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 majority 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


1) To determine the association of 31 biomarkers with pre-radiographic and radiographic symptomatic knee OA compared to asymptomatic 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: Study population: 40-79 years with knee pain were assembled, stratified by age decade and gender, in a cross-sectional population-based study and evaluated with MRI, xray and biomarkers. MR 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, 2, 4, 6, 7, 8, 10, 17A, tumor necrosis factor, c-telopeptide of type I collagen (CTX-I), adipoenectin, resistin, etoxan, 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 biomarker factor, incorporating stratum sampling weights. Standardized (i.e. per standard deviation change) odds ratios (ORs) were reported to enable comparison of the ORs across biomarker variables.

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 1.32-2.95). 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.

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.

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

L. Patel, W. Sun, S. Glasson, K.E. Georgiadis, E.A. Morris, P.S. Chockalingam

Wyeth Res., Cambridge, MA

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.

A large 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 upregulated 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 arthriitis (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 transection (ACLT), and the contra lateral limb had a ‘sham’ surgery with a miniarthrotomy but no generation of instability. TN-C was analyzed in 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