

by anterior cruciate ligament transection (ACLT) under general anesthesia, and 16 controls. Pre-operative radiographs of the femorotibial joints were performed to exclude animals with pathology. Rabbits were anesthetized and ACLT performed through a medial parapatellar incision. Serum was collected at -14 (baseline), 7, 14, 28, 42, 56, 70 and 84 days post-ACLT and aliquots were frozen at -20°C until analysis. Eight μ L of the diluted thawed serum (1:1 in 4% KSCN) was placed in 5 mm diameter circular wells on an adhesive-masked silicon microplate. Once dry, the microplate was mounted in a Fourier-transform infrared spectrometer (FTIR; Tensor 37, Bruker Optics, Milton, Canada) for the acquisition of IR absorbance spectra (400–4000 cm^{-1}). For each acquisition, 512 interferograms were signal averaged and Fourier transformed to generate a spectrum with a nominal resolution of 4 cm^{-1} . Spectra generated from each sample were averaged, differentiated and smoothed to resolve and enhance weak spectral features and to remove variation in baselines, using spectral manipulation software. Following preprocessing, partial least squares discriminant analysis (PLS-DA) was applied to the IR spectra at each time point using script written in MatLab[®]. Variables contributing to the separation of groups were selected using t-statistic, PLS weights and regression coefficients. Monte-Carlo methods were employed to determine the empirical significance of variables for separating groups, by randomly permuting the class membership 5000 times to obtain null distributions, and comparing the observed statistic for each variable with the null distribution. The PLS-DA optimal model was considered valid for Q2 (one minus the ratio of the prediction error sum of squares over the total sum of squares of the response vector) values significant at $P < 0.05$.

Results: Infrared spectroscopic spectra combined with a PLS-DA classification strategy, generated IR-based serum biomarker profiles that successfully discriminated between ACLT and control rabbits. Differences were significant at 7, 14, 28, 42, 56, 70 and 84 days post-ACLT. The distribution of t-statistic, PLS weights and regression coefficients varied significantly between time points, indicating the potential of the approach for monitoring the progression of osteoarthritis.

Conclusions: “Multiplexed” approaches have emerged in recent years for the identification and verification of novel OA biomarkers, employing genomic, proteomic and metabolomic tools. Although IR spectroscopy has been used for rheumatoid arthritis, this study is the first to employ IR spectroscopy and PLS-DA to screen serum for a biomarker signature reflective of OA, encompassing both known and unknown markers of disease. The ideal biomarker approach should be rapidly and simply performed, yield accurate results, identify progression, differentiate between early and late stages of disease, and use small volumes of easily accessible body fluid. The method described here achieves many of these ideals with fewer technical and less expensive infrastructure requirements than others. Nevertheless access to a larger number of well characterized biological samples is required for more extensive validation.

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BASELINE FIBULIN3 CONCENTRATIONS ARE ASSOCIATED WITH INCIDENCE OF CLINICAL KNEE OA AFTER 30 MONTHS IN OVERWEIGHT AND OBESE WOMEN

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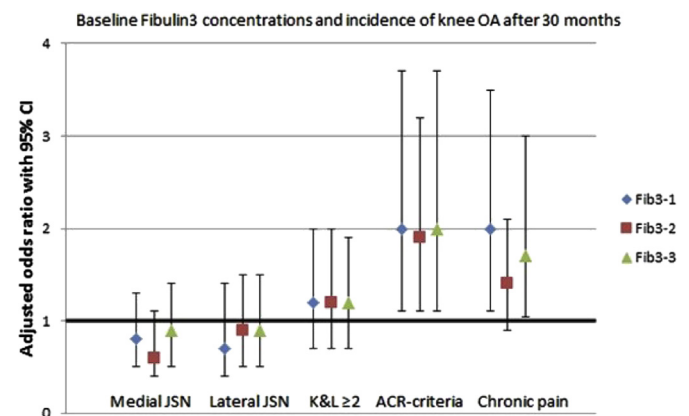
Purpose: Fibulin3 is highly expressed in osteoarthritic cartilage and inhibits angiogenesis and chondrocyte differentiation. It has been demonstrated to interact with the tissue inhibitor of metalloproteinase (TIMP)-3, which is a matrix bound inhibitor of matrix metalloproteinases (MMPs) and stimulates the expression of TIMP-1 and TIMP-3, but inhibits the expression and activities of MMP-2, MMP-3, and MMP-9. In the present study, the association between three Fibulin3 peptides identified by proteomics and the incidence of radiographic and clinical knee osteoarthritis (OA) in middle-aged overweight and obese women, free of radiographic and symptomatic knee OA at baseline, was tested.

Methods: Women between 50 and 60 years, with a BMI ≥ 27 kg/m², free of knee OA were recruited through their general practitioner. At baseline, physical examination including serum collection was performed and radiographs and questionnaires on knee complaints

were obtained. Using binary logistic regression, the association between baseline concentration of Fibulin(Fib)3-1, Fib3-2 and Fib3-3 and incidence of clinical and radiographic knee OA after 30 months of follow-up was tested, adjusting for age, BMI, and other potential covariates that were identified in multivariable regression analyses.

Results: Baseline serum samples and follow-up measurements were available for 242 women. Mean age was 55.9 ± 3.2 years, mean BMI was 31.6 ± 3.6 kg/m² and 70% was postmenopausal. All subjects were free of clinical and radiographic knee OA at baseline, but 24% had a unilateral K&L score of 1 and 33% bilaterally. Mild symptoms were present in 24% and 17% of the subjects, uni- and bilaterally respectively. Baseline concentrations of all Fib3 fragments were log-transformed for a normal distribution and z-transformed for uniformity reasons. Correlation coefficients for the baseline concentrations of the three Fib3 fragments ranged from 0.13 to 0.58. Neither of the concentrations of the three Fib3 fragments were associated with incidence of medial or lateral joint space narrowing ≥ 1.0 mm. or incidence of K&L grade 2. All three Fib3 fragments were associated with incidence of the clinical and radiographic ACR-criteria and Fib3-1 and Fib3-3 also with chronic pain at follow-up (see figure). When adjusted for the other Fib3 peptide concentrations, only Fib3-1 was significantly associated to the incidence of the ACR criteria (OR 2.5 [1.0-6.2]) and chronic pain at follow-up (OR 2.6 [1.1-5.9]).

Conclusions: Baseline Fibulin3 concentrations are associated to the incidence of clinical knee OA among middle-aged overweight and obese women. Therewith, they meet the criteria of a prognostic biomarker according to the BIPED biomarker classification for OA. Further validation of the Fibulin3 fragments seems warranted in order to better distinguish subgroups of individuals at increased risk for knee OA development.



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DETECTION OF CTXII IN THE RAT KNEE VIA MAGNETIC BIOMARKER CAPTURE

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Purpose: Osteoarthritis (OA) biomarker development often begins with rodent models; however, methods to recover OA biomarkers from rodent joints are not ideal. In large human joints, synovial fluid can be aspirated for biomarker analysis, but synovial fluid aspiration from a rat knee is limited by complex joint geometries and small fluid volumes. Instead, saline is often used to lavage synovial fluid from the joint, but lavage has limitations, including: 1) Lavage fluids dilute biomarker concentrations; 2) Mixing saline with highly viscous synovial fluid is not trivial and verifying fluids are well-mixed prior to removal from the rodent knee is practically impossible; and 3) Estimation of joint-level biomarker concentrations is susceptible to joint effusion. To address these limitations, our group has developed a magnetic nanoparticle-based technology to collect OA biomarkers from synovial fluid in situ, termed magnetic capture. Using polymeric particles that contain superparamagnetic iron oxide nanoparticles (SPIONs) and are surface-functionalized with a targeting molecule, OA biomarkers can be magnetically collected from a rodent knee without