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for non-signal knee with OV as predictors ($R^2 > 0.09$). Over 80% of the correlations between ΔThCtAB and ΔOutcome examined had p-values <0.05 at 1 and 3 Years, with most (13 of 22 at 1 Year and 12 of 20 at 3 Years) non-significant correlations observed with Incidence cohort. Correlation between ΔThCtAB at 1 Year and ΔOutcome at 3 Years was significant ($p < 0.05$) after adjusting for ΔOutcome at 1 Year for nearly all outcomes.
Conclusions: ΔThCtAB at 1 year helps predict ΔOutcome at 3 Years even after adjusting for ΔOutcome at 1 Year. The use of ordered values (OV) as predictors produced larger R^2 than use of subregion ΔThCtAB Results. Non-signal knees of Progression cohort had larger R^2 than signal knees.

156
ENHANCED COMP CATABOLISM DETECTED IN SERUM OF PATIENTS WITH ARTHRITIS AND ANIMAL MODELS THROUGH A NOVEL CAPTURE ELISA

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Purpose: There are several commercial enzyme-linked immunosorbent assay (ELISA) kits available for measuring COMP levels in human serum; however, these reagents lack specificity for degraded COMP fragments, which may hinder the usefulness of the test for the clinical monitoring of arthritis. The goal of this study is to develop a COMP fragment ELISA to measure levels of proteolytic COMP fragments in serum, and to determine whether detection of COMP degradative fragments will be a more sensitive biomarker of arthritis.
Methods: We generated a panel of monoclonal antibodies (mAbs) against COMP fragments and then characterized the mAbs domain specificity. We developed a novel capture ELISA for detecting COMP fragments and utilized this new test to measure the amount of degradative COMP fragments in patients with osteoarthritis (OA) and rheumatoid arthritis (RA). This test was also developed as an assay for the possible monitoring of the effects of interventions. This new test was then used to monitor OA progression in surgically induced OA animal models as well as the alteration in COMP fragment levels in response to biological treatments in collagen-induced arthritis (CIA) and TNF transgenic animal models.
Results: A panel of murine mAbs against recombinant COMP fragments was generated and one of these clones, 2127F 5B6, was identified as a mAb preferentially recognizing the COMP C-terminal degradative fragments. A novel COMP fragment ELISA was then developed using mAb 2127F 5 as a detection antibody. Compared with a commercial COMP ELISA kit that detected no significant difference in total COMP levels between symptomatic knee OA (SKOA) and non-OA control groups, a significant increase of the COMP fragments was noted in the serum of SKOA patients assayed by this newly established COMP ELISA. In addition, serum COMP fragment levels were well correlated with the Kellgren-Lawrence grade score in OA patients and the progression of surgically induced OA in murine models. Furthermore, the levels of COMP fragments in the serum of RA patients, mice with CIA, and TNF transgenic mice were significantly higher when compared with their corresponding controls. Interestingly, treatments with TNFα inhibitors and methotrexate led to a significant decrease of serum COMP fragments in RA patients. Additionally, administration of Atsttrin, a progranulin-derived engineered protein that binds to TNFR and has potent anti-inflammatory activity (Tang, et al, ***Science***, 2011 Apr 22; 332(6028):478–484), also resulted in a significant reduction in serum COMP fragments in several inflammatory arthritis mice models.
Conclusions: Using recombinant COMP fragments we have generated a series of mAbs that recognize different domains on the COMP protein. One of these antibodies that bind to the C-terminal portion of the molecule was then used to develop a novel sandwich ELISA capable of reproducibly measuring the levels of COMP fragments (COMP catabolism) in the body fluids of both arthritic patients and murine arthritis models. In addition, this new test provides a valuable means to utilize serum COMP fragments as a biomarker for monitoring the effects of therapeutic interventions on arthritis.

157
FEASIBILITY OF LARGE-SCALE ASSESSMENT OF BIOCHEMICAL MARKERS FOR OSTEOARTHRITIS: AN ANALYSIS IN CHECK

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Purpose: Osteoarthritis (OA) is currently regarded as a complex joint disease; multiple articular and peri-articular structures being involved, probably in a variable sequence and dominance between individuals. However, current literature on biochemical OA markers mainly concerns small-scale assessments of limited numbers of biochemical markers and is thereby discordant with this view. Instead, large-scale assessment of multiple biochemical markers is preferable. Large-scale assessment might be regarded unfeasible because of complex logistics, high financial burden, and the increased risk of inducing technical variability. This publication analyzes the feasibility of such a large-scale assessment of a wide spectrum of biochemical markers.
Methods: *Eleven biochemical markers* (CTX-II, COMP, CS846, PIIANP, C1,2C, CTX-I, NTX-I, OC, PINP, PIIINP, HA) were assessed in urine or serum samples of *1002 participants* (Cohort Hip & Cohort Knee; CHECK) by means of ELISA and RIA in an *academic laboratory* and *commercially-independent* setting. The assessment was necessarily performed by multiple technicians, in multiple assay plates, and during multiple days. Therefore, specific attention to minimizing technically-induced variability was vital. In addition to evaluation of several technical aspects, reliability of single instead of multiple assessments per sample was evaluated. A standard sample was repeatedly included in all assay plates throughout the assessment period to quantify technically-induced variability (3 levels: within plates, within all plates on a day, in all plates throughout the assessment period) and evaluate adjustment for between-plate variability.
Results: The additional variation that was introduced by *single instead of multiple sample assessments* was limited and considered acceptable in the context of logistics, scarcity of samples, and costs (triplicate assessment in a subset of samples showed high correlation between biochemical marker concentrations in the first sample and the mean concentration in triplets; r_s 0.841–0.996, P 0.000). Of the 11,000 assessments that were performed, 27 and 19 assessments showed concentrations that were *below or above the standard curve*, respectively. Re-assessment in adjusted dilutions induced (unpredictable) non-linear dilution effects and was considered inappropriate. Instead, artificial concentrations (0.8*minimum, 1.2*maximum concentration) were used. *Technically-induced variability* increased stepwise between the 3 levels (intra-plate < within day < total), but not statistically significantly. *Correction for technically-induced between-plate variability* on the basis of the standard sample assessment that was performed in all plates decreased the CV% of median biochemical marker concentrations between plates (mean ratio after/before adjustment 0.96; SD 0.3). Three of the 11 biomarkers showed *unanticipated results*. One could be re-assessed successfully, the other 2 remained unsolved, despite thorough analyses in consultancy with the manufacturer.
Conclusions: Large-scale biochemical marker assessments pose specific challenges. However, this study shows that large-scale assessment is feasible for the vast majority of the studied biochemical markers.
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158
BIOMARKERS OF KNEE OSTEOARTHRITIS: CORRELATION WITH OUTERBRIDGE CLASSIFICATION

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Purpose: To determine if an association exists between circulating concentrations of serum cartilage oligomeric matrix protein (COMP) and fatty acids, and degree of osteoarthritic degradation of the medial and lateral condyles of the tibial plateau graded according to the Outerbridge Classification, in patients undergoing total knee replacement (TKR) surgery. Identification of an easily accessible biomarker can aid in determination of progressive osteoarthritis and in drug development.
Methods: Sera was collected from 113 individuals presenting for TKR, representing both male (n = 51) and female (n = 62) patients with an

age range of 41–90 years. COMP levels were analysed by monoclonal antibody enzyme linked immunosorbent assay in duplicate. The fatty acid profile of collected serum provided information on over 57 fatty acids determined by Gas Chromatography, though this report focuses on the n-3, n-6 and n-9 types. The tibial plateau excised during surgery was imaged and both medial and lateral condyles were graded individually according to the Outerbridge Classification by two experienced orthopaedic surgeons.

Results: COMP values, serum fatty acid profiles and Outerbridge Classifications were compared, and significant positive correlations exist between Outerbridge Classification and total n-9 fatty acids ($r = 0.235$, $p < 0.05$), and Outerbridge Classification and 18:2(n-6) (linoleic-acid) ($r = 0.287$, $p < 0.05$). There was a significant negative correlation between Outerbridge Classification and 20:4(n-6) (arachidonic acid) ($r = -0.336$, $p < 0.01$). Significant correlations were also found to exist between the various fatty acid groups and also within the n-6 and n-3 groups, as would be expected.

Conclusion: Significant correlations between the degree of tibial condyle degradation and the level of overall n-9, linoleic-acid and arachidonic acid show these to be of potential use as biomarkers of disease progression. The finding of both positive and negative correlations between the Outerbridge Classification and n-6 fatty acids might be explained by the ability of mammals to convert linoleic acid to arachidonic acid. It is acknowledged that early-stage OA and controls are lacking from this data-set, and that Outerbridge Classifications are obtained at end-point OA.

159
ANALYSIS OF CARTILAGE BIOMARKERS OF TURNOVER AND AGING IN THE OSTEOARTHROPATHY OF ALKAPTONURIA

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Purpose: Alkaptonuria is a rare autosomal recessive condition resulting from deficiency of homogentisate 1,2 dioxygenase (HGD) causing inability to metabolise homogentisic acid (HGA). HGA levels in the body become elevated and show high affinity for collagenous tissues, primarily articular cartilages of load bearing joints, where it polymerises and deposits as a dark pigment; this process is ochronosis. Over time ochronosis causes rapid and early onset joint arthropathy, mimicking osteoarthritis (OA) in presentation. Little is known about how HGA and pigment affect cartilage matrix. This study aims to examine the effects of HGA and the pigment on matrix turnover of osteoarthritic AKU joints.

Methods: AKU ($n = 6$), OA ($n = 12$) and normal (NL from acute trauma, $n = 6$) articular cartilages were obtained as surgical waste with IRB approval. The soluble (GuHCl extracted) protein fractions were analysed for glycosaminoglycan (GAG) content quantified by the dye-binding assay with dimethylmethylene blue, total Cartilage Oligomeric Matrix Protein (T-COMP) with monoclonal antibodies (mAbs) 16F12 and 17C10 (kindly provided by Dr. V Vilim) and for the new deamidated-COMP (D-COMP) with mAbs 61A12 and 17C10. GAG was normalized to total protein of each sample and D-COMP was analyzed as a ratio to T-COMP. Statistical analysis consisted of ANOVA with the Tukey-Kramer HSD post-hoc test using JMP (SAS, Cary) software.

Results: Analysis of extractable GAG revealed a highly significant association with disease/cartilage type: AKU, OA, or NL (ANOVA $p < 0.0001$). AKU cartilage shows a significantly lower amount of extractable GAG compared to both NL and OA ($p < 0.0001$). There was no significant difference between OA and NL although OA tended to be lower than NL. GAG was also significantly different by disease type for both hips ($p < 0.0001$) and knees ($p = 0.02$). The ratio of D/T COMP was also significantly associated with disease/cartilage type (ANOVA $p = 0.017$). The ratio of D/T COMP in AKU vs NL and vs OA was significantly different ($p = 0.028$ and $p = 0.016$ respectively), demonstrating that the extractable COMP from AKU cartilages is quite aged. The D/T ratios were not significantly different between NL and OA. There were no associations of GAG or D/T COMP with patient age.

Conclusions: These studies are the first to analyze biomarkers of cartilage matrix turnover in AKU tissues. Previous evidence shows cartilage matrix in AKU does not turn over in a normal fashion, appearing impervious to proteolytic enzymes. These results provide further insights into the severely deranged metabolism of AKU cartilages. Although AKU manifests as a rare OA phenotype, it is more extreme with much lower GAG and much higher D/T COMP ratio indicative of severe degeneration and absence of a repair response. Although we cannot be certain at this time that GAG cannot be extracted due to being bound into the ochronotic matrix, the significantly higher D/T COMP ratio in the extractable protein clearly indicates cartilage matrix in AKU is not turned over in a normal manner, or even in a manner consistent with the pathological turnover seen in OA. The clinical significance of these results is important as any potential therapeutic strategy would need to be given prior to pigmentation occurring in the cartilage matrix of these individuals on the basis of these results and would need to involve stimulation of a cartilage reparative response.

160
THE DYNAMICS BETWEEN CYSTEINE PROTEASES AND METALLOPROTEINASES IN HUMAN OA CARTILAGE

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Purpose: Currently, treatment of osteoarthritis (OA) is either pain relieving treatments or terminal surgery. Thus, the discovery and development of new treatment strategies is needed. Proteases, such as metalloproteinases and cathepsins, play a major role in the net degradation of cartilage, and are therefore a natural target for drug development. However, studies have suggested that inhibition of one protease leads to a compensatory effects from another. We aim to characterize the dynamics of the different proteases in normal and OA cartilage. Furthermore, by testing the ability of a library of proteases to degrade cartilage explants, and by inhibiting one type of proteases, we aim to find the proteases responsible for collagen type II and aggrecan degradation and possible compensatory effects by other proteases.

Methods: We used an array of metallo- and cysteine-proteases (MMP-1/3/7/9/13, ADAMTS-4/5, Cathepsin-K/B/D/L/S) to degrade the matrix from human OA cartilage, independent of chondrocytes. Human OA cartilage explants were metabolically inactivated by freeze-thaw cycles and incubated individually for 9 h with the proteases ± MMP-inhibitor (GM6001) or cysteine-inhibitor (E64), in an MMP- or cysteine-buffer optimal for respective proteases.Furthermore, we cultured normal bovine cartilage explants and murine femoral heads with the pro-inflammatory cytokines, OSM + TNF-α ± inhibitors for 22 days. Biomarkers of cartilage-degradation; AGNxII (aggrecan degradation), CTX-II (type II collagen telopeptide fragment), CIIM (type II collagen C-terminal helical fragment) and NBCII (type II collagen inter helical fragment) were used to evaluate effects of the proteases and inhibitors on cartilage and femoral head explants.

Results: MMP-3 and -7, and ADAMTS-4 and -5, all increased aggrecan degradation (AGNxII), in human metabolic inactivated OA cartilage incubated in buffer optimal for MMPs. GM6001 inhibited this release of the aggrecan fragments. We did not see any release of AGNxII fragments by MMP-1, -9 and -13. In addition, cathepsin-S increased aggrecan degradation, whereas Cathepsin-K, -B, -D and -L decreased the degradation of aggrecan. The cysteine inhibitor, E64, increased aggrecan degradation in both MMP and cysteine buffer, and did not abrogate the effects generated by the cathepsins on aggrecan. In the MMP buffer, collagen type II degradation was only degraded with MMP-7 (CTX-II) and MMP-9 (NBCII), which both could be inhibited by GM6001. In the cysteine buffer, none of the cathepsins degraded collagen type II, whereas GM6001 increased the degradation as assessed by all three markers (CTX-II, CIIM and NBCII). Catabolic induced bovine cartilage increased aggrecan (AGNxII) and collagen type II degradation (CIIM), which was inhibited by GM6001 but increased in the presence of E64. Catabolic induced femoral heads increased collagen type II degradation, which was inhibited by GM6001, but we did not see any increase in presence of E64.

Conclusions: The presence and role of endogenous proteases probably depend on the stage of OA, as we did not observe the same compensatory effects in normal cytokine-stimulated cartilage as in OA cartilage when incubated with E64. Thus more investigation on this topic may have significant value for development of new treatments for OA. Furthermore, our data indicate that proteases are very dependent on extracellular factors, as different buffers had significant different influence on the outcome.