Proteins differentially expressed in OA SF identified with gretare than 2 fold change and $P<0.05$

Protein	Anova (p)	Maximum fold change	Highest mean condition
S100-A10	0.00028	2.2	OA
CD109 antigen	0.00039	2.0	OA
Phospholipid transfer protein isoform 1	0.00098	2.7	OA
Complement component C8 gamma chain	0.0115	3.1	OA
Collagen alpha-1(I) chain	0.0459	2.8	OA
Calsyntenin-1	0.0485	2.1	OA
Integral membrane protein 2B	0.00068	2.1	Normal
mannan-binding lectin serine protease 2	0.0061	2.2	Normal
Keratin, type II cytoskeletal 7	0.0114	2.2	Normal
Cyclin D binding myb-like transcription factor 1	0.0240	6.2	Normal

101
SERUM BIOCHEMICAL MARKERS OF JOINT METABOLISM AND INFLAMMATION IN RELATION TO CLINICAL SYMPTOMS AND PHYSICAL FUNCTION IN ADULTS WITH SYMPTOMATIC KNEE OSTEOARTHRITIS

A.E. Connelly, A.J. Tucker, L.S. Kott, A.M. Duncan, A.J. Wright. *Univ. of Guelph, Guelph, ON, Canada*

Purpose: To date, the majority of osteoarthritis (OA) biomarker research has examined associations between biomarker levels and radiographic parameters. While radiography provides information on the structural burden of disease, it is insensitive to small changes in cartilage metabolism and does not always correlate with the most significant clinical expression in OA, pain. The purpose of this study was to investigate the associations between joint metabolism and inflammation with pain and physical function in adults with painful knee OA. This is in support of qualifying burden of disease biomarkers with clinical variables to support the study, management, and understanding of disease pathology in OA.
Methods: The study used baseline data from 54 adults with knee OA participating in a nutrition intervention study. The 100 mm visual analogue scale version of the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) was used to assess pain, stiffness, and physical disability. A minimum WOMAC pain score of 125 was used as a cutoff for participant inclusion. Physical function was assessed with a 6-minute walk test (:6MWT) and stair climb task (SCT). Serum concentrations of biomarkers included measures of cartilage degradation (cartilage oligomeric matrix protein [COMP]), cartilage synthesis (type-IIA collagen N-propeptide [PIIANP]), synovial metabolism (hyaluronic acid [HA]), cartilage degrading enzyme levels (matrix metalloproteinase 3 [MMP-3]), and inflammation (C-reactive protein [CRP]). COMP, PIIANP, HA, and MMP-3 were measured by enzyme-linked immunosorbent assay and CRP was measured by an immunoturbidimetric assay. Correlations between biomarkers and clinical variables were assessed using Spearman correlation coefficients.
Results: Participants (38 females and 16 males) had a mean age of 60 $\pm$ 11.9 years, BMI of 32.6 $\pm$ 7.5 kg/m2, disease duration of 86.6 $\pm$ 112.2 months, and WOMAC pain score of 193.4 $\pm$ 94.04 mm. Higher serum MMP-3 levels were significantly associated with higher WOMAC pain scores ($R = 0.27$, $p = 0.05$), controlling for age and body mass index (BMI). Higher levels of serum HA were significantly associated with decreased walking distance in the :6MWT ($R = -0.35$, $p = 0.01$) and longer time taken in the SCT ($R = 0.31$, $p = 0.02$), when controlling for age and BMI. Higher CRP levels were significantly associated with higher WOMAC physical disability score ($R = 0.34$, $p = 0.01$) and worse performance in the :6MWT ($R = -0.38$, $p = 0.005$) and SCT ($R = 0.44$, $p = 0.001$) when age was controlled for, but these associations were not significant when BMI was controlled for. COMP and PIIANP were not significantly associated with any clinical variables analyzed.
Conclusion: Serum MMP-3 levels were associated with pain and serum HA levels were associated with physical functioning in adults who suffer from mild to moderate pain and impaired physical functioning from knee OA. Previous studies have shown that HA and MMP-3 are related to structural knee parameters in OA. Therefore, results from this study demonstrate that serum HA and MMP-3 also have potential as qualified burden of disease biomarkers that are clinically meaningful. (Research supported by the Ontario Ministry of Agriculture, Food and Rural Affairs, project #200121).

102
FAST FIELD-CYCLING NMR OF CARTILAGE: A WAY TOWARD MOLECULAR IMAGING

L.M. Broche, B.W. Kennedy, C. MacEachern, G.P. Ashcroft, D.J. Lurie. *Univ. of Aberdeen, Aberdeen, United Kingdom*

Purpose: A previous pilot study (presented at OARSI 2012) showed that Fast Field-Cycling NMR (FFC NMR) can be used to characterise the dispersion curves of cartilage in the region 0.4 to 3 MHz proton Larmor frequency. One feature of these dispersion curves, quadrupolar peaks, arise from relatively well known interactions between water protons and the ^{14}N nuclei of certain immobilised proteins. We have also previously shown that osteoarthritic cartilage gives rise to smaller quadrupolar peaks than cartilage from healthy volunteers. However, the exact protein responsible for the quadrupolar peaks observed in cartilage samples is uncertain. This present work aims to determine the protein responsible for the quadrupolar peaks observed in cartilage and how these signals correlate with disease progression.
Methods: Cartilage samples from femoral heads and knee joints were obtained after consenting patients undergoing joint replacement surgery or above-knee amputation at NHS Grampian Hospitals. All work with human tissue was approved by the North of Scotland research ethic committee. First, a pilot study was conducted on a commercial FFC NMR scanner (Stelar s.r.l, Mede, Italy). We used a pulse sequence with a short acquisition time ($<$ 1 ms), and included 7 patients undergoing arthroplasty for osteoarthritis (OA) and 5 patients undergoing hemiarthroplasty for hip fracture. The hip fracture group had no clinical or radiological evidence of OA prior to the fracture being sustained and there was no macroscopic evidence of cartilage degeneration seen intra-operatively on femoral head inspection. In a second, larger study, we examined the cartilage from 50 patients with evidence of OA changes and 50 without using both long ($\sim$20 ms) and short acquisition times.
We also measured the quadrupolar signals from a variety of samples using both long and short acquisition times including: normal and osteoarthritic human cartilage; collagen preparations using distilled water and a commercially available porcine collagen sponge (Collatamp; Tribute pharamaceutical); glycosaminoglycan extracts from human cartilage (both liquid and lyophilised); and human cartilage which had been extracted of its glycosaminoglycan (GAG) content with 4M guanidinium chloride.
Results: Variations of the quadrupolar signals were visible between short ($<$ 1ms) and long (CPMG echo trains, 20 ms) acquisitions. Long acquisitions did not show any contrast between normal and diseased cartilage whereas significant differences in quadrupolar peak amplitude were observed using short acquisition time sequences ($3.6\ s^{-1}$ vs $2.2\ s^{-1}$, $p < 0.01$). We observed no quadrupolar peaks in glycosaminoglycan extracts from cartilage, whether liquid or lyophilised.
Preparations containing 50%w/w collagen showed quadrupolar peaks indicating that collagen may be the source of this signal in cartilage. However, the quadrupolar peaks observed using short acquisition times were only seen in cartilage which had not been extracted of its GAG content. This suggests that the quadrupolar peaks observed in cartilage are likely to be linked to the macromolecular collagen fibril network and are reduced with decreasing matrix integrity. Interestingly, the short-lived component of the FFC-NMR signal was not evident in GAG-extracted cartilage cores from healthy or diseased cartilage. However, the long-lived signal was unaffected and was not found to be correlated with OA changes.
Conclusions: GAG extracts of cartilage samples and analysis of collagen preparations have shown that GAGs are not likely to be the proteins responsible for quadrupolar peaks. The amide linkages on the backbone of collagen are therefore indicated as the source of quadrupolar peaks but the organisation of the macromolecular collagen network within the cartilage matrix also seems to play an important role in the interaction between collagen proteins and water protons.
When this matrix is disturbed, such as after GAG-extraction, the amplitude of quadrupolar peaks are significantly diminished and there is a loss of the short-lived FFC NMR signal. Therefore it is likely that the quadrupolar signals of cartilage report on the macromolecular

organisation of the collagen network within the cartilage. We are thus preparing methods to image this signal *in vivo* using FFC MRI and zero echo time acquisition techniques. We hope applications of this technique will allow new ways to non-invasively detect and stage osteoarthritic cartilage before morphological structural changes occur, as are appreciated with conventional MRI techniques.

103
RELEASED MACROPHAGE MARKERS AS PREDICTORS OF KNEE OA PROGRESSION

H.N. Daghestani †, C.F. Pieper ‡, V.B. Kraus †. † *Duke Inst. of Molecular Physiology, Durham, NC, USA;* ‡ *Duke Univ. Med. Ctr., Durham, NC, USA*

Purpose: Despite being one of the most common types of arthritis and a leading cause of disability worldwide, Osteoarthritis (OA) often goes undiagnosed until its later stages and little beyond palliative therapy is available. Biomarkers are particularly needed that can be used for prognosis or to monitor the efficacy of future therapeutics. There are many unanswered questions regarding the complex nature of joint tissue biology, but the role of innate immunity in the pathology of OA has become more evident in recent years. Specifically, synovial macrophage-produced cytokines drive aggrecanases, matrix metalloproteinases, and other destructive responses towards cartilage degradation. Moreover, the pattern of synovial fluid (SF) cytokines in knee OA is indicative of macrophage-mediated inflammasome activation. In a recent study, we showed that the quantity of folate receptor (FR-β+) macrophages in joints based on etarfolatide imaging correlated with radiographic knee OA. We also showed that sCD163 and sCD14, which are macrophage markers, correlated with inflammatory phenotypes and radiographic severity of OA. The goal of this study was to evaluate the effectiveness of using the macrophage marker, sCD163, as a prognostic tool for knee OA.
Methods: sCD163 was measured by ELISA in the synovial fluid of 86 subjects (128 knees) with knee OA from the Prediction of Osteoarthritis Progression (POP) cohort. The POP cohort provided baseline SF and serum samples and 3-year longitudinal follow-up to assess the predictive capability of sCD163 for radiographic OA structural progression based on either osteophyte formation or joint space narrowing. Models, using generalized estimating equations (to account for correlation within knees), were fitted with adjustment for age, gender, and body mass index (BMI), to evaluate associations of sCD163 with OA progression phenotypes. P values $\leq$0.05 were considered significant.
Results: Baseline synovial fluid sCD163 was associated with progression of knee OA; specifically SF sCD163 was associated with change in osteophyte severity ($p = 0.0107$), but not change in joint space narrowing. However, systemic sCD163 did not show any correlation with OA progression.
Conclusions: Shedding of CD163 from macrophages typically occurs in response to oxidative stress mediators and other inflammatory stimuli. The association of SF sCD163 with knee OA progression supports the growing inference that inflammation plays an important role in the progression of disease; this marker could prove useful for identifying a high-risk subgroup of knee OA patients. Furthermore, these results contribute to a growing literature identifying macrophages and macrophage-activation pathways as potential targets for new OA therapies.

104
DETECTING KNEE CARTILAGE THICKNESS CHANGE AT THREE AND SIX MONTHS

R. Buck †, M-P. Hellio Le Graverand ‡, M. Hudelmaier §,‖, W. Wirth ‖, F. Eckstein §,‖. † *StatAnswers Consulting LLC, Minneapolis, MN, USA;* ‡ *Pfizer Global Res. and Dev., New London, CT, USA;* § *Inst. of Anatomy, Paracelsus Med. Univ., Salzburg, Austria;* ‖ *Chondrometrics GmbH, Ainring, Germany*

Purpose: Previous work found large differences (> 10%/y and < -10%/y) in knee cartilage thickness in distinct subregions of the joint between annual visits and these were more frequent in osteoarthritis patients than in asymptomatic subjects. Given these large observed changes in as little as 12 months, it is likely that important changes in cartilage thickness may be detectable at even shorter intervals. The goal of this study is to explore the magnitude of femorotibial cartilage thickness changes in participants with radiographic osteoarthritis (ROA) and asymptomatic subjects over three and six month observation periods. Data are available at 12 and 24 month periods for the same subjects and make it possible, for the first time, to directly compare short, i.e., 3 month, versus long-term, i.e., 24 month, changes. We also present robustness studies to assess the potential impact of observed, but statistically insignificant, differences between ROA and asymptomatic knees in the measurement error of cartilage thickness change.
Methods: Coronal MR images (3 Tesla) were acquired in 145 women (71 with ROA, 74 without symptoms or ROA) at baseline, 3, 6, 12 and 24 months. Femorotibial cartilage thickness was determined for five tibial and three femoral subregions in each (medial/lateral) compartment at each visit. Rapid change in knee cartilage in individual regions was classified via false discovery methods and the frequencies of rapid change were compared between ROA and asymptomatic subjects. Ordered values of knee regional cartilage thickness change in ROA and asymptomatic cohorts were also compared. Differences between ROA and asymptomatic subjects in measurement error, as estimated from scan/rescan analyses, were not statistically significant, but standard deviations were slightly higher (average of 10%) in ROA subjects. To assess possible differences in variability between the ROA and asymptomatic knees, thickness change in asymptomatic subjects were normalized by a) increasing change values by up to 60% across all regions; or b) increasing change values by up to 60% in central and external regions, while increasing change values in remaining regions by 5%.
Results: Rapid cartilage thinning was found in 18.3% of ROA subjects at Month 3 and this increased steadily over time with 25.4%, 33.8%, and 40.8% at Months 6, 12, and 24. These frequencies were higher (p-value < 0.05) than observed in the asymptomatic cohort at all visits except Month 3. In contrast, The percentage of subjects in the ROA cohort with rapid cartilage thickening was also statistically larger than frequencies observed in the asymptomatic cohort, but were 28.2%, 21.1% and 33.8% for Months 3, 6, and 12 and dropped to only 7% at Month 24 showing no apparent trend over time. Even after inflating asymptomatic change by 15 to 45% the frequency of rapid progression was still significantly higher in ROA cohort than in asymptomatic cohort, except at Month 3; see figure. While the largest percentage of subjects with rapid progression (thinning or thickening) at a given visit was 59%, overall the percentage of subjects with rapid progression at some visit was 81.7%. Also, although less than 8% of regions were classified as having rapid progression they accounted for 34% (at Month 3) to 64% (at Month 24) of observed variation from zero in thickness change. Results from ordered values were similar, but not as robust to adjusting standard deviation.
Conclusions: Regions with rapid subregional (focal) progression are an important component of cartilage thickness change in subjects with osteoarthritis. This study reaffirms the method used for classification is reasonable for estimating rapid progression in an ROA cohort. The results show that rapid thinning and thickening is detectable at six months and may also be present at three months.

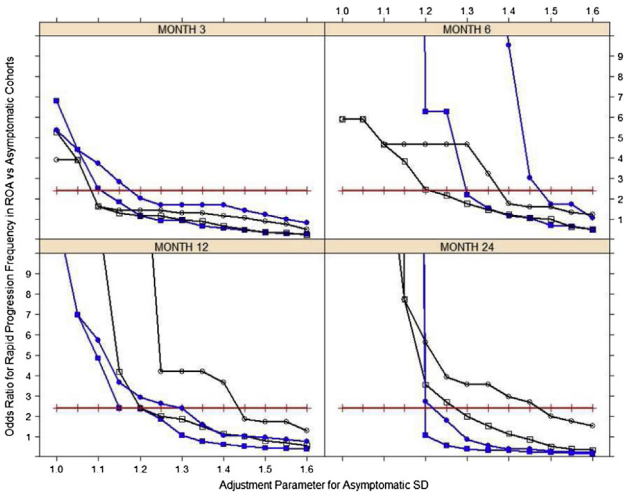

Figure 1. Odds ratio for Rapid Progression in ROA versus Asymptomatic Cohorts after various adjustments to underlying variability in Asymptomatic cohort.Black (open) = Thinning, Blue (closed) = Thickening, Red (dashes) represents $p = 0.05$ (approximately). square = SD adjustment equal for all regions, circle = adjustments differ between central and external regions and remaining regions.