and from this automatically to quantify a bone structure marker strongly associated with the presence of OA. The advantages of this approach are the use of low cost MRI equipments and a fully automatic computer-based framework, which makes the developed marker well suitable for large longitudinal clinical studies.

150 QUANTITATIVE AND HISTOPATHOLOGICAL ANALYSIS OF OSTEO-ARTHRITIC CARTILAGE USING MRI, HRMAS SPECTROSCOPY AND IMMUNOHISTOCHEMISTRY


Purpose: Osteoarthritis (OA) involves cartilage degradation resulting from biochemical changes. In this study we perform ex-vivo T1-rho weighted MRI followed by high resolution magic angle spinning (HRMAS) NMR spectroscopy at 11.7 T using knee articular cartilage acquired from OA patients during total knee replacement (TKA) surgery. HRMAS ensures tissue integrity and allows immuno-histochemistry (IHC) and histology of the same cartilage samples following spectroscopy. We use immuno-histochemistry and histology to show that the biochemical changes measured with HRMAS correlate with the histopathology of cartilage, and thus validate that HRMAS is a viable and powerful addition to our capabilities to study early and late OA, and it complements the T1-rho relaxation map acquired using ex-vivo MRI that depicts regions of increased cartilage degradation in OA specimens.

Methods: Osteoarthritic samples were excised from OA patients (n=4, range = 57–81 years) undergoing TKA during surgery. In case of each patient lateral and medial femoral condyles were acquired, flash-frozen in dry ice, then immediately stored at −80°C until experiments. The condyles were mounted and secured on a grid (Fig. 1) after thawing to obtain T1-rho MRI of the ex-vivo specimens using a 3 Tesla GE scanner. Semi-automatic segmentation software developed in-house was used to segment the cartilage overlaying the condyles and the mean+SD of T1rho was determined in each case. 8 cartilage punches were acquired per patient using a 3 mm punch resulting in a total of n=24 punches. The samples were then analyzed using HRMAS NMR spectroscopy using a Carr Purcell Meiboom Gill (CPMG) pulse sequence to determine the biochemical composition of the samples. Following HRMAS, histologic (hematoxylin/eosin and Safranin-O/Fast Green) and immunohistochemical evaluation were performed on the same samples using antibodies that recognize aggrecan cleavage neoepitopes G1-NITEGE (reflecting activities of ADAMTS) and G1-VDIPEN (reflecting activities of MMPs) as well as those that recognize the collagen-II cleavage neoepitopes (GPQG).

Results: Figure 2(A) shows a representative segmented ex-vivo T1-rho weighted MRI color map of the lateral inferior femoral condyle of an OA patient. The mean+ SD of T1-rho for the segmented cartilage was (60.4 ± 18.45) ms. The corresponding HRMAS spectra of the punch sample shown in 2(A) is indicated in Figure 2(B), with well resolved spectral peaks including N-acetyl (reflecting proteoglycan concentration) and alanine (reflecting collagen concentration). Figure 3 shows the H&E staining, Safranin-O staining and the immuno-histochemical labeling of anti-NITEGE of the same punch sample as was used for HRMAS acquisition.

Conclusions: This study is a multi-modality approach to understand the biochemical changes involved in OA. The use of HRMAS spectroscopy after performing ex-vivo T1-rho MRI allows the correlation of T1-rho relaxation with biochemical profiles of cartilage as determined by HRMAS. The fact that cartilage samples remain intact after HRMAS provides the ability to perform histo-pathological analysis of the same cartilage samples. Quantitative analysis of T1-rho values, HRMAS spectral quantification and histology/IHC staining grading will be performed with data collected from this study. This technique provides a validation of spectroscopy approach and helps establish potential biomarkers of the cartilage degradation process in OA.

Fig. 2. (A) Representative ex-vivo T1-rho weighted MRI of osteoarthritic femoral condyle and (B) the corresponding HRMAS spectra of a cartilage punch (indicated in A) acquired from the same femoral condyle indicating the resonance arising from the N-acetyl side-chain protons of proteoglycan and the alanine resonance corresponding to the collagen.

Fig. 3. Histological/immunohistochemical sections of cartilage (the same punch sample as in Figure 2) stained using (A) H&E, (B) Safranin-O, (C) anti-NITEGE.

151 COLLAGEN BIOMARKER RESPONSE TO ACUTE JOINT INJURY IN A NON-TERMINAL ANIMAL MODEL OF OSTEOARTHRITIS

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Purpose: Biomarkers of cartilage metabolism have potential to identify early stages of osteoarthritis (OA), before clinical evidence is present, and thus facilitate early diagnosis and therapy. The purpose of this study was to identify synovial fluid (SF) and serum biomarker changes that occur after acute joint injury using an equine model of early OA. We hypothesized that collagen biomarkers would identify early metabolic changes that occur secondary to acute osteochondral (OC) injury.

Methods: Twenty-two clinically and radiographically normal age- and sex-matched Quarter Horses were randomly divided into 2 groups: (1) horses (n=11) that had an OC fragment created arthroscopically on the dorsomedial aspect of the first phalanx in one randomly selected metacarpophalangeal (MCP) joint and a sham operation in the contralateral joint at week 0; and (2) unoperated exercise control horses (n=11). All horses were exercised on a high-speed treadmill 5 days/week from week 2 to week 16. Blood was collected from the jugular vein and SF samples were collected without lavage from both MCP joints of all horses at baseline (week 0), week 2, 8, and 16. Commercially available collagen degradation (CTXII [IDS/Nordic], C2C, C12C [IBEX]) and synthesis (CPII [IBEX]) ELISA assays were used to analyze SF and serum samples. All assays were previously validated for equine use. Data was assessed for normality and outliers were removed from further analyses. A repeated measures ANOVA with a Tukey’s test for multiple comparisons was used for all analyses. P<0.05 was considered significant. All procedures were approved by institutional animal care and use committees.
The evaluation of future therapies. Future application defining the onset and progression of OA and allowing the joint becomes more catabolic between weeks 2 and 8 after injury. Suggest that in spite of an early anabolic response in the injured joint, by week 8 while degradation (CTX II) concurrently increased. This may increased by week 2 after injury, as indicated by a peak in CPII such as cathespin K, or secondary to protease cleavage. Collagen synthesis that telopeptide breakdown is mediated by other biochemical pathways Conversely, CTX II continued to increase throughout the study, suggesting from collagenase cleavage, this may suggest that most of the collagenase but started to decline with chronicity. Since these neoepitopes result concentrations between OC injured horses and controls.

**A standardized scale has been used to show relative relationships.**

**Significant differences are not shown in the figure because of the standardized scale. Refer to the text for significant differences.**

**Results:** In SF, all collagen biomarkers significantly increased from baseline at weeks 2, 8, and 16 (P < 0.0001). In addition, SF from OC injured joints had significantly higher concentrations of all collagen biomarkers when compared to SF from control and sham joints (P < 0.05). The only exception was at week 8 with CPII, when OC injured and sham joints were not significantly different. Figure 1 compares all of the collagen biomarkers on a standardized scale to show the relative response over time. CPII and C2C concentrations peaked at week 2, C12C at week 8, and CTX II at week 16. The only significant change in serum biomarkers was a decreased C12C concentration at week 2 in both OC injured and control horses (P < 0.05). There were no significant differences in serum concentrations between OC injured horses and controls.

**Conclusions:** This study demonstrated that in an acute joint injury model of OA, SF is more sensitive than serum in determining changes that occur in collagen biomarkers. The relationships between multiple biomarkers over time helps to more accurately delineate the metabolic changes that occur after acute joint injury. We used 3 collagen degradation biomarkers to further elucidate changes in collagen metabolism after injury. C12C and C2C were elevated throughout most of the study period, but started to decline with chronicity. Since these neoepitopes result from collagenase cleavage, this may suggest that most of the collagenase damage to collagen occurs within the first 2 to 3 months of injury. Conversely, CTX II continued to increase throughout the study, suggesting that telopeptide breakdown is mediated by other biochemical pathways such as cathespin K, or secondary to protease cleavage. Collagen synthesis increased by week 2 after injury, as indicated by a peak in CPII concentration. However, CPII came back toward baseline concentrations by week 8 while degradation (CTX II) concurrently increased. This may suggest that in spite of an early anabolic response in the injured joint, the joint becomes more catabolic between weeks 2 and 8 after injury. Analysis of synovial fluid in an animal model of acute injury may have future application defining the onset and progression of OA and allowing the evaluation of future therapies.

**Fig. 1. MCP synovial fluid and serum biomarker results over 16 weeks.** A standardized scale has been used to show relative relationships. Significant differences are not shown in the figure because of the standardized scale. Refer to the text for significant differences.

**Purpose:** Total knee arthroplasty (TKA) is usually recommended for those with the most severe knee osteoarthritis (OA), as it has been established as a highly successful procedure for treating patients with severe knee OA. However, the decision whether to have a joint replacement or not is ambiguous. For the selection of patients receiving TKA, age, general health status, willingness to consider joint replacement, and severity of symptoms including in response to other treatment modalities are generally taken into account in addition to a radiographic severity of OA. The decision whether to have a TKA or not is a judgment call that has to be made by the physician and patient working together. For the physician, objective factors which can represent the past results of the patients who had already received TKA, if available, would be helpful to discuss with the patients for the decision. However, the characteristics of patients who will actually receive TKA are poorly understood.

**Methods:** This case-control study protocol was approved by the institutional review board of our university. One hundred twenty-one painful knee OA patients with K/L grade 4 were enrolled in this study. Serum samples were obtained from all subjects on the day that radiographs taken. A HA binding protein based latex agglutination assay that employed an ELISA format was used to measure the level of HA. Age, gender and body mass index (BMI) adjusted one way analysis of variance (ANOVA) was used for parametric comparisons. The AUC, which is analogous to the area under the the receiver operating characteristic (ROC) curve, was estimated for the discriminative value of prediction models. Odds ratios (ORs) were calculated to evaluate the shA cut-off score for receiving TKA. A p-value of less than 0.05 was considered to be statistically significant.

**Results:** In this study, patients were followed for 6 months on average. While 36 of 121 patients were received TKA (OP group), remaining 85 of 121 patients were not (NOOP group). No gender distribution (86.8% was female in total), age (72.4 y on average) and BMI (25.3 kg/m2) differences were observed between OP group and NOOP group, respectively. As the shA levels of the patients did not show normal distribution, the shA levels were logarithmically transformed (ln-shA), thus resulting in a normal distribution. The age, gender and BMI adjusted ln-shA levels at baseline of OP group were significantly increased in comparison to those of NOOP group (p < 0.05). The AUC in ROC curve of shA was 0.64 (95% CIs: 0.53, 0.74). The cut-off of shA level calculated by ROC curve was found at 55.0 ng/ml and the ORs at 55.0 ng/ml of shA level for receiving TKA was 2.95 (95% CIs: 1.1, 7.4).

**Conclusions:** These results suggest the potential of shA as not only a burden of disease marker but also a prognosis marker that can be useful for predicting the patients who will receive TKA among severe knee OA patients. In conclusion, shA could be one of the objective factors for the prediction of receiving TKA among the patients with severe knee OA.