When biomarker concentrations of all patient samples were plotted in chronological order of assaying, it appeared that there was a sufficient window of variation within a positively skewed distribution. In general there was no evident variation over time and between assays, except for two remarkable observations: For the sCOMP assay there was a clear difference in measured biomarker concentrations between the first 6 kits and last 8 kits. In the first kits measured biomarker concentrations were a 10-fold higher and showed significantly more variation than in the last kits. For the sC1,2C and sCS846 assays, there was a significant negative correlation between the measured biomarker concentration and sample order (chronologic/position) in the assay plates, repeatedly found for each of the assay plates.

Conclusions: Reliable biomarker measurement in this large sample set seems possible for the major part of the studied biomarkers. However, despite attempts to minimize variation, there are some striking challenges, presumably technical in nature. The remarkable observations in the sCOMP assay, and the sC1,2C and sCS846 assays need further evaluation (as is presently ongoing) before correlations with the clinical and radiographic data sets can be made.

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ANALYSIS OF 31 BIOMARKERS AND BIOMARKER FACTORS IN PRE-RADIOGRAPHIC AND RADIOGRAPHIC KNEE OSTEOARTHRITIS: RESULTS OF A POPULATION-BASED STUDY USING MRI

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1) To determine the association of 31 biomarkers with Synarc, Lyon, France; 5Boston Univ. Med. Ctr., Boston, MA

Purpose: 1) To determine the association of 31 biomarkers with pre-radiographic and radiographic symptomatic knee OA compared to symptomatic controls; 2) to determine if latent biomarker factors determined by principal components based factor analysis (PC-FA) are better able to distinguish OA stage compared to individual biomarkers. PC-FA, in addition to revealing potential underlying factors, may be useful to overcome problems of multiple comparisons and may help stabilize variance.

Methods: Subjects: 40-79 years, with knee pain were assessed, stratified by age decade and gender, in a cross-sectional population-based study and evaluated with MRI, xray and biomarkers. SF cartilage (MRC) defects (score 0-4) and x-rays (Kellgren-Lawrence [KL] grade 0-4) were read blinded. Subjects were classified as No OA (NOA) (KL<2, MRC=0), Pre-Radiographic OA (PROA) (KL<2, MRC>1) or Radiographic OA (ROA) (KL=2, MRC>1). Serum levels of matrix metalloproteinases (MMPs) 1, 3 and 9, tissue inhibitor of MMP, interleukins 1a, 1b, 4, 6, 7, 8, 10, 17a, tumor necrosis factor, c-telopeptide of type I collagen (CTX-I), adiponectin, resistin, etoxin, C-reactive protein, hyaluronic acid (HA) and other cytokines were evaluated. PC-FA was performed using individual biomarkers and ratios of markers previously measured, including urine c-telopeptide of type II collagen (uCTX-II), urine and serum type II and types I and II collagen cleavage neoepitopes (C2C and C1,2C respectively), c-propeptide of type II procollagen (CPII), 846 epitope, and urine n-telopeptide of type I collagen (uNTX-I). Multicategory logistic regression (adjusted for age, sex and BMI) was used to evaluate the association of OA category with each log transformed biomarker, biomarker ratio, and combinations of biomarkers. PC-FA, in addition to revealing potential unmeasured factors (determined by factor analysis) over individual biomarkers, is better able to distinguish OA stage compared to symptomatic controls; 2) to determine if latent biomarker factors (determined by factor analysis) over individual biomarkers or biomarker ratios resulted in similar findings. The strength of association with PROA and ROA was similar for individual biomarkers, biomarker ratios and biomarker factors.

Conclusions: In this population-based study, several biomarkers were significantly associated with PROA (MMP-9, uC2C and uC1,2C), and with ROA (uCTX-II, uC2C, uC1,2C). Discrimination of OA groups within this cohort was not improved using biomarker factors (determined by factor analysis) over individual biomarkers or biomarker ratios.

Results: Significant associations with PROA vs NOA were seen for MMP-9 (OR 1.60, 95% CI 1.00-2.57), uC2C (OR 2.34, 95% CI 1.34-4.09), uC1,2C (OR 1.99, 95% CI 1.24-3.19), and uC2C/sCPII ratio (OR 2.41, 95% CI 1.33-4.37). Risk of ROA vs NOA was significantly increased for uCTX-II (OR 2.97, 95% CI 1.45-6.08), uC2C (OR 2.28, 95% CI 1.23-4.22), uC1,2C (OR 2.08, 95% CI 1.21-3.56), uCTX-II/sCPII (OR 2.62, 95% CI 1.35-5.06) and uC2C/sCPII (OR 2.55, 95% CI 1.32-4.95). PC-FA identified 4 clinically interpretable factors, of which one factor (determined largely by uCTX-II, uC2C, uNTX-I, CTX-I, uC1,2C, and HA) was significantly associated with PROA (OR 2.08, 95% CI 1.04-4.14) and with ROA (OR 2.52, 95% CI 1.22-5.20). PC-FA with inclusion of biomarker ratios resulted in similar findings. The strength of association with PROA and ROA was similar for individual biomarkers, biomarker ratios and biomarker factors.

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TENASCIN-C LARGE, AN ELASTIC PROTEIN INDICATING JOINT DISEASE/INJURY IN HUMANS & PRECLINICAL ANIMAL MODELS

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Purpose: Tenascin-C (TN-C) is a modular, multifunctional, hexabrachion-shaped, elastic extra cellular matrix (ECM) glycoprotein with binding sites for other ECM proteins. It was discovered originally at the myotendinous junction and later found in the osteotendinous junction and superficial layers of articular cartilage. It plays a major role in cell adhesion & migration, and stretches several times its resting length due to its fibronectin type (FN) III domain. TN-C is abundantly expressed in musculoskeletal tissues during organogenesis and embryogenesis, its expression is very restricted in healthy tissues, and reappears as a high molecular weight splice variant in association with wound healing, inflammatory processes, or neoplasia. TN-C has been reported to be highly reexpressed in cartilage in diseased/injured joints. The objective of this study was to evaluate the potential of TN-C as a marker of joint disease/injury.

Methods: TN-C high molecular weight variants that include FN III A-D domains were specifically detected in synovial fluid (SF) samples by the Tenascin-C Large ELISA (IBL). Human SF from patients with end stage osteoarthritis (OA, 28), rheumatoid arthriti (RA, 8), anterior cruciate ligament rupture (ACL, 8), and knee-healthy reference subjects (Ref, 24) from NEBH/Northland Labs/NEBH/NDRI &Northland Labs, respectively were tested in the assay. Joint instability surgery was performed in male cross-bred hounds, right knee had a mini-arthrotomy and anterior cruciate ligament transected (ACLT), and the contralateral limb had a ‘sham’ surgery with a miniarthotomy but no generation of instability. At 1, 3 and 6 months post-surgery, 4-6 animals per timepoint were euthanized, and SF collected from ACLT/sham knees. Joint instability was induced in rats (n=4-6 per group) by median meniscal surgery in one knee with the other knee as a contralateral control. SF lavage samples collected at 1, 4, and 8 wks post surgery.

Results: TN-C levels were significantly elevated in SF from OA (7-fold), RA (5-fold), and ACL (3-fold) human patients as compared to reference individuals. There was a concomitant increase in TN-C levels in human OA cartilage (>60-fold) as compared to non-OA
cartilage extracts. The dog ACLT model of acute injury showed higher levels of TN-C in surgery knees at 1-month post surgery (73-fold) and 3-months post surgery (48-fold) as compared to control knees. Higher levels of TN-C were maintained in surgery knees 6-months post surgery in this dog model. In the rat meniscal tear model, there was a significant increase (40-fold) in TN-C in surgery knees at 1wk as compared to no surgery contralateral controls, and this increase was maintained at 4 and 8 wks, albeit with a smaller absolute difference from control.

Conclusions: The potential of TN-C Large as a unique biomarker of joint disease/injury has been demonstrated using synovial fluids from humans with various joint diseases and from preclinical animal models of joint injury. Being elastic, TN-C might play an important role in degenerative/regenerative processes where the normal biomechanical environment of musculoskeletal tissue is compromised by disease/injury. As a binder of several ECM proteins, release of TN-C could have a larger impact on the integral structure/function of other ECM proteins, and has the potential to be a marker of joint pathobiology and healing. Our preliminary results indicate that TN-C levels may be applicable to determining pharmacodynamic activity of chondro-protecting drugs in humans. Work is ongoing to study the levels of TN-C during degeneration in other joint tissues such as tendon. Understanding the functions of TN-C would provide insights for pharmacologic intervention of musculoskeletal diseases/injuries.

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DEGRADATION TO SYNTHESIS RATIOS OF TYPE II COLLAGEN BIOMARKERS IN SYNOVIAL FLUID AND SERUM IN THOROUGHBRED RACEHorses

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Purpose: Type II collagen biomarkers have shown promise in the study of osteoarthritis. CPII (cleaved C-propeptide of type II collagen) has been directly correlated with type II collagen synthesis. CTX II (crosslinked C-telopeptide fragments of type II collagen), C1,2C (neoepitope of types I and II collagen created after collagenase cleavage), and C2C (neoepitope of type II collagen created after collagenase cleavage) have been used to assess collagen degradation. The objective of the study was to compare type II collagen degradation to synthesis ratios in serum and synovial fluid (SF) from normal horses and those with osteochondral (OC) injury.

Methods: SF was taken from the carpal joints of 2 groups of Thoroughbred racehorses: (1) normal, adult horses > 3 years of age (2) OC injured horses 2-7 years of age undergoing arthroscopic surgery for removal of OC fragments resulting from racing injury. From group 1, serum was collected from 16 horses. SF was obtained from 10 middle carpal joints (MCJ), and 10 radiocarpal joints (RCJ). From group 2, serum was collected from 20 horses. SF was collected from 10 MCJ and 10 RCJ. SF was aseptically collected by needle arthrocentesis without lavage, centrifuged, and decanted. Commercially available ELISAs, were used to measure type II collagen markers (C1,2C, C2C, CPII, and CTX II). Differences between each group were evaluated using an unpaired t-test. P < 0.05 was considered significant.

Results: Concentrations of C2C; C1,2C; and CTX II were all significantly higher in SF from OC injured joints compared to normal joints (Table 1). Concentrations of CPII were also significantly higher in SF from injured joints compared to normal joints. Degradation to synthesis ratios in SF were significantly higher in OC injured carpal joints compared to normal joints for C1,2C:CPII and CTX II:CPII, but not for C2C:CPII. In serum, concentrations of C1,2C were significantly higher from OC injured horses compared to normal horses. Serum concentrations of CTX II were significantly lower from OC injured horses compared to normal horses. Serum ratios were significantly higher in horses with OC injured carpal joints compared to normal horses for C1,2C:CPII only. The ratio was significantly lower in serum from horses with OC injured carpal joints compared to normal horses for CTX II:CPII only.

Conclusions: Joint injury affects concentrations of type II collagen degradation and synthesis biomarkers and their ratios when compared to normal horses. In SF, C1,2C:CPII and CTX II:CPII ratios demonstrate that degradation predominates over synthesis when the joint is injured because the ratios are higher than normal joints. The serum C1,2C:CPII ratio suggests that there is higher degradation after injury compared to normal horses with no difference in the amount of synthesis. The CTX II:CPII ratio suggests that the synthesis of type II collagen stays steady with less degradation. However, increasing or decreasing degradation to synthesis ratios must be interpreted in light of the known effect of injury on biomarker concentrations in both SF and serum. Injury may cause increase in SF concentrations, but may at the same time cause an increase or decrease in serum concentrations. Thus, SF biomarkers may be more indicative of degradation or synthesis in a single joint than serum biomarkers.

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SYNOVIAL FLUID URIC ACID AS A MARKER OF JOINT TISSUE DEGRADATION IN OSTEARTHritis

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Purpose: Uric acid (UA) is constitutively present in normal cells, increased in concentration when cells are injured and released from dying cells. The products of cell stress and tissue damage may represent “danger signals” that function as endogenous adjuvants recognized by the immune system. UA has been identified as one of these principal endogenous “danger signals” released from injured cells. We sought to determine whether elevated synovial fluid (SF) UA might be a potentiating factor in osteoarthritis (OA).

Methods: Patients: A total of 159 participants were enrolled in the Strategies to Predict Osteoarthritis Progression (POP) study. Informed consent was obtained from all subjects and the entire study was approved by the Duke University IRB. Participants met...