Purpose: Detecting cartilage damage immediately following trauma could allow for early intervention and the subsequent prevention of post-traumatic osteoarthritis (PTOA) development. Current clinical imaging modalities do not have cellular resolution to detect subtle structural changes or chondrocyte viability. Multiphoton microscopy (MPM) can acquire micron resolution structural and biological information in live tissues. The purpose of this study was to utilize MPM imaging with FDA-approved angioscopic fluorescein dye to characterize the viability of early cartilage damage to further understand the early pathophysiology of PTOA.

Methods: Osteochondral blocks from the distal metacarpals of horses aged 4-6 years were placed in a custom-designed holder on an EnduraTEC ELF3200 mechanical test frame (EnduraTEC). The articular surface of each medial condyle was injured with a single compressive load of 30 MPa via a 2.25 mm flat ended cylindrical indenter within 1 sec to model traumatic injury. The lateral condyle served as the control. After 1 hour, blocks were placed in 1 μM fluorescein sodium (AK-FLUOR 25%, Akorn) and imaged with MPM. Emission spectra was collected and quantified using custom image analysis code in MATLAB (MathWorks) to determine chondrocyte viability and spatial distribution.

Results: Within one hour of damage, MPM imaging demonstrated significantly increased chondrocyte death (p<0.001, as assayed by dye penetration through ruptured membranes), autofluorescent signatures, and micro-cracks when compared to controls (Figure 1). Reconstructed MPM data revealed that cell death (Figure 2) occurred in either a circular pattern corresponding to a region around the perimeter of the indenter, or in an elliptical pattern (Table 1). 

Conclusions: MPM reveals cellular and matrix damage in live cartilage and reveals novel findings relevant to early PTOA pathophysiology. The reconstructed 3-D pattern of death-dissemination and the development of autofluorescent signatures following trauma could serve as optical biomarkers for early PTOA and are below the resolution of other validated scoring methods.

349 RADIOLOGIC PROGRESSION IN HAND OSTEOARTHRITIS (OA) OVER 2.6 YEARS - DATA FROM THE SEKOIA TRIAL

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Purpose: Hand OA is a frequent polyarticular disease. Few is known with respect to its radiological progression over time, which in addition is difficult to assess, considering that no radiographic scoring method has, today, proved being superior to another. The goal of this study was to assess hand OA radiological progression over 3 years using three validated scoring methods.

Methods: Data came from an international 3-year, randomized, placebo-controlled phase III trial designed to assess the effect of strontium ranelate compared to placebo on the radiographic progression of knee OA which included symptomatic primary knee OA patients (ACR criteria) at a Kellgren-Lawrence (KL) grade II or III, with a minimal joint space width (JSW) between 2.5-5 mm. During this trial, baseline and final postero-anterior radiographs of each hand were performed. Symptoms were assessed using the functional index for Hand OA (FIHOA; range 0-30) and the AUSCAN (0-300). Two independent readers scored half of the pairs of radiographs obtained each, blinded to treatment and time sequence, using the KL (range 0-128), Kallman (0-204) and Verbruggen anatomical phase (0-218) scoring methods with a good inter-rater reproducibility. Hand OA radiographic progression was studied in the placebo group by looking at 1/ baseline-end changes in global scores, 2/ the numbers of progressors (progression defined for each global score by a change over each reader’s smallest detectable difference (SDD)), and 3/ the number of patients in whom at least 1 joint showed a deterioration (from KLO-1 to KL≥2; progression of ≥1 phase for Verbruggen score).

Results: Of 1669 patients included in the SEKOIA trial, 1371 had baseline KL score and 1299 had Verbruggen score. 72% were female, mean age 64±7 years, body mass index 29.5±5 kg/m², and initial knee JSW 3.5±0.8 mm. Baseline hand OA radiologic severity was mild: KL score 21±13, Kallman score 24±21 and Verbruggen score 13±14. FIHOA score was 4±5, AUSCAN global score was 96±80. Mean time interval between baseline and final radiographs was 315 months. Hand OA radiographic progression over 2.6 years was modest with a mean change of 2.4±3.3 for KL score, 3.7±5.3 for Kallman score and 2.0±4.0 for Verbruggen score.

The numbers (%) of progressors (change≥SDD) were 7 (2%), 17 (6%), and 21 (7%) respectively.

The numbers (%) of patients with at least 1 worsened joint were 169 (57%) for KL and 139 (47%) for Verbruggen score, with respective means of 2.0±1.3 and 1.7±1.1 worsening joint.

Conclusions: Whatever the radiological scoring method used, and the kind of analysis performed, mild radiographic hand OA patients showed a very weak global radiological progression over 2.6 years. In future structure-modification trials in hand OA, analysing the number of patients with at least one joint worsening could be the most sensitive method.

350 RATES AND SENSITIVITY OF KNEE CARTILAGE LOSS IN RADIOGRAPHIC DISEASE STRATA - CENTRAL VERSUS SITE READINGS FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: Previous studies showed a weak relationship of mild vs. moderate joint space narrowing (specifically OARSI JSN grades 1/2) with knee pain and the body mass index (BMI). In the Osteoarthritis

Table 1

<table>
<thead>
<tr>
<th>Load (N)</th>
<th>Displacement (µm)</th>
<th>Axes; Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circular 117.7±0.02</td>
<td>367.1±50.8</td>
<td>15.2±1.8</td>
</tr>
<tr>
<td>Elliptical 117.7±0.02</td>
<td>372.5±29.7</td>
<td>74.4±32.7</td>
</tr>
</tbody>
</table>

Figure 1. MPM of control and injured samples. Control samples typically contained few fluorescein-stained cells, while injured samples typically contained fluorescent stained cells, autofluorescent structures, and cracks.

Figure 2. 3-D reconstruction of control and injured cartilage from n=1 sample. Each point represents a single dead chondrocyte. The total volume scanned was a checkerboard of volumetric Z-scans, with 10 images at 10 µm-step intervals. An image such as that in Figure 1 represents 1.6% of the area in Figure 2.
Initiative (OAI), the site radiographic readings used for recruitment collapsed OARSI JSN grades 1/2 into a single grade, but central readings that provide the full spectrum all 3 OARSI JSN grades were released recently by the OAI. The correlations between JSN grades and cartilage loss has not been well studied; Hence, we test the hypothesis that knees with medial JSN grades 1 and 2 display different rates of cartilage loss and that their differentiation is important in predicting structural progression. Further, we explore whether rates of cartilage loss within KLG1 and 2 depend on the presence [+ or -] of JSN.

Methods: We studied a sample of 836 right knees from the OAI (Table 1); 112 were healthy (age 55±7.7; BMI 24.3±3.0), and 724 were selected based on presence of definite radiographic OA at recruitment (i.e., definite osteophytes and OARSI JSN=siteKLG2, JLSN 1/2=siteKLG3, JSN3 =siteKLG4; age 63±9.3; BMI 29.6±4.7). Site readings were performed by 3-4 trained readers at each of 4 sites, and the central reading by a team of trained readers at Boston University. Segmentation of the weight-bearing femorotibial cartilages in baseline and 1 year follow-up MRI was performed by 12 readers (Chondrometrics GmbH) with blinded to acquisition order, using 3T coronal FLASH water-excitation sequences. Thickness change in the medial compartment (MFTC) and ordered value (OV1=the 1 of 16 subregions with the greatest loss in each knee) was used for the analysis.

Results: The 112 healthy reference subjects with bilateral siteKLG0 showed OV1 rates of change of -115±55 mm. Of these 101 had bilateral radiographically normal knees based on the central readings (centKLG0) and similar OV1 rates of change (-111±54 mm). Of the 724 siteKLG2-4 knees, 6 were excluded from site reading analysis because they displayed JSN without a definite osteophyte. Of these, 93 were centKLG0 (-120±58 mm), 61 centKLG1 -JSN (-150±101 mm), 40 centKLG1 +JSN (-119±77 mm), 98 centKLG2 -JSN (-134±100 mm), 210 centKLG2 +JSN (-172±152 mm), 161 centKLG3 (all +JSN: -220±140 mm), and 61 centKLG4 (-195±114 mm). OV1 rates of change were greater in centKLG2 knees with than in those without JSN (p=0.03) but did not differ significantly between centKLG1 knees (+/- JSN). A comparison of MFTC rates of change between site and central readings is shown in Table 1: rates in cent_med_JSN1 knees were substantially greater than those in cent_med JSN1 knees (p=0.02) and of similar magnitude as those of cent_med JSN3 knees.

Conclusions: We observe substantially greater rates of cartilage loss in knees with medial OARSI JSN2 than in those with JSN1. Within KLG2 (but not KLG1) knees, presence of JSN was associated with greater rates of cartilage loss. Differentiating between knees with mild (grade 1) vs. moderate (grade 2) medial JSN (and KLG2 knees with vs. without JSN) is important in predicting structural progression (i.e. cartilage loss).

Table 1: rates in cent_med_JSN2 knees were substantially greater than those in cent_med_JSN1 knees (p=0.02) and of similar magnitude as those of cent_med_JSN3 knees.

<table>
<thead>
<tr>
<th>MFTC</th>
<th>Site readings</th>
<th>Central readings (BU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= mean±SD (mm)</td>
<td>n= mean±SD (mm)</td>
</tr>
<tr>
<td>Healthy</td>
<td>112 +2±82</td>
<td>101 -2±85</td>
</tr>
<tr>
<td>medJSN 0</td>
<td>428 -15±107</td>
<td>-0.14</td>
</tr>
<tr>
<td>medJSN 1</td>
<td>217 -43±129</td>
<td>-0.34</td>
</tr>
<tr>
<td>medJSN 2</td>
<td>(collapsed with gr. 1)</td>
<td>116 -70±159</td>
</tr>
<tr>
<td>medJSN 3</td>
<td>73 -86±152</td>
<td>-0.57</td>
</tr>
</tbody>
</table>

MFTC= medial femorotibial compartment
SRM= standardized response mean = mean change/SD of change

351 CONCURRENT CRITERION AND CLINICAL VALIDATION OF A RAPID, SEMI-AUTOMATED QUANTITATIVE METHOD OF MRI-DETECTED BONE MARROW LESIONS IN KNEE OSTEOARTHRITIS

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Purpose: Bone marrow lesions (BML) have been associated with pain, cartilage thinning, and meniscal pathology. Most measurement methods are semi-quantitative, (ordinal) and relatively expensive. Imaging methods that rapidly and accurately measure BML volume may be valuable in detecting longitudinal change in large osteoarthritic (OA) trials and observational studies. We recently developed a rapid, semi-automated quantitative measurement of femoral BML volume and validated it against the Whole-Organ Magnetic Resonance Imaging Score (WORMS). We have now extended the method to include BMLs in the tibia. The purpose of this study was to compare this tibial BML quantitative measure with WORMS, and to test the hypothesis that quantitative BML volume in the tibial and femur is associated with weight bearing pain in knee OA.

Methods: One hundred and fifteen subjects from the baseline data of the OAI Progression Cohort whose knees had been WORMS-scored by OAI central imaging were included. Sagittal turbo spin echo fat saturated (TSE FS) (0.357 x 0.357 x 3.0 mm, TR 3200ms, TE 30ms) intermediate-weighted MRI were obtained on a 3T Siemens Trio MR system. A reader (CR) used semi-automated software to segment the subchondral BMLs in the proximal tibia using the same method and on the same MRI scans that femoral BML volumes were previously measured and validated. The software applies a grayscale thresholding algorithm to the raw image and provides the reader with regions for potential segmentation. Reader judgment is used to select, usually with 1 or 2 mouse clicks, the clinically appropriate region(s) of BML adjacent to subchondral bone and to reject irrelevant areas. Intra-reader and inter-reader reliability was assessed on a random sample of 20 subjects using the intraclass correlation coefficients and were 0.96 (95% CI, 0.93-0.98) and 0.97 (95% CI, 0.93-0.99) respectively. The second reader was an experienced radiologist (CV). We used the WOMAC pain sub-scale and defined the primary outcome of knee pain dichotomously as moderate to severe pain (scores 2-4) on any of the 3 weight-bearing WOMAC pain questions (pain on walking, pain on climbing stairs, pain on standing), acquired at the same baseline OAI visit as the MRI scans.

Results: The sample was 84% white and 52% male. The distribution of K/L grades was 34% with grade 2, 55% with grade 3 and 7% with grade 4. A box and whisker plot showing BML volumes in the tibial medial compartment against categories of WORMS is presented in the Figure. The software method required an average of approximately 5 minutes per knee, once images had been screened by an MSK radiologist for differential diagnoses. Significant positive associations between the volumetric measure and WORMS score were found in the tibial medial (Spearman’s correlation 0.71, p<0.001) and lateral (Spearman’s correlation 0.70, p<0.001) compartments. Spearman’s correlation for the femoral medial (0.73) and lateral (0.85) compartments of the same knee were previously reported. We found significant positive associations between weight-bearing pain and total volume of BML (femur and tibia combined) and femoral BML volume, while there was not a significant association in the tibia.

Conclusions: We have documented a moderately strong correlation between tibial BML volume from the semi-automated method and the semi-quantitative WORMS BML scores, similar to data for the femur, providing evidence of criterion validity. The hypothesis that weight-bearing pain was associated with BML volume was confirmed for total femoral BML volume but not for total tibial BML volume. The lack of association between tibial BML volume and pain requires further investigation.

Figure – Box and whisker plots showing mean, median, lower and upper quartiles, and outliers of BML volume from semi-automated quantitative assessment, by WORMS scores, for medical tibial compartment.