NZW rabbit 8 weeks after surgery. Black arrows indicate areas of PG loss that correspond with areas of increased MD. Intact cartilage surface can be observed histology.

Methods: We used an established animal model for PTOA. Skeletally mature male New Zeeland white (NZW) rabbits develop a mild form of OA around 4 weeks after surgery, which then develops into a moderate OA within 8 weeks after surgical transection of the anterior cruciate ligament (ACLT).[4] 8 skeletal mature (9-months-old) NZW rabbits underwent ACLT on one of the hindlimbs and sham surgery in the contralateral limb. NZW rabbits were sacrified at 4 weeks (n=4) and 8 weeks (n=4) after surgery. Both hindlimbs of each animal underwent MRI, micro-CT and histology. MRI was performed ex vivo on a 7 T Bruker horizontal magnet with 700 mT/m gradient strength using a 30-mm diameter birdcage transmit-receive coil. MRI protocol included a fat-saturated PD-w turbo-spin echo (TSE) (TE/TR=10/3500 ms, matrix=500×275, echo train length=3,resolution=80×80×300 μm3, 3 averages, acquisition time (tacq)=21 min), and a diffusion-weighted spin-echo (DWSE) sequence (TE/TR=18/2500 ms, resolution=100×100×900 μm^3, diffusion time (Δ)=8 ms, diffusion gradient duration (δ)=3.5 ms, 10 diffusion-weighted directions, b-values=10, 650 s/mm^2, 2 averages, t_{acq}=3:20 h). Micro-CT (Bruker, Skyscan 1076) had isotropic voxel size of 27μm (voltage=100 kV, current=100 μA, 0.5 mm aluminum filter, 0.3° intervals, 10 averages, t_{acq}=2 h). Serial 5-μm histology sections were dyed with safranin O to assess GAG content. Clinical images were evaluated for bone marrow edema (BME), meniscal tears, ACL integrity and cartilage damage. DTI images were segmented and maps of mean diffusivity (MD) and the fractional anisotropy (FA) were calculated. For each limb averaged values of MD and FA were calculated in each cartilage region (medial and lateral femoral condyles [MFC, LFC], medial and lateral tibia [MT, LT] and femoral trochlea [FT]). Micro CT was evaluated for osteophytes, subchondral bone thickening and integrity of the trabecular bone. Histology was used as a gold standard for cartilage integrity. Areas of abnormal staining or signs of fibrillation were correlated with the DTI maps.

Results: Clinical imaging: Figure 1 shows examples of a PD-weighted image of a rabbit stifle joint 8-weeks after ACLT. At 4 weeks three ACLT joints showed BME in the clinical images. Meniscal tears were evident in two of the four animals. There were no signs of osteophytes or cartilage damage at 4 weeks. At 8 weeks there were no signs of BME in either sham or surgery limbs. Two ACLT joints presented meniscal tears. No clear sign of cartilage damage was present at 8 weeks.

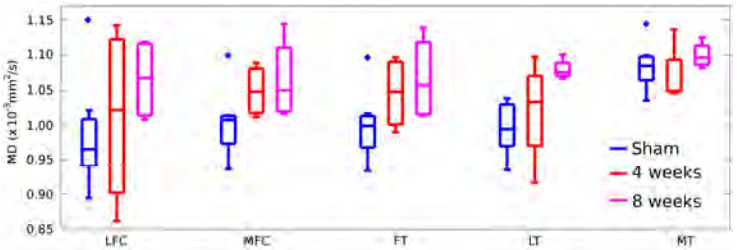

Figure 2: Boxplot of MD values for sham (4 and 8 weeks) and for ACLT hindlimbs.

Diffusion tensor imaging: Figure 1 shows examples of parameter maps at 8 weeks. MD values in al cartilage regions are represented in Figure 2. Sham joints showed no significant difference ($p<0.05$) in MD or FA between 4 and 8 weeks. The ACLT joints had a trend of increased MD with time to surgery in the FT, LFC, MFC and LT. At 4 weeks there were no significant difference in any cartilage plate in MD as compared to the sham joints (Wilcoxon test, $p>0.10$). At 8 weeks MD was significantly (Wilcoxon test, $p<0.05$) higher in the MFC, FT and LT regions. FA did not show a trend with time to surgery and there were no statistically significant differences either at 4 weeks ($p>0.34$) or 8 weeks ($p>0.42$) compared to the sham operated joints.

Micro-CT: There were no signs of osteophytes formation in the micro-CT images (Figure 1). There were no significant differences in subchondral bone thickness or bone volume between the controls and the 4 and 8 weeks joints.

Histology: An example of histology is shown in Figure 1. Histology analysis showed almost intact cartilage at 4 weeks with partial loss of PG in two ACLT limbs mostly in the FT and MFC. Areas of PG loss were more prominent at 8 weeks. Areas of increased MD correlated with areas of PG loss in histology (e.g. Fig. 1). There were no signs of cartilage fibrillation at 4 weeks and some minor superficial damage in cartilage regions at 8 weeks. Overall, histology showed very mild OA changes in the joints both at 4 and 8 weeks.

Discussion: Our experiments were design to test DTI in the early phases of PTOA, where DTI has potential to outperform standard clinical imaging. In our experiments clinical images did not show signs of cartilage damage, while ACLT joints showed increased MD values compared to sham joints. Histology showed only moderate degenerative changes that were more evident at 8 weeks than at 4 weeks. We found evidence of PG loss here were no signs of cartilage loss in any of the limbs and there were almost no sign of cartilage fibrillation that can evidence damage of the collagen network. The DTI parameters correlate with the integrity of articular cartilage observed in histology. Areas of decreased PG content were associated with areas of high MD. Despite the low number of animals per group (n=4) we could identify significant increases in MD at 8 weeks. The lack of a clear trend in the FA values is in line with the low damage of the cartilage surface observed in histology. The micro-CT did not show signs of bone remodeling thus suggesting that, at least in this animal model that does not involve trauma, bone remodeling do not precede changes in articular cartilage.

Conclusion: Our results identify MD as a biomarker with potential to detect cartilage damage before it becomes apparent in clinical MRI.

References: [1] Lohmander LS, et al. Am J Sports Med. 2007;35:1756, [2] Raya JG et al. Invest Radiol 2011;46:401, [3] Raya et al. Radiology 2012;262:550, [4] Yoshioka M et al. Ostoearthritis Cartilage 1996;4:87.